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FROM INDIVIDUALS TO GROUPS AND BACK: INTERACTIONS BETWEEN INDIVIDUAL VARIATION IN BEHAVIOUR AND GROUP PERFORMANCES IN HOUSE SPARROW

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Abstract

Stark differences in individual behavioural responses are a well-known feature of animal diversity. Even within a social group many distinct strategies coexist, and this variation has been recently found out to play a significant role in resource exploitation, social learning and various collective behaviours. How the entire group performs can therefore depend on various characteristics, all linked to its members' behaviour and the relationships that connect them. While there are theorical analyses focusing on the consequences of systems where individual variation and group environment influence each other by interacting through feedbacks, most of the assumptions and the effects hypothesized by these models have rarely been experimentally studied in controlled conditions.

My aim was thus to test if the interactions between variation in behavioural strategies and the social environment might have an effect on the performance of single individuals within the group and of the group itself. I approached the complex issue by performing a series of experiments on a captive population of House Sparrow (*Passer domesticus*). I started by examining the effect of predation on a potential proxy for life-history traits, i.e. relative telomere length, and the connection of the latter to various behavioural traits. In the second experiment I investigated if social connections between individuals within a group might have an influence on the measurable benefits obtained by its members. Expanding on this topic, I questioned if previous familiarity with a companion might be a factor strong enough to affect exploration of a novel environment, or if the presence of any conspecific would allow social facilitation. This investigation was also a necessary step to take before testing any group-related effect, as an attachment to one own's group was a necessary prerequisite for the next experiment. In fact, I then assessed the performances of two flocks facing each other over limited resources. While there have been studies comparing groups' performances, it has rarely been taken into account how two groups would interact together, even if in the natural environment groups sharing resources are quite common. For my last experiment I focused on one of the most well-studied dichotomous behavioural strategy, i.e. the leader/follower dynamic. I decided to investigate this variable strategy not only during exploration but also in a different situation, one of the most crucial in the life of any animal: the attack of a predator and the split-second reactions to it.

The first experiment showed no influence of predation on telomere dynamics: relative telomere length however changed with successive samples. In the second experiment results showed that

social connections affected the rate of discovery of a novel food source, with individuals more closely connected to the first feeder foraging before the others. In the following experiment I discovered that averaging over familiarity and sex the presence of a companion strongly increased exploratory behaviour. Familiarity with the companion however had an influence on the social exploration of female sparrows: they explored faster and consumed more resources only when accompanied by a familiar individual.

In the experiment where two groups of sparrows faced each other we found out a that group membership affected the outcome of the confrontation, as the group that foraged first ended up almost always consuming more of the limited resource. This meant that whoever shared the group with a risk-averse individual, one that foraged first at a novel food source, gained benefits regardless of their own behavioural traits. Finally, individuals that led movements during exploration were followers during a simulated attack and vice versa, showing that social positions in this species are context dependent.

In conclusion, these experiments shed light on interactions between variation in behavioural strategies and the social environment. Our results underscored how various assumptions made by theoretical models on the potential role of complex feedbacks between individual traits and the performance of the entire group were indeed correct and testable in a controlled setting. In the future, in order to keep investigating how social animals trade off costs and benefits in crucial contexts such as novel environment exploration and predator attacks it will be imperative to account for the role of diversity within the social environment.

Abstract (Italian)

Le forti differenze nelle risposte comportamentali individuali sono una nota caratteristica della diversità animale. Anche all'interno di un gruppo sociale coesistono varie strategie, la cui variabilità svolge un ruolo significativo in molti processi importanti per il gruppo. Le prestazioni del gruppo dipendono quindi dai tratti comportamentali dei suoi membri e dalle relazioni che li uniscono. Varie analisi teoriche si sono incentrate su come la variabilità degli individui e l'ambiente di gruppo possano influenzarsi a vicenda, ma la maggior parte delle assunzioni e delle conseguenze ipotizzate sono state raramente testate sperimentalmente. Il mio obiettivo è stato quindi di studiare se l'interazione tra la variabilità nelle strategie comportamentali e l'ambiente sociale potesse avere un effetto sulle prestazioni degli individui e del gruppo stesso. Ho proceduto nell'affrontare questo problema complesso eseguendo una serie di esperimenti su una popolazione in cattività di Passero domestico (*Passer domesticus*). Ho iniziato esaminando l'effetto dello stress predatorio su un potenziale *proxy* per tratti di *life history*, vale a dire la lunghezza relativa dei telomeri e la loro correlazione con vari tratti comportamentali sociali. Ho quindi studiato se i legami sociali tra individui all'interno di un gruppo potessero influenzare le risorse ottenute dai suoi membri. A seguito di questo mi sono chiesto se la familiarità con un altro individuo potesse influenzare l'esplorazione di un nuovo ambiente. Era necessario portare a termine questo studio anche perché l'attaccamento al proprio gruppo era un prerequisito necessario per l'esperimento successivo. Ho infatti valutato le prestazioni di due stormi assieme, con risorse limitate. Per il mio ultimo esperimento mi sono concentrato su una delle duplici strategie comportamentali più studiate, ovvero la dinamica *leader*/*follower*. Ho deciso di studiarla non solo durante l'esplorazione, ma anche in una delle più cruciali situazioni nella vita di qualsiasi animale: l'attacco di un predatore e le successive reazioni di fuga. Il primo esperimento non ha mostrato alcun effetto della predazione sulle dinamiche dei telomeri; la loro lunghezza relativa tuttavia è cambiata con i successivi campionamenti. Nel secondo esperimento ho mostrato come i legami sociali abbiano influenzato con quanta rapidità venisse scoperta nuova fonte di cibo, con individui più strettamente legati al primo a mangiare che si nutrivano prima degli altri. Ho poi scoperto che la familiarità con la compagna ha avuto un effetto sull'esplorazione delle femmine di passero: hanno esplorato più velocemente e consumato più risorse solo se accompagnate da un individuo familiare. Nell'esperimento in cui si confrontavano due stormi di passeri abbiamo visto che l'appartenenza ad un gruppo piuttosto che ad un altro ha influenzato il risultato dello "scontro": chiunque fosse nello stesso gruppo di un individuo *risk-taker*, i.e. dell'individuo che per primo si era nutrito presso la

nuova fonte di cibo otteneva benefici indipendentemente dal proprio comportamento. Infine, gli individui *leader* durante l'esplorazione sono stati *follower* durante l’attacco simulato e viceversa, dimostrando che le posizioni spaziali sociali in questa specie dipendono dal contesto. In conclusione, questi esperimenti hanno fatto luce sulle interazioni tra la variabilità nelle strategie comportamentali e l'ambiente sociale. I nostri risultati hanno sottolineato come varie ipotesi fatte da modelli teorici sul ruolo potenziale dei feedback tra i singoli tratti e le prestazioni dell'intero gruppo fossero effettivamente corrette e verificabili in un ambiente controllato. In futuro, al fine di continuare a studiare in che modo gli animali sociali compensano costi e benefici in contesti cruciali come l’esplorazione dell'ambiente e gli attacchi dei predatori, sarà importante tenere conto del ruolo della diversità all'interno dell'ambiente sociale.

Chapter 1

General Introduction

Causes and selective pressures of social behaviour

Most moving animals throughout their lives interact with conspecifics at least at one life-stage, if only for mating. As such, associations of conspecifics range from random encounters, convergence at food sources or other locations of interest, to a number of more stable relationships, which might be the structuring backbone of social groups. Depending on the focus, the study of 'groups' of animals can thus refer to "breeding units, social networks, neighbourhoods, populations, and communities" (Farine et al. 2015). For the purposes of this thesis I defined 'groups' (or more specifically 'flocks') as social units composed by animals that actively seek and follow conspecifics at least during a part of their life cycle, i.e. that stay together more often that it would happen by chance. Social groups are thus cohesive groups of individuals whose organized relationships impact both survival and fitness (Alexander 1974). They do not encounter each other by chance, for example near clumped food sources: the continued coexistence of different individuals causes the development of stable relationships between group members. There are many possible selective advantages in joining groups of conspecifics as defined above, and in fact such grouping behaviour is very common in many different animals. In birds for example flocking behaviour is not a feature of a few taxa, but it is commonplace and has been acquired many times during birds' evolutionary history (Beauchamp 2002). The main reason behind the success of social behaviour is usually decreased predation pressure (Sorato et al. 2012; Thiollay & Jullien 1998) thanks to various distinct effects. In the presence of many individuals for example general vigilance increases (many-eyes effect), even if the amount of time that each individual spends looking out for potential dangers decreases (Beauchamp 2008). Another advantage of grouping concerns the moment when the predator strikes: escaping animals can count on the 'confusion effect', which reduces the predator chances to catch a prey during an attack. In short, anti-predation advantages granted by group-living are as diverse as the predators' and preys' life histories, specializations and strategies.

Another fundamental advantage of social behaviour is considered to be social learning, hereby defined as the transmission of information throughout the members of a group (Swaney et al. 2001; Laland 2004; Dukas 2013). Social learning helps avoiding a costly trial-and-error process when discovering food sources, potential stressors etc. Individuals can get the chance to internalize information and react better and faster in different situations without having to personally investigate them (Dukas 2013). Generally larger groups might also be better problem solvers and social learning is considered extremely important particularly in vertebrates, even insofar as

creating ‘cultural differences’ in different populations of animals (Laland 2004; Aplin 2015). Information about food and water sources, but also roosting places, threats and other features can in fact spread very quickly in a social group and give an important edge to its members (Aplin et al. 2013).

However, living in groups comes at some costs, as there are stressors and challenges peculiar to social living that can strongly influence individual fitness. Many selective pressures are considered density-dependent or at least affected by the number of conspecific individuals in an area (Zàvorka et al. 2015). Competition is a primary example, as it increases in groups, in certain cases even causing individuals lacking in dominance, aggressiveness or experience to suffer worse fitness than if they were alone (Vehrencamp 1983).

However, for species living in social groups usually gains are thought to outweight the costs (Alexander 1974). Social living results thus in a continuous trades-off of advantages and disadvantages, which in turn shape the selective pressures active on the members of the group (Alexander 1974; Harrison & Whitehouse 2011). An animal is then posed to evolve differently if it moves, forages and explore socially, as the other individuals become part of its environment and shape the selective pressures the individual is under (Odling- Smee et al. 2003; Harrison & Whitehouse 2011). In other words, as a consequence of actively seeking and spending time alongside conspecifics in all social species we can find morphological, physiological and behavioural traits selected by life in groups. Decreasing costs and increasing the benefits within a group is crucial for the survival and fitness of any animal: the social environment is thus a strong force of selection. For example, the presence of other conspecifics might relax a selective pressure: animals in a group can invest more time in other behaviours, while animals alone might need stay alert longer (Elgar 1989; Beauchamp 2008). Thus, group-living causes also the development of group-specific traits and strategies: passerine birds are one of the many taxa that developed alarm calls, even if their direct consequence could be an apparent decrease of the fitness of the alarming bird (Zahavi 2008). Another example of in-group coevolution is the synchronization of shoaling formation of fishes or flying flocks of birds (Landeau & Terbourgh 1986), a feat obtainable only with traits specific for that behavioural response. A species used to live and move socially would also develop traits that increase the amount of information that could be exchanged, as forms of communication or copying strategies (Mateos-Gonzalez & Senar 2012; King et al. 2015). The ‘following’ behaviour of House Sparrow (*Passer domesticus*) can be considered an example of the latter, as some individuals consistently follow others to food sources (Tóth et al. 2014): this behaviour has clearly its basis in the social environment. In a study on Rock Sparrow (*Petronia petronia*) it was even hypothesized that the carotenoid-based yellow breast patch could be

interpreted by conspecifics as an honest signal of health and access to food sources, thus carrying information about the trustworthiness of the foraging bouts initiated by the individual (Tóth & Griggio 2011; Mateos-Gonzalez & Senar 2012).

Traits selected in the social environment can thus all be considered outcomes of co-evolution processes. Many of these traits decrease costs of social living (e.g. signals of dominance to avoid physical confrontations) or increase benefits (e.g. collective hunting). Some of these traits represent emergent properties, as their fitness advantage is apparent only when the individual is not alone, otherwise it is nil. Many examples of collective behaviour fall into this category, as simple interaction rules followed by each individual create complex and precise patterns of movement and decision-making. Other group-specific traits might not influence the general survival of the collective but might increase the fitness of the single individual within a group, e.g. the capability to scrounge at food sources or aggressively overtake resources. Finally, many social traits are present with different variations within a same group, creating groups containing a strong diversity in phenotypes and behavioural responses.

Within-group diversity and the social environment

Phenotypic variation and its maintenance have always been a central topic in the study of evolutionary biology and behavioural ecology. In behavioural ecology the variability in behavioural traits and strategies has been extensively studied, as it has long been known that within the same population individuals show consistent differences in many behavioural traits (Carere & Eens 2005). As behaviour can be interpreted as an immediate and plastic reaction to stimuli, the presence of systematically consistent different suites of responses seems counterintuitive, as one or more might be non-adaptive (Duckworth 2006). Some of these behavioural traits have been referred to as "personality" traits, i.e. traits that show repeatable differences in behaviour across time and contexts in a population (Réale et al. 2007); a few have also been found to be inheritable (Bouchard & Loelinn 2001). Among the processes cited to explain the persistence of such variability are variation in selective pressures (Carvalho et al. 2013) and frequency-dependent processes (Dingemanse & Wolf 2010); another reason might be the presence of physiological and life history constraints, limiting the chance for some behavioural responses to be decoupled from some other. (Duckworth & Sockman 2012). In this last case there might be a 'state' – defined as any feature of the organism affecting the balance of costs and benefits of behavioural actions (Wolf & Weissing 2010) – that might influence the relative advantages of implementing a behavioural strategy. State has thus a wide definition, as variation in genetic qualities, early life influences, condition or even experience

might mark a difference in the state of individuals. For example, an individual lacking size or strong armaments might find itself at disadvantage if it tries to climb the ranks of its group through aggressive confrontations; another behavioural response might be more suitable to its pre-existent state – i.e., being weaker than most conspecifics –. In other words, the same strategy employed by two individuals might result in different payoffs, due to the intrinsic difference between the two. Thus, behavioural responses might be evolutionarily linked to an individual state, which in turn might be inherently stable (Dingemanse & Araya-ajoy 2015), influenced by early life experience (Stamps & Groothuis 2010) or even labile and only stabilized thanks to positive feedback with the behavioural response (Sih et al. 2015). An example of such a labile state might be individual experience: if an animal is knowledgeable about the location of food sources it might be rewarded if it moves on its own, while if it is not knowledgeable it might gain more benefits from following other individuals.

In recent years the role of social behaviour has come into focus regarding the problem of polymorphism maintenance, with many studies shifting towards the investigation of the relation between phenotypic variation in the population and group-linked processes that might maintain it. In social species conspecifics with differing behaviour, morphology and physiology coexist and elect to stay together over time. This might of course lead to differential benefits depending on which individuals compose the group, and which groupmates they interact with. To increase survival and fitness in a social group (i.e. to decrease costs and increase benefits) the first step might thus be to select which individual to associate with – which creates variable social relationships (Silk et al. 2010; Firth et al. 2015). However, as every individual in a group is able to choose, to influence the other individuals' choices and to react to them, the traits of every individual come into play in all events linked to the social environment. In short, the influence of the social environment on the selective pressures active on each individual strongly depends on the identity and phenotype of all their groupmates. The success of each individual is thus influenced by the characteristics of all other individuals. Thus, once individuals have been brought together by the advantages linked to group-living, they then adapt to life with each other, even if not all of them are bound to have the same success within a group.

A possible theoretical model for how this might happen is the "social niche hypothesis" (Bergmüller & Taborsky 2010; Montiglio et al. 2013). In a social environment to avoid costly competition social conflict might cause individual character displacement, with animals repeatedly using one alternative strategy over another. Choosing a behavioural strategy might have as consequence the exposition to different environmental influences, i.e. different selective pressures. In other words, repeated use of one strategy might increase experience for that strategy and even,

through positive feedback, select or stabilize traits that increase the success when using that strategy. Moreover, individuals might be "forced" to use a different strategy than the optimal, thus increasing their chance to specialize in that role through character displacement. Finally, as selection might favour consistency in behaviour for decreased social conflict, the role adopted by each individual might affect selection on its phenotype. Therefore, this mechanism sees the social environment acting as an active force increasing diversity among its members.

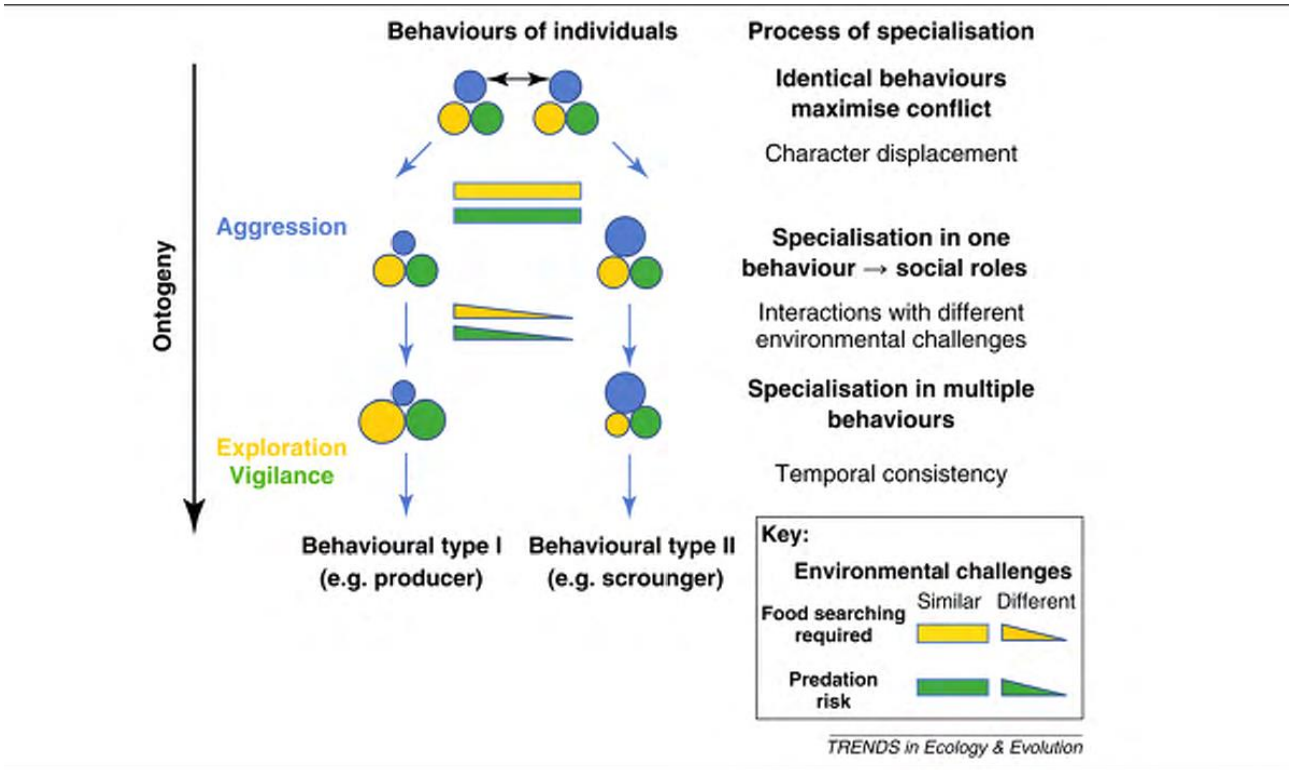

Figure 1. Social niche specialization alongside ontogeny: three circles represent one individual, each circle (and its relative size) represents the 'intensity of behaviours'. Other shapes represent environmental challenges. (from Bergmüller & Taborsky 2010).

Among-group diversity and group phenotypic composition

In a seminal paper on Trends in Ecology and Evolution, Damien Farine and collaborators (2015) spotlighted a very interesting way to see how individual differences within a group could influence the fitness of the members. In fact, not only individuals but also groups might have different success depending on the characteristics of their components. For example, a certain mixture of individuals sporting determined characteristics might be important to increase the overall speed of resource discovery, the synchronization of movement, or the appropriate response to environmental

conditions (Pruitt & Goodnight 2014). Thus, there might be an influence of the group phenotypic composition (GPC) on individual fitness. In turn, this allows not only for some phenotypes, but for some combination of phenotypes to remain stable in the population. If any process involving a group is ultimately shaped by the group own composition, then the relative benefits of each individual in the group might be linked both to their position within the group and with the success of the entire group. In other words, some intra-group differences might persist not because each phenotype net gain is equal within the group, but because thanks to the coexistence of different phenotypes the group itself has some advantage.

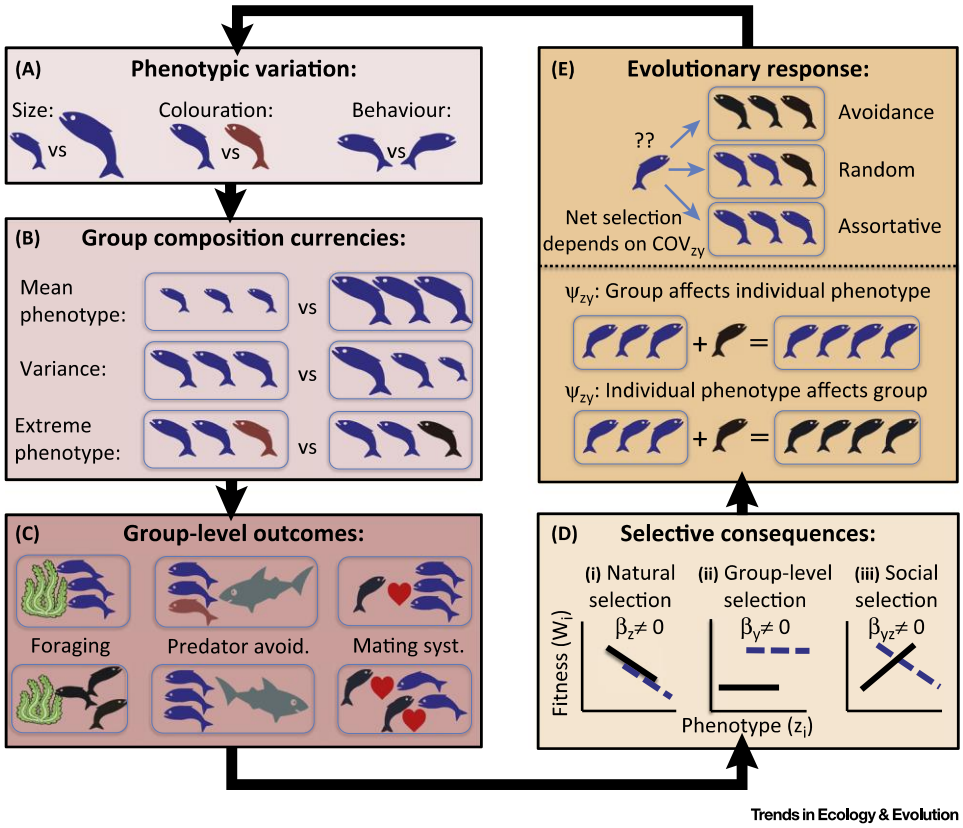

Figure 2. Graphical rappresentation of the evolutionary implications of Group Phenotypic Composition (GPC). (from Farine et al. 2015).

These are only some of the theoretical frameworks emerging from the study on the interaction between social environment and individual characteristics. However, most of them self-admittedly have gaps in evidence-based knowledge. Many of the referred processeses, while straightforward to imagine, have rarely been observed or tested. While theoretically situations like those presented might force some phenotypes to be assorted alongside others via selection or change in group

membership, everything would strongly depend on the rules of assembly of groups, which are different in every species. Thus, in order to study this exciting and novel field, the first thing that is needed are experiments proving that in controlled settings we might prove the value of the assumptions the theoretical work is based on. Afterwards, the second step is to test if there are actually measurable differences in individual gain and losses caused by to alternative strategies, social positions or social environment.

Study species

House Sparrows *Passer domesticus* (Linnaeus, 1758) are well-known passerines strongly associated with rural and urban habitats. They are fairly small (approx. 30 grams) and they belong to the Passeridae family. The species probably originated from Middle East and has followed the expansion of human settlements throughout history (Sætre et al. 2012). Thanks to their success as human commensals they are now widespread in most of the world, even if in some countries their numbers have recently dwindled for unknown reasons (De Laet & Summers-Smith 2007). It is one of the most abundant birds on earth, and given its almost cosmopolitan range the House Sparrow dwells in many different human-altered habitats, usually avoiding dense, thick undergrowth or much-open terrains, except for seasonal foraging in cornfields. It shows extensive morphological and physiological differentiation across its range, in agreement to Bergmann's rule, even in those continents where it has established only in the last century, as America. They are markedly opportunistic and although primarily granivorous, they are known to feed on a variety of food sources, strongly depending on which one is more available in different locations at different times of the year (Liebl & Martin 2014). House Sparrows are dimorphic: the male is boldly patterned, exhibiting a dark-streaked rich brown back, a black eye-stripe and a prominent grey crown. The most intensively studied male plumage trait is, however, the black bib (throat patch) sported on the throat and on the upper part of the breast (chest). It is a melanin-based trait of variable size, whose function appear to be that of "badge of status", since it was often found to be associated with dominance hierarchy: recently, however, the role of the breast-patch as a badge of status has been challenged by a thorough meta-analysis (Sanchez-Tojar et al. 2018), which highlighted how the generally accepted result was possibly more connected to publication bias than to actual evidence-based findings. The females are dull-coloured, presenting a buff supercilium and pale bill, as are juveniles, which resemble adult females and moult adult plumage starting with September.

The House Sparrow is a highly social species and a model species for avian sociality. In non-breeding season House Sparrows travel and forage in mixed-sex flocks with up to 10-30 individuals, which usually also roost together. Kinship can be an important component of a flock, as it is believed to affect various different aspects of House Sparrow social behaviour (Tóth et al. 2009).

It is a semi-colonial breeder, whose nests are often found clumped together: the start of the breeding season depends on the latitude, but it can last a few months and it is not unusual for central European sparrows to produce more than two broods each year. It is monogamous and usually faithful to the partner for life, but extra-pair paternity is also present. The House Sparrow forages usually on the ground, with initiator individuals that lead the foraging bout and can actively emit assembly calls to their companions (Elgar 1986). On the other hand, agonistic interactions often do occur in both sexes: flocks are characterized by a clear if not very steeped hierarchy, with dominant individuals having privileged access to resources.

In novel environment exploration experiments, and more generally in behavioural and personality tests Great Tits and Zebra Finches (*Taeniopygia guttata*) are two of the most-used model birds. However, we decided to use the House Sparrow instead, because of two main characteristics we were interested about. Firstly, the House Sparrow, as an opportunistic human commensal, is a species that often depends on clumped, novel and ephemeral food sources. In a context of rapid-changing environments, this is a key selective pressure: House Sparrows survive thanks to the knowledge of their spatial surroundings and of the food sources within it (Dukas 2013). Moreover, it has been already demonstrated that House Sparrows use their social environment to obtain clues about unknown food sources, and can perform complex foraging tasks to obtain food (Liker & Bokony 2009). They also tend to feed on novel food sources more when closer to the borders of their range, where they generally exhibit lower neophobia (Martin & Fitzgerlad 2005; Liebl & Martin 2014). House Sparrow also sometimes feed on dispersed and non-divisible food sources, but in such cases when an individual finds them it was found to emit less assembly calls. Thus, when competition is not a significant factor (clumped, overabundant and divisible food sources) both leader and follower House Sparrows actively promote following bouts (Elgar 1986).

The second characteristics that made the House Sparrow so interesting for this experiment was its adaptability and success: its range is still expanding in urban and human-modified habitats all around the world (Martin & Fitzgerlald 2005; Liebl & Martin 2012). Its success has been attributed to various factors, as for example its ecological niche of human commensal, behavioural flexibility but also a weak sense of neophobia. As an invasive bird that thrives into anthropic, and thus rapidly changing habitats, neophobia and low capacity to explore could be counter-selective, at least on the

border of its range. Finally, as selection requires variability, it was demonstrated that House Sparrows at the outer limits of their range have different levels of glucocorticoids receptors (GC) and different explorative behaviour than their conspecific residing in places where the population has been long established (Liebl & Martin 2013). The possibility that the House Sparrow is being selected for novel environment exploration at the border of its range makes it a perfect model for studying how social environment and individual behavioural traits might interact.

Aim of the study and methodology

The main purpose of my study was to investigate with a series of experiments the interactions between diversity in individual behaviour and the social environment, with a focus on their potential for influencing the performance – a proxy for survival and fitness – of entire social units. In order to deepen the knowledge about this topic I adopted an experimental approach, testing various hypothesis in a captive population of House Sparrows. The controlled setting of a captive population was fundamental to focus my experiments on certain relationships of cause and consequence, as pinpointing and describing the effects of the selected variables might turn out to be much more difficult in a wild population. In general, my hypotheses concerned i) the influence of different compositions or characteristics of the groups on the performance of individual House Sparrows', ii) the influence of the characteristics of each individual House Sparrow on the performance of its flock-mates, i.e. of its entire group. In order to do this, I first had to test some assumptions often made when studying this kind of interaction. This was necessary both to proceed in my research and to know if what I would find had biological significance. In chapters 3-5 – I laid the foundation necessary to the study of my chosen topic on the study species. This meant clarifying some crucial aspects of its behavioural ecology with respect to group living, testing my hypothesis in order to further proceed with the experiments.

Outline of the study

Chapter 2 details an experiment that examined one of the most important questions concerning the presence of behavioural diversity within a group, i.e. if this diversity is linked to some life-history or physiological trait. In particular we decided to investigate both if Relative Telomere Length (RTL) might be linked to specific behavioural traits, and if some environmental stressor could change the House Sparrows physiological state. Telomeres, the 'caps' at the ends of eukaryotic chromosomes, have been found in various studies to decrease with age and stressful early life

conditions, insofar as being used as proxies to predict the presumptive life span of individuals. However, no study has ever addressed the role that predation might have in shaping their dynamics, and evidence linking telomere shortening rate or length with behavioural traits are still scarce. Thus, we ran a three-parted experiment to investigate these questions: during the first part we measured RTL in a cohort of same-age individuals, alongside a number of individual and social behavioural traits. During the second part of the experiment we manipulated the sparrows' perception of predation threat by exposing half of them for 20 days to a mounted predator. Finally for the third part we collected three more measures of their RTL, one just after the end of the experiment and two later in the year. As glucocorticosteroid dynamics are thought to be strictly connected to telomere shortening rate we also tested whether before and after the experiment ther might be a change in corticosterone stress responses.

I investigated in **chapter 3** one of the basic assumptions made in social behaviour studies, i.e. that variable social relationships within a group (or, in this case, a flock) have consequences on the individual benefits of the members of the flock. Social network studies have highlighted how within each group individuals tend to have very different relationships with their group mates, i.e. interactions among individuals are non-random. Variable relationships might be caused by differences in behavioural traits, dominance rank and many other individual characteristics. The possible consequences of this differences in social connections however have been investigated far less. In fact, many studies focusing on the advantages and disadvantages of a social group treat it as homogeneous, without considering the deep implications that differences in the interactions' rate and direction might have on the transmission of information, discovery of resources etc. Starting from this theoretical framework we investigated whether social connections could affect the discovery (latency to forage) from hidden food patch in an artificial group of House Sparrows.

In **chapter 4** and **5** I focused on a crucial context in the life of House Sparrows, i.e. the social exploration of novel environments, testing with a single experiment two more assumptions that I considered fundamental in order to study social behaviour in this species. The experiment I performed had House Sparrows in three different social contexts (alone, with a familiar companion and with an unfamiliar companion) in a vast room of which they had no previous experience – i.e. open-field test –, in order to study i) the influence of a conspecific companion on the exploration of a novel environment ii) how familiarity with the companion could affect the exploration of a novel environment. While a positive influence (in terms of increased foraging and decreased alert time) of the presence of conspecifics during exploration had already been observed in fish and mammals, curiously results pointing in this direction had been lacking in birds. As one of the main assumptions in later chapters needed to be how the presence of conspecifics led to better

performances in social groups of this species, I focused on this first question in **chapter 4**. In this chapter I thus analysed the results by pooling together data from both unfamiliar and familiar social contexts. In **chapter 5** I investigated whether, aside from the difference between individuals exploring alone and individuals exploring with a companion, there might be an effect of previous familiarity with the companion. As flocks of House Sparrows might encounter each other while foraging or moving, studying group-specific features and benefits holds interest only as far as individuals are able to recognize and be affected by membership of one group over the other. In short, if familiarity among group-members has an influence on their performances then it might be more relevant to study a group as a meaningful unit. While both these questions deserved a proper focus in their treatment and discussion – and are thus treated separately in two chapters of this thesis – I decided to publish the results in one single paper (Chapter 5), as I wanted to avoid data overlapping.

In **chapter 6** I investigated if differences in group performances could be observed when two groups faced each other, competing for limited resources. In previous experiments on this topic group performances had been tested by measuring each group separately and then confronting their scores. However, groups encountering each other is commonplace in many taxa, both in species with population structures based on fission-fusion dynamics and species with stricter rules membership. The performance of one group might thus be influenced by the presence of another, and the two groups might gain or lose benefits in a completely different way when tested alone than when competing against each other. I was thus interested to discover if some group characteristics could play a role in a situation when small flocks were forced to compete with each other over with limited resources. I investigated the competition over exploitation of resources between two flocks via a novel experiment design; as far as I know this was the first experiment performed in a controlled setting investigating how two groups of animals would perform when facing each other. In particular, I hypothesized that the familiarity among flock members or their behavioural traits might influence the exploitation of the food sources, with a greater exploitation by one group leading to a decreased exploitation in the other.

In **chapter 7** I created an experiment on another aspect of the relation between behavioural strategies in a group, i.e. on how the behavioural role that each individual preferably employ might be context-dependent, and with it any possible benefit that the role might provide. In House Sparrow individuals moving together (e.g. exploring or foraging) either lead movements (leaders) or follow who does (followers). In this species this dichotomous behavioural strategy has been found to be consistent across time and linked to various individual behavioural characteristics; leaders are often risk-taker and explorative individuals, while followers are shy and risk-averse.

Moreover, it has been recently experimentally demonstrated that individuals leading movements might incur in greater predation risks (but still less than if they were alone) (Ioannou et al. 2019). However, as risk-averse individuals are thought to gain specific benefits when moving with risk-taking individuals, for the latter the association might bring a selective advantage in a different context. Interestingly, while there have been studies focusing on collective movement in condition of high predation risk (Ioannou et al. 2017), no research has ever investigated if during the escape flight from an attack – a crucial time for any animal – individuals in a group also employed different behavioural strategies. Studies on individual response to potential threats have discovered differences in the flight initiation distance from the attacker, with risk-averse individuals taking off first. We hypothesized that pairs of individuals would consistently adopt either leader or follower strategy during a social escape flight caused by a simulated attack; moreover, the ones behaving as leaders during exploration would be followers during a simulated attack and vice versa. Continuing after the experiment detailed in chapters 4-5 I designed a novel experimental set up in order to test if social position within a pair were context-dependent. The experiment is outlined in **chapter 7**.

In **chapter 8** I summarize the main discoveries of the thesis and briefly discuss their implication when synthesized together.

Chapter 2

Predation threat and telomere dynamics in captive House Sparrow

In preparation

PREDATION THREAT AND TELOMERE DYNAMICS IN CAPTIVE HOUSE SPARROW

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INTRODUCTION

Telomeres are highly conserved repetitive sequences of DNA that cap the ends of eukaryotic chromosomes, alongside with a number of proteins. Without the telomeres-restoration work of telomerase telomeres shorten during each round of normal somatic cell division. This happens because the RNA polymerase cannot fully replicate the lagging strand. Telomeres thus shelter the coding sequences from attrition, limiting also cell replicative potential: when telomeres reach a certain length, cells stop dividing and enter a state of replicative senescence. Accordingly, evidence has been accumulating indicating that both telomere length and rate of telomeres attrition could be predictive of individual lifespan, sometimes more accurately that age itself (Bize et al. 2009; Heidinger et al. 2012; Boonekamp et al. 2014). As even in the same age class there is a striking variation in telomere length, it was hypothesized that environmental factors could partially be responsible for telomere loss, particularly during early life (Angelier et al. 2018; Smith et al. 2016). Stressful conditions or energetically expensive events such as reproduction are in fact known to increase oxidative stress (Selman et al. 2012), which is thought to be a key mechanism involved in increasing telomere attrition (Kim & Velando 2015). In the last two decades various stressors and trade-offs that have been found to be linked to faster telomere attrition, such as sibling competition (Boonekamp et al. 2014; Nettle et al. 2013;), breeding and parental effort (Sudyka et al. 2014; Reichert et al, 2014a, but see Beaulieu et al. 2011; Sudyka et al. 2016), low quality habitats (Angelier et al. 2013) and urban noise (Meillère et al. 2015).

One of the most important stressors in the life of every animal is predation: chronic predation threat is known to have numerous negative effects on prey individuals (Lima, 1998; Slos & Stoks 2008), such as strongly affecting corticosterone levels (Silverin, 1998; Cockrem & Silverin, 2002). Glucocorticosteroid hormones in high concentrations for their part have been linked to greater oxidative stress and down-regulation of telomerase activity (Choi et al. 2008; Costantini et al. 2011;

Young et al. 2016). However, the relation between perceived predation threat and telomere dynamics has rarely been investigated (Olsson et al. 2010; McLennan et al. 2016): the stress and the increase of vigilance brought by a constant predator threat would cause higher level of glucocorticosteroid hormones, which might increase rate of telomere attrition in young individuals.

Individuals with different telomeres length have also been demonstrated to vary in their strategies according to life history theory: birds with shorter presumptive lifespan showed greater impulsivity and lesser fear of the unknown (Bateson et al. 2015) and fishes with shorter telomeres tended to be more fecund (Selman et al. 2012) and bolder (Adriaenssens et al. 2016). It would be in fact counter-selective if an individual with a shorter life expectancy behaved in the same way of another with a high chance of surviving longer. Moreover, certain behavioural strategies exact a greater cost in terms of oxidative stress and stress hormones levels: for example, it has recently been found that dominant Meerkats (*Suricata suricatta*) have greater telomeres attrition than subordinates (Cram et al. 2018). House Sparrows (*Passer domesticus*) have been shown to have a finely tuned social system and great variability in personality and tendency to interact with others. Differences in telomere length or rate of telomere shortening could be thus linked with differences in boldness and/or dominance.

We thus hypothesized that i) telomere length would decrease in an environment with high perceived predation threat ii) dominant individuals would have greater telomere attrition, as dominance is often linked with impaired antioxidants and elevated exposure to stress hormones (Creel 2011; Cram et al. 2015). In order to test these hypotheses we recorded various individual behavioural variables in a captive population of house sparrows: we also collected blood samples in order to measure relative telomere length before and after repeated presentations of a predator. In order to focus on the effect of the experiment and to rule out a possible constant and gradual telomeres erosion we also collected blood sample after one month and after one year. As the level of glucocorticosteroid hormones is one of the main actors of increased telomeres attrition during chronic stress, we also measured corticosterone stress response before and after the repeated presentation of the predator.

METHODS

Housing and study subject

We conducted this study during the 2018 breeding season at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine), in Vienna, Austria. All 72 House Sparrow

individuals (36 males and 36 females) were born during the previous breeding season (2017) and reared by their parents in the same aviaries where they were born. We used birds all of the same age in order to decouple telomere length from possible age effects.

Eight weeks before the start of the tests (five weeks before the start of the observations), individuals were moved from their original aviaries to the experimental ones, where they would remain for the rest of the experiment. These aviaries were outdoor enclosures measuring 2 x 3.9 and 2.6 m high, each equipped with a feeder (a metal bowl on a wooden pedestal 1.2 m from the ground) small pine trees and branches. All aviaries were provided with food (a mixture of millet, canary seeds, wheat, sunflower seeds, protein-based mash, apple slices and millet sprays hanging from the branches) and water (in a dish on the ground) ad libitum. We moved 3 males and 3 females in each aviary, creating 12 social groups in 12 different aviaries: no birds tested in the same aviary were siblings. Six aviaries were assigned to the 'predation' treatment while 6 aviaries were the 'control'. Treatment aviaries were acoustically and visually isolated from control aviaries; each aviary was equipped with a cardboard box on the ground. Each bird was made recognizable by coloring a small part of their plumage with a marker (Marabu®, see Tóth et al. 2017).

Experimental design – before predator presentation

Before the experiment began every individual was measured and, using a method akin to that used by Carvalho (2013), we evaluated its boldness by assessing the number of movements, rate of pecks and vocalization in the hand. After three weeks of habituation to the new social groups we started monitoring the aviaries in order to collect baseline behavioural observation of individual social behaviour. Starting at 08:00 ± 15' and finishing at 11:45 ± 15' each day, we collected 7h30' of observation per aviary divided in two days. Observations were made from a mimetic tent outside of the aviary, positioned 15' before the start of the observation. We recorded the following variables: i) the number of aggressive interactions each individual was involved in, and if they resulted in a win or in a loss; ii) the number of following bouts, i.e. any movement where two or more birds departed within 3 seconds of each other from the same perch and arrived to land within 3 seconds of each other in another perch, and if they were leader (they initiated the movement) or followers; iii) the number of times an individual flew alone, in order to correct the previous measure for individual activity.

Experimental design – predator presentation

Once the observation period was over the predator exposition part of the experiment started. We exposed the 'predation' aviaries to a simulated predation threat for 20 consecutive days. We used two mounted sparrowhawks (*Accipiter nisus*) as predators. We presented one of the two sparrowhawks to every aviary once a day, between 06:30 and 13:00. The hour of presentation was randomized among all aviaries. 45 minutes before each presentation an experimenter entered the aviary and, without letting the sparrows see the mounted predator, they put it into the cardboard box on the ground and attached the pulley. The predator was raised with the pulley, which allowed it to spring fast towards the ceiling as if the predator was taking off from within a cardboard box (which was always placed on the ground). The predator was kept swaying next to the ceiling for 15 seconds and then it was lowered back to the ground. After the presentation an experimenter entered the aviary and recovered the predator: the procedure was then repeated for the next aviary. In the 'control' aviary an experimenter entered twice at an interval of 45 minutes, as to make sure that the only thing that differed between the two set of aviaries was the predator presentation. Birds in these aviaries are fed by caretakers, who enter inside to deliver the food: thus the experimenter entering inside, while it might have had an influence on the immediate behaviour of the birds, was probably not enough to elicit a stress response (Huber et al. 2017).

DNA extraction

We collected blood from each individual four times throughout the entire experimental procedure. Each time 100-200 µl of blood were collected from the brachial vein into a 2 ml microtube containing 500 µl of Queen Lysis Buffer (Seutin et al. 1991) and then stored frozen at -20° C. The blood samples were collected: i) two weeks before the start of the experimental treatment, just after the observation period ('first sampling') ii) right after the experimental treatment ('second sampling') iii) one month after the experimental treatment ('third sampling') iv) twelve months after the first sampling ('fourth sampling). We extracted Genomic DNA with the EuroGOLD Blood DNA Mini Kit PLUS (EuroClone S.p.A., Pero, MI, Italy). We placed approximately 100 µl of each sample into a microcentrifuge tube alongside 200 µl of Lysis Buffer and 25 µl of Proteinase K solution 20 mg/ml. The following step was mixing and incubating at 60 °C for 10 min, and after that we added 350 µl of Binding Solution to the lysate. The resulting solution was stripped of the residual clot and transferred into a PerfectBind DNA Column and centrifuged at 11.000 g for 1 min. We then discarded the flow-through liquid and washed three times the solution, with 400 µl of Wash Buffer I once and with 600 µl of Wash Buffer II twice. We then added 100 µl of Elution

Buffer, and DNA elution was performed by centrifuge at 6.000 g for 1 min. The extracted DNA samples were stored a temperature of -20 °C.

Relative telomere length (RTL) measurement

We used a protocol developed by Cawthon (2002) to measure the Relative Telomere Length (RTL from now on) of each sample via Real-Time quantitative PCR (qPCR). As DNA samples of reference we used pooled samples from multiple individuals unrelated to the experiment. As internal control we chose Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Telomere primers sequence were (from Criscuolo *et al.*, 2009):
Tel1b (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3').
Tel2b (5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3').

GAPDH primers sequence were (from Eastwood *et al.*, 2018):
GT2-GAPDH-forward (5'- CCATCACAGCCACACAGAAG-3').
GT2-GAPDH-reverse (5'- TTTTCCCACAGCCTTAGCAG-3').

qPCRs were performed in a BioRad C1000 TouchTM Thermal Cycler coupled to a BioRad CFX384 TouchTM Real-Time PCR Detection System, using BioRad Hard-Shell® 384-Well PCR Plates sealed with BioRad Microseal® 'B' seals. Each well volume was 20 µl, including 4 µl of 5x HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne, Tartu, Estonia), 5 ng of genomic DNA and 200 nM of both forward and reverse primers. qPCR profile was 95 °C for 12 min (to activate the polymerase), followed by 40 cycles of 95 °C for 20 s, temperature of annealing for 18 s (58°C for telomere primers, 56°C for GAPDH primers) and 72°C for 1 min, followed by the melting curve cycle consisting of increments of temperature of 0.5°C for 5 s from 65 to 95°C. We used CFX ManagerTM Software (Bio-Rad, version 3.1,) to visualize data and to correct and analyze Baseline and Cq we used LinRegPCR software (version 2017.1, Ruijter *et al.*, 2009). Cq correction across plates were done using a common threshold for Cq calculation. Thresholds were obtained for each amplicon using Cq means of the two reference DNA samples from all the plates (TEL log threshold=2,333; GAPDH log threshold=2,189).

Relative telomere length was obtained, considering mean plate efficiency, as a ratio between the quantity of telomere (variable among individuals) and the quantity of control gene (present in the

same constant amount in individuals, as it is a single-copy gene), referred to the calibrator measurements, following the equation proposed by Pfaffl, 2001:

$$RTL = \frac{E_{TEL}^{CqTEL(calibrator)-CqTEL(sample)}}{E_{GAPDH}^{CqGAPDH(calibrator)-CqGAPDH(sample)}}$$

Where:

- E_{TEL} = mean efficiency of telomere plate
- E_{GAPDH} = mean efficiency of GAPDH plate
- *CqTEL(calibrator)* and *CqGAPDH(calibrator)* = mean Cq value of the average of the two reference DNA samples in the plate, respectively for telomere and GAPDH
- *CqTEL(sample)* and *CqGAPDH(sample)* = mean Cq value for the triplicate of each sample in the plate, respectively for telomere and GAPDH.

Handling protocol and hormone assay for CORT levels

In order to detect a change in circulating CORT levels during a stress response we sampled birds before and after the experimental treatment following a well-established stress protocol based on capture/handling induced stress (Wingfield & Ramenofsky 1999). After starting the stopwatch, we entered an aviary and started capturing the birds with hand-nets. Due to time constraints we collected only one blood sample from every individual (and then a second one after the experimental treatment). All blood samples were either taken i) less than 3 minutes after entering the aviary complex; ii) 15 minutes after entering the aviary complex; iii) 30 minutes after entering the aviary complex. As there were 6 birds in each experimental aviary, each aviary provided approximately 2 measurements per time slot. Thus, while we could not provide individual-specific stress-response CORT concentration curves, we could provide averaged CORT concentration curves for the two treatment groups. The hormone assay to quantify plasma levels of total Corticosterone used a commercially available $CORT^{125}$ I radioimmunoassay kit (catalogue number 07-120102; MP Biomedicals, Solon, OH, USA). The protocol followed was that of the company alongside the modifications detailed in Washburn and Millspaugh (2002). All samples were analyzed in duplicates.

Statistical analysis

All data were analyzed using R version 3.4.1 (R Core Team, 2014). We analyzed changes in telomere length with Generalized Linear Mixed Models (GLMM) implemented with the 'glmer' function in package 'lme4' (Bates et al. 2015). The four individual RTL measurements were the dependent variable; we fitted as categorical fixed effect 'sampling' (first sampling before the experimental treatment, second sampling right after the experimental treatment, third sampling one month after the experimental treatment, fourth sampling 1 year after the first sampling), 'treatment' (exposed to predator or control group), 'sex' and all of their interactions. As random effects we fitted 'individual' (to account for repeated measures) and 'aviary' (each of the 12 bird aviaries, i.e. their social environment). Interactions found to be non-significant were excluded from the model. We square-root-transformed the dependent variable and modelled it with gamma distribution (log link). Estimates and significance of fixed effects were obtained using the 'Anova' function within the 'car' package (Fox & Weisberg, 2011). To differentiate among three or more groups we performed post-hoc analyses of contrasts with the 'lsmeans' function within the package 'lsmeans' (Lenth, 2016) applying the Tukey's method adjusted for multiple comparisons. We obtained dominance rank using the package 'AniDom' to compute wins and losses during aggressive interactions (Sánchez-Tójar et al. 2017), and we calculated leadership as the percentage of following bouts performed as leader, adjusted for individual activity (number of movements performed alone). We ran a full correlation array between all behavioural variables (number of aggressive interactions, percentage of wins in aggressive interactions, number of following bouts, percentage of following bouts performed as leader, general number of movements) and the RTL measurement obtained in the first sampling. Finally, we analyzed the hormone assay result using GLMM with CORT concentration as the dependent variable and 'time slot', 'sex', 'treatment' and 'sample' (before and after the experimental treatment) as categorical fixed effects. The dependent variable was square-root-transformed and modelled with gamma distribution (log link).

RESULTS

Of 72 individuals at the start of the experiment only 56 could be sampled all 4 times, across one entire year of experiments. The other individuals either returned one or more inconclusive samples or were excluded from the experiment due to sickness/death. Relative telomere length was not repeatable across all four samples ($R = 0.06$, $p = 0.19$); however, it was weakly but significantly repeatable if the first sample was excluded ($R = 0.17$, $p = 0.018$). We did not find any effect of predation threat on the relative telomere length of house sparrows ($df = 1$, $\chi^2 = 0.005$, $p = 0.945$), while females showed a non-significant tendency to have shorter relative telomere length than

males (df =1, χ^2 = 3.3257, p = 0.068). Relative telomere length was strongly affected by the time of sampling, with the sample collected before the start of the experiment showing much higher relative telomere length than the others (df =1, χ^2 = 59.003, p < 0.0001, Figure 1). All samplings after the first one (second, third and fourth) did not differ significantly from each other (Post-hoc Tukey: all p > 0.071, Figure 1). No interaction was found to be significant. Relative telomere length was not correlated with any behavioural measure (all tau < 0.232, all p > 0.128).

The concentration of corticosterone increased as expected during the stress response, starting from baseline level and peaking after 30 minutes. Both the baseline level and the stress response were lower after the experimental period (df =1, χ^2 = 19.410, p < 0.0001, Figure 2); while this happened for sparrows both in the control and in the predation aviaries, the decrease was greater in the latter (significant interaction between the sampling and the treatment, df =1, χ^2 = 7.472, p = 0.006, Figure 2). The effect of the treatment on the response was, however, non-significant (df =1, χ^2 = 2.778, p = 0.096).

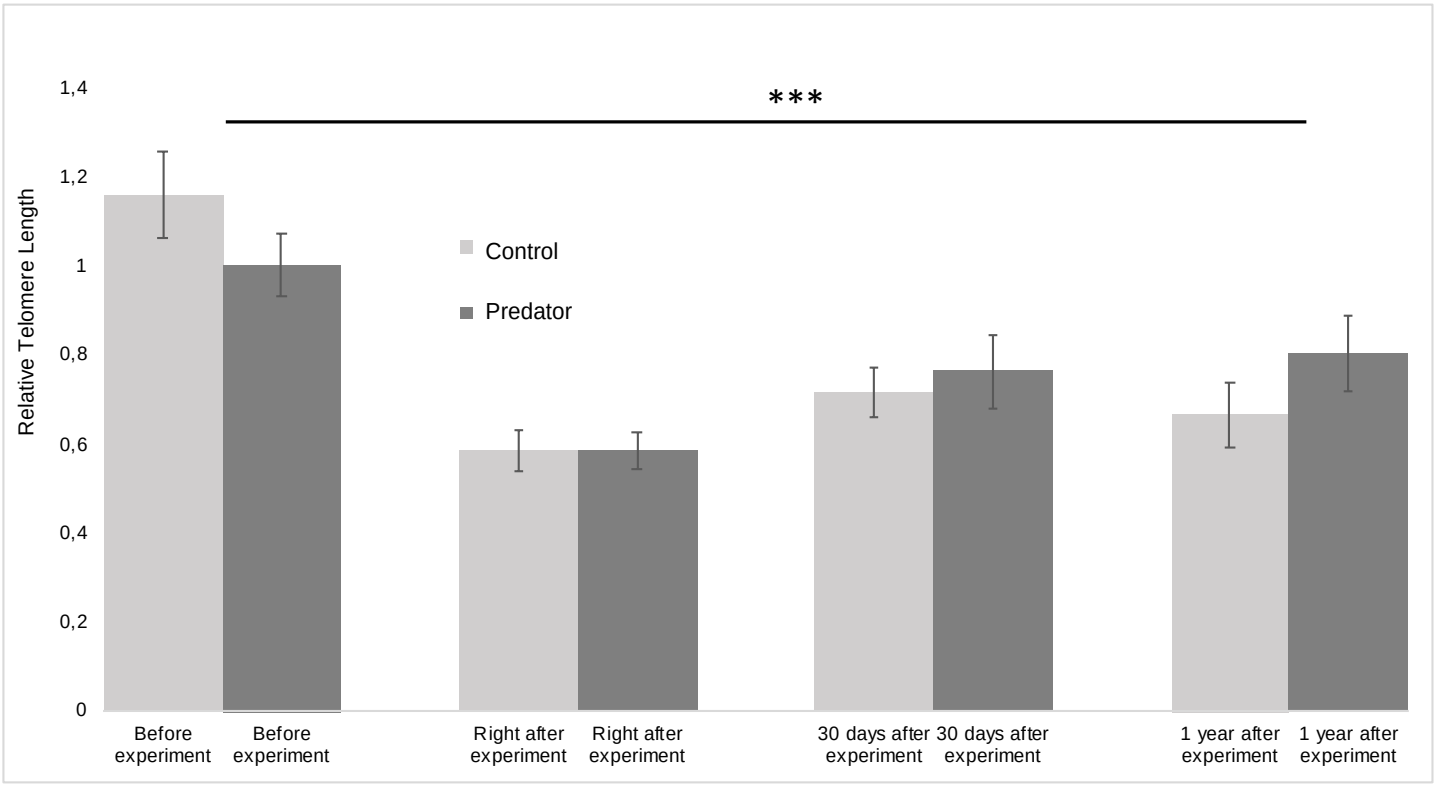

Figure 1. Effect of sampling and experimental treatment on RTL in house sparrows. While there were no differences between control (light grey) and predation (dark grey) treatments, RTL was higher in the first sampling. Means and standard errors of the mean are shown. *P < 0.001.

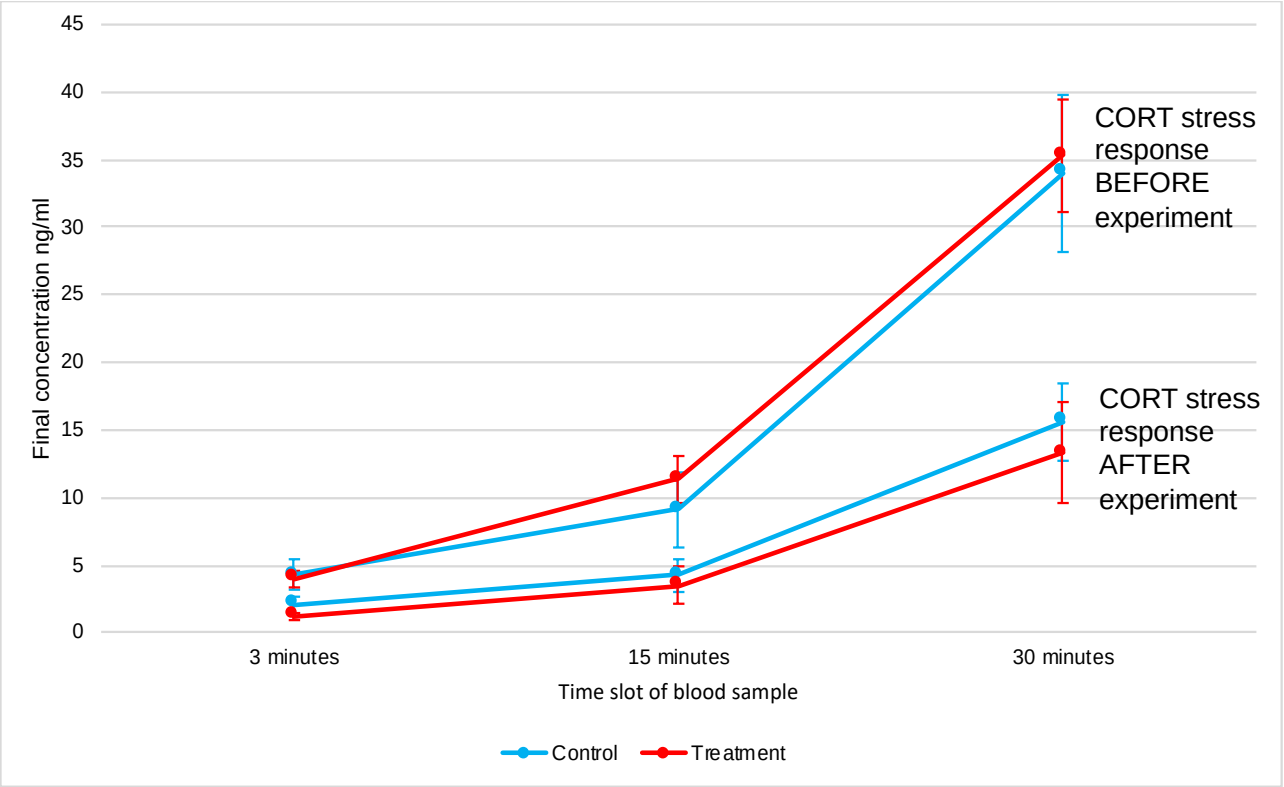

Figure 2. Corticosterone (CORT) stress response curve during the first sampling, before the experiment (lines above) and during the second sampling, after the experiment (lines below). The CORT stress response detected in the second sampling was lower. There was no difference between CORT levels in individuals belonging to the predation (red) and control (blue) treatment, the decrease from first sampling to second sampling was greater in the predation treatment. Means and standard errors of the mean are shown.

DISCUSSION

During our study we did not find an effect of predation threat on relative telomere length. RTL was however affected by the time of sampling: before the experimental procedure RTL was in fact longer than in the successive samplings, both in treatment and control groups. We also found a non-significant increase in relative telomere length one month after the experiment, and one year later RTL had not decreased any further. The greatest changes in telomere dynamics are thought to happen during early life but in our experiment we assisted to a massive decrease in first-year birds: a -32.3% yearly change, quite above the yearly rate usually estimated for passerine birds (12% as in Sudyka et al. 2016b, Tricola et al. 2018). However, while our study consisted of multiple samplings during one year, other studies spanned multiple years and reported results calculated cross-

sectionally among individuals of different age: this might be a possible reason why the results they reported diverged from those we obtained.

There may be two explanations for the unforeseen drop in relative telomere length after the first sampling. Firstly, in our study we could not take into account the role of the enzyme telomerase, as it is difficult to obtain accurate measures of the activity of this enzyme (Monaghan et al. 2018). Additionally, telomerase activity has rarely been studied in vertebrates different than rodents, with few exceptions (Taylor & Delany 2000, Haussmann et al. 2004, Wirthlin et al. 2018), which however did not investigate neither seasonal nor stress-related fluctuations in its expression. It might be possible that regulation of telomerase is linked with seasonal variation (Turbill 2013,) or with certain taxing physiological events (Reichert et al. 2014b) and thus that our results might be connected to a change in the regulation of the expression of this enzyme.

Another possible explanation concerns the timing of our experiment. In fact, the experimental period coincided with the breeding season, which is known to increase stress: while our individuals did not breed, they still increased activities such as nest material collection, territorial defence via competitive interactions and possibly mate courting. All these activities are energetically expensive, and the costs might have been traded off with telomeres maintenance. This in turn would cause telomere length to be impacted: other studies have found that reproductive effort increased telomere attrition (Sudyka 2014, Reichert et al. 2014a). Another possibility is that seasonal changes in glucocorticosteroids (Romero & Wingfield 1999, Romero 2002) might have increased oxidative stress (Costantini et al. 2011) which in turn might have heightened the rate of telomere attrition. While the weak change that we detected in the baseline corticosterone level was in fact a decrease, this was possibly due to downregulation of the stress response caused by chronic stress (Rich & Romero 2005). In fact, in this species usually the level of plasma corticosterone is higher in breeding season, both because of enhanced stress and because of seasonal variation (Breuner & Orchinik 2001; Huber et al. 2018). However, when we recorded the corticosterone stress response after the experimental period we found that it was greatly decreased. This might in effect be another evidence of the general stressfulness of the time frame chosen for the experimental period. Prolonged times of amplified stress are in fact associated with either a downregulation of the response or a physiological habituation (Cockrem & Silverin 2002; Cyr & Romero 2009). Indeed, in the predator treatment the drop in the stress response was significantly greater, meaning that birds in the predator regime might have been more stressed than those in the control: the sighting of a predator in fact usually elicits an even greater corticosterone stress response than isolation in a cage or in a cloth bag (Canoine et al. 2002).

Finally, we did not find any evidence supporting a connection between telomere dynamics and individual behavioural traits. While there are few results linking telomere dynamics and individual behavioural traits, the expression of individual traits of social behaviour is complex and strongly dependent on the entire social environment. Thus, while theory predicts that individual behaviour might be linked to a stable 'state' (Bergmüller & Taborsky 2010), such as a physiological state or a life-history strategy, from our results it appears that more research is required before using relative telomere length as a proxy for measuring this relationship. Telomere dynamics are a topic still greatly unexplored, with many studies reporting results contrary to the initial predictions (McLennan et al. 2018; Danzer et al. 2019; McLennan et al. 2019). In particular, future studies in this species might address how the expression of telomerase is regulated across seasons, and how telomere dynamics might be impacted by the expected and seasonal variation in glucocorticosteroids.

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Chapter 3

The effect of social connections on the discovery of multiple hidden food patches in a bird species

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The effect of social connections on the discovery of multiple hidden food patches in a bird species

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Social foraging is thought to provide the possibility of information transmission between individuals, but this advantage has been proved only in a handful of species and contexts. We investigated how social connections in captive flocks of house sparrows (*Passer domesticus*) affected the discovery of (i.e. feeding for the first time from) two hidden food patches in the presence of informed flock-mates. At the first-discovered and most-exploited food patch social connections between birds affected the order of discovery and presumably contributed to a greater exploitation of this patch. However, social connections did not affect discovery at the second food patch despite its close spatial proximity. Males discovered the food sources sooner than females, while feeding activity was negatively related to patch discovery. Age had no effect on the order of discovery. Birds that first discovered and fed at the food patches were characterized by higher level of social indifference, i.e. followed others less frequently than other birds in an independent context. Our findings provide experimental evidence for the importance of variable social connections during social foraging in house sparrow flocks, and suggest that social attraction can contribute differently to the exploitation of different patches when multiple food sources are present.

Animals often rely on social information when foraging: the presence of a conspecific individual at a food patch can transmit information about patch location, resource quality or accessibility[1–3]. The use of social information obtained from the observation of conspecifics' behaviour[1,4] can lead to improved foraging opportunities and increased rate of food intake[5–8], while also resulting in greater competition at the food source[9]. Models assuming the existence of social attraction among conspecifics however often do so without incorporating an underlying mechanism, simply expressing this effect as a function of the number of foragers present at the patch [e.g. refs 10–13, but see ref. 14]. While this can be true for individuals that are not socially associated with each other crowding at the same location (i.e. when animals aggregate at clumped and superabundant resources)[15], in many species social groups are characterized by non-random associations between group-mates and in particular, members of the same social group may often follow each other or move together to a food source. In this case one individual exploiting a food patch can facilitate associated individuals to join that patch sooner than they or any non-associated group-mates otherwise would: resource discovery and exploitation are thus mediated by the presence of close social connections between certain group members[1,16].

The presence of variable social connections between group-mates can have far-reaching implications, because the social transmission of information, novel techniques or foraging skills is often not a function of the number of individuals present, but determined by complex interactions between individuals in the group[17–20]. Recent studies on such animal societies have provided evidence for the presence of cultural transmission of foraging innovations in mammals[19,21,22], spread of experimentally induced foraging techniques and learning of foraging skills in birds[20,23,24], discovery of prey patch locations and spread of foraging information in fish[25,26] and in insects[27]. Moreover, individual differences in the sensitivity to these social attraction effects or 'social indifference' [sensu 28] have also been proposed to play an inherent role in collective decision-making in animal aggregations [e.g. refs 2, 13, 29–31], similarly to the influential role of different "needs" due to individual differences in personal goals or motivation[28,30].

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However, the generality of these social effects, especially in less complex foraging scenarios but in the presence of multiple food sources, has been rarely addressed. We carried out our experiment on house sparrows, as it is an opportunistic and highly gregarious species[32].

In this study we collected data on individual latencies to discover (i.e. first feed) from two hidden food patches in captive house sparrow flocks. In these flocks individuals did not have experience of these novel food sources, except for two informed flock-mates, which had been habituated to eat from similar hidden food patches, each marked with a colourful spot. These informed individuals were more knowledgeable about the location of the resource than other birds in the experimental flock, so their role was to speed up the discovery and exploitation of the hidden food patches during the trial. Using the first feeding events (i.e. patch discoveries), we investigated how the time of the discoveries predicted the extent of exploitation of the two food patches and how previously established social connections between flock-mates, measured in an independent context, and individual characteristics such as age, sex and feeding activity affected the order of discoveries among the naïve birds. Social connections were estimated from following networks constructed from the recorded following interactions between individuals and correspondence between patterns of association and food acquisition was tested using a modified version of network-based diffusion analysis (NBDA; [e.g. see in refs 1, 20, 24 and 33]). NBDA estimates the effect of social transmission on individuals' acquisition of a trait or information based on the social connections previously measured in a social network, and quantifies how acquisition rate is accelerated when connected group-mates demonstrate the new trait. The applied variant of this analysis (OADA) has the advantage that it is insensitive to the shape of the baseline function (i.e. allows the baseline rate of acquisition to increase or decrease as the diffusion proceeds), and measures the relative rate at which individuals acquire the trait[34]. Aside from the following-based networks, we also generated homogeneous networks to model the situation when all individuals have equal opportunity to learn from each other [e.g. refs 24 and 26]. In this case, solely the increasing number of informed individuals may exert an acceleratory effect on the rate of acquisition at the food patches[34]. With this set-up, we aimed to explore the effect of social attraction on patch discovery in foraging house sparrows and test whether variable social connections influence individuals' foraging decisions.

Methods

Study subjects. The experiment was carried out from October 2013 to April 2014 on a total of 108 house sparrows (54 males and 54 females) originating from a captive population at the Konrad Lorenz Institute of Ethology (University of Veterinary Medicine, Vienna, Austria)[35,36]. Prior to the experiment, birds were kept in eight unisex outdoor aviaries (3.5 m × 3.5 m × 3 m; approx. 20 individuals per aviary; 'initial flocks' henceforward). Two weeks prior to the experiment, tutor flocks were formed from two groups of four individuals (2 males and 2 females randomly selected from the initial flocks) and were allocated into two outdoor aviaries (3.9 × 1.9 × 3 m; 'tutor flocks' henceforward). The experimental flocks, formed one at a time during the course of the study, consisted of ten adult individuals (5 males and 5 females) randomly chosen from the initial flocks (each individual was used only once) and were housed in an indoor aviary (2.8 × 2.7 × 2.1 m). In all flocks (initial, tutor and experimental), aviaries were equipped with a roosting tree, several perches and a water basin, and nest-boxes for resting were also added to the initial and the tutor flocks. Commercial food for granivorous passerines was provided to all flocks according to the experimental design, which is described with further details in the Supplementary Information.

Experimental procedure. *Pre-training period.* After the formation of the experimental flock, the pre-training period lasted for two days in which 250 g of food were provided every day on the central feeder at 8:00 am and removed at 18:00 pm. We recorded the foraging activity of the birds at the central feeder during the entire period when the food was present using the software iSpy 6 (video resolution 960 × 544 pixels, 7 frames per second). Individuals were unambiguously identified by the colour ring combination and the coloured marks on the head. We noted the time when birds arrived at the feeder and had access to food. Using these video recordings, two different foraging events were specified: following events (for a similar method see refs 37 and 38) and the total number of visits on the feeder. A following event occurred when an individual arrived at the feeding place and was followed by one or more group-mates within 5 seconds. The former individual was described as the 'initiator' and the latter(s) as the 'follower(s)'. The total number of visits was defined as the sum of all foraging events by which an individual arrived at the central feeder (i.e. arrived alone, by following another group-mate or by arriving in a group without a specific initiator). This latter measure was used as a proxy for feeding activity during the analysis.

Training period. After the pre-training period, in the morning we randomly caught one male and one female from the experimental flock. These trainee individuals (the future informed birds in the hidden food patch trials) were inserted in the two tutor flocks by randomly assigning one sex to a specific tutor aviary (and as a consequence to a specific coloured marking). In the tutor aviaries trainee birds had access to food only under a marked box (out of two) on the ground, and had not previously encountered this novel food location. This habituation period lasted for several consecutive days (3.0 ± 0.47 day) during which the trainee birds visited the food source with the same frequency of the tutor birds (authors' personal observations). This measure was used as an indication that the trainee birds foraged at the box with food similarly to tutor birds. We also waited until we observed the trainee birds visiting the marked box on their own initiative, not only by following the tutors. For the rest of the birds in the experimental flock, food was always provided *ad libitum* on the central feeder throughout this period, thus only the trainee birds had experience in feeding from a food patch under the box.

Trials. Once both trainee individuals foraged at the food source hidden under the marked boxes at the same frequency as the tutor birds, the trial started on the following day. In the morning the central feeder from the

experimental aviary was removed and the two trained birds were re-introduced into the experimental flock. The four cardboard boxes were removed and under two of them 52.14 ± 3.35 g of millet spray were anchored to one of the inner sides. The boxes were then placed inside the aviary; as the positions of the food-hiding boxes were *a priori* assigned, the two webcams were already positioned in front of them. The coloured markings were added and randomly associated to a box with food. All birds were familiar with the boxes as these were added to the aviary of experimental flocks from the beginning of the pre-training period, but food was placed under two of such boxes only when the trial started, with a light blue or magenta marking placed on the top of each box serving as a visual cue for the informed birds. Informed birds and the rest of the experimental flock were food deprived for the same time period (from 8:00 until the video recordings in the experimental aviary started). Hidden food contained inside the two boxes was the only food source available during the trial. Once the food was placed inside the aviary, the trial and the video recording lasted until 18:00. As the video recordings did not start exactly at the same time in all flocks, the maximum duration of the trial was set to 30420 seconds (shortest duration among the flocks) to standardize the time frame for patch discovery.

Video recordings at the hidden food sources were collected during the trial when food was present under the two boxes in the experimental flock. The two cameras were time-synchronized and set to record when movement in front of the cameras was detected by using the software iSpy 6 (with the same settings as above). Using the colour ring combination and the coloured marks on the head, we identified each individual on the video recordings that ate directly from the food source and measured its latency to feed for the first time at each food patch ('latency to feed'). For each food patch, we also recorded the first time when an individual approached it by hopping toward the millet spray and visually inspecting it ('first approach'), and the number of aggressive interactions between birds during the trial. At the end of the day the remaining amount of food at both patches was measured as the difference in weight of the millet sprays before and after the trial at each patch.

Constructing social networks. We used following events collected in the pre-training period as direct interactions between individuals to characterize social connections and construct directed weighted social networks. Nodes in these following networks represented individuals in the flock, and edges represented following rates, i.e. the number of followings per hour, which were calculated as the total number of occasions when one individual followed another divided by the duration of the trial in hours (which is not necessarily the same as the number of followings per hour by which the other bird followed the first one). We used these following networks test in the NBDA to test whether social transmission of information about the hidden food patches follows the pattern of associations in the house sparrow flocks. We also calculated in- and out-strength network metrics for each individual from these following networks, which denote the frequency of being followed by other flock-mates (per hour) and the frequency of following others in the flock (per hour), respectively[38,39].

Statistical analysis. We used R 3.3.2 for all statistical calculations[40]. We applied Approximative Wilcoxon-Pratt Signed-Rank Tests, Approximative Wilcoxon-Mann-Whitney Tests and Approximative Spearman Correlation Tests with 19999 iterations from the 'coin' R package[41] to estimate between- and within-flock differences and correlations; 'flock' was used for stratification in all two-sample comparisons and correlation tests. Directed weighted following networks were constructed and individuals' in- and out-strength were calculated using the 'tnet' R package[42]. We applied a modified version of the order of acquisition diffusion analysis (OADA) variant of NBDA[34,43], which is fitted to the collected data on the order in which individuals acquire a behavioural trait relative to other naïve individuals. In the modified OADA, the computation of the social transmission parameter did not differ from that of the original OADA[34], but instead of using standard Cox proportional hazard models we applied Cox mixed-effect proportional hazard models during optimization and model fitting. This subtle change in the calculation routine allowed us to include the necessary random term, i.e. 'individual identity' nested into 'flock', into the models for the investigation of trait acquisition at the two patches within each flock. Also, we combined the acquisition diffusions into a single dataset, which allowed us to take different baseline rates at the two patches into account through stratification of the data (for more details, see Supplementary Information). Since this method preserves information about which individual comes from which diffusion, we could test whether or not social transmission rates differed at the two patches. To fit separate parameters for social transmission for different tasks, we used a specific argument (sParam in the NBDA context) and fitted models in the following scenarios: no social transmission at either patch (i.e. asocial models), same rate of social transmission at both patches, different rates of social transmission at the two patches, social transmission only at the first-discovered patch, social transmission only at the second-discovered patch ('model categories'). Into the models we incorporated the presence of informed birds, which means that social connections of informed individuals to naïve flock-mates created opportunity for social transmission to operate already at the very first discovery event in a flock. We also added individual transmission weights to each bird to control for the possibility that individuals may perform the acquired trait (i.e. feeding from a hidden food patch) at a different rate in their flocks due to their differences in foraging activity; weights were calculated as the total number of visits to the central feeder in the pre-training period scaled to the maximum value in the given flock. We tested the effects of individual characteristics such as feeding activity, sex and age; the latter two were previously found to affect individuals' position in following networks in house sparrows[38]. Social connections were based on either the constructed following-based or homogeneous networks (i.e. all connections set to 1). We tested all potential combinations of the above scenarios, explanatory variables and type of social connections, and then ranked the models according to their predictive power using Akaike Information Criteria corrected for small sample sizes (AICc[44]) and corresponding Akaike weights[44,45] (Table S2). Preliminary analysis of OADA models fitted with following-based networks separately at the two patches indicated a stronger support for the multiplicative models (ref. 34, Table S3), thus in our final analysis social and asocial acquisition processes could interact multiplicatively (i.e. only multiplicative models were fitted). Conditional 95% confidence intervals for the social

	First-discovered food patch	Second-discovered food patch
Time of first approach (s)	6263.9 ± 2807.23	7838.9 ± 4039.47
Time of discovery by the first-feeder birds (s)	6807.1 ± 2948.77	13490 ± 8591.43
Time of discovery by an average bird (s)	11554.53 ± 3424.98	16544.33 ± 4517.11
Number of birds discovered the patch	9.5 ± 0.71	5.9 ± 3.57
Amount of seed taken (g)	36.5 ± 10.44	14.66 ± 10.95
Frequency of aggressive interactions	33.7 ± 39.86	9.8 ± 13.60

Table 1. Mean ± SD of the investigated parameters at the two food patches in the house sparrow flocks.

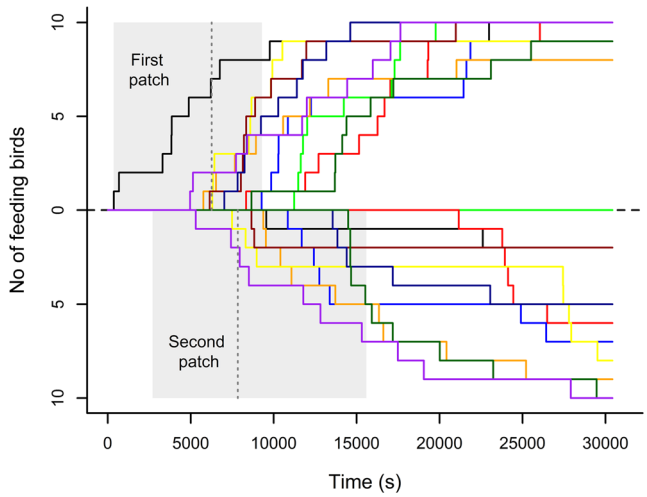

Figure 1. Diffusion curves showing the latency of individuals to feed from the two hidden food patches in the flocks. Each coloured line represents one flock, and the same colour denotes the same flock at the first-exploited patch (upper half of the panel) and the second-exploited patch (lower half of the panel). Time to first approach is indicated by a grey bar at each patch, with the dashed lines showing the mean values.

transmission rate and the explanatory variables were calculated using profile likelihood technique[46,47]. We based our inference on these estimations in the 'best models' set (within 4 ΔAICc with the best-fitting model), as we could not calculate conditional standard errors for model-averaging from the numerical estimate of the Hessian matrix in those models which contained different transmission parameters for the two food patches. More details about the applied analysis, together with the constructed R script, are provided in the Supplemental Information. NBDA was performed using the code provided on The Laland Lab's website (NBDA code v1.2.13; http://lalandlab.st-andrews.ac.uk/freeware.html) with the modifications detailed above.

Ethics Statement. Capture, housing and handling of birds were in accordance with the relevant Austrian laws and were licensed by the government of Vienna (MA 22) license number 424/2011. The experiment reported in this study complies with current laws on animal experimentation in Austria and the European Union. This study was approved by the institutional ethics committee (University of Veterinary Medicine, Vienna) and the national authority according to 8ff of Law for Animal Experiments Tierversuchsgesetz - TVG, licence number GZ 68.205/0220-II/3b/2012.

Results

In 9 out of 10 flocks birds fed from both hidden food patches during the trials, but the two patches were exploited differently (Table 1): from the first-approached patch (Approximative Wilcoxon-Pratt Signed-Rank Test: $Z = -2.09$, $P = 0.038$) individuals obtained food earlier than from the other patch; this was true for those birds that fed first from this patch (i.e. first-feeders; $Z = -2.80$, $P = 0.002$) and also in the case of an average bird ($Z = -2.55$, $P = 0.009$). By the end of the trial more birds fed from the first-discovered patch ($Z = 2.15$, $P = 0.036$; Fig. 1) and more seeds were taken by the end of the trial from this patch than from the second-discovered patch

($Z = 2.40$, $P = 0.014$). During feeding, the frequency of aggressive interactions was also higher at the first than at the second patch ($Z = 2.14$, $P = 0.028$). There was a strong and significant negative correlation between the average time of first feeding (with birds that did not eat from one of the two patches being excluded) and the amount of food loss at the first patch ($r_S = -0.71$, $N = 10$, Approximative Spearman Correlation Test with 'flock' used for stratification: $Z = -2.13$, $P = 0.026$), but this relationship was weak and non-significant at the second patch ($r_S = -0.23$, $N = 9$, $Z = -0.66$, $P = 0.555$). These findings indicate that although uncertainty regarding patch location was likely to decrease over time as both patches became utilized by more and more individuals, first-feeding latencies predicted the extent of exploitation at the first, but not at the second patch. Also, the first-approached food patch was exploited to a higher extent by more individuals under a higher competition regime by the end of the trial compared to the second-approached patch despite their close spatial proximity (~0.5–1.5 m).

In the OADA we found that the order of discovery was affected by the presence of variable social connections: models fitted with following-based networks had a 2.67 × higher overall support than those fitted with homogeneous networks (72.78% vs. 27.22%; Table S3). Asocial models had very low relative support in general (<0.01%; Table S3). In the 'best models' set (i.e. those within 4 ΔAICc with the best-fitting model; 69.11% overall support), the social transmission parameter at the first patch was estimated to be higher than zero in all models, while at the second patch it was either constrained to or not different from zero (Table 2). This result indicates that social transmission of information between naïve birds and any of those individuals that already discovered the patch could occur at the first-discovered food patch if these individuals were connected even only by a few followings within an hour (or by a single following per hour if the bird that already discovered the patch had the highest transmission weight in the flock). A likelihood ratio test between the best-fitting model and its corresponding asocial model also indicated a significant effect of social transmission at this patch ($\chi^2_1 = 29.71$, $P < 0.001$). Both sex and feeding activity had a significant effect on the order of patch discovery in all models in the 'best models' set (Table 2), implying that males discovered the food patches sooner than females and more active birds discovered the food patch later. This latter finding may indicate that the measured feeding activity was rather related to the number of 'feeding alone' events in birds than to their tendency to forage in groups. Age was found to have a negligible effect on patch discovery in these models (Table 2).

Being informed did not predict to be a first-feeder more frequently than expected by chance (6 first-feeder informed birds out of 19 events [the second patch remained unexploited in one flock]; Binomial test: $P = 0.246$), although informed individuals gained access sooner to the first (informed birds: 9765.5 ± 3193.91 s, other flock-mates: 13760.66 ± 7730.91 s, Approximative Wilcoxon-Mann-Whitney Test with 'flock' used for stratification: $Z = 2.67$, $P = 0.008$), but not to the second patch (informed birds: 24796.75 ± 9992.03 s, other flock-mates: 23467.56 ± 9843.88 s, $Z = -0.67$, $P = 0.503$), compared to other birds. The coloured marks did not have any effects whatsoever on the time of patch discovery during the trial (data not shown). Sex of the informed birds did not affect the average latency of flock-mates to feed from a patch (male informed birds: 15895.87 ± 5635.65 s, female informed birds: 19325.18 ± 7493.21 s, Approximative Wilcoxon-Pratt Signed-Rank Test: $Z = -0.87$, $P = 0.433$). However, first-feeder birds, i.e. individuals that fed first from a hidden patch in their flock, were characterized by lower out-strength compared to the other individuals in their flock (first-feeder birds: 4.62 ± 4.74, other flock-mates: 8.63 ± 7.82, $Z = 2.34$, $P = 0.017$), so they followed others less frequently in the flock than other birds (Fig. 2A). On the other hand, these individuals did not elicit more followings in the pre-training period than other birds in their flock, i.e. in-strength of the first-feeders did not differ from that of their flock-mates (first-feeder birds: 6.91 ± 5.69, other flock-mates: 8.17 ± 6.80, $Z = 0.48$, $P = 0.633$; Fig. 2B).

Discussion

In this study we tested how previously established social connections between house sparrows affected the discovery of hidden food patches, and showed that information about the first-discovered food patch transmitted through the established social networks in the flocks. This result is in line with previous works that demonstrated the importance of social connections in the context of group foraging in various animal species[1,19,21], and stresses the importance of social connections to knowledgeable or experienced individuals within a group when accessing and exploiting novel food sources. However, this pattern was true only for the first-discovered patches, while at the second-discovered patches social attraction was not related to the measured social connections between individuals. This difference arose despite the very small distance between the two patches (0.5–1.5 m) which were similarly accessible and profitable to the birds, reflecting that social reinforcement processes operated in individual decision-making during social foraging even at this spatial scale[2]. Presumably as a consequence of differences in both the time of first discoveries and the level of social attraction at the two food patches, first-discovered patches were exploited by more individuals and also to a greater extent by the end of the trial, which possibly was the cause for a higher number of aggressive interactions at these patches. This may indicate that social attraction caused birds to perceive the first patch as having higher quality than the second one, because of either a lower perceived predation threat when they foraged in close-knit groups or facilitation in foraging due to the quicker dismantling of the millet spray.

Another interesting result is that first-feeder birds were characterized by lower out-strength, i.e. these individuals followed others at a lower frequency compared to their flock-mates. This implies that first-feeder birds were socially more indifferent compared to others, thus less affected by social attraction effects. One could speculate that first feeder individuals could also be more explorative than their flock-mates and/or characterized by reduced neophobia, which idea is in accordance with recent works where personality differences between individuals were found to substantially affect the use of social information and group decision-making[13,48,49]. Similarly to these studies, where proactive individuals were found to rely less on social information and have generally weaker social bonds, we found that first-feeder birds were less motivated to follow others in their flock. Thus, our finding supports the idea that social indifference and exploratory behaviour are often positively related individual traits[49].

Model category	AICc	ΔAICc	w_{Akaike}	Parameter estimates [95% CI]				
				s_1	s_2	Age	Sex	Feeding activity*
Social transmission only at patch 1 (support: 45.91%)	**1037.81**	**0.70**	**0.16**	**1.633 [0.550–5.161]**	**constrained to 0**	**0.182 [−0.112–0.471]**	**0.415 [0.078–0.753]**	**−0.004 [−0.005–0.002]**
	1050.37	13.26	0.00	0.572 [0.185–1.811]	constrained to 0	0.284 [−0.083–0.586]	0.366 [0.032–0.707]	—
	1037.11	***0.00***	***0.23***	***1.571 [0.527–4.986]***	***constrained to 0***	—	***0.431 [0.095–0.768]***	***−0.004 [−0.005–0.002]***
	1041.08	**3.97**	**0.03**	**1.191 [0.402–3.703]**	**constrained to 0**	**0.204 [−0.089–0.492]**	—	**−0.003 [−0.005–0.002]**
	1052.56	15.44	0.00	0.442 [0.136–1.438]	constrained to 0	0.286 [−0.039–0.585]	—	—
	1050.81	13.69	0.00	0.564 [0.171–1.890]	constrained to 0	—	0.394 [0.057–0.739]	
	1040.80	**3.69**	**0.04**	**1.090 [0.367–3.540]**	**constrained to 0**	—	—	**−0.004 [−0.005–0.002]**
	1053.57	16.46	0.00	0.411 [0.112–1.452]	constrained to 0	—	—	—
Different social transmission rates at the two patches (support: 26.17%)	**1039.00**	**1.89**	**0.09**	**1.835 [0.626–5.852]**	**0.120 [0–0.601]**	**0.169 [−0.125–0.459]**	**0.446 [0.109–0.784]**	**−0.004 [−0.006–0.002]**
	1052.56	15.44	0.00	0.572 [0.185–1.811]	0 [0–0.188]	0.284 [−0.083–0.586]	0.366 [0.032–0.707]	—
	1038.05	**0.94**	**0.14**	**1.803 [0.613-5.760]**	**0.137 [0–0.644]**	—	**0.464 [0.129–0.800]**	**−0.004 [−0.006–0.003]**
	1042.89	5.78	0.01	1.251 [0.425–3.908]	0.061 [0–0.424]	0.197 [−0.096–0.485]	—	−0.004 [−0.005–0.002]
	1054.71	17.59	0.00	0.442 [0.136–1.438]	0 [0–0.161]	0.286 [−0.039–0.585]	—	—
	1052.96	15.84	0.00	0.564 [0.171–1.890]	0 [0–0.196]	—	0.394 [0.057–0.739]	—
	1042.43	5.32	0.02	1.163 [0.395–3.798]	0.075 [0–0.457]	—	—	−0.004 [−0.006–0.002]
	1055.69	18.58	0.00	0.403 [0.112–1.453]	0 [0–0.168]	—	—	—

Table 2. Model parameter estimates and their conditional profile likelihood 95% confidence intervals in the two best supported categories of models fitted with following-based networks. *As feeding activity was measured as the total number of visits at the central feeder, the estimated decrease in log odds of discovery corresponds to one unit increase in feeding activity. The model with the lowest AICc is written in italics, while the 'best models' set (within 4 ΔAICc) on which we based our inference is written in bold. Other model categories (same social transmission rate at both patches, social transmission only at the second patch, no social transmission at either patches) had very low overall support (≤0.70%). AICc values and corresponding Akaike weights of all models (both fitted with following-based and homogeneous networks) are shown in Table S3.

House sparrows are a highly adaptable and opportunistic species for which discovering and exploiting novel food sources is crucial to survival. In areas where their range is still expanding, they have been shown to be bolder and to exhibit lower levels of neophobia in an asocial context[50,51]. However, house sparrows usually forage in flocks, which are often composed of individuals with phenotypic differences in boldness, social indifference and also presumably in following behaviour. The difference in phenotypic composition of a group has been recently emphasized as having a potential impact on fitness[52,53], also in this species during social foraging[54]. In our study, the measured social connections within the flocks were likely to be robust (similarly as in ref. 38), and also variable enough to affect the order of discovery of the hidden food source, along with individual characteristics such as sex and foraging activity, a few days after the social network was first assessed. Our results therefore also implicate that the discovery of new food sources, a fundamental aspect for the survival of house sparrows, is strongly influenced by the presence of phenotypic polymorphisms within the flocks[48]. An important question that consequently arises is to what extent the social environment within a house sparrow flock could influence the fitness of its members, or even favour certain group compositions or polymorphisms combinations compared to others[55,56].

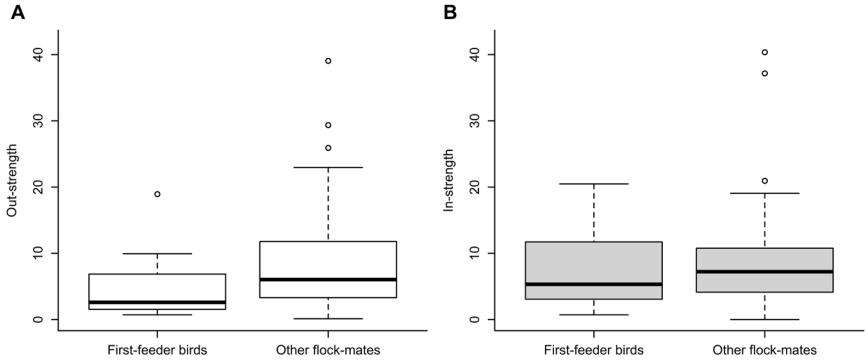

Figure 2. Differences in out-strength and in-strength between the first-feeder birds and other birds in the flocks. Out-strength (**A**) was calculated from the number of followings per hour when the focal bird followed a flock-mate to the feeder during the pre-training period, whereas in-strength (**B**) was derived from the number of followings per hour during which the focal bird was the initiator individual. These metrics were derived from the constructed following networks and reflect individuals' social position (in terms of their tendency to follow others and elicit followings from others, respectively) in their flock. Horizontal lines are medians, the boxes and the whiskers show the interquartile ranges and the data ranges, respectively.

This would be particularly interesting to investigate in a landscape of novel and ephemeral food sources and unexpected threats, such as the environment where house sparrows and other invasive species usually thrive[50].

In the studied sparrow flocks, patch discovery could be a stochastic event[12], but as soon as one patch was discovered by a socially less sensitive bird and then utilized by more and more individuals, it was likely to remain the major food source for many individuals throughout the trial. Our results provide experimental evidence for the influential effect of social connections on foraging decisions in the presence of multiple food patches in the house sparrow. We propose that similar studies should investigate how acquisition rate at one food source may directly affect and lead to the change of the acquisition rate at other food sources (e.g. as patch discovery become faster at one patch, discovery rate decelerates at another patch). Also, using a Bayesian estimation of decision making rule proposed by Arganda and collaborators[3] additional experiments could further scrutinize the consequences of subsequent foraging choices of individuals on resource exploitation at different food sources, linking animal social foraging and individual decision making to the framework of information cascades[57,58].

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Supplementary Information

The effect of social connections on the discovery of multiple hidden food patches in a bird species

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Tutor flocks

Two weeks prior to the experiment, two groups of four individuals (two males and two females) were allocated into two outdoor aviaries (3.9 × 1.9 × 3 m; 'tutor aviaries' henceforward). These aviaries were equipped with a roosting tree, several perches, nest boxes and a water basin. On the floor two same sized brown cardboard boxes (33 × 21 × 12 cm) were placed, equidistant to the roosting tree and perches, about 0.5 m from each other. The boxes were open only on one of the long sides opposite to the roosting site. The only food source in these aviaries was approx. 60 g of millet spray provided daily under one of the boxes, anchored on an inner side so that the food was only visible and accessible when the birds approached the box from the front. On the top of the box containing food a small coloured marking (a 5 cm diameter circle; for a similar approach, please see [1]) was placed and alternated between boxes on consecutive days. The colour of the markings, light blue and magenta, differed between the two tutor aviaries. Before food was added every morning, the boxes were temporarily removed with any leftovers from the aviary, and the floor around the boxes was carefully cleaned. Then, both the empty box and the one hiding the food was put back into the aviaries, and the coloured marking was always associated with the box containing the food so tutor individuals could rely only on the coloured marking and on approaching the boxes to identify the presence of food. These aviaries were used to train informed individuals for the experimental flocks.

Experimental flocks

The experimental flocks consisted of ten adult individuals (five males and five females) randomly chosen from the eight unisex outdoor aviaries. Each individual was used once during the study. The experimental individuals were transferred into an experimental aviary and individually banded with metallic and coloured rings. To facilitate the identification of birds from video recordings, the crown feathers of all the individuals were painted with non-toxic coloured markers (Deco painter matt, Marabu GmbH & Co. KG, Germany). Tarsus, wing and tail length (to the nearest 0.1 mm) were measured as well as body mass before and after the experiment (to the nearest 0.1 g). The experimental aviary (2.8 × 2.7 × 2.1 m) was equipped with a roosting tree, several perches, and a water basin situated at the back of the aviary. A single feeder was situated at the center of the aviary on a small platform approx. 10 cm off the ground, and served as the main food source. Commercial food for granivorous passerines was provided on the feeder, but the amount differed according to the experimental design. In the front side of the experimental aviary, similarly to the tutor aviaries, four same sized brown cardboard boxes (identical to the ones placed into the tutor aviaries) were placed on the floor, about 20 cm distant one another. These boxes were only open on the side opposite to the roosting site and were fixed on the floor.

Three webcams (Microsoft Lifecam Studios, model Q2F00015) were placed inside the aviaries throughout the entire experimental period, one recording the activity at the central feeder, and the remaining two positioned in front of those two cardboard boxes (approx. 30 cm distant) which were *a priori* randomly selected for hiding the food during the trial. Other than natural light from different windows, artificial light was also provided with 12:12 h light:dark periods (07:00-19:00). The experimental indoor aviary was maintained at a temperature of about 20 Celsius degrees. At the formation of the experimental group, birds were allowed to become familiar with the environment of the experimental aviary for 1 day, during which food was provided *ad libitum*

on the central feeder. The evening before the onset of the trial the feeder was removed and the floor carefully cleaned from seeds.

Network-based diffusion analysis (NBDA)

NBDA was initially developed by Franz & Nunn [2] and extended by Hoppitt et al. [3] (for additional extensions see also 4, 5-7]. We used the order of acquisition diffusion analysis (OADA) variant of NBDA [3], where the model is fit on the order of individual acquisitions, thus measures the relative rate at which individuals acquire the trait. OADA has the advantage that it is insensitive to the shape of the baseline function, and is recommended to be used if the baseline rate of acquisition changes over time [3]. However, a weakness of OADA is that this method can detect social transmission only if it results in substantial differences between the rates of acquisition by which individuals acquire the trait [3]. In a standard OADA, the baseline rate of acquisition is unspecified with the assumption that each diffusion has its own baseline rate. Alternatively, different diffusions or tasks may be included in the same stratum, in which case they are treated as a single diffusion with zero connections among individuals from different diffusions and the same baseline rate function can be assumed in all diffusions within each stratum. Stratifying by food patch in our study also allowed us to estimate different social transmission parameters for each stratum, i.e. for each food patch in the flocks. With this set-up, the potential influence of social connections in homogeneous networks on patch discovery could also be tested. Individual-level variables influencing the rate at which an individual acquires a trait can be incorporated into an OADA using an additive model:

$$\mathrm{R}_{i,l}(n) = \big(1 - z_i(n)\big)\left(s_l \sum_{j=1}^{N}(\alpha_{i,j}\, z_j\,(n)) + (1 - s_l)\exp\left(\sum_{k=1}^{V}\beta_k x_{k,i}\right)\right), \qquad (1)$$

or individual-level variables can be incorporated using a multiplicative model:

$$R_{i,l}(n) = \left(1 - z_i(n)\right)\left(s_l \sum_{j=1}^{N}\left(\alpha_{i,j}\, z_j\,(n)\right) + (1 - s_l)\right)\exp\left(\sum_{k=1}^{V}\beta_k x_{k,i}\right), \qquad (2)$$

where $R_{i,l}(n)$ is individual i's relative rate of acquisition of the trait immediately prior to the nth acquisition event in stratum l, $z_i(n)$ is the status of individual i prior to the nth acquisition event, $s_l \geq 0$ is a parameter determining the rate of social transmission between individuals per unit of network connection in stratum l ($s_l = 0$ indicates that all acquisition is by asocial means in stratum l), $\alpha_{i,j}$ is the network connection leading from individual j to i, $z_j(n)$ is the status of j prior to the nth acquisition event (1 indicates informed and 0 indicates naïve), N is the number of individuals, β_k is the coefficient determining the effect of variable k, $x_{k,i}$ is the value of variable k for individual i, and V is the number of individual level variables in the model [3,8].

Table S1. Observed foraging events in the house sparrow flocks during the pre-training period. Identified foraging events represent those observed events at the central feeder for which all participants were successfully identified. The total number of visits for an individual was calculated as the sum of the number of arriving at the central feeder alone, by following a flock-mate and in groups without a specific initiator; the flock-level measure of this variable was obtained by summing the individual-level data across all birds in a given flock.

Flock	# of identified foraging events	Identification accuracy (%)	# of followings	Total # of visits
1	1419	97.26	678	1855
2	2093	92.32	1242	2704
3	426	93.83	101	512
4	2362	87.35	543	3471
5	1420	82.80	758	1892
6	1645	82.41	937	2229
7	1578	97.23	730	2068
8	1143	98.79	394	1398
9	880	97.56	351	1108

10	1014	99.12	217	1175

Table S2. Type and relative support of the fitted 72 models. Present: social model (i.e. social transmission is present at least at one patch), Absent: asocial model (i.e. no social transmission); Same *s* at the patches: social transmission rate is the same at the two patches, Different *s* at the patches: social transmission rate is different at the two patches, *s* only at patch 1: social transmission rate is estimated only at the first-discovered patch, *s* only at patch 2: social transmission rate is estimated only at the second-discovered patch; F: fitted with following-based networks, H: fitted with homogeneous networks; ILV: individual-level variable (i.e. 'sex', 'age', or 'feeding activity'). Models in the 'best models' set (i.e. models fitted with the following-based networks and within 4 ΔAICc to the best-fitting model) are written in bold.

Model order	Social transmission	Model category	Type	ILV	AICc	ΔAICc	w_{Akaike} (%)
1	**present**	**s only at patch 1**	**F**	**sex, feeding activity**	**1037.11**	**0.00**	**0.23**
2	**present**	**s only at patch 1**	**F**	**sex, age, feeding activity**	**1037.81**	**0.70**	**0.16**
3	**present**	**different s at the patches**	**F**	**sex, feeding activity**	**1038.05**	**0.94**	**0.14**
4	**present**	**different s at the patches**	**F**	**sex, age, feeding activity**	**1039.00**	**1.89**	**0.09**
5	present	same *s* at the patches	H	sex	1040.10	2.99	0.05
6	present	same *s* at the patches	H	-	1040.41	3.30	0.04
7	**present**	**s only at patch 1**	**F**	**feeding activity**	**1040.80**	**3.69**	**0.04**
8	**present**	**s only at patch 1**	**F**	**age, feeding activity**	**1041.08**	**3.97**	**0.03**
9	present	different *s* at the patches	H	sex	1041.66	4.55	0.02
10	present	same *s* at the patches	H	sex, feeding activity	1041.74	4.63	0.02
11	present	same *s* at the patches	H	feeding activity	1041.85	4.74	0.02
12	present	different *s* at the patches	H	-	1041.94	4.83	0.02

13	present	same *s* at the patches	H	sex, age	1042.25	5.13	0.02
14	present	different *s* at the patches	F	feeding activity	1042.43	5.32	0.02
15	present	same *s* at the patches	H	age	1042.50	5.39	0.02
16	present	different *s* at the patches	F	age, feeding activity	1042.89	5.78	0.01
17	present	different *s* at the patches	H	sex, feeding activity	1043.37	6.25	0.01
18	present	different *s* at the patches	H	feeding activity	1043.45	6.34	0.01
19	present	different *s* at the patches	H	sex, age	1043.82	6.71	0.01
20	present	same *s* at the patches	H	sex, age, feeding activity	1043.92	6.81	0.01
21	present	same *s* at the patches	H	age, feeding activity	1044.00	6.89	0.01
22	present	different *s* at the patches	H	age	1044.02	6.91	0.01
23	present	same *s* at the patches	F	sex, feeding activity	1045.24	8.12	0.00
24	present	different *s* at the patches	H	sex, age, feeding activity	1045.58	8.47	0.00
25	present	same *s* at the patches	F	sex, age, feeding activity	1046.81	9.70	0.00
26	present	different *s* at the patches	H	age, feeding activity	1048.06	10.94	0.00
27	present	same *s* at the patches	F	feeding activity	1048.40	11.28	0.00
28	present	same *s* at the patches	F	age, feeding activity	1049.79	12.67	0.00
29	present	*s* only at patch 1	F	sex, age	1050.37	13.26	0.00
30	present	*s* only at patch 1	F	sex	1050.81	13.69	0.00
31	present	*s* only at patch 1	H	sex	1050.82	13.71	0.00
32	present	*s* only at patch 1	H	-	1050.96	13.85	0.00
33	present	*s* only at patch 1	H	feeding activity	1051.68	14.57	0.00

34	present	*s* only at patch 1	H	sex, feeding activity	1051.80	14.68	0.00
35	present	*s* only at patch 1	H	age	1052.29	15.18	0.00
36	present	*s* only at patch 1	H	sex, age	1052.31	15.20	0.00
37	present	*s* only at patch 1	F	age	1052.56	15.44	0.00
38	present	different *s* at the patches	F	sex, age	1052.56	15.45	0.00
39	present	different *s* at the patches	F	sex	1052.96	15.84	0.00
40	present	*s* only at patch 1	H	age, feeding activity	1053.38	16.27	0.00
41	present	*s* only at patch 1	F	-	1053.57	16.46	0.00
42	present	*s* only at patch 1	H	sex, age, feeding activity	1053.60	16.49	0.00
43	present	different *s* at the patches	F	age	1054.71	17.59	0.00
44	present	different *s* at the patches	F	-	1055.69	18.58	0.00
45	present	*s* only at patch 2	H	-	1056.10	18.99	0.00
46	present	*s* only at patch 2	H	sex	1056.55	19.43	0.00
47	present	*s* only at patch 2	H	feeding activity	1056.56	19.45	0.00
48	present	*s* only at patch 2	H	sex, feeding activity	1057.31	20.20	0.00
49	present	*s* only at patch 2	H	age	1057.97	20.85	0.00
50	present	same *s* at the patches	F	sex, age	1058.08	20.97	0.00
51	present	same *s* at the patches	F	sex	1058.09	20.98	0.00
52	present	*s* only at patch 2	H	sex, age	1058.50	21.38	0.00
53	present	*s* only at patch 2	H	age, feeding activity	1058.66	21.55	0.00
54	present	same *s* at the patches	F	age	1059.32	22.21	0.00
55	present	*s* only at patch 2	H	sex, age, feeding activity	1059.46	22.34	0.00
56	present	same *s* at the patches	F	-	1059.52	22.40	0.00

57	absent	-	-	feeding activity	1063.62	26.51	0.00
58	absent	-	-	-	1064.37	27.26	0.00
59	absent	-	-	sex, feeding activity	1064.55	27.44	0.00
60	absent	-	-	sex	1064.95	27.84	0.00
61	absent	-	-	age, feeding activity	1065.05	27.94	0.00
62	absent	-	-	age	1065.11	28.00	0.00
63	absent	-	-	sex, age	1065.81	28.70	0.00
64	present	*s* only at patch 2	F	feeding activity	1065.83	28.72	0.00
65	absent	-	-	sex, age, feeding activity	1066.02	28.91	0.00
66	present	*s* only at patch 2	F	-	1066.53	29.42	0.00
67	present	*s* only at patch 2	F	sex, feeding activity	1066.82	29.71	0.00
68	present	*s* only at patch 2	F	sex	1067.17	30.05	0.00
69	present	*s* only at patch 2	F	age, feeding activity	1067.33	30.22	0.00
70	present	*s* only at patch 2	F	age	1067.33	30.22	0.00
71	present	*s* only at patch 2	F	sex, age	1068.09	30.98	0.00
72	present	*s* only at patch 2	F	sex, age, feeding activity	1068.36	31.25	0.00

Table S3. Relative supports for the OADA models fitted separately at the first- and second-exploited food patches. The number of models in each category is written in brackets; values in bold indicate the best supported category at each patch. Relative support was calculated by summing Akaike weights across the set of models. The 'No ILV' models are those which did not include any individual-level variables (i.e. 'sex', 'age', or 'feeding activity').

Food patch	Asocial models	Models with social transmission		
First-discovered patch	0.03% (8)	99.97% (15)	**Multiplicative**	**80.77% (7)**
			Additive	17.39% (7)
			No ILV	1.81% (1)
Second-discovered patch	34.93% (8)	65.07% (15)	**Multiplicative**	**38.00% (7)**
			Additive	27.06% (7)
			No ILV	<0.01% (1)

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Chapter 4

Social context when facing a novel environment is paramount in a passerine species

SOCIAL CONTEXT WHEN FACING A NOVEL ENVIRONMENT IS PARAMOUNT IN A PASSERINE SPECIES

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ABSTRACT

Exploratory behaviour in a novel environment has been extensively investigated in many taxa and different contexts, as it is one of the traits most studied in relation to animal personality. It presents in fact consistent between-individual differences within a population and it is considered inheritable. How an individual explores its environment can moreover be paramount for the location of food sources and its capability to disperse, particularly for species invading or currently living in anthropized environments. However, the few previous studies on the influence of social context during exploration did not return consistent results in birds, which are among the most-studied organisms in this field: conspecific presence may result in a faster and more extensive exploration, but evidence for this social facilitation effect is still lacking. During this experiment we released 96 house sparrows (*Passer domesticus*) in a large novel room (8.3 x 8.7 m and 2.5 m high), both alone and with a companion. Both males and females exploring with a companion spent more time eating, had shorter latencies to land on the ground and forage and visited both more food sources and sectors. Such a difference during the invasion of a new environment could prove crucial for their survival, as they would secure more resources in shorter time. We argue that the social context had such a strong effect in our experiment because of various factors, such as the potentially stressful and natural-like appearance of the experimental room and the ecology of the model species. These results underline the prominence of the social context for bird species facing a novel environment.

KEYWORDS: Exploration, house sparrow, invasive species, novel environment, open-field test, *Passer domesticus*, personality, social behaviour, social exploration.

INTRODUCTION

Exploratory behaviour is one of the most commonly studied behavioural traits (Perals, Griffin, Bartomeus, & Sol, 2017), with novel environment tests performed across many different taxa (Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013) and contexts (Webster & Ward, 2011; Thys et al., 2017). Exploring a novel area can become unavoidable for different reasons, such as during colonization, dispersal or for a sudden change of the environment, with food sources disappearing in some areas and becoming available in others (Fretwell & Lucas, 1970). The behavioural response to a novel environment is thus considered paramount for the individuals' survival and fitness (Clobert, Galliard, Cote, Meylan, & Massot, 2009; Korsten, van Overveld, Adriaensen, & Matthysen, 2013): differences in this behaviour have in fact been linked to numerous life history traits, from natal dispersal to reproductive success (Dingemanse, Both, van Noordwijk, Rutten, & Drent,, 2003; Dingemanse, Both, Drent, & Tinbergen, 2004; Cole & Quinn, 2012; Snijders et al., 2014). Novel environment exploration (NEE from now on) has hence become a signature trait for the study of personality (Arvidsson, Adriaensen, van Dongen, Stobbeleere, & Matthysen, 2017), as it presents striking variability among individuals within a single population (Dingemanse, Both, Drent, van Oers, & van Noordwijk, 2002). It is often used to assign individuals to a point along the proactive-reactive continuum, with fast-exploring animals considered to have bolder personalities than slow-exploring ones (Aplin, Farine, Mann, & Sheldon, 2014). This behavioural trait has thus been demonstrated to be repeatable, inheritable (Dingemanse et al., 2002; Van Oers, de Jong, van Noordwijk, Kempenaers, & Drent, 2005a; Korsten et al., 2013), and consistent across contexts in many species (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Theoretical models underline its importance especially for opportunistic species living in unstable habitats (Wright, Eberhard, Hobson, Avery, & Russello, 2010) or for populations at the border of their range (Canestrelli, Bisconti, & Carere, 2013), that are more often faced with novel resources or stressors (Liebl & Martin, 2014). In house sparrows (*Passer domesticus*) individuals living in recently colonized areas were found to be bolder during novel

environment exploration and to exploit novel foods faster than birds in long-colonized parts of their range (Martin & Fitzgerald, 2005; Liebl & Martin, 2012). This was interpreted as an adaptive behavioural shift due to a greater chance of incurring in novel resources and stressors in an unfamiliar and unstable environment. Moreover, in many bird species populations living in urbanized areas have been shown to present bolder personality traits (Atwell et al., 2012; Lowry, Lill, & Wong, 2013; Ducatez, Audet, Rodriguez, Kayello, & Lefebvre, 2016), possibly indicating that faster exploration and reduced neophobia represent an advantage in such unpredictable environments.

However, NEE appears more and more as one of the many behavioural traits that in natural conditions are often influenced by social context (Webster & Ward, 2011). In fact, many of the species whose exploratory behaviour has been studied extensively are unlikely to explore alone, as they usually forage in groups (Tóth, Tuliozi, Baldan, Hoi, & Griggio, 2017). This is not surprising, as benefits usually associated with social living could greatly increase the individuals' chances of survival when faced with novel stressors or novel potential resources (Skandrani, Bovet, Gasparini, Baldaccini, & Prévot, 2017). The possibility for social transmission of information for example is considered fundamental for quick exploiting of novel food sources, a critical feature during NEE (Laland, 2004; Aplin, 2016). Animals in fact are thought to highly value information provided by other individuals, even more so when confronted with an unknown situation (Webster & Laland, 2008). At the same time as novel environments are potentially very stressful (Banerjee & Adkins-Regan, 2011), the presence of other individuals could be particularly important in decreasing alert time, neophobia and latency to exploit food sources (Devries, Glasper, & Detillion, 2003; Ward, 2012). For example, social context in fish has indeed been associated with social facilitation, or the change in the rate of certain behavioural responses (*sensu* Webster & Ward, 2011). In particular individuals swimming in groups decrease their individual latency to emerge from a shelter and to approach objects, and generally increased their speed of exploration in a wide variety of contexts (Krause & Ruxton, 2002; Webster, Ward & Hart, 2007; Magnhagen & Bunnefeld, 2009; Ward, 2012).

Yet, surprisingly, the influence of social context on personality traits in birds is much less clear. Female Great Tits (*Parus major*) were found to increase their latency to feed after a startle test in a social context, while a decrease in the latency to feed in the same experiment was possibly attributed to the fact that individuals had a previous experience of a similar test (van Oers, Klunder, & Drent, 2005b). Zebra Finches (*Taeniopygia guttata*) either exploited more feeders when alone than when a companion was in view (Mainwaring, Beal, & Hartley, 2011) or were influenced by the exploration score of their companion (Schuett & Dall, 2009). Evidence of conformity (suppression of individual variation within a group) was found also in Gouldian Finches (*Erythrura gouldiae*), as birds adopted the strategy of their companion, but on average did not have significantly shorter latencies to approach objects or forage (King, Williams, & Mettke-Hofmann, 2015) while in corvids the social context was either found to increase (Stöwe et al., 2006; Miller, Bugnyar, Pölzl, & Schwab, 2015) or decrease (Chiarati, Canestrari, Vera, & Baglione, 2012) the latency to approach a novel object. Only in an experiment by Kuo, Lee, & Chu (2014) it was found that tree sparrows (*Passer montanus*) landed on the ground sooner when in a group of five than when alone. These contrasting results could be due to many factors, as for example differences between experimental set-ups. Birds in fact have been recently demonstrated to behave differently when tested in rooms or cages of variable shape and size (Arvidsson et al., 2017). Furthermore, the identity of the companion(s) appeared to have an influence on the exploration strategy of the focal individual, underlining a possibly complex behavioural response influenced by phenotype and experience (Schuett & Dall, 2009; Ilan, Katsnelson, Motro, Feldman, & Loten, 2013). Some companions could for example represent a source of ulterior stress because of competition or dominance (Stöwe et al., 2006), thus slowing the exploration and increasing the alert time of the focal individual. However, the roles that social context plays in other circumstances in birds, such as reducing anxiety (Apfelbeck & Raess, 2008), increasing activity and speeding up problem-solving (Liker & Bókony, 2009) would lead us to predict that during a potentially stressful situation such as NEE the presence of a companion could induce social facilitation, i.e. decreasing latencies to feed and drink and increasing the fraction of environment

visited. The influence of the social environment may be particularly conspicuous in a species that strongly relies on social cues to detect ephemeral food sources in an unpredictable habitat (Elgar, 1986; Tóth et al., 2017). In this study we investigated the effect of a companion on NEE in house sparrows, focusing on resource acquisition and efficiency of exploration. During the exploration of an unknown area quicker access to resources and novel sectors could prove crucial for survival, particularly for an invasive species. While during the experiment we tested not only birds exploring with a familiar companion and alone, but also birds exploring with an unfamiliar companion, in this chapter I will not discuss the latter treatment, in order to solely focus on the consequences that a social companion might have on exploratory behaviour. All three treatments are discussed together in chapter 5 (Tuliozi, Fracasso, Hoi, & Griggio, 2018).

While exploratory behaviour has long been studied via novel environment tests conducted in relatively small rooms (Aplin et al., 2014), tents (Liebl & Martin, 2012) or cages (Mainwaring et al., 2011; King et al., 2015; Perals et al., 2017), we decided to conduct our tests in a vast room (183 m^3), with numerous branches as perches providing different levels of cover. This was done in order to simulate more faithfully a natural environment that would require a longer time to be properly assessed for its size, novelty and thus possibly also perceived risk, and where individuals could be able to follow each other to food sources. For this reason we also decided to run 2-hours long tests, as we did not consider shorter tests to be informative for our purposes. In order to investigate the different aspects of social and individual exploration we recorded various behavioural responses that are generally considered to represent various aspects of NEE. Latency to land and time spent on the ground, as it is potentially more dangerous than branches or other elevated perches, can be considered indicators of reduced anxiety (Schuett & Dall, 2009), while the number of sectors visited gives a measure of exploratory behaviour proper. Finally, the time spent eating during NEE is an important proxy for survival in a novel environment. We predicted that the presence of a companion would lead to social facilitation in house sparrow individuals: they would visit more sectors in a shorter time, decrease latencies to exploit resources and visit sooner areas that could be perceived as risky.

METHODS

Study species

The house sparrow is an adaptable human commensal, and thus a species that often depends on clumped, novel and ephemeral food sources. It is a highly sociable species, a semi-colonial breeder that during the non-breeding season travels, forages and roosts in mixed-sex flocks. It has been already demonstrated that this species uses its social environment to obtain clues about unknown food sources, with individuals leading the foraging bout actively emitting assembly calls to their companions (Elgar, 1986; Liker & Bókony, 2009). For these reasons – being both highly sociable and opportunistic invaders – the house sparrow constitutes an ideal model species to examine the role of social environment in relation to exploratory efficiency during NEE.

Housing and study subjects

The study was conducted between March and June at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine) in Vienna, Austria (48˚13' N, 16˚17'). All 96 house sparrow individuals (48 males and 48 females) used in the experiment were born during the previous breeding season (252.46 ± 26.57 days of age at the beginning of the experiment, reported henceforward are mean ± se) and reared by their parents in the same aviaries where they were born. We used only one age-class in order to avoid age-related variations during tests (Miller et al., 2015). The birds were kept in mixed-sex outdoor enclosures (from now on "housing aviaries"), measuring 2 × 3.9 m and 2.6 m high. Each housing aviary was equipped with a feeder (consisting of a metal bowl on a wooden pedestal, 1.2 m from the ground), small pine trees, which were usually used to roost, and branches as additional perching places. All aviaries were provided with food (a mixture of

millet, canary seeds, wheat, sunflower seeds, protein-based mash, apple slices and millet sprays hanging from the branches) and water (in a dish on the ground) ad libitum (Griggio & Hoi, 2010; Griggio, Biard, Penn, & Hoi, 2012; Griggio, Fracasso, Mahr, & Hoi, 2016). All the study subjects were housed together in 5 housing aviaries. No birds tested together were siblings.

Temporary housing during the experiment

Two days before the start of the tests 4 groups of study subjects (each group consisting of 6 same-sex birds from the same aviary) were moved from the housing aviaries to new temporary aviaries, where they would remain until all individuals in their group had been tested once (7.08 ± 1.31 days). When all individuals belonging to the first 4 groups had been tested they were returned to their housing aviaries and the next 4 groups were moved to the temporary aviaries. We did not return the birds to the housing aviaries until every bird in the 4 groups had been tested, in order to maintain the social groups consistent between tests. The reasons for the momentary transfer from housing to temporary aviaries were both practical and experimental. An aviary with only six individuals made the management, selection and capture process much less stressful for the entire group: moreover, it allowed a closer inspection of the birds' state and behaviour. The temporary aviaries were not meant to stress the birds with novelties, and thus were similarly but more homogeneously equipped than the housing aviaries: they measured 3.7 x 1.9 m and 2.5 m high, with a metal bowl on a pedestal (1.2 m from the ground), branches on the corners of the roof, one roosting trees and a water dish on the ground. To ensure that transferring the birds was not in stressful all individuals were closely monitored after each relocation. Features of these temporary aviaries were, as far as possible, of the same size and in the same position in all four temporary aviaries. Birds in the temporary aviaries were fed daily (in the morning) with ad libitum (roughly 300 g) standard mixture of seeds (wheat, canary seeds, sunflower seeds). After two days of habituation to the temporary aviaries (enough time for

captive house sparrows to adjust to a new environment, Tóth, Baldan, Hoi, & Griggio, 2014), on the morning of the third day we started with the tests.

Experimental protocol and experimental room

We recorded the exploratory behaviour of all 96 individuals (48 males and 48 females) while in same-sex familiar pairs ("social context") and alone ("non-social context") in a novel environment. The pairs were formed by individuals that had always been housed in the same aviary, hence flock-mates familiar with each other. We also recorded the behaviour of same-sex unfamiliar pairs, which will be treated in the next chapter (Tuliozi et al., 2018). The order of the tests was randomized across contexts: after all 96 birds had been tested in one of the three contexts we started a new round of tests. At the end of the experiment for each 48 pairs tested one bird was randomly chosen as "focal", while the other ("companion") was not included in the analysis. Thus, every bird was tested both in the social and in the non-social context, independently from all the other individuals considered. The two tests of the same birds were separated by 36.82 ± 13.3 days, which is considered enough to avoid potential learning effects (Schuett & Dall, 2009). We generally performed 3.55 ± 1.13 tests a day, (between 1 and 5 tests each day), usually testing every day at least one bird from each of the 4 groups in the temporary aviaries. All tests were conducted between 2 hours after sunrise and 1 hour before sunset: the hour of the test was randomized between contexts.

2 hours prior to each test the food bowl was removed from the temporary aviaries of the individual(s) scheduled for the test, in order to standardize the feeding motivation. As soon as the individual(s) scheduled for the test were captured, food was returned to the temporary aviaries for the other individuals. We assessed the exploratory behaviour in a vast indoor novel environment, which measured 8.3 x 8.7 and 2.5 m high, with wood shavings on the floor, as in all the outdoor aviaries. Light was both natural, coming from the semi-transparent roof, and artificial (9 neon lights, always turned on). All tests were also observed via a one-way plastic window on the left wall of the room.

All tests were recorded via three webcams (LifeCam Studio, Microsoft. Article number: Q2F-00015 and Q2F-00016). All video data were processed through iSpy, a free open source software (version 6.3.0.0. See at www.ispyconnect.com). The environment was equipped with a number of features, in order to quantitatively test the exploratory behaviour of focal individuals and to simulate a natural novel environment: an ample part of the approximately 72 square m of the experimental room was covered by branches, providing cover more or less dense in different areas. There were also multiple food sources and water was positioned on the ground, as in in the living and temporary aviaries. The branches and the other perching areas were differentiated in 10 sectors, corresponding to spatial locations independent from one another (we rarely observed birds hopping back and forth from different sectors, as moving from one to the other usually required at least a brief flight).

At the beginning of every test the study subject(s) were captured with hand-nets as quickly as possible, and then transferred via a small cloth bag to the two-parted cage (200 x 50 cm and 50 cm high) inside the room. All focal individuals were unable to see their companion in this situation, but they were able to see the exploration room. After 10 minutes of habituation into the cage the lid(s) of the cage were opened from outside the room, using a system of strings. As soon as the lid(s) were open the test started. Each test, both social and non-social, lasted 2 hours, after which we recaptured the bird(s) from the room and we released them back in their temporary aviaries. For all individuals (both focal and companion) we recorded a number of variables usually recorded in NEE tests (Perals et al., 2017), such as i) latency to exploit (take the first bite from) the first food source, ii) latency to touch the ground, iii) fraction of sectors visited iv) fraction of food sources visited v) time spent on the ground vi) time spent eating. This last measure was taken by measuring specifically how much time each bird spent pecking at the millet sprays. We also recorded vii) if the visits to the ground were in the proximity (within 50 cm) of the water saucer or in any other area. Birds that did not leave the cage (3 out of 96 individuals in the non-social test, 0 in the social test), that did not eat or touch the ground were assigned a latency of 7201 s (van Oers et al., 2005b).

Statistical analyses

All data were analysed using R version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). All statistical tests were two-tailed. The significance threshold was set at $\alpha = 0.05$. All statistical tests were conducted using as subjects only focal individuals: companion individuals were completely excluded from the analysis in order to avoid the influence that two individuals tested together could have on each other. Exploratory behaviour in a novel environment was analysed using General Linear Mixed Models (GLMMs). GLMMs are often used wherever data are non-normally distributed and random effects possibly account for part of the variance. The models were fitted using the 'glmer' function within the package lme4 (1.0.5) for 3.2.1 (Bates, Maechler, Bolker, & Walker, 2015). Each dependent variable was analysed using a separate model. Our dependent variables were i) fraction of sectors visited (out of a maximum of 10), ii) fraction of food sources discovered (out of a maximum of 4), iii) time spent foraging iv) latency to forage, v) latency to touch the ground vi) time spent on the ground. We analysed the first two using logistic regression for proportion (logit link), time spent foraging was normally (Gaussian) distributed, while the latter three were modelled with gamma distribution (log link). The log link was chosen because the use of the canonical (inverse) link often caused models to fail to converge. The gamma models that did converge with the inverse link had similar results to the ones with the log link. Variables were weakly correlated (Pearson coefficients range from -0.389 to 0.313) apart from areas visited and food sources visited, which presented a medium correlation (Pearson coefficient: 0.574), and foraging latency and time spent foraging, which presented a strong inverse correlation (Pearson coefficient: -0.651).

Sex, context (social and non-social) and their interaction were fitted as the categorical fixed effects, whose estimates and significance were obtained using the 'Anova' function within the 'car' package (Fox & Weisberg, 2011). All results reported are from models containing both main effects: however, no interaction term was found significant ($p > 0.05$) and all were thus removed from the models. As

random terms in the analysis we entered the identity of the focal bird, as each focal bird participated in both social and non-social tests.

Ethical note

Capture, housing and handling of birds were in accordance with the relevant Austrian laws and were licensed by the government of Vienna (MA 22) license number 424/2011. The experiment reported in this study complies with current laws on animal experimentation in Austria and the European Union. This study was approved by the institutional ethics committee (University of Veterinary Medicine, Vienna) and the national authority according to 8ff of Law for Animal Experiments Tierversuchsgesetz - TVG, licence number GZ 68.205/0220-II/3b/2012. The condition and health of experimental birds were monitored on a daily basis by means of behavioural observation at the aviaries. No individual died during the 5-months long experiment.

RESULTS

Out of 96 sparrows tested in the non-social context, 7 did not forage (7.3%, 5 males, 2 females), while all sparrows tested in the social context ate from at least one food source. There was no significant difference between the sexes both in social and non-social contexts (GLMM, all $p > 0.15$, Table 1). There was a significant effect of social context on exploratory behaviour: individuals with a companion had shorter foraging (Fig. 1a, Table 1) and ground latencies (Fig. 1b, Table 1). They also exploited more food sources (Fig. 1d, Table 1) and explored a higher proportion of sectors (Fig. 1c, Table 1). House sparrows with a companion spent more time eating (Fig. 2, Table 1); they also spent more time and on the ground (Table 1) where, however, in both contexts individuals stayed for a very short time (10.44 ± 3.11 s in the non-social context, 14.69 ± s in the social context, N=48). Moreover, only 11 out of 193 total visits to the ground in both contexts were not in the immediate

proximity of the water. All companion birds were excluded from the analysis in order to avoid the influence that two individuals tested together could have on each other.

DISCUSSION

The presence of a group-mate during the exploration of a novel environment (or open-field test) has never been unambiguously demonstrated to cause a faster and more thorough exploration in birds as it has generally been in fish (Webster & Ward, 2011; Mainwaring, et al., 2011). In fact, while both previous social connections (Tóth et al., 2017) and personality type (Schuett & Dall, 2009) have been found to variably influence group-mates' exploratory and foraging behaviour, a generalized effect of social context in itself has not often been demonstrated during NEE. We found out that the presence of a group-mate had a strong influence on all aspects of exploration: individuals released alongside a companion visited more sectors and food sources, started foraging sooner and had shorter latency to land on the ground. They also spent more time eating and hopping on the floor when in company than alone.

In recent years the identification of the behavioural traits measured during an open-field test has been the subject of debate and care should be taken when differentiating between them (Arvidsson et al., 2017; Perals et al., 2017). We found indeed only weak correlations between most variables recorded during our study: for example the correlation between the fraction of sectors visited and foraging latency was low, which could indicate that they were the expressions of different behavioural traits. It is also necessary to consider that the same variable can possibly measure two different traits in two different contexts: for example, latency to visit a novel sector could measure neophobia when the individual is alone and propensity to follow another individual when it is tested in a social context. This emphasizes the importance of focusing on the general influence that a different context (in our case, the presence of a companion) could have on different behavioural traits.

One of the main ways in which the presence of a group-mate can influence individual behaviour is the reduction of neophobia and anxiety (Apfelbeck & Raess, 2008; Banerjee & Adkins-Regan, 2011). Perceived anti-predator defence and its consequent decrease in individual alert time are advantages reputed fundamental for the development of social behaviour (Sorato, Gullett, Griffith, & Russell, 2012), alongside with social learning (Dukas, 2013). But while the latter could not have played a huge role in our experiment, as individuals did not have the chance to learn from each other, we have indications that group mates tested together had a fear-reducing effects on each other. The amount of time spent eating has often been used as a proxy of the perceived safety of a situation (Beauchamp, 2008) as an individual concentrating more on the food sources spends less time alert, observing its surroundings. In our experiment house sparrows in the social context spent a significantly longer time foraging, thus exploiting the new environment more efficiently than individuals alone. Such a difference during dispersal or the invasion of a new environment could prove crucial for their survival, as they would secure more resources in shorter time.

The latency to land on the ground could also be considered evidence of the anxiety-reducing effect of the social context. Birds perceive the floor as a higher-risk area compared to branches and other higher perches (Schuett & Dall, 2009): in our study this part of the room was apparently visited as little as possible, rarely for reasons different than going to the water, and even in that occasion for very short time (see Results). Individuals with a companion in the room not only had much shorter latencies to land, but they also spent more time on the floor. Another neophobia-reducing effect of the presence of a conspecific is the decrease in the latency to touch objects, to explore areas and start foraging (Ward, 2012; Galhardo, Vitorino, & Oliveira, 2012). Surprisingly either no such change or the opposite was found in similar experiments both in birds and mammals (van Oers et al., 2005b; Ilyina, Ivankina, & Kerimov, 2010; Mainwaring et al., 2011; King et al., 2015; Weiss, Segev, & Eilam, 2015; Dorfman, Nielbo, & Eilam, 2016), suggesting that in some situations the social context could function instead as a distraction or as an ulterior stressful element. This was not the case in our experiment, as the presence of another individual reduced all latencies and increased the number of

sectors visited and food sources exploited. Behind these results there could be a number of not-excluding factors.

Firstly, our experimental room was quite vast and complex and thus potentially more stressful than a relatively smaller cage or room where individuals could assess quickly the entirety of their surroundings. This could have downplayed possible anxiogenous effects of social context, such as fear of dominant or aggressive individual, by overshadowing them with a greater fear of the novel environment (Stöwe et al., 2006; Banerjee & Adkins-Regan, 2011). A second reason for which the social context in our test appeared to have such a straightforward effect could have been that all individuals tested together were already knowledgeable of each other. This is a rarely investigated variable of the social environment during NEE in birds (Kohn, Meredith, Magdaleno, King, & West, 2015; Kabasakal et al., 2017) but the unfamiliarity between individuals could account for a strong part of the potentially anxiogenous effect of the social environment. This topic is discussed at length in the next chapter (chapter 5), where alongside the results presented here we analyse also the influence of an unfamiliar companion (Tuliozi et al., 2018). Lastly, the species subject of this study is presumably particularly influenced by social context because of its behaviour and ecology. In our experiment we demonstrated that house sparrows in a social context got quicker access to resources and started foraging earlier. This species is strongly sociable and relies on other individuals on information about its surroundings, food sources (Tóth et al., 2017) and stressors (Elgar, 1989). Moreover, in our experiment the food sources were clumped (non-dispersed) and overabundant: in this situation birds are thought to gain an advantage from the presence of conspecifics, because they gain anti-predator benefits with competition being purportedly less problematic (Beauchamp, 2002). The presence of another individual could thus allow birds to exploit resources quicker, longer, and yet without perceiving an increased predation risk. Ultimately this is a key advantage, particularly for an urban species. Recently urban birds have been found to be generally more explorative and bolder (Lowry et al., 2013) than their rural or wilderness counterparts, which is consistent with the idea that bolder and more explorative individuals are favoured in a combination of novel and fast-changing

environments (Liebl & Martin, 2012; 2014). For this reason the social context could provide greater efficiency, decreasing alert time and increasing the chance of exploiting novel food sources (Skandrani et al., 2017).

The sum of these results underlines the prominence of the social environment for bird species released in a novel territory. This in turn could have several major evolutionary consequences. The social environment could act as a buffer, allowing birds invading new areas in social groups to be subject to different selective pressures than if they were outside of the social environment. Thus some traits – like neophilia and boldness – could be less strongly selected for, and for this reasons population of individuals could present greater variability (Cote, Fogarty, Tymen, Sih, & Brodin, 2013). Moreover, newly colonized or urban landscapes could in fact grant better survival chances not only to bolder or innovative individuals, but also to more sociable ones. Further studies may thus focus on the differences in social tendency presented by populations in urban and wild areas. The possibility that house sparrows and other invasive species are being selected for traits linked to NEE at the border of their range makes them perfect model for studying how social environment during NEE can influence the selective pressures on different behavioural traits.

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Tables

Table 1. Table shows dependent variable, AIC of the General Linear Mixed Model, model terms (fixed factors), test statistic (Wald) and *P* value. For all models "bird identity" was retained as random factor and all fixed factors had 1 degree of freedom. Significant test results are indicated in bold.

	AIC	Fixed factor	Wald (χ^2)	p-value
Foraging latency	753.8	Social context	**8.870**	**0.003**
		Sex	0.013	0.909
Ground Latency	1721.7	Social context	**16.950**	**< 0.0001**
		Sex	0.019	0.892
Sectors visited	479.2	Social context	**13.955**	**< 0.0002**
		Sex	1.972	0.160
Food sources exploited	243.1	Social context	**8.292**	**0.004**
		Sex	0.696	0.404
Time spent foraging	1318.4	Social context	**5.255**	**0.022**
		sex	0.056	0.812
Time spent on the ground	626.8	Social context	**16.110**	**< 0.0001**
		Sex	0.238	0.626

Figure Legends

Figure 1. Behavioural response to the novel environment in non-social and social context. (a) Latency to forage (s) for birds tested alone and with companion (*N=48*). (b) Latency to land on the ground (s) for birds tested alone and with companion (*N=48*). (c) Number of room sectors visited for birds tested alone and with companion (*N=48*). (d) Number of food sources exploited for birds tested alone and with companion (*N=48*).

Figure 2. Time spent eating. Amount of time spent foraging (s) during novel environment exploration in non-social and social context.

Figure 1.

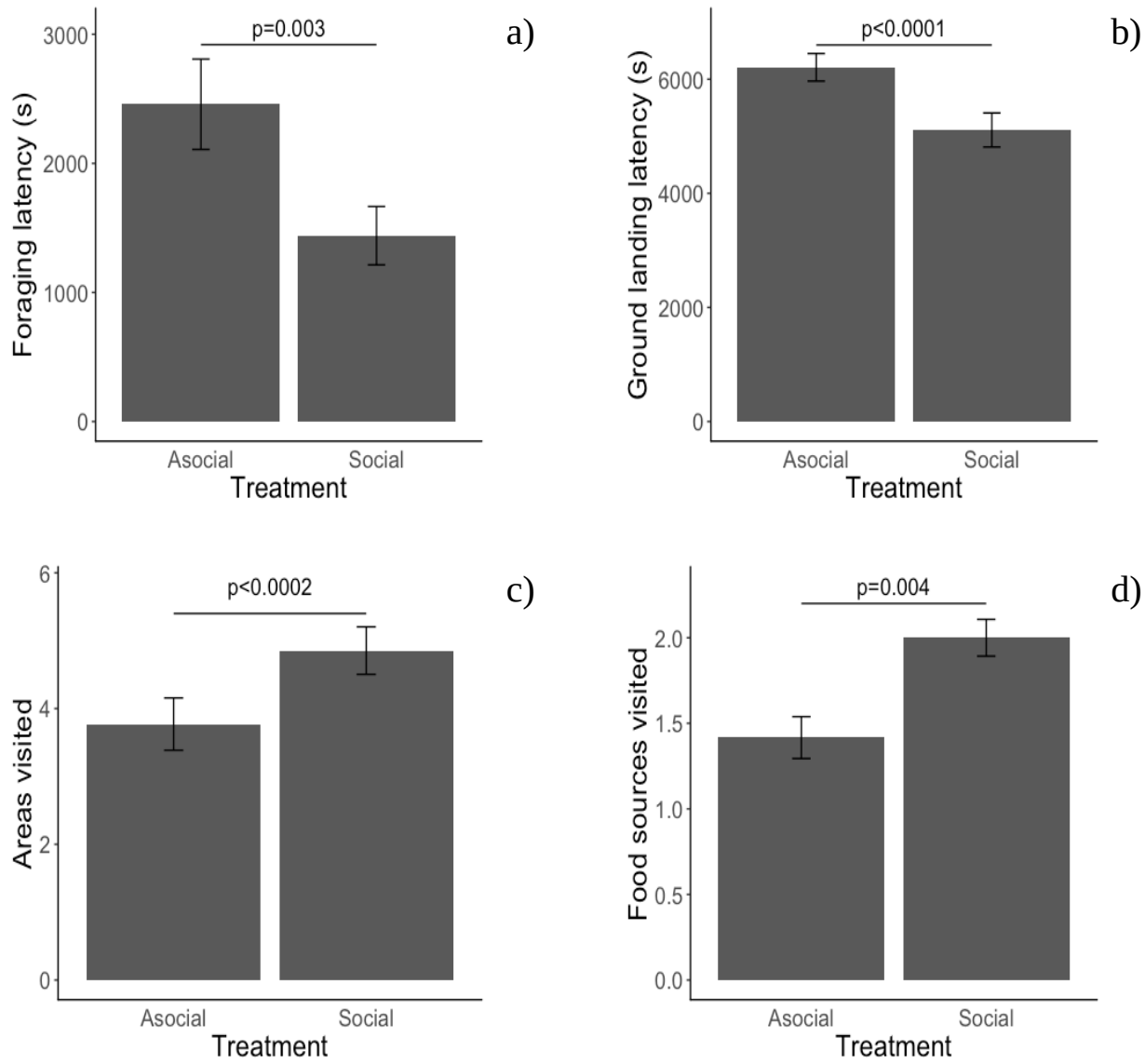
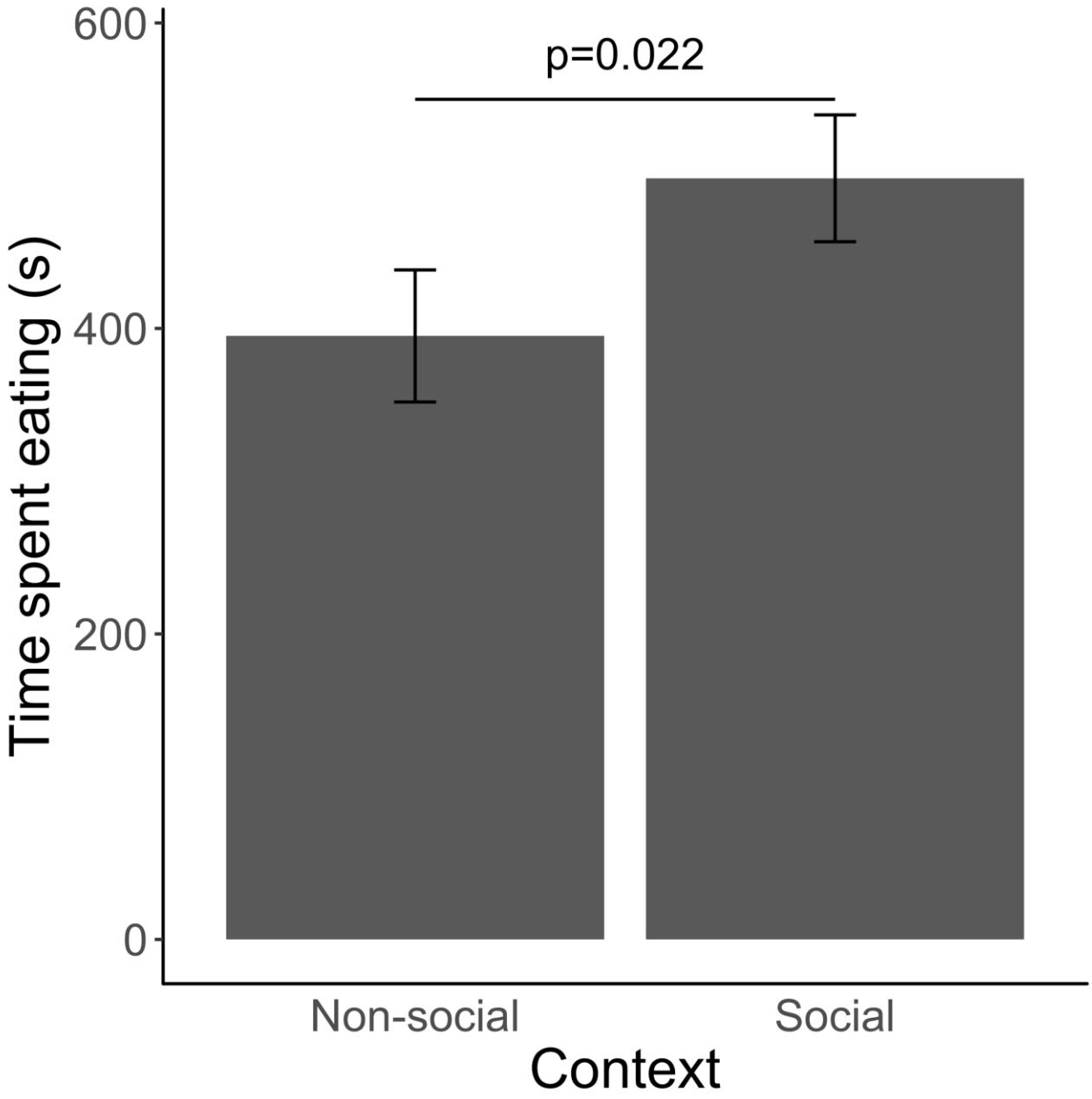

a)
p=0.003
Foraging latency (s)
3000
2000
1000
0
Asocial
Social
Treatment
b)
p<0.0001
Ground landing latency (s)
6000
4000
2000
0
Asocial
Social
Treatment
c)
p<0.0002
Areas visited
6
4
2
0
Asocial
Social
Treatment
d)
p=0.004
Food sources visited
2.0
1.5
1.0
0.5
0.0
Asocial
Social
Treatment

Figure 2.


600
400
200
0
p=0.022
Time spent eating (s)
Non-social
Social
Context

Chapter 5

House Sparrows' (*Passer domesticus*) behaviour in a novel environment is modulated by social context and familiarity in a sex-specific manner

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House sparrows' (*Passer domesticus*) behaviour in a novel environment is modulated by social context and familiarity in a sex-specific manner

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Abstract

Background: Exploratory behaviour is one of the best-investigated behavioural traits. However, little is known about how differences in familiarity, i.e. in the knowledge and previous experience with a companion can influence the exploration of a novel environment. However, to our knowledge, such a critical feature of the social environment has never been the target of a study relating it to exploratory behaviour in birds. Here we examined if familiarity with a conspecific could affect behavioural responses of individuals confronted with a novel environment. We recorded the latency to land on the ground, latency to feed, time spent feeding and number of sectors visited of 48 female and 48 male house sparrows (*Passer domesticus*) in an indoor aviary in three contexts: alone (individual context), with an unfamiliar and with a familiar same-sex companion.

Results: House sparrows landed sooner on the ground when in the familiar context than when in the individual context. Birds in unfamiliar pairs followed each other less than familiar birds, but this difference diminished with time spent exploring. Moreover, males and females differed in their behavioural responses in the unfamiliar context. Females with a familiar companion landed sooner than when they were paired with an unfamiliar conspecific, whereas only the presence of a companion but not familiarity reduced males latency to land on the ground. Finally, when considering the unfamiliar context males had shorter latencies to forage and thus spent more time eating than females.

Conclusions: The presence or absence of a companion and its familiarity with the focal individual influenced differently the behavioural responses of male and female house sparrows in a novel environment. As house sparrows are strongly sociable, the influence of the social environment is likely to be of paramount importance to understand the selective pressures acting on them, particularly in recently colonized areas with ephemeral food sources. Our results shed light on the complex influence that the social environment has on the behavioural responses of a cosmopolitan bird.

Keywords: Exploration, Familiarity, House sparrow, Invasive species, Novel environment, Open-field test, *Passer domesticus*, Personality, Sex-difference, Social behaviour

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Background

Behavioural responses to novel environments (such as exploratory behaviour and neophobia) are considered critical targets of selective pressures [1–3]. While animals exposed to unfamiliar environments generally perceive them as less predictable and more dangerous than familiar places and situations [4–6], they are often forced to explore, disperse and colonise new areas [7, 8]. However, exploratory traits are often investigated using animals in an individual context, while the presence of conspecifics can modulate the expression of behavioural responses, for example through social facilitation (change of rate of certain behavioural responses, sensu [9]).

Indeed, in recent years the social environment has been more and more often recognized to play an important role in shaping the evolution of various behavioural and physiological traits [10] and individuals facing a novel environment can gain various benefits from being in a group. The presence of conspecifics could result in social buffering, with individuals reacting better and faster to stressful experiences such as exploring a new environment [11]. This could result in decreased neophobia that would allow, for example, to visit areas perceived as risky or approach and acquire novel food sources [12, 13]. Early discovery, examination and securing of resources could prove crucial for survival, particularly for invasive species that rely on novel and ephemeral food sources. For individuals of such species it is conceivable that covering ground rapidly and having short latencies to forage and drink, i.e. the characteristics of a fast exploration, could prove advantageous in a novel environment [14–16]. Exploring with a conspecific could allow to spend less time alert without sacrificing cautiousness, as alert time can be split between companions. Moreover, some species strongly rely on social cues to detect clumped food sources in an unpredictable habitat [17, 18]; when different and often novel food sources are available, such as during a colonisation event, a group can allow greater flexibility (coping faster with new situations) and better performances than individuals alone (see for example [19]). Apart from the conspecific presence in itself, attention has recently been given to the influence that the characteristics of the conspecifics have on the behaviour of a focal individual [3, 10]. Among other things, individuals were discovered to behave differently depending on their companions' boldness [20], kinship [21], and social dominance [22]. Some aggressive or dominant individuals could for example be perceived as a stressor for their group-mates, thus increasing alert time and neophobia in difficult situations [23, 24], while other conspecifics could have the very opposite effect, decreasing neophobia and alert time [13]. This underlines a system of conspecific recognition and flexibility in behavioural responses that may be affected by differences in behavioural traits and experiences [25, 26].

The phenotype of the companion is not the only aspect that could influence an individual's behaviour in a social context. One distinction to be made between conspecifics is if they are familiar or unfamiliar with one another, namely if they have learnt to recognize group mates with which they had repeated interactions or not [27–29]. The behavioural response to the presence of a familiar conspecific can be different from the response to the presence of an unfamiliar conspecific [30]. Firstly, antagonistic interactions are often less common among familiar than among unfamiliar conspecifics as the unfamiliar conspecifics may use such interactions to establish a new social dominance hierarchy [31]. Secondly, since animals living in social groups are prone to competition and other within-group stressors, familiarity between group-members has been argued to be an important factor keeping groups together, avoiding a continuous fission-fusion process that could be costly in the long run [28, 29, 31, 32]. Thirdly, an unfamiliar conspecific could represent an unknown risk and source of stress until the potential threats it presents are fully assessed [33]. Lastly, immediately trusting an unfamiliar individual from the first encounter could prove maladaptive, since an unfamiliar conspecific could exploit the newly formed connection without giving anything in return [34]. In this scenario, the unfamiliar individual would simply not be trustworthy enough to be used as a reliable source of vigilance or social information, and thus either be ignored or mistrusted [35–37].

The effects of conspecifics familiarity on animal behavioural responses, i.e. the difference in behavioural responses due to conspecific presence being either familiar or unfamiliar, has been mostly studied in fish [30], where it has been found to facilitate social learning, decrease stress and reduce aggression in social groups [30, 38, 39]. In the few studies available on birds it was shown that couples consisting of birds that had been familiar with each other for a long time were sometimes found to have higher fitness, possibly due to greater coordination and cooperation ([40–42], but see [43]). Other studies focused instead on the difference between familiar conspecific presence and conspecific absence [44]. In this study we extended the comparison to unfamiliar conspecifics, focusing on the effect of conspecific familiarity which, to our knowledge, has never been studied in relation to exploratory behaviour in birds.

Therefore, we argue that behavioural responses such as latency to forage and visit the ground in a novel environment, or time spent foraging and fraction of the novel environment visited, could be influenced by i)

presence or absence of conspecific individuals; ii) relationship between conspecific individuals, i.e. if they are familiar or unfamiliar with one another. We argue that the presence of any conspecific could act by itself as a social buffer, lowering neophobic behaviours and resulting, for example, in shorter latencies to forage and more explored areas. However, as assessing the potential threats that an unknown conspecific provides could take time and be potentially stressful, it is possible that an unfamiliar conspecific could not be an effective social buffer. In contrast, a known companion would be a familiar feature in an unfamiliar situation: being alongside it during novel environment exploration could reduce neophobia, which would be particularly useful for a species with a rapid-expanding range or unpredictable habitat, as it would encounter many novel resources, stressors and social environments [45].

To address these questions we decided to use the house sparrow (*Passer domesticus*), as it is an opportunistic human commensal, and thus a species that often depends on clumped, novel and ephemeral food sources. House sparrows have been studied for processes of urbanization [46], dispersal [47] and range expansion [14, 45], as it is an invasive species in many areas of the world. Moreover, it is a highly sociable species, which has already been shown to use its social environment to obtain clues about unknown food sources, with individuals leading the foraging bout actively emitting assembly calls to their companions [18, 19]. For these reasons – being both highly sociable and an opportunistic invader – the house sparrow constitutes an ideal model species to examine the role of social environment in relation to exploratory efficiency (i.e., during novel environment exploration). During winter house sparrows reunite in mixed-sex flocks and often forage in small sub-flocks in urban areas. In this period of the year their social life is thus characterized by continuous fission-fusion dynamics, that allow them to come in contact with both familiar and unfamiliar individuals [48]. Moreover, while generally sedentary, first-year birds (like the ones that we used in our experiment) undergo extensive dispersal [49], while changes in local condition can force them to colonize new areas alongside human settlements [50, 51]. It is not uncommon for them to separate in same-sex couples or small groups, or even move alone for short periods of time ([52, 53], Authors unpublished observations).

We tested the exploratory behaviour of first-year house sparrows in an indoor aviary in three different social contexts: alone, in same-sex familiar pairs and in same-sex unfamiliar pairs. In the current study we did not test mixed-sex pairs. In the novel indoor aviary the sparrows could find food sources, water (on the ground), branches divided in ten sectors. We predicted that birds in the individual context (i.e. tested alone) would be the least bold, having longest latencies to exploit resources (i.e., forage at any food source for the first time) or visit potentially risky areas (i.e., the ground). They would also visit the fewest number of sectors in the novel environment. On the contrary, individuals in the familiar context would behave the most exploratory, having shortest latencies to forage and touch the ground and spending more time eating than when in the other contexts. Finally, individuals would explore differently when alongside an unfamiliar companion from when alongside a familiar one. The unfamiliar context could either cause a decrease of exploratory behaviour under the levels of individuals alone [54, 55], or result in an intermediate level of exploration, i.e. between the familiar and individual context [30].

Methods

Housing and study subjects

The study was conducted between March and June at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine) in Vienna, Austria (48°13' N, 16°17' E). All 96 house sparrows (48 males and 48 females) used in the experiment were born during the previous breeding season (252.46 ± 26.57 days of age at the beginning of the experiment. Measures reported here and henceforward are mean ± standard error of the mean) and reared by their parents in the same aviaries where they were born. We used only one-year-old birds to avoid age-related variations during tests [56, 57]. The birds were kept in mixed-sex outdoor enclosures (from now on "housing aviaries"), measuring 3.9 × 2 × 2.6 m (m) (l × w × h). Each housing aviary was equipped with a feeder (consisting of a metal bowl on a wooden pedestal, 1.2 m from the ground), small pine trees, which were usually used to roost, and four branches as additional perching places. Pine trees had the same size, shape and height (1.5 m) while branches came from trees near the research institute. All aviaries were provided with food (a mixture of millet, canary seeds, wheat, sunflower seeds, protein-based mash, plus apple slices and millet sprays hanging from the branches) and water poured in a dish on the ground [58, 59]. All the study subjects were housed together in 5 housing aviaries and all individuals not belonging to the age-class of the study subjects were removed from the 5 housing aviaries 50 days before the start of the experiment, leaving 19.2 ± 1.8 sparrows in each aviary (range: 15–25 sparrows). Sparrows from different aviaries had never been housed with each other (were completely unfamiliar with each other). Conversely, sparrows from the same housing aviary were either born in the same aviary or were kept together

for at least 50 days before the start of the experiment (were thus familiar with each other). Different housing aviaries were located in four different corners of the Institute, and thus separated by trees and buildings and not in visual or acoustic contact. Two housing aviaries were in the same corner of the institute but were at the two extremities of a row of 12 aviaries, thus separated by ten other aviaries (25 m distant), all housing other birds unrelated to the experiment.

Temporary housing and sub-flocks

The study subjects were further divided in 16 groups (8 groups of males and 8 groups of females) of 6 birds each: all groups were randomly composed of same-sex familiar individuals. There was no difference in body mass, wing length and tarsus length between groups of the same sex (data not shown). Two male groups and two female groups were then moved into 4 temporary aviaries, which were visually and acoustically isolated from the other temporary aviaries and only visually isolated from their own housing aviary. When all the birds in the first 4 groups had been tested once (7.08 ± 1.31 days), the birds were returned to their housing aviaries and the next 4 groups were moved to the temporary aviaries. The reasons for this transfer from housing to temporary aviaries were both practical and experimental. The temporary aviaries were not meant to stress the birds with novelties, and thus were similarly but more homogeneously equipped than the housing aviaries. Furthermore, the management, selection and capture of a bird inside a temporary aviary (containing only 6 birds) was much easier and less stressful than in the housing aviaries where more birds were housed together. A short food deprivation was also necessary for the experimental design and would have been difficult to achieve in the housing aviaries (see Exploration aviary and experimental protocol). Above all, a flock of six birds likely resulted in all birds in each temporary housing aviary closely interacting with one another. Hence, we considered the birds being familiar to one another for the purpose of the experiment. The temporary aviaries measured $3.7 \times 1.9 \times 2.5$ m ($l \times w \times h$), and were equipped with a metal bowl on a pedestal (1.2 m from the ground), branches on the corners of the roof, one or two roosting trees (depending on the amount of roosting places provided) and a water dish on the ground. Features of these novel aviaries were, as far as possible, of the same size and in the same position in all four temporary aviaries. Birds in these aviaries were fed daily (in the morning) with ad libitum (roughly 300 g) standard mixture of seeds (wheat, canary seeds, sunflower seeds). To ensure that transferring the birds was not causing excessive stress all individuals were closely monitored after each relocation by observing them for a minimum of 3 h or until all birds drank and fed if that took longer. Moreover, we made sure that no bird was showing injuries or atypical behaviour, such as prolonged time spent on the floor or flying issues. The birds were left for two days to habituate to the temporary housing aviaries (enough time for captive house sparrows to habituate to a new environment; [17]) before testing began on the morning of the third day.

Test order

We recorded the exploratory behaviour of all 96 individuals (48 males and 48 females), testing them in three social contexts, namely alone (individual context), with a familiar individual and with an unfamiliar same-sex individual. The total number of tests performed was 192: 96 individual tests, 48 familiar tests (each one with two individuals, 96 individuals tested) and 48 unfamiliar tests (each one with two individuals, 96 individuals tested) (Additional file 1: Figure S1). Thus, every bird was tested thrice, once in the familiar, once in the unfamiliar and once in the individual context, independently from all the other individuals considered. For each bird the order of the three tests was randomized across contexts: after all 96 birds had been tested in one context (32 of them in the individual context, 32 in the familiar and 32 in the unfamiliar context) we started two new rounds of tests where we tested each bird in the two remaining contexts. Each round had then 32 individual tests, 16 familiar tests (32 individuals tested) and 16 familiar tests (32 individuals tested). Successive tests of the same birds were separated by 37.24 ± 13.9 days, a period that is considered more than sufficient to avoid learning effects [20]. We did not return the birds to the housing aviaries until every bird in the 4 groups had been tested, in order to maintain the social groups consistent among tests. All tests were conducted between 2 h after sunrise and 1 h before sunset: the hour of the test was randomized between individuals and contexts.

At the end of the experiment half of the birds (48 individuals, 24 males and 24 females) were randomly chosen as “focal”. In each social test individuals were either focal or companion: no individual was both focal and companion (Additional file 1: Figure S1). No siblings were tested together.

Exploration aviary and experimental protocol

Two hours prior to each test the food bowl was removed from the temporary aviaries of the individual(s) scheduled for the test, in order to normalize the foraging motivation [60]. After the birds undergoing testing were capture and removed from the temporary housing aviary, food was returned to the other individuals.

Exploratory behaviour has long been studied via novel environment tests conducted in relatively small rooms [61], tents [45] or cages [54, 62, 63]. We decided to assess exploratory behaviour in an indoor novel environment (exploration aviary), which measured 8.3 × 8.7 × 2.5 m (l × w × h) and was equipped with a number of features to simulate a natural environment. Due to its size, novelty and thus possibly also perceived risk the exploration aviary required a longer time to be properly assessed by house sparrows. Thus, we decided to run 2-h long tests. Light was both natural, coming from the semi-transparent roof, and artificial (9 neon lights, always turned on); the floor was covered with wood shavings, as in all the outdoor aviaries. A quarter of the 72.21 square meters of the exploration aviary was covered by branches. There were also four food sources, of which 3 were sprays of millet hanging from the branches and one was a food bowl on a pedestal with a mixture of seeds and a spray of millet inside. Water was positioned on the ground, as in the housing and temporary aviaries. The branches and the other perching areas were differentiated in 10 sectors, corresponding to spatial locations independent from one another. We rarely observed birds hopping back and forth from different sectors, as moving from one to the other usually required at least a brief flight. All observations were done via a one-way see-through plastic mirror on the left wall of the exploration aviary. All tests were recorded using three webcams (LifeCam Studio, Microsoft. Article number: Q2F-00015 and Q2F-00016). Video data were processed through iSpy, a free open source software (version 6.3.0.0). The birds were also visually monitored by one of the authors (B.T.) through the one-way see-through plastic mirror previously mentioned. After carefully measuring every feature of the exploration aviary we reviewed all video footage to estimate total travel distance [64]. One fifth of the individuals were reviewed by both G.F. and B.T. to account for possible effects of subjectivity.

At the beginning of every test the study subjects were captured at the temporary aviaries with hand-nets as quickly as possible (usually less than 4 min), and then transferred via a small cloth bag to a two-parted cage (2 × 0.5 × 0.5 m (l × w × h)) inside the exploration aviary. All individuals were unable to see their companion in this cage, but they were able to see the exploration aviary. After 10 min of habituation, the cage was opened from outside the exploration aviary using a remote system. As soon as the cage was open the test started. Each test lasted 2 h, after which we captured the birds from the exploration aviary and we released them back to their temporary aviaries. For all individuals (both focal and companion) we recorded a number of variables, such as i) latency to forage; ii) latency to touch the ground; iii) number of sectors visited and iv) time spent foraging. The latter was defined as time spent by birds pecking at the food: any pause in the pecking longer than 3 s was recorded. Birds that did not eat or touch the ground were assigned a latency of 7201 s [65]. Through the analysis of the video footage for each test in the familiar and unfamiliar context we also recorded, for all conspecific pairs: v) number of aggressive interactions (i.e. biting and chasing); vi) number of following bouts. Following bouts were defined as the flights of both birds from one sector to another, taking off within 3 s of each other (similarly as in [17]).

Ethical note

Capture, housing and handling of birds were in accordance with the relevant Austrian laws and were licensed by the government of Vienna (MA 22) license number 424/2011. The experiment reported in this study complies with current laws on animal experimentation in Austria and the European Union. This study was approved by the institutional ethics committee (University of Veterinary Medicine, Vienna) and the national authority according to 8ff (rules) of Law for Animal Experiments Tierversuchsgesetz - TVG, licence number GZ 68.205/0220-II/3b/2012.

The condition and health of experimental birds were monitored on a daily basis. No individual died during the 5-month long experiment.

Statistical analyses

All data were analysed using R version 3.2.1 [66]. All statistical tests were two-tailed. The significance threshold was set at $\alpha = 0.05$. Exploratory behaviour in a novel environment was analysed using Generalized Linear Mixed Models (GLMMs). GLMMs are often used wherever data are non-normally distributed and random effects possibly account for part of the variance. The models were fitted using the 'glmer' function within the package 'lme4' (1.0.5) for R version 3.2.1 [67]. Each dependent variable was analysed using a separate model. Sex, context (alone, i.e. individual context, with a familiar conspecific, with an unfamiliar conspecific) and their interaction were fitted as categorical fixed effects. We also added test order (i.e. if the test took place during the first, second or third round of tests) and part of the day when the test took place (i.e. morning or afternoon) as fixed effects. The dependent variables relative to each individual were i) fraction of sectors visited (out of a maximum of 10); ii) latency to forage (seconds); iii) latency to touch the ground (seconds). We analysed the fraction of sectors visited using logistic regression for proportion (logit link), while the latency to forage and the latency to touch the ground were modelled with gamma distribution (log link). The log link was chosen because the use of the canonical (inverse) link often caused models to fail to converge. The gamma models that did converge with the inverse link had similar

results to the ones with the log link. Total distance travelled was correlated with number of sectors visited, and time spent foraging was correlated with latency to forage (see Results); analysis of both these variables are shown in Additional file 1: Table S1 and S2. We also analysed a variable that was not related to individuals alone, but to each pair, i.e. iv) number of following bouts in each hour of test. In order to focus on differences in this behavioural response between the first and the second hour of each test we used as dependent variable the number of following bouts relative to each of the two hours that comprised a test. Thus, for this dependent variable we also fitted as categorical fixed effect 'hour', i.e. if the number of following bouts corresponded to the first or second hour of experimental observation. As the two hours of the same test could not have been considered independent we added a random factor 'test'. We analysed this variable using Gamma distribution (log link). Aggressive interactions could not be analysed as their number was too low to be informative (see Results). We also tested for correlation using the 'Kendall' package [68] applying a false discovery rate correction.

Estimates and significance of the fixed effects were obtained using the 'Anova' function within the 'car' package [69], while the 'confint.merMod' function within the 'lme4 package was used to obtain intervals of confidence. To differentiate among three or more groups we performed post-hoc analyses of contrasts with the 'lsmeans' function within package 'lsmeans' [70] applying the Tukey method adjusted for multiple comparisons. Results were back-transformed and compared to those obtained with 'glht' in the 'multcomp' package [71], to which they were very similar. We entered as random effects the identity of the bird (as every bird participated once in all three tests).

Social context was also entered as a repeated measure to account for the non-independence of birds' behavioural response to each context [63].

Results

Latency to touch the ground

House sparrows in the individual context had longest latency to touch the ground. The main effect 'social context' had a significant influence on the latency to touch the ground ($df = 2$, $\chi^2 = 22.380$, $p < 0.0001$). The social context × sex interaction was also significant ($df = 2$, $\chi^2 = 8.751$, $p = 0.013$). However, the main effect 'sex' was not significant ($df = 1$, $\chi^2 = 0.191$, $p = 0.663$), even if females in the unfamiliar context had longer latency to touch the ground than males in the unfamiliar context (Table 1).

Table 1 Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) and interaction between 'social context' and 'sex' on latency to touch the ground

Fixed effect	Comparison	Estimate	2% CI	98% CI	*P* value
Part of the day	Morning vs afternoon	− 0.183	**− 0.290**	**− 0.076**	**0.0004**
Sex	Female vs male	0.051	−0.185	0.287	0.6581
Round	First vs second	0.160	**0.045**	**0.276**	**0.0021**
	First vs third	0.200	**0.085**	**0.315**	**0.0001**
	Second vs third	0.039	0.075	−0.154	0.6817
Social context	Individual vs unfamiliar	−0.114	− 0.228	0.0006	0.041
	Individual vs familiar	− 0.224	**−0.338**	**− 0.109**	**<.0001**
	Familiar vs unfamiliar	−0.110	−0.224	0.004	0.050
Social context × sex	Female: individual vs unfamiliar	0.024	−0.136	0.185	0.9283
	Female: individual vs familiar	−0.170	**−0.330**	**− 0.009**	**0.0283**
	Female: familiar vs unfamiliar	0.194	**0.031**	**0.356**	**0.0101**
	Male: individual vs unfamiliar	−0.252	**−0.413**	**− 0.089**	**0.0005**
	Male: individual vs familiar	−0.278	**−0.440**	**− 0.116**	**0.0001**
	Male: familiar vs unfamiliar	0.026	−0.135	0.188	0.9163
Sex × social context	Individual: female vs male	0.077	−0.183	0.338	0.815
	Unfamiliar: female vs male	−0.199	**−0.460**	**0.063**	**0.009**
	Familiar: female vs male	−0.031	−0.292	0.230	0.534
Random effect		**Variance**	**± SE**		
Individual identity		0.090	± 0.303		

Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. *P* values obtained with Tukey method adjusted for multiple comparisons. Results are in the log (not in the response) scale. 'Individual identity' is fitted as random effect; variance associated with it is shown

Females touched the ground earliest in the familiar context, significantly sooner than in both the individual and unfamiliar context (Table 1, Fig. 1). Males did not differ between the unfamiliar and familiar contexts but touched the ground last in the individual context, significantly later than in both the unfamiliar and familiar context (Table 1, Fig. 2).

The main effect 'test order' had a significant influence on the latency to touch the ground (df = 2, $\chi^2 = 19.998$, $p < 0.0001$). Individuals visiting the room for the second and third time touched the ground sooner than when at the first experience, but they did not differ in their latency to touch the ground between the second and third tests (Table 1). Finally, the main effect 'time of day' significantly influenced the latency to touch the ground (df = 1, $\chi^2 = 12.323$, $p = 0.0005$), with individuals touching the ground sooner in the afternoon than in the morning (Table 1).

Latency to forage

House sparrows in the individual context had longest latency to forage. The main effects of 'social context' was significant (df = 2, $\chi^2 = 24.109$, $p < 0.0001$) and so was the social context × sex interaction (df = 2, $\chi^2 = 7.319$, $p = 0.026$): males had longer foraging latency in the individual context than both in the unfamiliar and familiar context (Table 2). Females had shorter foraging latency in the familiar than in the individual context but did not differ between unfamiliar and individual context and between unfamiliar and familiar context (Table 2). In contrast to males, female foraging latency in the unfamiliar context was intermediate between the individual and familiar contexts though the difference was not significant. Accordingly, there was also a significant sex difference limited to the unfamiliar context, with males having significantly shorter latency to forage than females (Table 2). The main effect of 'sex' was not significant (df = 1, $\chi^2 = 0.168$, $p = 0.682$). The main effect 'test order' had a marginally significant influence on the latency to forage (df = 2, $\chi^2 = 5.992$, $p = 0.050$), with sparrows foraging marginally sooner in the third round of tests (Table 2).

Number of sectors visited

House sparrows in the individual context visited the least sectors. The main effect of 'social context' was significant (df = 2, $\chi^2 = 10.481$, $p = 0.005$): birds in the individual context visited less sectors than birds in both unfamiliar and familiar contexts (Table 3). The main effect of 'sex' showed a non-significant tendency (df = 1, $\chi^2 = 3.279$, $p = 0.070$) and the greatest difference being in the unfamiliar context (Table 3). The interaction between the two factors was not significant; however, we kept it in the model as it made theoretical sense in the context of our question. The main effect 'test order' had a significant influence on the number of sectors visited (df = 2, $\chi^2 = 42.796$, $p < 0.0001$, with individuals visiting less sectors with increasing test order, i.e. visiting the most sectors when they first entered the room and the least the third time. Finally, individuals visited more sectors in the morning than in the afternoon (df = 1, $\chi^2 = 9.849$, $p < 0.002$, Table 3).

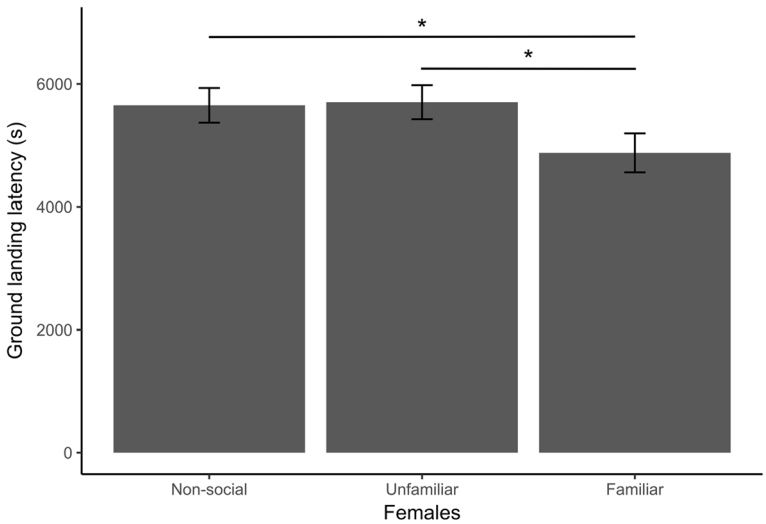

Fig. 1 Social context influence on ground landing latency in female house sparrows in a novel environment. Females exploring with a familiar companion had significantly shorter latencies to land on the ground than females exploring with an unfamiliar companion or alone. Means and standard error of the mean are shown. *$P < 0.05$

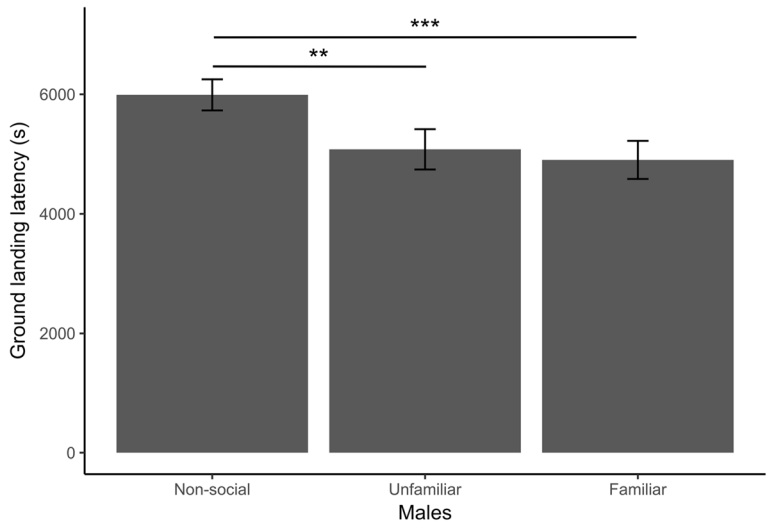

Fig. 2 Social context influence on ground landing latency in male house sparrows in a novel environment. Males exploring alone had significantly longer latencies to land on the ground than males exploring with an unfamiliar or a familiar companion. Means and standard error of the mean are shown. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$

Table 2 Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) and interaction between 'social context' and 'sex' on latency to forage

Fixed effect	Comparison	Estimate	2% CI	98% CI	*P* value
Part of the day	Morning vs afternoon	−0.125	− 0.431	0.181	0.403
Sex	Female vs male	−0.264	− 0.667	0.137	0.177
Round	First vs second	0.010	−0.358	0.377	0.998
	First vs third	−0.318	− 0.686	0.049	0.088
	Second vs third	−0.328	−0.700	0.044	0.082
Social context	Individual vs unfamiliar	−0.711	**−1.075**	**− 0.348**	**0.0001**
	Individual vs familiar	−0.614	**−0.974**	**− 0.253**	**0.0001**
	Familiar vs unfamiliar	0.098	−0.270	0.465	0.534
Sex × social context	Individual: female vs male	0.060	−0.467	0.587	0.815
	Unfamiliar: female vs male	−0.692	**−1.234**	**− 0.149**	**0.009**
	Familiar: female vs male	−0.162	− 0.698	0.374	0.534
Social context × sex	Female: individual vs unfamiliar	−0.336	− 0.842	0.171	0.241
	Female: individual vs familiar	−0.503	**−1.007**	**−0.002**	**0.041**
	Female: familiar vs unfamiliar	−0.167	−0.687	0.354	0.716
	Male: individual vs unfamiliar	−1.087	**−1.608**	**−0.567**	**<.0001**
	Male: individual vs familiar	−0.725	**−1.236**	**−0.214**	**0.0016**
	Male: familiar vs unfamiliar	0.362	−0.155	0.880	0.205
Random effect		**Variance**	**± SE**		
Individual identity		0.495	± 0.703		

Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. *P* values obtained with Tukey method adjusted for multiple comparisons. Results are in the log (not in the response) scale. 'Individual identity' is fitted as random effect; we show the variance associated with it

Table 3 Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) and interaction between 'social context' and 'sex' on number of sectors visited

Fixed effect	Comparison	Estimate	2% CI	98% CI	*P* value
Part of the day	Morning vs afternoon	−0.328	**−0.542**	**−0.113**	**0.002**
Sex	Female vs male	0.313	−0.040	0.665	0.069
Round	First vs second	−0.370	**−0.609**	**− 0.130**	**0.005**
	First vs third	−0.642	**−0.882**	**− 0.403**	**<.0001**
	Second vs third	−0.272	**−0.512**	**− 0.032**	**0.0161**
Social context	Individual vs unfamiliar	0.284	**0.044**	**0.524**	**0.011**
	Individual vs familiar	0.268	**0.031**	**0.506**	**0.017**
	Familiar vs unfamiliar	−0.016	−0.255	0.222	0.985
Sex × social context	Individual: female vs male	0.286	−0.136	0.708	0.165
	Unfamiliar: female vs male	0.455	**0.031**	**0.880**	**0.028**
	Familiar: female vs male	0.197	−0.224	0.618	0.337
Social context × sex	Female: individual vs unfamiliar	0.199	−0.138	0.536	0.3215
	Female: individual vs familiar	0.313	−0.024	0.649	0.0621
	Female: familiar vs unfamiliar	0.113	−0.223	0.450	0.6917
	Male: individual vs unfamiliar	0.369	**0.028**	**0.709**	**0.023**
	Male: individual vs familiar	0.224	−0.111	0.558	0.236
	Male: familiar vs unfamiliar	−0.145	−0.483	0.193	0.549
Random effect		**Variance**	**± SE**		
Individual identity		0.552	± 0.743		

Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. *P* values obtained with Tukey method adjusted for multiple comparisons. Results are in the log (not in the response) scale. 'Individual identity' is fitted as random effect; variance associated with it is shown

Social behaviour variables

The number of following bouts recorded during the entire duration of the test (2 h) was influenced by the familiarity of the pair, with familiar pairs performing more following bouts than unfamiliar pairs ($df = 1$, $\chi^2 = 4.619$, $p = 0.032$). Sparrows performed more following bouts during the second hour in both contexts ($df = 1$, $\chi^2 = 5.964$, $p = 0.015$); however, the difference was much more pronounced in the unfamiliar context. Accordingly, the treatment × hour interaction was significant ($df = 1$, $\chi^2 = 4.905$ $p = 0.027$): sparrows in the familiar context performed significantly more following bouts than those in the unfamiliar context, but only in the first hour (Table 4). The 'social context' × sex interaction was not significant and was excluded from the model. The main effect 'sex' was also not significant ($df = 1$, $\chi^2 = 0.024$, $p = 0.877$). The main effect 'test order' was significant ($df = 2$, $\chi^2 = 9.174$, $p = 0.010$), with birds in the first round performing more following bouts than in the third (Table 4). The total number of aggressive interactions recorded was very low, as we recorded 44 aggressive interactions in 48 tests in the unfamiliar context and 36 aggressive interactions in 48 tests of familiar context (0.42 aggressive interaction per hour).

Correlation between the dependents variables

Total distance travelled was highly correlated with sectors visited (Kendall Rank Correlation, tau = 0.668, $p < 0.001$). Such correlation was strongest in unfamiliar (tau = 0.700, $p < 0.001$) and familiar (tau = 0.681, $p < 0.001$) contexts: in individual context however it was much weaker and not significant after correction. Time spent foraging was negatively correlated with foraging latency (tau = − 0.423, $p < 0.001$). Ground latency was weakly but significantly negatively correlated with fraction of sectors visited (tau = − 0.298, $p < 0.001$): this correlation was stronger when considering only the individual (tau = − 0.452, $p < 0.001$) or the unfamiliar context (tau = − 0.434, $p < 0.001$), but very weak when considering the familiar one (tau = − 0.184, $p = 0.16$). A full correlation matrix is provided in the (Additional file 1: Table S3).

Discussion

Our experiment analysed numerous variables in three contexts for both sexes and provided various results. We provide a short summary of the most relevant results below.

1) Both sexes in the individual context had longer latency to land on the ground than in the familiar

Table 4 Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar), 'hour' (first or second hour of the test) and interaction between 'social context' and 'hour' on number of following bouts recorded in one hour

Fixed effect	Comparison	Estimate	2% CI	98% CI	*P* value
Part of the day	Morning vs afternoon	0.043	−0.029	0.116	0.221
Sex	Female vs male	0.007	−0.085	0.098	0.877
Hour	Second vs first	−0.063	**−0.089**	**− 0.037**	**<.0001**
Round	First vs second	0.060	−0.033	0.154	0.259
	First vs third	0.121	**0.024**	**0.219**	**0.007**
	Second vs third	0.061	−0.040	0.162	0.307
Social context	Unfamiliar vs familiar	0.059	**0.001**	**0.121**	**0.045**
Social context × hour	First hour: unfamiliar vs familiar	0.074	**0.003**	**0.144**	**0.032**
	Second hour: unfamiliar vs familiar	0.018	−0.045	0.081	0.554
Hour × treatment	Familiar: second hour vs first hour	−0.035	**−0.064**	**− 0.006**	**0.014**
	Unfamiliar: second hour vs first hour	−0.091	**−0.134**	**− 0.048**	**<.0001**
Random effect		**Variance**	**± SE**		
Individual identity		0.008	± 0.087		
Test		0.007	± 0.085		

Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. *P* values obtained with Tukey method adjusted for multiple comparisons. Results are in the log (not in the response) scale. 'Individual identity' and 'test' are fitted as random effects; variances associated with them are shown

context. Also, house sparrows visited less sectors in the individual context than either in the familiar or unfamiliar contexts when averaging across sexes.

2) Familiar pairs performed more following bouts than unfamiliar pairs, with the difference being more pronounced in the first hour of testing and non-significant in the second hour.
3) Females landed on the ground sooner when in the familiar context than either when in the unfamiliar or individual context. They also foraged sooner in the familiar than in the individual context. Females behavioural responses did not differ significantly between the individual and the unfamiliar contexts. Males' behavioural responses, on the other hand, significantly differed between the unfamiliar and the individual contexts (they foraged and landed on the ground sooner, spent more time foraging and visited more sectors when coupled with a companion); latency to forage and to go to the ground also differed between familiar and individual contexts.
4) When considering only the unfamiliar context males foraged sooner (and thus for longer, Additional file 1: Table S1) than females. Males in general visited also marginally more sectors than females, with this difference being more pronounced in the unfamiliar context.
5) 'Test order' had an effect on every dependent variable while 'part of the day' affected ground latency and number of sectors visited.

The first result is consistent with the social facilitation effect on exploratory behaviour [9, 12, 13]. Interestingly, we did not find any difference between the total distance travelled between sparrows moving in pairs and in the individual context (Additional file 1: Table S2). As sparrows in pairs visited more sectors, we can assume that they covered more ground even if travelling the same distance than when in the individual context. We may thus speculate that even if the presence of a companion increases the number of sectors visited, it could possibly have no effect on the energy spent in movement, as the same distance would still be travelled in the individual context, even if perhaps in a more restricted area. However, there was also a strong correlation between sectors visited and distance travelled, meaning that across all three contexts there is a relationship between the two measures.

The second result, the difference in the number of following bouts between familiar and unfamiliar pairs is interesting for three reasons. Firstly, as the difference was highly significant when confronting the first hour and non-significant when confronting the second, we could be seeing a quick process of habituation to the unfamiliar conspecific [31]. Both contexts performed more following bouts in the second hour of the test – as it is to be expected, as they gain confidence with the environment and start foraging and going to the ground: this suggests that familiarity in this species could have an influence on behavioural responses only on a relatively restricted temporal scale. Secondly, this result offers an insight in how two birds move together in a novel environment: following one another

from one sector to the other might be the cause of social facilitation, i.e. increase in the number of sectors visited. Lastly, the higher number of following bouts in familiar pairs could be a clue on how familiarity influences behavioural responses: being used to move with another individual would be, for example, the reason for quicker coordination in case of attack or discovery of a food source [72]. We encourage future studies to investigate how differences in the behavioural responses of familiar and unfamiliar pairs fade after a certain amount of time. Also, it is still unclear if pairs or groups of birds move differently according to their familiarity when in a novel environment.

The third result underscores how the behavioural response to unfamiliar individuals differed depending on the sex of the individuals. Females in the familiar context had significantly shorter latency to visit the ground than when in the unfamiliar context. This result is in line with previous studies performed in fish where familiarity was associated with increased time spent exploring a novel object, latency to emerge from a refuge and faster habituation to a novel environment [30, 73, 74]. The latency to visit the ground is particularly important, as birds usually perceive the soil as a higher-risk area compared to perches that were higher off the ground [20, 60], and in our set-up the birds rarely visited the ground for reasons different than going to a water source [BT unpublished observation]. For these reasons we argue that a shorter latency to venture on the ground provides a strong indication of reduced perceived predation risk. It is worth noting that this result could also have been due to distraction due to a higher frequency of aggressive interactions in the unfamiliar context. However, the total number of aggressive interactions was very small and thus unlikely to have a significant effect on other behavioural traits.

Males did not differ in their behaviour between the unfamiliar and familiar contexts. The behavioural responses either differed from between the individual context and whenever they moved with a companion, independently of its familiarity (latencies to touch the ground and forage) or differed only between the individual and the unfamiliar context (sectors visited, time foraging). Conversely, females significantly decreased their latencies to forage and to touch the ground (and slightly increased the number of sectors visited, even if not significantly) only when released alongside a familiar group-mate.

Hence the fourth result: males exploring with an unfamiliar companion visited more sectors, spent more time eating and started foraging sooner than females with an unfamiliar companion. Only in recent years has the role of sex been taken into consideration in familiarity studies [29, 75, 76]. In a parallel work on Mediterranean killifish (*Aphanius fasciatus*) it was found that in exploring same-sex pairs only females showed reduced latency to emerge from a refuge if their companion was familiar instead than unfamiliar, whereas males showed the opposite trend [73]. Moreover, a study on brown-headed cowbirds (*Molothrus ater*) found that females spent more time interacting with familiar conspecifics than unfamiliar conspecifics [29], which is consistent with our current results. There is thus a growing body of evidence suggesting that females of different taxa value the familiarity of conspecific individuals differently than males, with our findings strengthening this hypothesis.

Differences in how the two sexes approach unfamiliar conspecifics could have a number of non-exclusive explanations. A different response to novel environments between females and males has already been shown in some previous studies [20, 54]. The lack of prior interactions between two unfamiliar females could have left them with very limited information about each other's reliability as a source of vigilance [34–36]. In this case, unfamiliar females could have failed to reduce the anti-predation alertness of the other conspecific because they did not consider each other a reliable source of information. On the other hand, male house sparrows have been shown to be quicker than females to habituate to a potential disturbance (i.e. human disturbance near an unfamiliar object) [77], and less risk-averse than females [78]. This could also be the case of our study, as there is the chance that males could have habituated to the new companion quicker, without giving importance to previous experiences with it.

There are also a number of potential functional explanations. As house sparrow males are the ones picking and defending the nest site it would be paramount for them to assess and utilize the resources of a novel area as quickly as possible; even if this means exposing themselves to risks, such as novel predators or stressors [79]. In house sparrows, females were found to follow their companions to food sources, while males on the contrary were more consistently followed [80]. Because of this, females would have an advantage in carefully evaluating their companions, since they would depend more on the social information they provide.

Another possible explanation for our results would be that females value familiarity with their flock-mates because it could lead to help (decreased harassment, conjunct mobbing, shared alarmed behaviour) especially during the semi-colonial breeding season. In particular, it was recently shown that female cowbirds that preferred familiar connections laid more eggs during the breeding season [75]. Social instability can be costly due to increased rate of aggression, higher stress and lower reproductive output [75, 81] and in particular, stronger social bonds between females may lead to higher fitness compared to conspecifics with weaker social bonds, as shown in social mammals [75, 82, 83]. Moreover, birds are more likely to mob possible predators with familiar conspecifics than with unfamiliar conspecifics [36].

Finally, our work shows that, depending on the sex of the individuals, a familiar companion can strongly influence exploration in a social passerine bird, a situation particularly important for invasive and range-expanding species, such as the house sparrow and the brown-headed cowbird. Exploring a new area can indeed result in the chance of encountering unfamiliar conspecifics and in such a situation it would be important to fine-tune behavioural responses between familiar individuals and newly met strangers. The tendency to behave differently according to conspecific familiarity could prove to have a key role in the social environment structure, possibly as a factor keeping groups cohesive when exploring new territories [22, 75]. We may speculate that females behaving differently according to conspecific familiarity may be a factor in the social structure of sociable passerine bird flocks [75, 76], and maybe also of other different taxa [72, 73].

The test order had a strong influence on the house sparrow behavioural response to the novel environment. In particular, individuals had shortest ground latencies during the first round of tests, and significantly longer ground latencies in the second and third test – which did not differ between them. The pattern was the same also for total distance travelled (shortest in the first round of tests, Additional file 1: Table S2) and time spent foraging (longest time spent foraging in the first round of tests, Additional file 1: Table S1). The number of sectors visited was not only greater during the first round of tests with respect to both the others, but the second round of tests also saw a fewer number of sectors visited with respect to the third. Thus, all behavioural responses showed a slower, less extensive exploration after the very first round of tests [84] which has been known to happen also for house sparrows [77]. We cannot completely exclude the possibility that the effect was not due to habituation to the experimental aviary, but to the progress of the season [85]. However, in that case we would have possibly seen a greater difference in behavioural responses not between the first round of tests and the other two, but between the second and the third round of tests due to the onset of the breeding season.

A possible limit of our study was that we did not control for acute physiological stress responses, as all birds after being rapidly captured had only 10 min in the habituation cage. Future studies could definitely try to integrate stress responses analysis when investigating the effect that familiar and unfamiliar conspecifics have on the focal birds. Also, we encourage future studies to address the reasons behind this sex difference in the response to familiarity, for example by seeing if more exploratory males can be more or less attractive to females [86]. Moreover, it could be interesting to test how the fission-fusion structure of winter flocks of house sparrows could vary according to the sex of the individuals, and verify if females are more nuclear to the subgroups than males.

Conclusions

We found evidence that pairs of familiar female house sparrows released in a novel environment landed faster on the ground than both in the unfamiliar and individual contexts. Males on the other hand did not differ in their behavioural responses between unfamiliar and familiar contexts, but had shorter latencies to land and forage, ate more and visited more sectors when in the unfamiliar context than in the individual one. Bird species are an important model in the field of exploratory behaviour, which nonetheless has been rarely considered in relation to the social environment. We provided evidence of the complex effects of social context on novel environment exploration. In particular, to the best of our knowledge this is the first evidence of the effect of conspecific familiarity on a behavioural response during novel environment exploration in birds: for the first time we tried to determine the effects of unfamiliar conspecifics alongside the usual comparison between familiar conspecifics and no conspecifics. Differences in the social context (i.e. alone, with an unfamiliar or with a familiar conspecific) impacted how both sexes exploited resources in a novel environment, an effect possibly paramount for invasive and opportunistic species.

Additional file

Additional file 1: Table S1. Output of LMM with 'time spent foraging' as dependent variable. Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) and interaction between social context and sex on time spent foraging. Fixed effect with significance obtained with 'car' package are presented. Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold P values obtained with Tukey method adjusted for multiple comparisons. **Table S2.** Output of GLMM with 'total distance travelled' as dependent variable (family Gamma, link = log). Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) on total distance travelled. Interaction between social context and sex was excluded as not significant. Fixed effect with significance obtained with 'car' package are presented. Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. *P* values obtained with Tukey method adjusted for multiple comparisons. **Table S3.** Correlation matrix between all dependent variables. Tau values obtained through Kendall Rank correlation. Results in bold are significant. False discovery rate correction was applied to value of α. **Figure S1.** An example of our test sorting. Boxes with the same colour (either red or blue) represent sparrows from the same aviary (familiar with each other). Each curved double arrow is a familiar context test, each straight double arrow is an unfamiliar context test, each point is an individual context test. Colours of arrows/points represent the test round: green first round of tests, yellow second round of tests, black third round of tests. (DOCX 139 kb)

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
MG conceived the project. MG, BT, GF and HH designed the experiment. BT and GF collected the behavioural data, MG directed the research. BT analysed the dataset and wrote the original draft. Funding Acquisition and Resources: MG and HH. All authors contributed critically in preparing the manuscript and gave final approval for publication.

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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SUPPLEMENTARY MATERIAL

Sex difference in the role of familiarity during novel environment exploration in an urban species

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Table s1. Output of LMM with 'time spent foraging' as dependent variable. Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) and interaction between social context and sex on time spent foraging. Fixed effect with significance obtained with 'Anova' function in 'car' package are presented. Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. P values obtained with Tukey method adjusted for multiple comparisons.

Fixed effect	Comparison	Estimate	2% CI	98% CI	P value
Part of the day df = 1, χ_2 = 1.200, p = 0.273	Morning vs afternoon	32.47	-28.408	93.339	0.2743
Sex df = 1, χ_2 = 0.216, p = 0.642	Female vs male	18.562	-64.566	101.690	0.643
Round df = 2, χ_2 = 21.466, p < 0.0001	First vs second	-78.73	**-131.345**	**-15.014**	**0.0241**
	First vs third	-136.90	**-209.617**	**-64.177**	**<.0001**
	Second vs third	-58.17	-131.345	15.014	0.1281
Social context df = 2, χ_2 = 3.573, p = 0.168	Individual vs unfamiliar	38.853	-33.868	111.575	0.392
	Individual vs familiar	54.437	-18.268	127.143	0.161
	Familiar vs unfamiliar	15.584	-57.137	88.305	0.859
Sex × social context	Individual: female vs male	-53.313	-161.950	55.325	0.3118
	Unfamiliar: female vs male	112.186	**3.519**	**220.853**	**0.034**
	Familiar: female vs male	-3.188	-111.825	105.450	0.952
Social context × sex df = 2, χ_2 = 8.185, p = 0.017	Female: individual vs unfamiliar	-43.90	-146.720	58.928	0.5485
	Female: individual vs familiar	29.37	-73.449	132.199	0.7635
	Female: familiar vs unfamiliar	73.27	-29.553	176.094	0.1906
	Male: individual vs unfamiliar	121.603	**18.734**	**224.471**	**0.012**

Male: individual vs familiar	79.500	-23.324	182.324	0.1428
Male: familiar vs unfamiliar	-42.103	-144.971	60.766	0.576

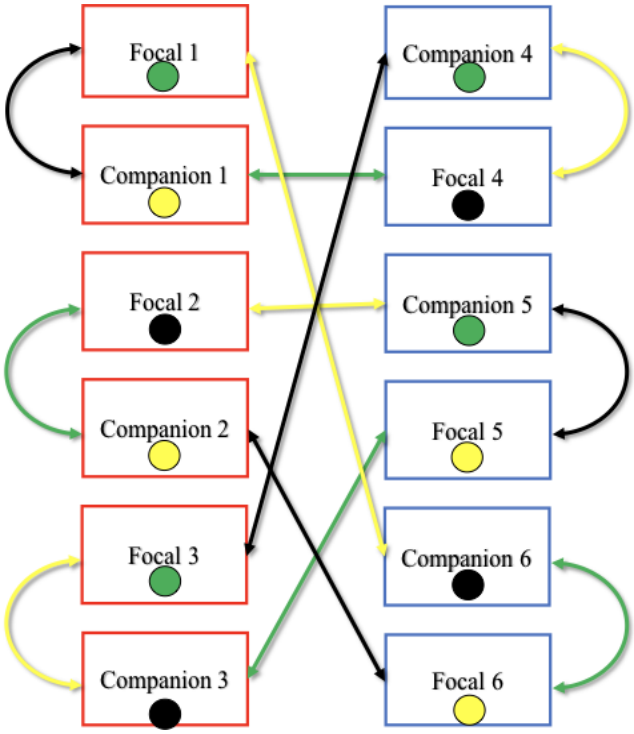
Table s2. Output of GLMM with 'total distance travelled' as dependent variable (family Gamma, link=log). Effect of 'part of the day' (morning or afternoon), 'round of tests' (first, second or third), 'sex' (female or male), 'social context' (individual, unfamiliar, familiar) on total distance travelled. Interaction between social context and sex was excluded as not significant. Fixed effect with significance obtained with 'Anova' function in 'car' package are presented. Coefficients and 96% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. P values obtained with Tukey method adjusted for multiple comparisons.

Fixed effect	Comparison	Estimate	2% CI	98% CI	P value
Part of the day df = 1, χ_2 = 2.209, p = 0.137	Morning vs afternoon	-0.210	0.500	-0.080	0.137
Sex df = 1, χ_2 = 1.536, p = 0.215	Female vs male	0.223	-0.146	0.592	0.215
Round df = 2, χ_2 = 22.452, p <0.0001	First vs second	-0.445	**-0.790**	**-0.101**	**0.005**
	First vs third	-0.670	**-1.022**	**-0.320**	**<.0001**
	Second vs third	-0.225	-0.572	0.121	0.255
Social context df = 2, χ_2 = 2.671, p = 0.263	Individual vs unfamiliar	0.067	-0.288	0.422	0.890
	Individual vs familiar	0.228	-0.128	0.585	0.264
	Familiar vs unfamiliar	0.161	-0.173	0.495	0.470

Table s3. Correlation matrix between all dependent variables. Tau values obtained through Kendall Rank correlation. Results in **bold** are significant. False discovery rate correction was applied to value of α.

	Areas visited			Foraging latency		
	Individual	Unfamiliar	Familiar	Individual	Unfamiliar	Familiar
Foraging latency	**-0.233**	-0.075	-0.086			
Ground latency	**-0.452**	**-0.434**	-0.184	**0.270**	0.162	**0.253**

Figure s1. **An example of our test sorting.** All birds performed three tests (individual context, familiar and unfamiliar context). Focal individuals were focal in both their familiar and unfamiliar tests and had two different companions (one they were familiar with and one they were not familiar with). Companion individuals were companions of two different focal individuals, one they were familiar with and one they were not familiar with. Boxes with the same colour (either red or blue) represent sparrows from the same aviary (familiar with each other). Each curved double arrow is a familiar context test, each straight double arrow is an unfamiliar context test, each point is an individual context test. Colours of arrows/points represent the test round: green first round of tests, yellow second round of tests, black third round of tests.


Focal 1
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Companion 4
Focal 4
Companion 5
Focal 5
Companion 6
Focal 6

Chapter 6

Flock-dependent exploitation of a limited resource in House Sparrow

Scientific Reports (in review)

Flock-dependent exploitation of a limited resource in House Sparrow.

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ABSTRACT

The performances of different social groups can depend on various characteristics, such as their phenotypic composition or familiarity among their members. However, it has rarely been investigated how groups perform during an encounter with other conspecifics, even if in the natural environment social groups often run into each other and compete for resources. We investigated whether a certain characteristic of the group (i.e., familiarity) could benefit its members when they are confronted with another group. We designed a novel experimental set-up, creating triads of captive house sparrows *(Passer domesticus)* and examining whether in a situation of competition for limited resources one triad could gain benefits over the other (consume more mealworms, *Tenebrio molitor*). While we did not find effect of previous familiarity among triad members on the triads' performances, we discovered a group-based difference in the number of mealworms eaten per capita. Group-mates of the very first individual to eat a mealworm (first feeder) ate more mealworms than those in the opposing triad. While individual behavioural traits did not influence the birds' performances, first feeder individuals foraged sooner and more than other birds in a subsequent prey consumption assay. Our results suggest that individual performances were influenced by group membership, even when groups were exploiting the same resource together: group performance was, in turn, possibly infuenced by the characteristics of certain individuals.

INTRODUCTION

The variable interactions among individuals living, moving or foraging in a group play a significant role in resource exploitation, disease or information transmission [1-3]. In recent years, increasing attention has been given to the possible differences in performance (i.e. resource use, survival) not only within but also among social groups [4-6] and their consequent impact on individual fitness [7, 8]. How social groups perform can depend on various characteristics, such as the phenotypes of the individuals composing the group [9-12], the role assumed by particular individuals (*i.e.* keystone individuals [13, 14]) or other group properties such as familiarity [15] or sex-ratio [16-17]. Because of such existing variability among them, groups could enjoy differential benefits according to their characteristics in a particular situation [17, 18], which would translate into benefits for all individuals belonging to that particular group [8, 19]. For instance, asocial and bolder individuals can have an advantage during dispersal, as they settle in a novel environment and exploit resources before others, leading to a faster spread of their entire group as well [20]. In other cases, a particular group composition can lead to higher fitness advantages for all its members [7]: for example, in mixed shoals of bold and shy guppies *(Poecilia reticulata)*, individuals approached faster a novel feeder and fed more than in groups of only shy or bold individuals, possibly because of mutual phenotype-dependent benefits [6].

An additional factor influencing groups' performances is familiarity among its members [21-23]: previous experience of groupmates with each other has been shown to give fitness advantages over short [24] and long [25] periods of time, particularly in unstable and/or novel environments with scarce resources. Antagonistic interactions are

73 less common among familiar conspecifics [26]; moreover, assessing the threats that
74 unfamiliar individuals might pose can be time-consuming, possibly leading to an
75 increase in individual alert time and stress [27]. In the context of resource acquisition in
76 a novel environment, familiarity has been known to increase the rate of social
77 transmission [28] and exploratory behaviour and to facilitate social foraging [29, 30].
78 While there have been studies comparing groups' performances [31, 32], it has rarely
79 been taken into account how two groups would interact together. In the natural
80 environment, however, it is unlikely that groups would not come into contact with each
81 other [33], or at least share the same resources [34]. While it could happen that groups
82 encountering each other fuse quickly or immediately, thus decreasing the importance of
83 starting out in a specific group, familiarity among group-members could still cause
84 group-linked patterns of movement or foraging [24, 35] strong enough to have an
85 impact on resource acquisition and survival [25]. In this case, not only the performance
86 of one group could be better or worse, but it could also influence the performance of the
87 other group. For example, one group gaining a resource first would mean that
88 individuals of the other group would lose it.
89 Multiple studies already focused on how the process of groups' fusion in fission-fusion
90 societies influences individual social rank [36, 37], associations [38] and social learning
91 [1]. Therefore, our novel experimental set-up attempted to test whether there might be a
92 measurable group-specific advantage in terms of resource acquisition – i.e., a difference
93 in group performance – during a direct confrontation between two groups (but see [39]
94 for an example of interspecific colony-level confrontation). Moreover, as in the natural
95 environment resources can be a limiting factor, we also implemented a limited resource,
96 so that a benefit gained by one group would create a disadvantage to the other group.

We thus created a number of artificial flocks of female House sparrows *(Passer domesticus)*, half entirely composed of already-familiar individuals (familiar flocks) and half entirely composed by unfamiliar individuals (unfamiliar flocks): we then paired them together and examined whether the familiar flocks could gain benefits over the unfamiliar ones in an invasiveness context and on a small-time scale. Afterwards, we tested samples of our population through two different repeated assays, in order to investigate if group performances might be linked to measurable behavioural traits. The house sparrow is an opportunistic and sociable passerine, invasive in many areas of the world [40, 41]. In winter they reunite in variable-sized flocks that often forage in small sub-flocks, particularly in urban areas. Their social life is thus characterized in this period by repeated fission-fusion dynamics [42], forcing them to move in small groups and encounter both familiar and unfamiliar individuals, often over clumped and/or limited resources [43].

In general, we expected to see a flock-based difference in the amount of resource consumed (i.e., an individual would eat more or less depending on its flock). In particular, we set out to test four major hypotheses. Firstly, we hypothesised that familiar flocks would have an advantage over unfamiliar flocks (i.e., they would exploit sooner the food source and consume more of it) because their stronger social connections would facilitate their social exploration. Secondly, we hypothesised that flocks containing individuals faster at finding and exploiting the food source might partake in more of the resource. Following others to food sources and novel areas is in fact a paramount behavioural strategy in house sparrows, particularly for females: individuals within a flock strongly differ in their propensity to lead and follow [44]. The presence of a particularly enterprising individual might thus have consequences on the

121 actions of its flock-mates, that if alone would not otherwise venture to certain areas or
122 food sources as quickly [44]. Consequently, if instances of social facilitation were
123 stronger among flock-mates than between individuals from different flocks [24], there
124 might be an effect of being in the same flock of the first individual to move to the
125 central aviary or of the first individual to acquire the resource (mealworms, *Tenebrio*
126 *molitor*). Thirdly, we hypothesized that behavioural traits such as greater activity and
127 risk-taking behaviour might influence both individual and flock performances: active
128 and risk-taking individuals might be the first ones to enter the aviary and forage, while
129 triads foraging first might contain more risk-taker individuals. Lastly, we hypothesized
130 that roles assumed during the main experiment (such as first to cross into the central
131 aviary or first to forage) might be linked to individual behaviour such as activity and
132 boldness, e.g. first feeder individuals might be more risk-taking [45].
133 We decided to use three sparrows per flock, as a greater number of individuals might
134 have made flock-level phenomena more diluted and difficult to detect. We also decided
135 to use only female individuals, as it has been demonstrated that when tested in a novel
136 environment two female house sparrows familiar with each other differ in key aspect of
137 exploration from a pair of unfamiliar females [22]. Male house sparrows, on the other
138 hand, do not appear to be influenced by familiarity with their companion [22], a trend
139 that is found in other passerine species [25, 46]. Female house sparrows also show a
140 greater tendency to follow other individuals to food sources [47], and finally, this
141 species disperses during the first year showing a female-biased dispersal pattern [48,
142 49]. As various behavioural traits involved with foraging and exploration have been
143 linked to differences in age, we also decided to test separately adults and juveniles [50],

predicting that, as in other passerine species [51], younger birds might be faster to explore and acquire food sources, and less neophobic.

METHODS

Housing and study subject

The study was conducted at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine) in Vienna, Austria (48˚13' N, 16˚17'). The house sparrows originated from a population kept in mixed-sex outdoor enclosures (mean number of birds/aviary: 10.95 ± 6.80. Measures reported here and henceforward are mean and standard error of the mean), measuring 2 m × 3.9 m and 2.6 m high. We used a total number of 102 female birds. Of these, 42 were born in captivity during the previous breeding season (149 ± 14 days) and had already undergone their post-juvenile moult; the remaining 60 individuals were mature adults (2-3 years old) also born and raised in the same aviaries. Each aviary (from now on "housing aviary") was equipped with a feeder (consisting of a metal bowl on a wooden pedestal, 1.2 m from the ground), small pine trees, which were used as roosting sites, and branches as additional perching places. All aviaries were provided with food (a mixture of millet, canary seeds, wheat, sunflower seeds, protein-based mash, apple slices and millet sprays hanging from the branches) and water *ad libitum* [52].

Experimental design

The trials were conducted in a three-parted outdoor arena, which consisted of three adjoining aviaries linked to each other by two remotely-opened small windows (50x50

168 cm, 1.4 m from the ground). All birds had previously experienced similar windows in
169 their own housing aviaries and were all able to cross them. The aviaries composing the
170 arena, while identical to the housing aviaries in size and similar in roosting equipment,
171 were however novel to all individuals. The central aviary of the arena was the only one
172 that had a food source, novel to all individuals, consisting of 9 live mealworms placed
173 in three small cups (3 mealworms/cup) above a wooden pedestal. The three aviaries
174 were visually but not acoustically isolated.
175 All the 102 female individuals were randomly assigned to one of 34 "triads", i.e.
176 artificially-composed flocks of three individuals of the same age; all triads were tested
177 two at a time, in order to simulate an encounter between two flocks. The two triads
178 facing each other were always of the same age: there were thus 7 trials with opposing
179 triads composed by first-year birds and 10 trials with opposing triads composed by
180 mature individuals. However, triads tested together differed in familiarity: one of the
181 two was composed by individuals that had always (since hatching date) been housed
182 together, hence flock-mates familiar with each other, while the other one was composed
183 by individuals which had never been in contact before the trial (nor visual nor acoustic)
184 hence unfamiliar with each other. No bird was tested with siblings, no bird was familiar
185 with any individual of the opposing triad and no individual was tested twice.
186 The afternoon (1700 hours) before the experiment, the food bowl was removed from
187 the housing aviaries of the individuals scheduled for the trial, in order to standardize the
188 feeding motivation. The trial started the following day at 0800: all study subjects of the
189 two opposing triads were quickly captured with hand-nets and transferred via a small
190 cloth bag to the lateral aviaries, randomly assigning either the left or the right lateral
191 aviary to the familiar triad and the opposite lateral aviary to the unfamiliar triad (Figure

192 1A). Here they were given 5 minutes to habituate (the habituation time needed to be
193 short in order to avoid a decrease in the level of unfamiliarity among individuals of
194 newly-formed flocks). After this time, windows opened and the trial started (Figure 1B).
195 All trials lasted 4 hours as we did not consider a shorter trial to be informative for our
196 purposes [22]. Individuals had in fact to locate and pass through the windows in order to
197 move from the aviary where they had been released to the central aviary. For all
198 individuals we recorded i) their latency to cross the window, ii) their latency to feed, iii)
199 the number of mealworms eaten.
200
201 *Individual behaviour assays*
202 In order to measure individual behavioural traits, we tested house sparrows using two
203 different assays: the first one aimed at measuring activity in isolation ('activity assay')
204 [53] while the second one was performed in a group setting, where we tested daily prey
205 consumption rate ('consumption rate assay'). The first test aimed to provide a measure
206 of activity, while the second aimed to provide a measure of risk-taking behaviour. We
207 performed both behavioral assays after the main experiment in order to avoid that
208 experience accumulated during these tests might influence their behavior in the main
209 experiment. Activity assays were conducted first, starting a month after the main
210 experiment: as two aviaries had been previously scheduled for different purposes we
211 tested 48 mature birds (80% of all the mature birds tested in the main experiment), and
212 33 one-year old birds (79% of all the one-year old birds tested in the main experiment).
213 The activity assays were conducted in a cage (Montana-Terenzo, 1 m x 0.5 m and 0.5 m
214 high) equipped with water and seeds on two cups on the front. Each cage was also
215 equipped with two wooden perches, going from the back to the front of the cage. We

recorded for all individuals the number of hops within the cage for 10 minutes (from perch to perch or from perch to front), starting 5 minutes after release. Individuals were re-tested after one month in order to measure behavioural repeatability [54].

The second assay (consumption rate assay) was performed only with one-year old individuals, as we preferred not to disrupt the mature individuals' social composition any further. The 33 one-year old females were assigned to 11 mixed-sex groups of 6 sparrows each, 3 one-year old males and 3 females. After two months of habituation to their new social groups (habituation started six weeks after the end of the main experiment) we started with the assays. In this assay we measured the amount of resource that each individual would consume in a social setting, just after the introduction of a food source by the experimenter. This measure could be thus interpreted as a proxy of risk-taking behaviour, not unlike the "startle test" widely used in personality research to measure risk-taking behaviour [55-57], which is based on the latency to go back to a food source after a startle. In our case the startling event was the experimenter entering the aviary and placing the food source inside, which caused birds to fret and fly in the farthest corners; the vicinity of the food source could moreover be considered the riskiest area, as it was where the experimenter had just been. We presented each aviary in the morning (0630 – 1130; hour of test was randomized across groups every day) with 6 mealworms in a cup. We observed each aviary for 45 minutes, while in that period every other food source was removed from the aviary. We recorded the number of worms that each individual ate and the hour of each feeding event. As the resource was limited, the only individual(s) that could feed were the ones with the shortest latency to approach the food source after the experimenter had left. At the beginning of this assay all birds had already experienced cups with mealworms inside,

240 which could thus not be considered novel food sources: therefore, differences in latency
241 to approach the cups could not be due to differences in experience. The same procedure
242 was repeated for each aviary for 10 days.
243
244 *Statistical analysis - Main experiment*
245 All data were analyzed with R version 3.2.1 [58]. The significance threshold was set at
246 $\alpha = 0.05$. We used Generalized Linear Mixed Models (GLMMs) to analyze the three
247 dependent variables (individual latency to cross, individual latency to feed, individual
248 number of mealworms eaten). All models were fitted using the 'glmer' function within
249 the package lme4 (1.0.5) for R version 3.2.1 [59]. Estimates and significance of the
250 fixed effects were obtained using the 'Anova' function within the 'car' package [60],
251 while the 'confint.merMod' function within the 'lme4 package was used to obtain 95%
252 confidence intervals via bootstrapping. Each dependent variable was analyzed using a
253 separate model. As random factors we fitted 'triad' nested within 'trial' (each trial saw
254 two opposing triads) in all three models. In order to test our hypothesis that individuals
255 belonging to a familiar triad would outperform individuals belonging to an unfamiliar
256 triad we fitted as categorical fixed effects i) age (first-year or third-year) and ii)
257 familiarity (belonging to a triad composed of either familiar or unfamiliar individuals)
258 and their interaction in all three models. To test our second hypothesis, i.e. that
259 individuals would have an advantage if they belonged to the first triad to cross into the
260 central aviary and/or to the first triad to eat a mealworm we had to take into account the
261 effect of social influence on flock-mates behaviour. We thus determined the identity of
262 the very first individual that in every trial crossed the window ('first crosser') and the
263 very first individual that ate a mealworm in each trial ('first feeder', as in [44]). We

created a dummy variable, assigning "1" to each individual in the first feeder and/or first crosser triad and a "0" to every individual in the opposing triad. Thus, we added as independent categorical variables in the models iii) belonging to the triad of the "first crosser" and iv) belonging to the triad of the "first feeder" and their interactions with all other fixed factors. In order to maintain independency of our data we excluded: first feeder individuals from our analysis of the number of mealworms eaten and the latency to eat the first mealworm, and first crosser individuals from the analysis of crossing latency. We analyzed the number of mealworms eaten using poisson distribution (log link), while the latency to cross and the latency eat were modelled with gamma distribution (inverse link). In the models with 'poisson' distribution we checked for over-dispersion and whenever it was significant we included an observation-level random effect as detailed in [61]. Birds that did not cross the window or did not eat were assigned a latency of 14400 s.

Influence of individual behaviour (as measured by behavioural assays) on performance during the main experiment

To test if individual characteristics had an influence on performance during the main experiment (our third hypothesis) we used the same methodology of the previous section and part of the same dependent variables: however, in this analysis we included also individual behaviour (as measured by subsequent behavioural assays) as a factor in all the models. We decided to present both analyses alongside each other as the one in the previous section is more comprehensively run on all individuals, while those in this section focus only on the selection of individuals for which we had measurement of behavioural assays. We utilized two sets of models, each set testing the influence of one

behavioural trait on all three dependent variables (individual latency to cross, individual latency to feed, individual number of mealworms eaten). In the first set of models we analyzed all individuals that had been tested in the activity assay. We analyzed all three dependent variables with models fitted with the previously mentioned fixed effects ('age', 'familiarity', 'first feeder triad', 'first crosser triad') plus adding 'activity' (i.e. the number of hops within the cage during the first test) as a fixed factor in all three models. In the second set of models we analyzed the individuals (all first-year) that had been tested in the consumption rate assay. We analyzed all three dependent variables with models fitted with the previously mentioned fixed effects ('familiarity', 'first feeder', 'first crosser') plus adding 'average number of mealworms eaten' as a fixed factor to all three models. In both sets of models we analyzed the number of mealworms eaten using poisson distribution (log link), while the latency to cross and the latency eat were modelled with gamma distribution (inverse link). Birds that did not cross the window or did not eat were assigned a latency of 14400 s.

Relation between membership during main experiment and individual behaviour

As the main experiment was performed before the two behavioural assays we decided to test also if being a first feeder or a first crosser in the main experiment had any relation with the expression of individual behavioural traits in the two subsequent behavioural assays (our fourth hypothesis). We used one dependent variable (number of hops) for the activity assay, and two variables (number of mealworms eaten per day and number of days as first feeder) for the consumption rate assay. Each variable was analyzed using a separate model. As both assays were repeated we fitted as random factors 'identity' in both models, plus 'day of test' (1-10) and 'aviary' in the models concerning the

consumption rate assay. As categorical fixed effects we fitted i) being or not a first-crosser individual nested within ii) belonging to a first-crosser triad and iii) being or not a first-feeder individual nested within iv) belonging to a first-feeder triad and vi) age (only in the model analyzing the hops). We analyzed number of mealworms eaten per day using poisson distribution (log link), level of activity using a Gamma distribution (inverse link) and number of days as a first feeder using binomial distribution (logit link). We also tested for correlation between all dependent variables using the 'Kendall' package [62] applying a false discovery rate correction. To test for repeatability in the individual behavioural traits tested we used package 'rptR' [63], which uses parametric bootstrapping to estimate confidence interval and standard errors. We used 'day' as fixed effect and 'group' as random effect for the consumption rate assay and 'age' as the fixed effect for the activity assay.

ETHICAL NOTE

During the course of the study no experimental bird was injured or died. Capture, housing and handling of birds were in accordance with the relevant Austrian laws and were licensed by the government of Vienna (MA 22) license number 114/2012. The experiment reported in this study complies with current laws on animal experimentation in Austria and the European Union. This study was approved by the institutional ethics committee (University of Veterinary Medicine, Vienna) and the national authority according to 8ff (rules) of Law for Animal Experiments Tierversuchsgesetz - TVG, license number GZ 68.205/013-WF/V/3b/2014. The condition and health of experimental birds were monitored on a daily basis.

RESULTS

Main experiment

Birds fed in every trial except one, which was thus excluded from the analysis. In the remaining 16 trials 11 birds (11.46%) did not cross during the trial; 42 birds (43.75%) did not eat any mealworm during the trial. In 3 trials the first feeder took the first mealworm when no bird of the other triad had already crossed; however, in only 1 of these 3 trials individuals in the first feeder triad were the only ones to feed. During the 13 other trials when the first feeder took the first mealworm on average 1.362 individuals of its triad and 1.509 individuals of the other triad had already crossed. Birds ate on average 1.438 ± 0.170 mealworms per capita. We found a medium positive correlation between latency to cross and latency to eat the first mealworm (tau = 0.358, $p < 0.001$), while the number of mealworms eaten was strongly correlated with latency to eat the first mealworm (tau = -0.644, $p < 0.001$) but only weakly correlated with latency to cross (tau = -0.243, p = 0.001).

Age had no significant effect on the number of mealworms eaten (df = 1, $\chi^2 = 1.062$, p = 0.303; Table 1) while there was a non-significant trend for mature birds to cross (df = 1, $\chi^2 = 2.964$, p = 0.085; Table 2) sooner than first-year individual. Previous familiarity with the other triad members did not affect the number of mealworms eaten (df = 1, $\chi^2 = 0.794$, p = 0.372; Table 1), the latency to eat (df = 1, $\chi^2 = 0.023$, p = 0.879; Table 3) or the latency to cross (df = 1, $\chi^2 = 0.039$, p = 0.845; Table 2) of individual birds.

Out of 144 mealworms available in total 138 were eaten; first feeders ate 48 mealworms (on average 3.000 ± 0.442 mealworms per capita; Figure 2), individuals belonging to the first feeder's triad ate 55 mealworms in total (on average 1.719 ± 0.324

mealworms per capita; Figure 2) and individuals belonging to the other triad ate 35 mealworms in total (on average 0.730 ± 0.174 mealworms per capita; Figure 2).

Having the first feeder as a group-mate increased significantly the number of mealworms eaten per capita (df = 1, χ^2 = 6.480, p = 0.011; Table 1, Figure 3), but did not affect the latency to take the first mealworm (df = 1, χ^2 = 1.713, p = 0.191; Table 3). On the other hand, belonging to the first crosser triad did not affect the crossing latency (df = 1, χ^2 = 0.157, p = 0.692; Table 2) or had any effect on the number of mealworms eaten (df = 1, χ^2 = 0.572, p = 0.449; Table 1) or on the latency to feed (df = 1, χ^2 = 0.223, p = 0.637; Table 3).

Influence of individual behaviour (as measured by behavioural assays) on performance during the main experiment

The level of activity each individual fell in was weakly but significantly repeatable (R = 0.196, p = 0.028). Individual activity did not have any influence on the number of mealworms eaten during the main experiment (df = 1, χ^2 = 0.794, p = 0.372; Supplementary Table S1), nor on their latency to forage (df = 1, χ^2 = 0.920, p = 0.337; Supplementary Table S2). However, we found a non-significant tendency for more active individuals to cross first into the central aviary (df = 1, χ^2 = 3.147, p = 0.076; Supplementary Table S3). Results concerning the other predictors (age, familiarity, first crosser and first feeder triad) did not differ from those obtained in models that did not included 'activity' as a fixed factor (Supplementary Tables S1-S3).

The number of mealworms eaten during the consumption rate assay was repeatable across the 10 days (R = 0.442, p < 0.001). Individual consumption rate did not have any influence on the number of mealworms eaten during the main experiment (df = 1, χ^2 =

384 0.194, p = 0.660; Supplementary Table S4), nor on their latency to forage (df = 1, χ^2 =
385 1.307, p = 0.253; Supplementary Table S5) or cross into the central aviary (df = 1, χ^2 =
386 0.148, p = 0.700; Supplementary Table S6). Results concerning the other predictors
387 (familiarity, first crosser and first feeder triad) did not reach significance, as the analysis
388 was performed only on a selection of first-year individuals (Supplementary Table S4-
389 S6).
390
391 *Relation between membership during main experiment and individual behaviour*
392 Triads did not differ in their composition of behavioural traits, i.e. there was no
393 difference in the number of hops between first-crosser individuals, first-feeder
394 individuals and all the others, nor there was any difference in the number of hops
395 between individuals belonging to first-feeder, first-crosser, familiar or unfamiliar triads
396 (df = 1, all χ^2 < 0.884, all p > 0.347).
397 In the consumption rate assay however birds eating more worms were also first feeders
398 more often than their flock-mates (tau = 0.971, p < 0.001). Birds that were first feeders
399 in the main experiment ate more mealworms (df = 1, χ^2 = 4.705, p = 0.030, Figure 4)
400 and showed a tendency to be first feeders also in the consumption rate assay (df = 1, χ^2
401 = 3.521, p = 0.063) while first-crosser individuals, birds belonging to first-crosser triads
402 or first-feeder triads did not differ on average from their counterparts in the opposing
403 triads in neither variable (df = 1, all χ^2 < 0.198, all p > 0.239).
404
405
406
407

DISCUSSION

To our knowledge, our experiment gives possibly the first evidence of a variable performance between two social groups facing each other in captivity. While we did not find any effect of previous familiarity among triad members on the triad's performances, we discovered a group-based difference in the number of mealworms eaten per capita: birds belonging to the triad of the first feeder ate significantly more mealworms than those in the opposing triad. As the resource was limited and easily depletable, if a triad consumed more of the resource individuals belonging to the opposite one would have less of it to exploit. We found no difference in the composition of the opposing triads relatively to two individual behavioural traits; moreover, individual behavioural traits did not appear to influence birds' performances during the main experiment. However, birds that were first feeders during the main experiment consumed more mealworms and tended to forage first also in the consumption rate assay.

During our trials, first feeders on average acquired also the most mealworms per capita: as individuals virtually never took more than one mealworm at once (Authors' personal observation), this means that these individuals returned to the feeder more than the others. Nevertheless, the first feeders' triad-mates also consumed more food items than the individuals in the opposite triad: interestingly, the two triad-mates of the first feeder ate on average more mealworms than all three sparrows in the other triad combined. However, while first feeder birds consistently acquired more worms also in the subsequent repeated consumption rate assay, their triad-mates did not (Figure 4); they consumed more mealworms than the other sparrows only during the main experiment.

This could be due to several non-excluding factors, depending on the dynamic of the social interactions and following movements. It could be argued that a triad entering first in the central chamber acquired more of the resource before the opposing triad could even enter. However, this happened only in three out of 17 trials; instead, in the remaining 14 trials when the first feeder took the first mealworm approximately the same number of individuals of both triads had already crossed into the central aviary. Moreover, belonging to the triad of the first crosser did not have any influence on the number of mealworms acquired; crossing into the central aviary appeared to happen because either of greater individual activity, which in fact had a non-significant influence on the crossing latency or by sheer chance. Acquiring mealworms on the other hand was possibly a more purposeful activity, linked to decreased neophobia [64]. Thus, the difference in mealworms per capita shown in the main experiment might be attributed to the triad-mates of the first feeder following it to the food source more readily than the individuals of the other triad, even if both triads were already present in the central room. This might mean that, with respect to following behaviour, there was a difference between how individuals regarded their triad-mates and those in the opposing triad.

As the average crossing latency was quite long, individuals may have interacted with each other during that time, thus developing a familiarity with their triad-mates [65] sooner than expected. This possibly canceled out the effects of previous unfamiliarity with their triad-mates, which in fact did not influence our results: in Tuliozi et al. 2018 [22] pairs of unfamiliar house sparrows habituated to each other within the second hour of interaction. On the other hand, the opposing triads were composed of individuals completely unfamiliar to each other when both groups entered the central aviary. This

456 would explain why only triad-mates of the first feeder ate more mealworms: they
457 possibly reacted more strongly to the cue of their triad-mate landing on the pedestal and
458 approaching the food source, as it was an individual that they had – even if relatively
459 briefly – already developed a relationship with and started following. This is consistent
460 with what is known from other studies of this species: social facilitation and following
461 behaviour in house sparrows are vital activities and familiarity among individuals
462 moving together does play a significant role during exploration [22]. Several studies
463 have shown that in other species the decision to move is conditioned by the network of
464 social relationships and the decision of close partners when joining group-mates [66,
465 67]. In particular, a study on three-spined stickleback found that individuals tended to
466 discover a food patch sooner if a familiar individual from their group had previously
467 done so [24]. Moreover, in a previous experiment with an unlimited hidden food source
468 the individuals closely associated with the first feeder gained access to the food source
469 before the others [44]. In our experiment, resources were limited and in fact association
470 with the first feeder led to a difference in the quantity of resource consumed, i.e. to a
471 definite benefit. Consequently, the opposing triad found itself at disadvantage: a greater
472 number of mealworms consumed by one triad meant a lower number of mealworms
473 consumed by the other. We also cannot exclude the possibility of a monopolization of
474 the feeding cups by the first-feeder triad [68]: while aggressive interactions were rarely
475 observed (Authors' personal observations), the presence of an individual of another
476 triad on the feeder might have been a deterrent for the opposite triad to start foraging.
477 The variability in individual phenotype (i.e. personality traits such as boldness,
478 exploratory behaviour) is deeply linked to the individual latency to feed in a social
479 context [6, 69]. Bolder individuals are often shown to display greater moving initiative,

480 whereas shyer individuals tend to follow conspecifics more [70, 71]. Nevertheless, we
481 did not find any influence of individual behavioural traits on the sparrows performances
482 in the main experiment, and we did not find any evidence for a difference in activity
483 between first feeders and other birds when we tested them in the activity assay.
484 However, during the consumption rate assay one-year old birds that were first feeders in
485 the main experiment again consumed more mealworms and foraged sooner. As the
486 consumption rate assay was conducted after the main experiment it is also possible that
487 individual experience might have had a role influencing the acquisition of resources;
488 individuals that foraged successfully in the main experiment could have been more
489 eager to take advantage of the food source. However, the consumption rate assay was
490 performed three months after the main experiment (which lasted only one morning) and
491 in the meantime all birds had had access to cups with mealworms inside: for this reason
492 we reckon that the variability was not due to differences in experience with the cup but
493 rather to a specific behavioural trait.
494 First feeders individuals were thus faster to acquire the food source in both social
495 contexts, suggesting a consistency in their role within the two very diffferent groups.
496 Approaching and exploiting a food source after the experimenter had tampered with it
497 can be considered a proxy for risk-taking behaviour (the sooner an individual
498 approaches a potentially "risky" food source, the more risk-taker it is) [55], a trait often
499 linked with exploratory behaviour [56]. However, the influence that this behavioural
500 trait had on the performance of the triads during the main experiment was not
501 significant. There might be two possible explanation as to why first feeders were greater
502 risk-takers during the consumption rate assay but we did not find any influence of
503 individual risk-taking behaviour on the sparrows' performances in the main experiment.

Firstly, in the main experiment the sample size might have been too low to detect an effect of individual behaviour (as each individual was tested once it provided one performance score, while in the consumption rate assay the test was repeated for ten days). Secondly, the triads' performances might not be a function of the number of bold individuals within them; highly performing groups might be often composed of a mix of different traits [6]. We can thus hypothesize that the factor explaining the difference between the triads during the main experiment could have been the personality of only some of the individuals within them [13]. As having precedence to eat gave an advantage in both scenarios, following closely a bold group-mate might have considerably sped up the feeding process [10]. We might thus speculate that in this species differences among groups might be linked to differences in the phenotype of their boldest individuals [72]. In fact, while we detected a posteriori that first feeder birds were indeed either bolder or less risk-averse [68, 57], their entire triads were not, on average, composed of more risk-taker individuals than the opposite ones.

In conclusion, our results appear to suggest a foraging pattern based on both individual characteristics and flock membership [69, 73]. Bolder individuals led their entire group to acquire a resource sooner [74], but boldness alone did not influence resource acquisition; moreover, we showed that differences at flock level can lead to variable individual benefits when two flocks are briefly together and competing for the same limited resource, even in fission-fusion societies such as the house sparrows'. We tested this only on a short time-scale, that is, however, the time scale at which many feeding events on limited resources happen. It must also be taken into account that flock size in this species is highly variable: the effect that we observed might be linked to the low

number of individuals (three) in each group, while in larger groups dynamics might differ [75].

These results indicate the necessity to focus not only on individual characteristics and traits when considering the processes of novel environment exploration [76], dispersal [77, 78] or invasion [79] in a sociable species, but also on how these characteristics and strategies interact in a context of multiple flocks. In the future it might be worthwhile to investigate how different phenotypes within the group can change the outcome of similar experiments, to test if manipulation of group composition (different mixes of individual characteristics within a group) could determine or influence the performance of an entire group during a merging scenario.

DATA AVAILABILITY: https://figshare.com/s/821920b86773adcc65dc

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AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization, B.T, M.G. and E.L.; Methodology, B.T. and E.L.; Investigation, E.L.; Validation, B.T. and E.L.; Formal Analysis, B.T.; Writing – Original Draft, B.T. and E.L.; Writing – Review & Editing, B.T., E.L., M.G. and H.H.; Funding Acquisition, M.G., E.L. and H.H.; Resources, M.G. and H.H.; Supervision, M.G. and B.T.

Competing interests:

The author(s) declare no competing interests.

FIGURE LEGENDS

Figure 1.

Schematic representation of experimental set-up, allowing two triads of house sparrows to face each other in a central, novel aviary. The novel aviary was equipped with a coveted and limited food source arranged in a novel manner (nine worms in three cups). (A) Both triads started in aviaries adjactent to the central aviary, (B) Once the windows opened sparrows could freely enter the central aviary. We recorded each individual latency to cross to the central aviary, its latency to feed and the number of mealworms that each individual consumed.

Figure 2.

Per capita average number of mealworms consumed during the main experiment. On the left, mealworms consumed on average by individuals of the first feeder triad; on the right, mealworms consumed on average by individual of the opposing triad. Note that first, second and third to feed refer to the ordinal feeding position within the triad. Mean and standard error of the mean are shown.

Figure 3.

Total number of mealworms acquired by each triad. Bars laying next to each other represent triads tested together (same trial). In dark grey, number of mealworms consumed by first feeder triads. In white, number of mealworms consumed by their opposing triad.

Figure 4.

Per capita daily average number of mealworms consumed during the consumption rate assay. On the left, mealworms consumed on average by individuals of the first feeder triad (relative to the main experiment); on the right, mealworms consumed on average by individual of the opposing triad. Note that first, second and third to feed refer to the ordinal feeding position within the triad during the main experiment. Mean and standard error of the mean are shown.

TABLES

Table 1. Effect of ‘age’ (first-year versus mature), ‘familiarity’ (familiar versus unfamiliar), ‘triad of the first crosser’ (first crosser triad versus other triad), ‘triad of the first feeder’ (first feeder triad versus other triad) on the number of mealworms eaten per capita. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. ‘Group’ and ‘day’ are fitted as random effects; we show the variance associated with them. The mealworms eaten by first feeders were excluded by the analysis in order to maintain data independence.

875 **Table 1.**

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		0.268	-0.486	1.190	0.418
Age	First-year versus mature	-0.330	-1.033	0.217	0.303
Familiarity	Familiar versus unfamiliar	0.334	-0.394	1.137	0.373
Triad of the first crosser	First crosser triad versus other triad	-0.286	-1.021	0.651	0.449
Triad of the first feeder	First feeder triad versus other triad	-0.826	**-1.629**	**-0.049**	**0.011**
Random effect		**Variance**	**± SD**		
Group		0.001	0.001		
Day		0.001	0.001		
OLRE		0.751	0.767		

876

Table 2. Effect of ‘age (first-year versus mature), ‘familiarity’ (familiar versus unfamiliar), ‘triad of the first crosser’ (first crosser triad versus other triad) on the individual latency to cross into the central chamber. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. ‘Group’ and ‘day’ are fitted as random effects; we show the variance associated with them. The mealworms eaten by first crossers were excluded by the analysis in order to maintain data independence. Results are in the log (not in the response) scale.

901 **Table 2.**

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		8.599	7.821	9.382	0.001
Age	First-year versus mature	0.402	-0.590	1.218	0.085
Familiarity	Familiar versus unfamiliar	-0.036	-0.882	0.888	0.844
Triad of the first crosser	First crosser triad versus other triad	-0.073	-0.845	0.784	0.692
Random effect		**Variance**	**± SD**		
Group		0.046	0.216		
Day		0.282	0.531		

902

903

Table 3. Effect of 'age (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'triad of the first feeder' (first feeder triad versus other triad) on the individual latency to eat the first mealworm. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group' and 'day' are fitted as random effects; we show the variance associated with them. The mealworms eaten by first feeders were excluded by the analysis in order to maintain data independence. Results are in the log (not in the response) scale.

928 **Table 3.**

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		9.085	0.302	10.116	0.001
Age	First-year versus mature	0.257	-1.091	1.230	0.149
Familiarity	Familiar versus unfamiliar	-0.025	-1.257	1.066	0.879
Triad of the first crosser	First crosser triad versus other triad	-0.077	-0.738	1.019	0.191
Triad of the first feeder	First feeder triad versus other triad	0.187	-1.629	1.198	0.637
Random effect		**Variance**	**± SD**		
Group		0.044	0.210		
Day		0.015	0.121		

929

Figure 1.

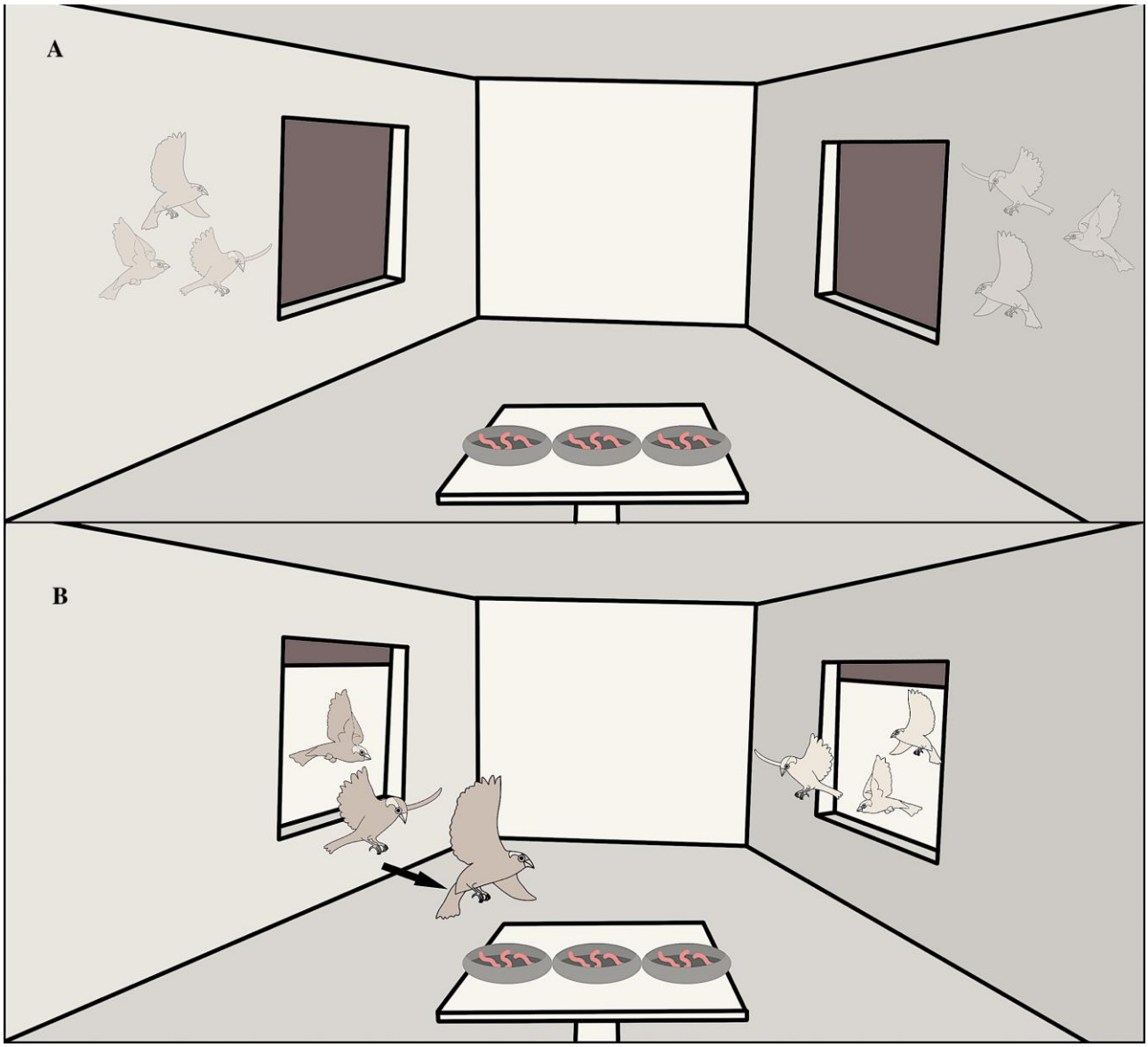

Figure 2.

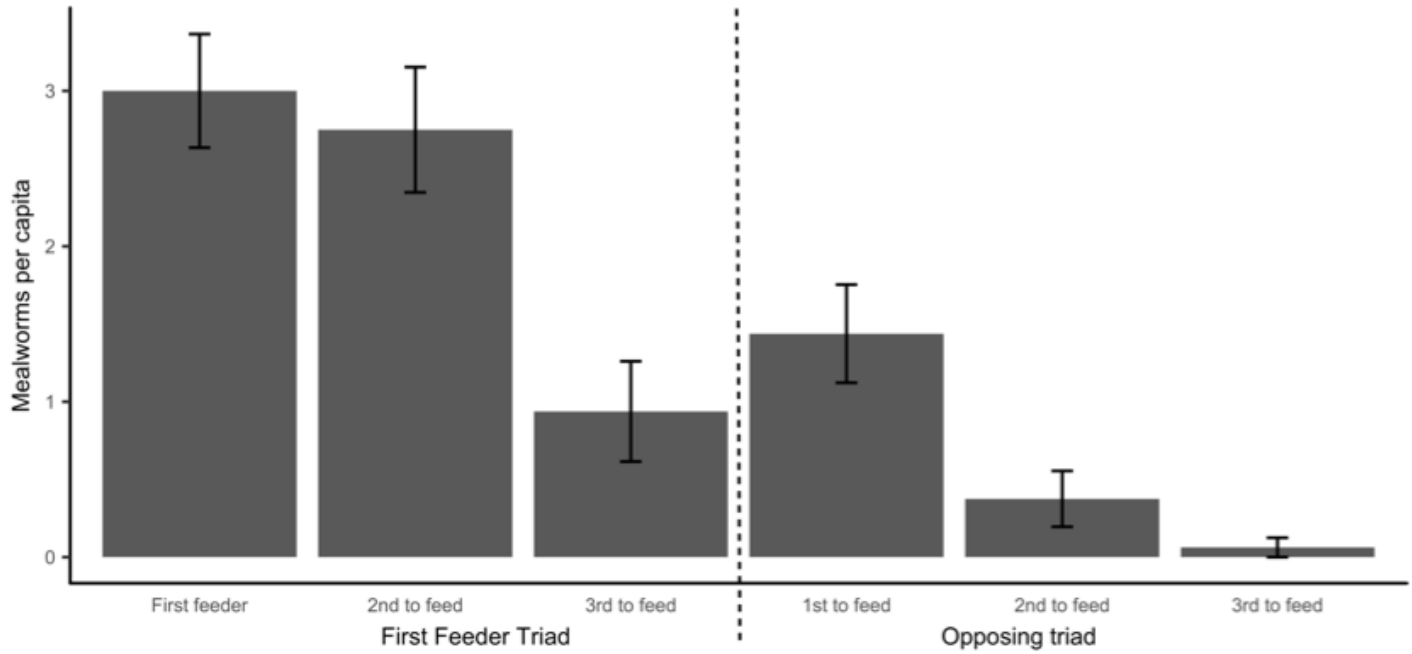

Figure 3.

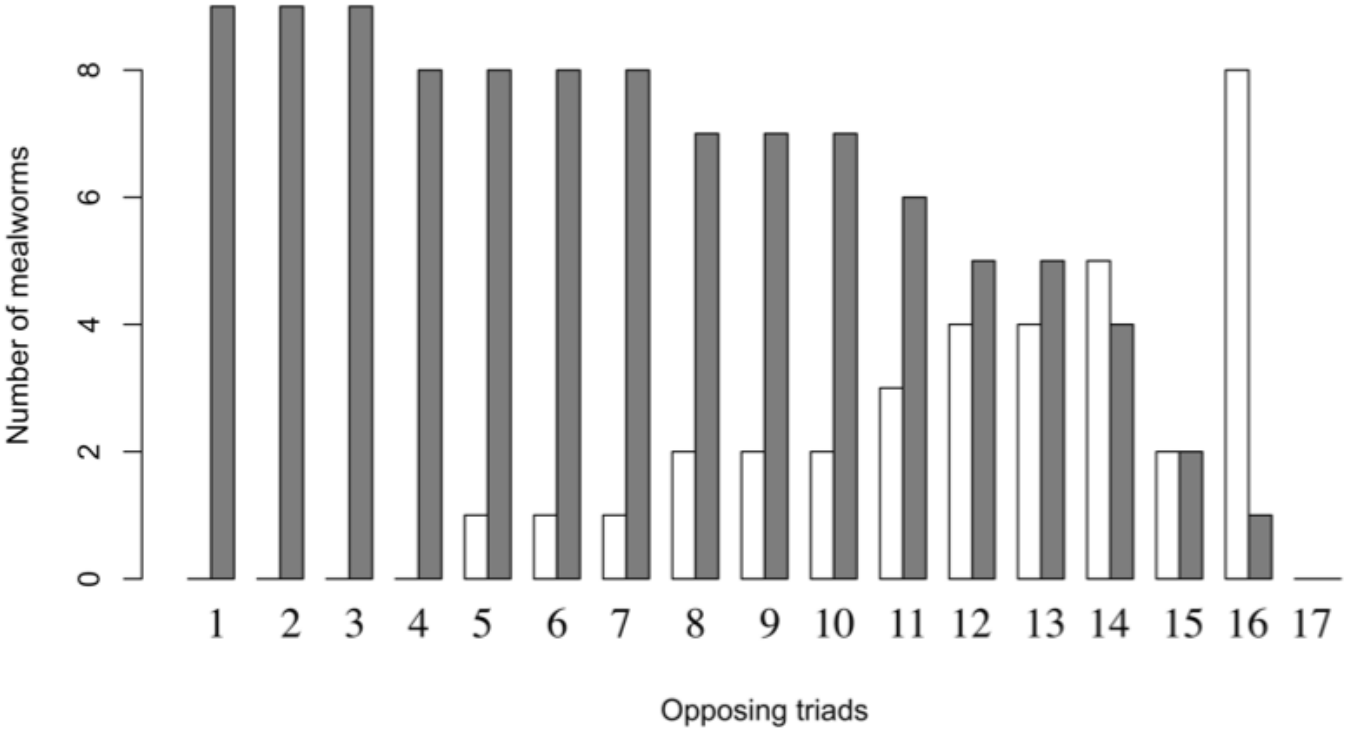

Figure 4.

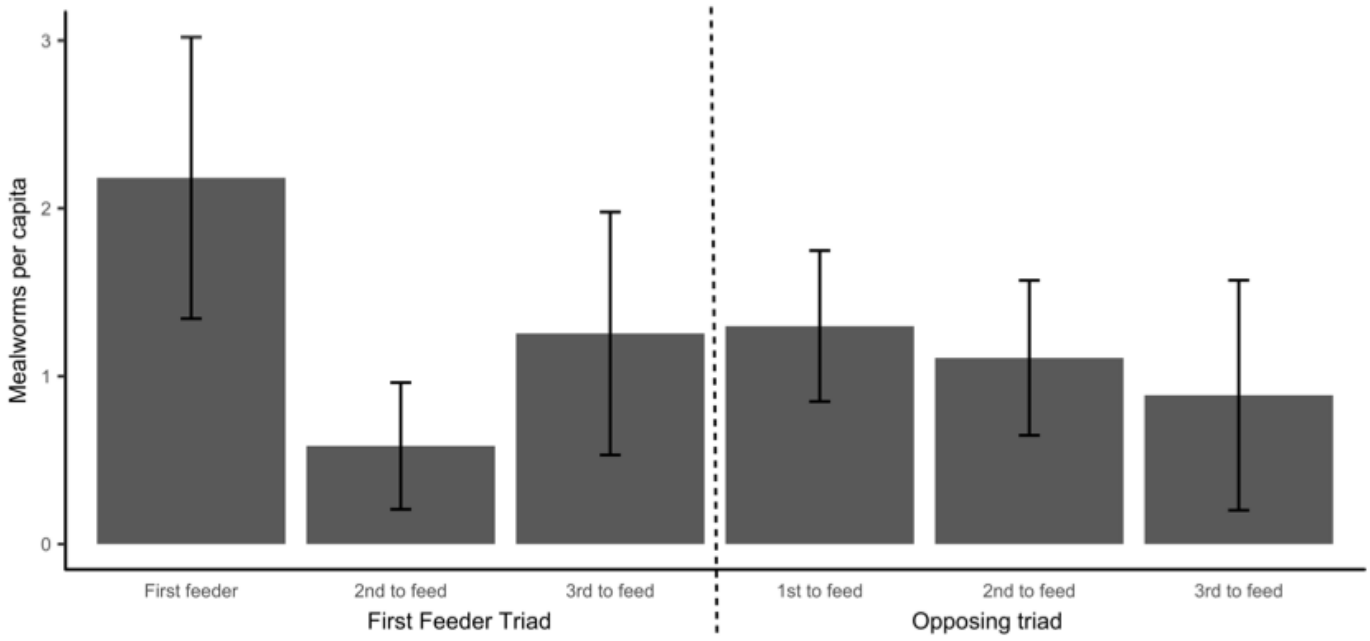
Mealworms per capita
3
2
1
0
First feeder
2nd to feed
3rd to feed
1st to feed
2nd to feed
3rd to feed
First Feeder Triad
Opposing triad

Table S1.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'triad of the first feeder' (first feeder triad versus other triad), 'activity' (number of hops during activity assay) on the number of mealworms eaten per capita. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group', 'day' and 'OLRE' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the activity assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		0.527	-0.092	0.891	0.032
Age	First-year versus mature	-0.095	-0.667	0.345	0.692
Familiarity	Familiar versus unfamiliar	0.144	-0.417	0.612	0.610
Activity		0.001	-0.001	0.001	0.787

Triad of the first crosser	First crosser triad versus other triad	-0.086	-0.751	0.396	0.761
Triad of the first feeder	First feeder triad versus other triad	**-1.103**	**-1.614**	**-0.563**	**0.001**

Random effect	**Variance**	**± SD**
Group	0.001	0.001
Day	0.001	0.004
OLRE	0.489	0.598

Table S2.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'triad of the first feeder' (first feeder triad versus other triad), 'activity' (number of hops during activity assay) on the individual latency to eat a mealworm. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group' and 'day' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the activity assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		8.886	7.772	9.904	0.001
Age	First-year versus mature	0.271	-0.864	1.217	0.128
Familiarity	Familiar versus unfamiliar	0.027	-1.128	1.142	0.863
Activity		0.001	-0.001	0.001	0.338

Triad of the first crosser	First crosser triad versus other triad	-0.078	-1.019	1.009	0.620
Triad of the first feeder	First feeder triad versus other triad	0.325	**-0.238**	**-0.073**	**0.018**

Random effect	**Variance**	**± SD**
Group	0.039	0.199
Day	0.018	0.136

Table S3.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'activity' (number of hops during activity assay) on the individual latency to enter the central aviary. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group' and 'day' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the activity assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		8.665	-0.092	0.891	0.001
Age	First-year versus mature	0.430	-0.362	1.208	0.054
Familiarity	Familiar versus unfamiliar	0.044	-0.780	0.814	0.833
Activity		-0.001	-0.001	0.0004	0.076
Triad of the first crosser	First crosser triad versus other triad	-0.073	-1.386	1.171	0.730

Random effect	Variance	± SD
Group	0.077	0.278
Day	0.026	0.162

Table S4.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'triad of the first feeder' (first feeder triad versus other triad), 'average consumption rate' (number of mealworms eaten on average during consumption rate assay) on the number of mealworms eaten per capita. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group', 'day' and 'OLRE' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the consumption rate assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		0.908	0.375	1.603	0.002
Familiarity	Familiar versus unfamiliar	0.041	-0.797	0.737	0.904
Consumption rate		-0.001	-0.001	0.001	0.660
Triad of the first crosser	First crosser triad versus other triad	-0.675	-1.964	-0.406	0.052
Triad of the first feeder		**-1.072**	**-1.614**	**-0.563**	**0.003**

First feeder triad

versus other triad

Random effect	**Variance**	**± SD**
Group	0.001	0.001
Day	0.001	0.001
OLRE	0.263	0.513

Table S5.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'triad of the first feeder' (first feeder triad versus other triad), 'average consumption rate' (number of mealworms eaten on average during consumption rate assay) on the individual latency to eat a mealworm. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group' and 'day' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the consumption rate assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		9.139	7.149	0.103	0.001
Familiarity	Familiar versus unfamiliar	0.071	-1.285	1.428	0.503
Consumption rate		0.001	-0.001	0.002	0.253
Triad of the first crosser	First crosser triad versus other triad	0.099	-1.950	1.791	0.337
Triad of the first feeder		0.154	-1.833	-1.546	0.136

First feeder triad

versus other triad

Random effect	**Variance**	**± SD**
Group	0.003	0.059
Day	0.014	0.119

Table S6.

Effect of 'age' (first-year versus mature), 'familiarity' (familiar versus unfamiliar), 'triad of the first crosser' (first crosser triad versus other triad), 'average consumption rate' (number of mealworms eaten on average during consumption rate assay) on the individual latency to enter the central aviary. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in bold. P values obtained with Tukey method adjusted for multiple comparisons. 'Group' and 'day' are fitted as random effects; we show the variance associated with them. Individuals that were not tested in the consumption rate assay were excluded from the analyisis.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		8.667	7.847	9.421	0.001
Familiarity	Familiar versus unfamiliar	0.064	-0.768	1.223	0.760
Consumption rate		0.001	-0.001	0.001	0.700
Triad of the first crosser	First crosser triad versus other triad	0.205	-0.707	0.870	0.315
Random effect		**Variance**	**± SD**		

Group	0.001	0.001
Day	0.032	0.181

Chapter 7

Dynamic duos: social roles across different contexts

Behavioral Ecology (submitted)

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Data accessibility. Data related to this study may be accessed through Figshare digital repository: https://figshare.com/s/447d5acbd783da2b9e59.

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Dynamic duos: social roles across different contexts

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Abstract

Stark differences in individual strategies are a well-known feature of animal diversity. The leader/follower dichotomous strategy in particular has been reported in many taxa of moving animals; follower individuals are thought to be more risk-averse, while leaders may incur greater predation risks. We decided to investigate dichotomous strategies not only during exploration but also in a vastly different situation, one of the most crucial times in the life of any animal: during the attack of a predator and the split-second reactions to it. We thus tested captive house sparrows (*Passer domesticus*) dyads both during an open-field trial and during a simulated attack. As expected, we discovered that during the open-field trial individuals behaved consistently either as leaders or followers. However, during the simulated attack individuals in the dyads switched roles, with 'followers' leading the escape flights and 'leaders' tailing them. This evidence for the mirror positioning of the two individuals during such a frantic movement underlines the importance of the coordination of individual strategies in a social group. Moreover, it suggests that as certain roles are linked across different situations, some dichotomous strategies might be advantageous for both individuals by providing or allowing to gain benefits depending on the context.

Introduction

A key focus in the study of social behavior has been how animals within a group physically position themselves relative to their groupmates and what are the reasons and consequences of employing these formations [Nagy et al. 2010]. Several theoretical models and investigations in many taxa have underlined how a spatial organization of the group can naturally emerge as the result of simple individual-level interactions, i.e. how each animal reacts to its closest groupmates [Couzin and Krause 2003; Attanasi et al. 2015; Herbert-Read et al. 2015]. Structuring within a group might thus reflect the feedback between each individual's characteristics and the surrounding social environment – the characteristics of its groupmates and their influence on each other [Farine et al. 2015]. Consequently, in every group might exist differential benefits linked to each spatial position [Krause 1994; Webster and Ward 2011] and yet, as groups encounter suites of different challenges and contexts, these benefits may greatly vary [Jolles et al. 2018].

One of the best-studied examples in this field is the leader-follower dichotomy [King and Cowlishaw 2009], researched extensively in humans [King et al. 2009], other vertebrates [Bevan et al. 2018], invertebrates [Hodgkin et al. 2014] and even in artificial entities [Wang et al. 2017]. Whenever an individual initiates a movement and is followed by others it can be defined as the "leader", while those following it are "followers" (however, for further debate on the definition see [King 2010]). The differentiation in these two roles is known to emerge in dyads [Harcourt et al. 2009], small social groups [Sasaki et al. 2018] and huge assemblies [Couzin et al. 2005]. In the simplest scenario, leaders are thought to be the individuals with greater motive (either hunger [Nakayama et al. 2012; Webster 2017], or knowledge [Hodgkin et al. 2017]) or propensity [Harcourt et al. 2009] to move independently, while their group-mates preferentially employ a copying strategy. Certain behavioral phenotypes and strategies have been thus linked to leadership more often than others: greater boldness [Bevan et al. 2018], tendency to explore [Sasaki et al. 2018], low sociability and producer strategy [Jolles et al. 2017; Tóth et al. 2017], while conversely

behavioral traits such as shyness [Pruitt et al. 2018], high sociability [Aplin et al. 2014] and scrounging strategy [Kurvers et al. 2009; González-Bernal 2014] have been connected to following. For example, black-headed Gouldian finches (*Erythrura gouldiae*), which have been found to be more risk-taking and exploratory, act as leaders more than red-headed individuals, that are consistently shyer but more aggressive [O'Reilly et al. 2019].

Moreover, feedback mechanisms are thought to reinforce the social niche of both leaders and followers [Johnstone and Manica 2011; Nakayama et al. 2012], with individuals using a preferred strategy that "forces" their groupmates into the other [Harcourt et al. 2009; Pruitt et al. 2018]. Both leader and follower strategies have thus been found to be repeatable within a group [Santos et al. 2014] and are usually believed to differ starkly in relation to their respective costs and benefits [Krause et al. 1998; Webster and Ward 2011]. For example, during a foraging bout a leader individual might arrive first at a food source and hence have a greater chance to exploit it; however, it might also incur greater costs [Krause et al. 1998], particularly as it risks failing to initiate a following event and might find itself foraging alone [Ioannou et al. 2015], exposing itself to a higher risk of predation.

Nevertheless, the distinction between followers and leaders can become especially clear in environments where animals are exposed to high predation threat. Ioannou et al. [Ioannou et al. 2017] demonstrated that fish reared in high-predation habitats tend to differentiate in leaders and followers instead of moving more homogenously. However, although a central role of predation is always hypothesized when explaining these groups' structure [Herbert-Read et al. 2017], very few studies actually focused on one of the most crucial moments in the lifetime of any animal: the attack of a predator and the split-second reaction to it [Herbert-Read et al. 2017]. The main reason for this is apparent: predator attacks in nature are not uncommon to observe, but are by necessity quick and frantic, with scarce chance for recognition of individuals or for observations of their reaction to conspecifics. Studies on individual behavioral responses to a potential predation threat have shown that animals vary in their individual flight initiation distances (a repeatable behavioral trait

79 associated with other risk-taking behaviors [Cooper and Blumstein 2015]) and that risk-taking
80 individuals might be more vulnerable to predation [Santos et al. 2015; Lapiedra et al. 2018].
81 However, the importance of the social component of escape behavior has been increasingly
82 recognized in group-living species, as in-flight cues given by conspecifics could provide
83 information on distance and direction of danger as well as a safe path or flight trajectory [Evans et
84 al. 2019]. During predator attacks, prey in fact strive to move together, as an instant of un-
85 synchronization could lead to isolation and increase the chances of becoming a target [Ioannou
86 2017]. As there is strong individual variation in prey anti-predator responses, it is hence
87 conceivable that even during panicked flights a spatial organization of the group could emerge as a
88 consequence of individuals' characteristics. For example, certain group members could dart in front
89 of the flock while others keep behind, with the former determining the escape direction and the
90 latter following. This differentiation in flight strategies could be linked to individual traits [Carrete
91 and Tella 2009; Cooper and Blumstein 2015], individual condition, or also to the social role already
92 assumed within the flock, for example as leaders or followers during normal movements.
93 We investigated the behavioral roles – leader or follower – employed by captive house sparrows
94 (*Passer domesticus*) in two different contexts: during the exploration of a novel environment
95 [Tuliozi et al. 2018] and during a simulated predator attack. We utilized a novel experimental set-up
96 to simulate a relatively prolonged chase, split into brief separated movements that allowed quick
97 recognition of flight direction and individual identity. We hypothesized that sparrows within each
98 pair would differentiate themselves into leaders and followers in each context, depending on their
99 individual behavioral characteristics. We then further considered two alternative hypotheses to be
100 plausible. First, if there are individuals that always initiate group movements, regardless of context,
101 we predicted that the leaders during the open-field stage of the trial and the predator attack would
102 always be the same individuals and their companions would unchangingly follow. Conversely, if
103 traits associated with following behavior during exploration are better linked to faster flight

initiation during a predator attack, we predicted that leaders during the open-field stage would behave as followers during the attack and vice versa.

Methods

Housing and study subjects

This study was conducted between March and June at the Konrad Lorenz Institute of Ethology (KLIVV, University of Veterinary Medicine) in Vienna, Austria (48°13' N, 16°17' E). We selected 96 house sparrows (48 males and 48 females) for the experiment, all born during the previous breeding season and reared by their parents in the same aviaries where they were born. The birds were kept in 5 mixed-sex outdoor enclosures (from now on "housing aviaries"), measuring 3.9×2×2.6 m (length × width × height, from now on implied), each one holding 19.2 ± 1.8 individuals (measures reported here and henceforth are mean ± standard error of the mean), range: 15–25 sparrows. For further information on the housing aviaries set-up and feeding regime, see Tuliozi et al. [2018]. The study subjects were further divided in 16 groups (8 groups of males and 8 groups of females) each group consisting of 6 same-sex birds from the same housing aviary. When testing began two male groups and two female groups were moved into four temporary aviaries, where they remained until all individuals in their groups had been tested once (7.08 ± 1.31 days). After two days of habituation to the temporary aviaries (enough time for captive house sparrows to habituate to a new environment; [Tóth et al. 2017]), trials started on the morning of the third day. The temporary aviaries were similarly equipped than the housing aviaries: they measured 3.7×1.9×2.5 m. Birds in the temporary aviaries were fed daily (in the morning) with ad libitum (roughly 300 g) standard mixture of seeds (wheat, canary seeds, sunflower seeds). When all individuals belonging to the first 4 groups had been tested they were returned to their housing aviaries and the next 4 groups were moved to the temporary aviaries. We tested all 96 individuals (48 males and 48 females) in three social contexts, namely alone (individual context), with a

familiar same-sex individual (familiar context) and with an unfamiliar same-sex individual (unfamiliar context). The total number of trials performed was thus 192, of which 96 individual trials, 48 familiar trials (each one with two individuals, 96 individuals tested) and 48 unfamiliar trials (each one with two individuals, 96 individuals tested). Thus, every bird was tested three times, once in the familiar, once in the unfamiliar and once in the individual context, independently from all the other individuals considered. For each bird the order of the three trials was randomized across contexts, with successive trials of the same bird separated by 37.24 ± 13.9 days. All trials were conducted between 2 h after sunrise and 1 h before sunset: the hour of the trial was randomized between individuals and contexts. All social trials consisted of two consecutive stages.

Trial procedure

Exploration aviary and open-field stage of the trial

The procedure for the open-field stage of the trial is more thoroughly described in Tuliozi et al. [42]; therein we also investigate house sparrow behavioral responses across the three different social contexts. We assessed exploratory behavior in an indoor novel environment (exploration aviary), which measured 8.3 × 8.7 × 2.5 m and was equipped with a number of features to simulate a natural environment. The open-field stage of the trial ran for 2 hours. A quarter of the 72.21 square meters of the exploration aviary was covered by branches. The branches and the other perching areas were differentiated in 10 sectors, corresponding to spatial locations independent from one another. We rarely observed birds hopping back and forth from different sectors, as moving from one to the other usually required at least a brief flight. There were also four food sources, including three sprays of millet hanging from the branches and one food bowl on a pedestal with a mixture of seeds and a spray of millet inside. Water was positioned on the ground, as in the housing and temporary aviaries. All observations were done via a one-way see-through plastic mirror on the left wall of the exploration aviary. All trials were recorded using three webcams

156 (LifeCam Studio, Microsoft. Article number: Q2F-00015 and Q2F-00016). Video data were
157 processed through iSpy, a free open source software (version 6.3.0.0). The birds were also visually
158 monitored by one of the authors (BT) through the one-way see-through plastic mirror previously
159 mentioned. At the beginning of every trial, the study subjects were transferred to a two-part divided
160 cage (2 × 0.5 × 0.5) m inside the exploration aviary. After 10 min of habituation, the cage was
161 opened from outside the exploration aviary using a remote system. As soon as the cage was opened,
162 the trial started. For all conspecific pairs tested (both in the familiar and unfamiliar context) we
163 recorded the following social behavioral variables during the open-field stage: i) number of
164 aggressive interactions; ii) winners/losers of aggressive interactions (such as biting and chasing –
165 losers were defined as the individuals that after a confrontation retreated and were chased away
166 from the branch they were on); iii) number of following bouts (defined as the flight of both birds
167 from one sector to another, taking off within 3 s of each other; similarly as in Tóth et al. 2017); and
168 iv) the identity of the leader in each following bout. The leader in a following bout was defined as
169 the bird that departed first when followed by the other. As reported and discussed in Tuliozi et al.
170 [42], for all individuals in all contexts we also recorded a number of variables such as i) latency to
171 forage; ii) latency to touch the ground; iii) number of sectors visited; iv) number of food sources
172 visited; and v) time spent foraging. Birds that did not eat or touch the ground were assigned a
173 latency of 7201 s [Van Oers et al. 2005].
174
175 Simulated attack stage
176 Right after the open-field stage of the trial in the two social contexts (both familiar and unfamiliar
177 contexts) we began the second part of the experiment, i.e. the simulated attack stage of the trial
178 ("SA stage of the trial"). As soon as the 120th minute ended an experimenter entered the exploration
179 aviary (always the same person). This part of the experiment was also performed in the exploration
180 aviary: branches were differentiated in 10 clearly defined sectors corresponding to spatial locations
181 independent from one another. This resulted in sparrows flying from one to the other when

182 escaping, landing and then departing again whenever the experimenter was close. During this part
183 of the experiment, we never saw birds hopping around, as they limited their movements to flights
184 that usually brought them from one side of the exploration aviary to the other. The experimenter
185 moved at a brisk pace with the hand-net raised, always towards the closest individual, pretending to
186 chase the bird. The hand-net is routinely used for capturing them in the aviaries and birds strongly
187 react to it; moreover, they always try to avoid being captured as much as possible, with this method
188 having long been used to test individual capacity to escape [Moreno-Rueda 2003]. Whenever the
189 birds moved away, the experimenter redirected himself towards the closest individual, never
190 stopping walking for 60 seconds. As the room was quite extensive, after a flight the sparrows
191 usually stayed in the sectors where they had landed until the experimenter was again closer. Thus,
192 separate flights were clearly distinct, and we can exclude the possibility that the second sparrow to
193 land in a given sector was the second to depart simply because it was too startled from just having
194 landed. During the SA stage we recorded i) the number of following bouts (see above for definition)
195 and ii) the identity of the leader (and of the follower) in each following bout. All trials were
196 observed and recorded using the same methods as the open-field stage. After the SA stage was over,
197 a second person entered the exploration aviary and both individuals were quickly captured with
198 hand-nets and returned to their aviaries.
199
200 Statistical analyses
201 All data were analyzed using R version 3.5.3 [R Development Core Team 2015]. We investigated
202 our research questions using Generalised Linear Models, all with binomial error distribution (link =
203 logit). The models were fitted using the 'glm' function within the package 'lme4' (1.2.1) for R
204 version 3.5.3 [Bates et al. 2015]. Estimates and significance of the fixed effects were obtained using
205 'car' package, while the 'confint' function from the 'lme4' package was used to obtain confidence
206 intervals. Firstly, we analyzed the relationship between the behavioral strategies used in the open-
207 field stage and in the SA stage. As a dependent variable we fitted the proportion of following bouts

208 performed as a leader by each individual during the SA stage. The proportion of following bouts
209 performed as leader during the open-field stage was used as independent variable. In order to
210 maintain independence of our data we chose a random sample of one individual per pair (focal
211 individual) to be included in this analysis (as of course following bouts performed as leader by each
212 individual in the pair were inversely proportional). We re-sampled our data 50 times using a
213 random number generator (https://www.random.org) in order to ensure that our results were not
214 artefacts created by our sample of focal individuals. Thus, we decided not to include individual
215 identity as a random factor in the models: given that we selected one focal individual per pair in the
216 analysis and as not all trials provided viable data (see Results) the majority of the individuals were
217 present just once in each sample. Moreover, because this analysis investigated differences in the
218 proportion of following bouts performed as leader within the pairs, it was not necessary to fit
219 familiarity of the pair, sex of the pair or round of testing (first, second or third round) within the
220 model, as the two individuals in the pair did not differ in any of these factors.
221 Therefore, in order to investigate if familiarity, sex, previous experience with the exploratory room
222 (and the interactions between these factors) had any effect on the proportion of following bouts
223 performed as leader in both stages we fitted two separate models with binomial error distribution
224 with these variables as fixed effects. As dependent variables, we fitted the proportion of following
225 bouts performed as leader by one individual in each pair: in order to provide a consequential
226 between-pair comparison we always selected from each pair the individual with more following
227 bouts as the leader – any other option would have made the results indistinguishable from artefacts
228 created by uneven sampling.
229 Finally, we also compiled a full correlation matrix between all recorded behavioral variables,
230 condition (determined as weight/tarsus length^3) and the proportion of following bouts performed as
231 leader during the two stages using Kendall package [McLeod 2009].
232
233 **Results**

Out of 96 social trials, 19 did not provide viable data during the SA stage, as we did not manage to clearly determine the identity of both individuals at all times because of technical issues with the recording system. Of the remaining 77 trials, we analyzed only the 71 that reached the minimum threshold of four clearly-determined following bouts during the open-field stage and four clearly-determined following bouts during the SA stage. We established this threshold as we did not consider a lesser number of following bouts to be informative on the leader/follower relationship of the pair.

Individuals within pairs consistently tended to position themselves either as leaders or followers in both stages of the trial. Indeed, individuals performing more following bouts as leaders within each pair did so on average in 81.36% of the following bouts during the open-field stage (significantly higher than the expected 75%, pooled G-test, $G = 15.083$, $p < 0.001$) and in 81.07% of the following bouts during the SA stage (significantly higher than the expected 75%, pooled G-test, $G = 8.691$, $p = 0.003$).

Analyzing the frequency of following bouts performed as leader and follower we discovered that individuals changed their strategical position within the pair with respect to the context. Results obtained in the re-sampled datasets did not differ in significance or direction (supplementary material, table S1).

The difference between familiar and unfamiliar pairs in the number of following bouts recorded during the first hour of the open-field stage (result reported in [42]) did not influence our analysis, as there was no difference between familiar and unfamiliar pairs in the distribution of leadership neither during the open-field stage of the trial (82.0% vs 18.0% for familiar pairs versus 79.6% vs 20.4% of unfamiliar pairs) nor during the SA stage (82.9% vs 17.1% for familiar pairs versus 80.1% vs 19.9% for unfamiliar pairs). Additionally, there was no influence of pairs' familiarity in the number of following bouts in the SA stage (F-value = 0.006, $p = 0.939$) nor was there any

significant influence of any of the fixed effects (familiarity, sex, and previous experience and their interactions) on the proportion of following bouts performed by leaders in both stages (tables 1–2).

The probability of being a leader during the SA stage was not correlated with any behavioral response recorded during the asocial context (supplementary material, table S2), while the proportion of movements performed as a leader during the open-field stage showed a significant but weak negative correlation with latency to touch the ground both in the asocial context (tau = 0.32, p = 0.003, supplementary material, table S2) and in the social context (tau = 0.27, p = 0.024, supplementary material, table S2).

During the open-field stage, while the number of aggressive interactions was low (80 aggressive interactions total), most of them showed a clear winner (individual chasing the other away) and loser (individual being chased off). Out of 96 social trials, 30 had aggressive interactions, with all of them having one individual consistently winning over the other (83.3% of the trials with aggressive contests showed one individual winning all contests). Individuals winning aggressive contests were also individuals usually following during the open-field trial (tau = -0.44, p = 0.002) and leading during the SA stage (tau = 0.36, p = 0.012).

Discussion

To our knowledge our study shows possibly the first evidence of i) the employment of a leader-follower dichotomous strategy during an escape flight ii) the individual-specific use of such strategies during this critical context iii) individuals' link to previously-adopted behavioral roles within the pairs.

Animals living in groups often rely on their groupmates' actions to detect predation threats, trusting alarm calls, sudden initiation of flights [Hingee and Magrath 2009] and other social cues to escape predators safely [Ioannou 2017]. During the actual attacks, individuals' behavioral responses are particularly crucial: a social component of escape flights has in fact been commonly found in

285 gregarious animals [Beauchamp 2012], with individuals often fleeing sooner when in flocks than
286 when alone [Boujja-Miljour et al. 2017; Morelli et al. 2019] for example through propagation of
287 escape waves [Hemelrijk et al. 2015; Herbert-Read et al. 2015].
288 During our experiment, as the room was large and complex [José de Anchieta et al. 2015], flights
289 varied greatly in their trajectories and length: birds taking off had multiple options in terms of
290 sectors where they could land, all equally distant from the attacker [Herbert-Read et al. 2017]. They
291 tended nevertheless to closely follow each other not only to the same sector, but usually to the same
292 branch, time after time rarely landing more than 50 cm apart (BT pers. obs.). Moreover, the
293 difference in departing times was always minimal, with the second individual almost always taking
294 off immediately after the first and – as far as we could observe – following it closely with its gaze.
295 We therefore find it much more plausible that the pairs' repeated flights (following bouts) in the SA
296 stage were coordinated by inadvertent social cues rather than solely ascribable to two birds
297 independently sharing an optimal flight path and differing only in their taking off and landing times.
298 Individuals showed a strong consistency in positioning themselves as either leaders or followers,
299 even in a frenetic context such as the SA stage. Birds have been demonstrated to vary in their
300 individual latency to flee an incoming potential predator, measured through their distance from the
301 attacker when taking off [Carrete and Tella 2009]. During our chasing experiment, this variability
302 was evident, as one of the two individuals repeatedly took off before its companion. A shorter FID
303 is a trait usually associated with more risk-taking behavioral phenotypes, while a greater FID is
304 considered a characteristic of risk-averse individuals [Møller and Garamszegi 2012]. In our
305 experiment the leader in the SA stage might thus be considered the lesser risk-taker of the two, as it
306 was the one that neither waited for the attacker to be any closer nor for a social cue before taking
307 off. Hence, this difference in behavioral response characterized the first bird to flee as a leader, as
308 its departure was the social cue that routinely prompted the second individual to dart after it (i.e.,
309 inadvertently eliciting a following bout). It would appear that a basic leader/follower dynamic was
310 still very much in play in this context, even if restricted to a greatly hastened time-frame. The

311 difference in individual response to the attack and its effect on the birds' positioning during the
312 flight could be seen as a natural consequence of the expected combined effect of behavioral
313 variability and social tendency (following instead of flying in another direction); however, this had
314 never been experimentally observed. In future studies of collective responses to predator attacks, it
315 might thus be interesting to focus on the possible role of individual-level differences in behavioral
316 strategy, and on the variable interactions that they could generate in these frantic but crucial
317 situations.

318 To our knowledge moreover our study is the first to show that while individuals always positioned
319 themselves as leaders or followers, they also switched their roles between the two stages. During an
320 open-field exploration, moving out first and leading a movement is usually considered a risky
321 option [Krause et al. 1998; Ioannou et al. 2019], at least compared to staying still in one place
322 [Wilson et al. 2010]. Risk-averse individuals might prefer to play the waiting game until their
323 companions move [Scheid and Noë 2010] in order to decrease their chance of encountering a threat
324 during exploration. Conversely, when facing a potential predator the safest option is actually fleeing
325 away sooner, as it increases the chance of surviving a predator encounter [Cooper and Blumstein
326 2015]. This is consistent with what we found, since in our experiment individuals following more
327 during the open-field stage were also initiators of movements during the attack, while individuals
328 that moved first in the open-field stage were followers in the SA stage.

329 We have some further evidence that individuals leading following bouts in the open-field stage and
330 following in the SA stage were greater risk-takers than their companions. Visiting water sources is a
331 behavior often investigated in relation to risk-taking [O'Reilly et al. 2019] as the ground is
332 considered inherently more dangerous than branches [Schuett and Dall 2009]. During our open-
333 field trials the latency to land on the ground was possibly the best proxy of individuals' perceived
334 threat and risk-taking behavior [Tuliozi et al. 2018]. Individuals that behaved as leaders in the open-
335 field stage went to the ground sooner when tested alone (the correlation was weaker in the social
336 trials, as there was a strong effect of social facilitation). On the other hand, the overwhelming

majority of the aggressive confrontations was initiated and won by individuals that were followers during the exploration and leaders in the SA stage. Dominance in social conflict is often considered a key trait of scroungers and follower individuals [Barta and Giraldeau 1998], particularly in this species [Liker and Barta 2002], as they find themselves more often in the situation to obtain resources that other individuals have already located or claimed [Liker and Barta 2002].

Whenever animals move or forage together followers can acquire information or resources at a lesser cost due to the presence of leaders [Tóth et al. 2017], which on the other hand were recently experimentally shown to suffer more predation than followers (but still less than lone individuals [Ioannou et al. 2019]). Our study might show a potential mechanism for leaders to benefit from associating specifically with followers, i.e. with individuals complementing their behavioral traits [Aplin et al. 2014]. Similarly to how risk-takers are known to decrease foraging latency and influence their companions' behavior during exploration, risk-averse individuals might be instrumental in increasing their groupmates' chances of surviving a predator encounter, particularly for more risk-taking individuals [Santos et al. 2015]. This could happen by increasing their flight initiation distance [Tätthe et al. 2018] or by nearing it to an optimum [Cooper Jr and Frederick 2007]. Individuals leading during escape flights could also provide a common trajectory to be followed and, by extension, inadvertently coordinate social flights that might otherwise become un-synchronized and uncoordinated. This context-dependent role switch might thus provide an example of the possible benefits given by behavioral diversity within a group [Delgado et al. 2018].

We might further speculate that groups with individuals employing different behavioral strategies and possessing variable characteristics might fare better than more homogeneous groups [Dyer et al. 2008; Hodgkin et al. 2014], particularly when performing across multiple contexts. Specifically, heterogeneous groups might be able to minimize the trade-off between high gain (fast and efficient movement or resource acquisition) and low predation risk by being collectively more flexible [Krause et al. 2010]. This flexibility in fact would not require individual plasticity, as the same simple mechanism, i.e. following another individual, could possibly benefit risk-averse individuals

during exploration and risk-taking individuals during an attack. While we did not have a concrete way to verify the extent of the advantage provided to risk-taking individuals by their following during the simulated chase, this might be one of the most interesting routes to investigate in following studies. We believe that our results open up a few more interesting possibilities for future research. Firstly, while the proportion of following bouts performed as leaders during the SA stage was significantly higher for individuals that followed more during the open-field stage, there were also a number of pairs that did not switch roles (25 pairs, 35% of the total), i.e. the leaders in the open-field stage of the trial were also leaders in the SA stage. Nevertheless, during the SA stage there appeared to be a strong strategy differentiation also in these pairs, with one of the two individuals performing on average 83% of the following bouts as leader (against the expected average of 75%). While in our study the number of pairs that did not switch was possibly too low to show meaningful patterns (for further details: supplementary material, table S3), in future studies it might be interesting to compare performances of pairs or groups of individuals that switch roles according to contexts with those that do not. It would be also worthwhile investigating exactly what individual characteristics are associated with certain behavioral responses during a chase, or if on the contrary, individuals are forced into determined positions by the social environment within every group.

Ethics. Capture, housing and handling of birds were in accordance with the relevant Austrian laws and were licensed by the government of Vienna (MA 22) license number 424/2011. The experiment reported in this study complies with current laws on animal experimentation in Austria and the European Union. This study was approved by the institutional ethics committee (University of Veterinary Medicine, Vienna) and the national authority according to 8ff (rules) of Law for Animal Experiments Tierversuchsgesetz - TVG, license number GZ 68.205/ 0220-II/3b/2012. The condition and health of experimental birds were monitored on a daily basis. No individual died or was injured during the 5-month long experiment. Furthermore, the chasing procedure was

389 purposefully time-restricted to minimize stress to well within the limits of a normal recapture
390 procedure.
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392 **Data accessibility.** Data related to this study may be accessed through the journal office.
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394 **Competing interest**. We have no competing interest.
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Table 1

Fixed effect	Comparison	Estimate	2.5% CI	97.5% CI	P value
Intercept		2.011	**1.468**	**2.593**	< **0.001**
Familiarity	Familiar vs unfamiliar	-0.173	-0.614	0.261	0.436
Sex	Female vs male	-0.237	-0.700	0.212	0.303
Previous experience	First round vs second round	-0.576	-1.109	-0.056	0.087
	First round vs third round	-0.408	-0.967	0.144	

Table 1. Output of GLM with 'proportion of following bouts performed as leader during the SA stage of the trial' (proportion) as dependent variable. Effect of 'familiarity' (familiar, unfamiliar), 'sex' (female or male), 'previous experience with the room' (first, second or third round of tests) on the proportion of following bouts performed as leader during the SA stage. All interactions between fixed factors were excluded from the model as not significant. The only individuals considered in the analysis are the ones that in their pair performed the majority of following bouts as leaders. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero

575 is not included in the interval) are in **bold**. Results are in the log (not in the response) scale. P
576 values obtained with Tukey method adjusted for multiple comparisons.
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Table 2

Fixed effect	Comparison	Estimate	2.5% CI	97.5% CI	P value
Intercept		1.582	**1.154**	**2.033**	< **0.001**
Familiarity	Familiar vs unfamiliar	-0.284	-0.671	0.098	0.146
Sex	Female vs male	-0.213	-0.600	0.166	0.272
Previous experience	First round vs second round	0.320	-0.133	0.790	0.301
	First round vs third round	0.265	-0.181	0.721	

Table 2. Output of GLM with 'proportion of following bouts performed as leader during the open-field stage of the trial' (proportion) as dependent variable. Effect of 'familiarity' (familiar, unfamiliar), 'sex' (female or male), 'previous experience with the room' (first, second or third round of tests) on the proportion of following bouts performed as leader during the open-field stage. All interactions between fixed factors were excluded from the model as not significant. The only individuals considered in the analysis are the ones that in their pair performed the majority of

following bouts as leaders. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. Results are in the log (not in the response) scale. P values obtained with Tukey method adjusted for multiple comparisons.

637 **Figure legend.**

638

639 **Figure 1.** Schematic representation of house sparrow leader-follower dynamics in two different
640 contexts. (*a*) Open-field stage: house sparrows released in the exploratory room freely moved for
641 two hours. During this time following bouts were recorded; individuals consistently assumed either
642 leader (on the right, arrow point end) or follower (on the left, arrow back end) positions. (*b*)
643 Simulated attack stage: house sparrows were chased with a hand-net for 60 seconds. During their
644 escape flights they consistently assumed leader and follower positions; generally, however,
645 individuals that had led more following bouts during the open-field stage were followers in this
646 context, and vice versa.

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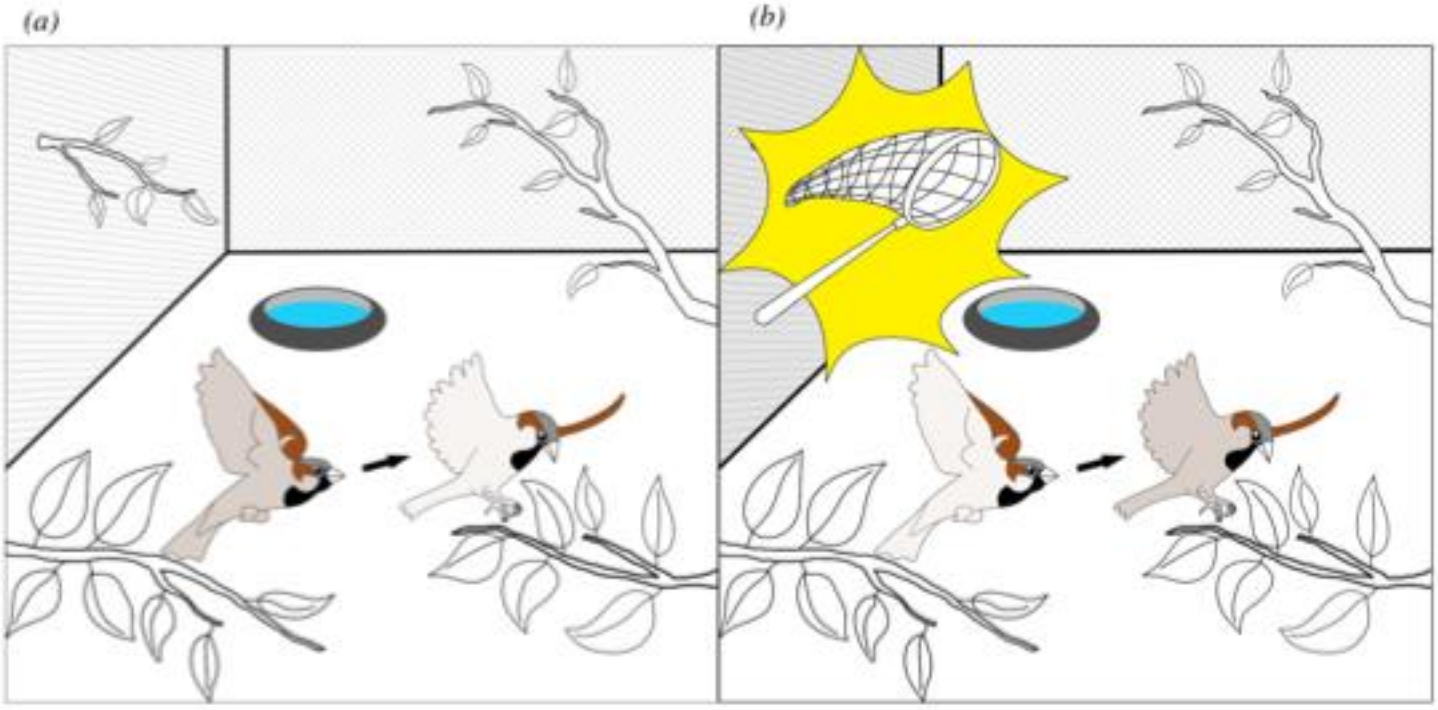
Figure 1.



SUPPLEMENTARY

Table S1. Output of GLMs with 'proportion of following bouts performed as leader by each individual during the simulated attack (SA) stage' as dependent variable and 'proportion of following bouts performed as leader during the open-field stage' as independent variable. We repeated the analysis re-sampling 50 times the identities of focal individuals, as only one per pair could be analysed in order to maintain data independence. Coefficients and 95% confidence intervals are presented; P values obtained with Tukey method adjusted for multiple comparisons.

Sample number	**Significance**	**Confidence intervals**
Model 1	$\chi2 = 18.42$, p = 1.772e-05	lCI = -1.873832, hCI = -0.8274647
Model 2	$\chi2 = 22.706$, p = 1.888e-06	lCI = -1.7767228, hCI = -0.7600668
Model 3	$\chi2 = 23.638$, p = 1.163e-06	lCI = -1.7431196, hCI = -0.7271307
Model 4	$\chi2 = 24.239$, p = 8.509e-07	lCI = -1.8133098, hCI = -0.7890303
Model 5	$\chi2 = 25.462$, p = 4.512e-07	lCI = -1.8155245, hCI = -0.7832375
Model 6	$\chi2 = 25.598$, p = 4.205e-07	lCI = -1.6387923, hCI = -0.6051331
Model 7	$\chi2 = 26.038$, p = 3.348e-07	lCI = -1.7290549, hCI = -0.7094674
Model 8	$\chi2 = 26.549$, p = 2.569e-07	lCI = -1.7708153, hCI = -0.7544235
Model 9	$\chi2 = 34.482$, p = 4.301e-09	lCI = -1.7865420, hCI = -0.7589584
Model 10	$\chi2 = 22.51$, p = 2.091e-06	lCI = -1.7580721, hCI = -0.7447512
Model 11	$\chi2 = 24.78$, p = 6.427e-07	lCI = -1.7960238, hCI = -0.7727081
Model 12	$\chi2 = 24.93$, p = 5.945e-07	lCI = -1.7518234, hCI = -0.7220174
Model 13	$\chi2 = 25.38$, p = 4.708e-07	lCI = -1.6405643, hCI = -0.6097188
Model 14	$\chi2 = 15.765$, p = 7.171e-05	lCI = -1.7617620, hCI = -0.7360733

Model 15	$\chi2$ = 17.472, p = 2.915e-05	lCI = -1.7488499, hCI = -0.7353167
Model 16	$\chi2$ = 18.621, p = 1.595e-05	lCI = -1.7551411, hCI = -0.7420844
Model 17	$\chi2$ = 21.514, p = 3.513e-06	lCI = -1.7339459, hCI = -0.7186384
Model 18	$\chi2$ = 21.572, p = 3.408e-06	lCI = -2.1928122, hCI = -1.073197
Model 19	$\chi2$ = 22.319, p = 2.31e-06	lCI = -1.7871879, hCI = -0.7669586
Model 20	$\chi2$ = 22.726, p = 1.868e-06	lCI = -1.7353506, hCI = -0.7172178
Model 21	$\chi2$ = 22.736, p = 1.859e-06	lCI = -1.7607221, hCI = -0.7442446
Model 22	$\chi2$ = 22.859, p = 1.743e-06	lCI = -1.844441, hCI = -0.7999364
Model 23	$\chi2$ = 22.953, p = 1.66e-06	lCI = -1.8124763, hCI = -0.7864389
Model 24	$\chi2$ = 23.116, p = 1.525e-06	lCI = -1.70618097, hCI = -0.6855841
Model 25	$\chi2$ = 23.289, p = 1.394e-06	lCI = -1.7223899, hCI = -0.7038864
Model 26	$\chi2$ = 23.348, p = 1.352e-06	lCI = -1.802332, hCI = -0.778843
Model 27	$\chi2$ = 23.359, p = 1.344e-06	lCI = -1.8083330, hCI = -0.7837972
Model 28	$\chi2$ = 23.389, p = 1.324e-06	lCI = -1.7205629, hCI = -0.6902038
Model 29	$\chi2$ = 23.463, p = 1.273e-06	lCI = -1.5944188, hCI = -0.5361125
Model 30	$\chi2$ = 23.681, p = 1.137e-06	lCI = -1.7684560, hCI = -0.7508703
Model 31	$\chi2$ = 23.731, p = 1.108e-06	lCI = -1.7498213, hCI = -0.7349197
Model 32	$\chi2$ = 23.811, p = 1.063e-06	lCI = -1.7455169, hCI = -0.7254883
Model 33	$\chi2$ = 23.962, p = 9.828e-07	lCI = -1.7324872, hCI = -0.7139502
Model 34	$\chi2$ = 23.967, p = 9.801e-07	lCI = -1.8156852, hCI = -0.7901945
Model 35	$\chi2$ = 24.002, p = 9.624e-07	lCI = -1.7461085, hCI = -0.7309449
Model 36	$\chi2$ = 24.068, p = 9.301e-07	lCI = -1.8378211, hCI = -0.8062956
Model 37	$\chi2$ = 24.102, p = 9.136e-07	lCI = -1.7882735, hCI = -0.7578002
Model 38	$\chi2$ = 24.136, p = 8.975e-07	lCI = -1.7585119, hCI = -0.7412167
Model 39	$\chi2$ = 24.203, p = 8.668e-07	lCI = -1.7382378, hCI = -0.7166086

Model 40	$\chi 2$ = 24.275, p = 8.353e-07	lCI = -1.7456174, hCI = -0.7291663
Model 41	$\chi 2$ = 24.388, p = 7.875e-07	lCI = -1.7558300, hCI = -0.7429011
Model 42	$\chi 2$ = 24.399, p = 7.831e-07	lCI = -1.7754066, hCI = -0.7543985
Model 43	$\chi 2$ = 24.591, p = 7.089e-07	lCI = -1.7629716, hCI = -0.7471472
Model 44	$\chi 2$ = 24.681, p = 6.763e-07	lCI = -1.7779446, hCI = -0.7572374
Model 45	$\chi 2$ = 25.118, p = 5.392e-07	lCI = -1.8236664, hCI = -0.7922076
Model 46	$\chi 2$ = 25.207, p = 5.148e-07	lCI = -1.8127572, hCI = -0.7865286
Model 47	$\chi 2$ = 25.389, p = 4.686e-07	lCI = -1.6138929, hCI = -0.5781817
Model 48	$\chi 2$ = 25.429, p = 4.589e-07	lCI = -1.7876200, hCI = -0.7656290
Model 49	$\chi 2$ = 25.457, p = 4.524e-07	lCI = -1.7510720, hCI = -0.7369498
Model 50	$\chi 2$ = 25.579, p = 4.246e-07	lCI = -1.7498779, hCI = -0.7309346

Table S2. Output of GLM with 'proportion of following bouts performed as leader during the SA stage of the trial' (proportion) as dependent variable. Effect of 'familiarity' (familiar, unfamiliar), 'sex' (female or male), 'previous experience with the room' (yes, no) on the proportion of following bouts performed as leader during the SA stage. All interactions between fixed factors were excluded from the model as not significant. The only individuals considered in the analysis are the ones that in their pair performed the majority of following bouts as leaders. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. Results are in the log (not in the response) scale. P values obtained with Tukey method adjusted for multiple comparisons.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		2.594	**1.603**	**3.945**	**< 0.001**
Familiarity	Familiar vs unfamiliar	-0.171	-0.704	0.246	0.443
Sex	Female vs male	-0.274	-0.871	0.226	0.240
Previous experience	Unexperienced vs experienced	-0.470	-1.037	0.005	0.052

Table S3. Output of GLM with 'proportion of following bouts performed as leader during the open-field stage of the trial' (proportion) as dependent variable. Effect of 'familiarity' (familiar, unfamiliar), 'sex' (female or male), 'previous experience with the room' (yes, no) on the proportion of following bouts performed as leader during the open-field stage. All interactions between fixed factors were excluded from the model as not significant. The only individuals considered in the analysis are the ones that in their pair performed the majority of following bouts as leaders. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. Results are in the log (not in the response) scale. P values obtained with Tukey method adjusted for multiple comparisons.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		1.312	0.680	1.966	< 0.001
Familiarity	Familiar vs unfamiliar	-0.313	-0.690	0.059	0.136
Sex	Female vs male	-0.242	-0.625	0.134	0.263
Previous experience	Unexperienced vs experienced	0.310	-0.057	0.679	0.125

Table S4. Correlation between the proportion of following bout performed as leader, either during the open-field stage or during the SA stage of the trials, and the behavioural responses obtained during the open-field stage trials [1]. The proportion of following bouts that individuals performed as leader in each stage of the trial were correlated either with the behavioural responses obtained during the open-field stage of the same trial (social context), or with the behavioural responses obtained by each individual when they explored alone (asocial context). Tau values obtained through Kendall Rank correlation. Results in **bold** are significant (after correction). False discovery rate correction was applied to value of α.

	Proportion of following bout performed as leader during the open-field stage		Proportion of following bout performed as leader during the SA stage	
Context	Asocial	Social	Asocial	Social
Time spent foraging	-0.018	0.032	0.081	0.001
Latency to forage	-0.016	-0.002	-0.109	-0.028
Number of areas visited	0.111	0.056	0.119	0.027
Number of food sources exploited	0.003	0.097	0.099	-0.021
Latency to touch the ground	**-0.218**	**-0.169**	-0.106	-0.060

Condition	-0.028	0.010

1. Tuliozi B, Fracasso G, Hoi H, Griggio M. 2018 House sparrows' (Passer domesticus) behaviour in a novel environment is modulated by social context and familiarity in a sex-specific manner. *Front. Zool.* **15**, 16. (doi:10.1186/s12983-018-0267-8)

Table S5. In order to investigate if familiarity, sex of the pair and previous experience with the exploratory room had any effect for the propensity of a pair to "switch roles", i.e. for individuals leading during the open-field stage to follow during the SA stage and vice versa, we fitted a model with familiarity, sex and round as fixed effects, and a binary response as dependent variable, i.e. if the pair switched role or not.

Output of GLM with 'switch' (yes, no) as dependent variable. Effect of 'familiarity' (familiar, unfamiliar), 'sex' (female or male), 'previous experience with the room' (yes, no) on the propensity of a pair to switch roles across stages. All interactions between fixed factors were excluded from the model as not significant. Coefficients and 95% confidence intervals are presented; statistically significant comparisons (zero is not included in the interval) are in **bold**. Results are in the log (not in the response) scale. P values obtained with Tukey method adjusted for multiple comparisons.

Fixed effect	**Comparison**	**Estimate**	**2.5% CI**	**97.5% CI**	**P value**
Intercept		2.162	**0.298**	**4.173**	**0.027**
Familiarity	Familiar vs unfamiliar	-0.434	-1.475	0.591	0.406
Sex	Female vs male	-0.169	-1.204	0.866	0.747
Previous experience	Unexperienced vs experienced	-1.437	**-2.514**	**-0.436**	**0.006**

Chapter 8

Synthesis and concluding remarks

The present thesis investigated various interactions between individual characteristics and the social environment. Its aim was to expand current knowledge on how variation in behaviour can influence performance of conspecifics in a social group, and how properties of the social environment can in turn influence the benefits and disadvantages of the single individuals. I adopted both correlational and experimental approaches, manipulating characteristics of the group (such as familiarity or distribution of knowledge) and testing them in various crucial contexts (novel environments, a situation of high perceived predation, a simulated predator attack) in order to see how different groups and individuals would fare with respect to each other.

The study presented in **Chapter 2** focused on the potential influence of perceived predation pressure on a potential proxy for life-history traits, telomere length, and on the relation of the latter with differences in social behaviour. Results showed a significant drop in relative telomere length after the first sampling, possibly an indication of a breeding season-related increase in stress. However, we did not find any effect of perceived predation pressure on telomere dynamics, nor a correlation between individual behaviour and relative telomere length. Glucocorticoid (corticosterone) analysis also showed a decrease in the stress response after the first sampling, possibly due to down-regulation of corticosterone responses, which might be a physiological answer to a period of chronic stress. In individuals exposed to predation pressure we detected a greater decrease in the corticosterone stress response. On the whole we did not find evidence for a possible link between telomere dynamics and individual variability in behaviour or experience; nonetheless, as very little is known about telomeres' possible role in connecting behavioural ecology and the life history of individuals, our novel finding of such a strong within-year decrease in relative telomere length is an interesting insight for further exploration on the topic.

Chapter 3 aimed to investigate the influence of social connections on the discovery of hidden food patches. Our results show that social information about the food patch transferred via the flocks' social networks, resulting in a measurable difference in the time of discovery of a resource due to previously established social connections. In addition, individuals that fed first from the novel food source were characterized by lower rate of following behaviour, providing evidence in support of the relation between social indifference and exploratory behaviour. Finally, we found out that social attraction shaped foraging individual decisions, as the second food source was usually left untouched whenever the first one was discovered. In this species locating and exploiting novel food sources is crucial to survival: experimental evidence that the order of discovery was affected by both individual

phenotype and social connections thus implicates an influential effect of social environment composition on the access to benefits decisive for both survival and fitness.

Chapters 4 and **5** dealt with how different social contexts affected resource exploitation and exploration in a vast novel environment. Despite the widespread use in behavioural studies of open-field tests and the importance that exploring novel areas holds during the life of moving animals, the influence of different social environments on exploration had rarely been addressed in birds; in fact, the effect of familiarity had hitherto never been considered in the context of novel environment exploration. Whenever paired with a familiar same-sex companion both male and female house sparrows showed a strong increase of exploratory behaviour. Experimental evidence of such a strong effect of the social environment might have key significance for a species that is still expanding its range in many parts of the world (and that is studied in relationship to differences in behavioural characteristics between the borders and the center of its range). While male house sparrows increased their exploration rate also in the presence of unfamiliar conspecifics, female individuals did not. Female house sparrows moving alongside an unfamiliar companion landed on the ground later than females moving with a familiar companion. In the unfamiliar context moreover female house sparrows ate less than males. This difference might be due to several different factors: females in this species follow more often than males and thus might need to be more careful when evaluating their flock-mates, as they are more dependent on social cues to find food and search novel areas. Another explanation might be that females value familiarity with their flock-mates more than males because it might give fitness advantages during breeding season (Kohn 2017). Finally, familiar pairs performed more following bouts, but the difference was significant only during the first hour of the test. This result is particularly interesting as it might show a timeframe for the development of familiarity in this species, with pairs formed by unfamiliar individuals habituating to each other with time.

While the experiment described in the previous two chapters had tested separately the performances of pairs of house sparrows, in **Chapter 6** we investigated the following step, i.e. the possibility to investigate group performances when two groups are facing each other. In order to do this, I created and implemented a novel experimental design which allowed two triads of sparrows to come into contact in a central aviary and compete for limited resources. We did not find an effect of previous triad familiarity on the performance of the triads: the long time spent together before entering the central aviary might have allowed them to become familiar with each other. However, we still found a strong group-linked advantage, as individuals belonging to the triad of the first individual to eat (first feeder) ate more than those in the opposing triad. This effect was not influenced by individual latency to cross into the central aviary, as when the first feeder started eating usually both triads had

already entered in the central aviary. Nonetheless, once all individuals had arrived, only triad-mates of the first feeder followed it to the food source, possibly because they had habituated to each other before entering. We did not find, on the other hand, evidence for an influence of individual behavioural traits on the amount of resource consumed and latency to forage. However, first feeder individuals tested in a subsequent experiment were shown to be more risk-taking. As far as we know, this is the first experimental evidence of variable group performances between two flocks of conspecifics facing each other. It proves that even in a species with known fission-fusion population dynamics processes accounting for differences in group characteristics might still be relevant (at least on a short time-scale) when the groups share the same area or compete over the same resources. Moreover, while group performance was not based on its composition, the behaviour of certain individuals – the first feeders – was determinant in increasing the resource consumption of their groupmates.

Finally, the work presented in **Chapter 7** tackled the issue of individual strategies within a group and their possible context-dependence. Our aim was to test experimentally if a pair of individuals would assume dichotomous leader/follower social positions not only during exploration but also in a seldomly investigated but critical situation, i.e. the (simulated) attack of a predator. Individuals, as expected in this species, indeed behaved consistently either as leader or as follower during the open-field test (the same as Chapter 4). However, we were also able to show that individuals moved as coordinated pairs of leader and follower during a simulated attack, providing the first evidence for a consistent social position during this kind of event. Finally, our results indicate that individuals which were followers during the open-field test behaved as leaders during the simulated attack and vice versa. This confirmed our hypothesis that risk-taking individuals would be the ones initiating movements during exploration (i.e., the leaders), but would also be the ones braving the approach of a threat for longer during a simulated attack; on the other hand risk-averse individuals, usually following during typical movement (i.e., the followers), would be the ones departing first and directing the social escape flight during a simulated attack. Risk-taking individuals, moreover, while usually associated to greater social indifference, demonstrated in the specific context of an approaching threat to be able to follow social cues and direct their flight towards the individual that had already taken off. To our knowledge our experiment contributes the first evidence for a differential social strategy during an attack; in addition, we show that social strategies – leader and follower – are employed by different individuals according to the situation. Our result suggests a possible mechanism for distribution of advantages among group members with different characteristics, with risk-taking individuals possibly causing their followers to increase their rate of

exploration while risk-averse individuals provide direction and an increase in the flight initiation distance when a threat approaches.

Conclusion

The various studies collected in this thesis showed the strong influence that the social environment can have on the performance of the individuals composing it. This thesis tested various widely accepted assumptions that, however, had rarely or ever been thoroughly tested in a controlled setting. Moreover, the results hereby presented indicate that it is possible to obtain experimental evidence of some of the most elusive processes in social behavior research, such as variable performances in groups competing for the same resources, context-specific advantages of certain behavioral roles and benefits linked to variable social relationships within a flock. Finally, the investigation of the different hypotheses was almost always performed via novel or heavily modified experimental designs, as the questions asked were generally not answerable through classic experimental set ups.

House sparrows had access to tangible benefits (foraged more and sooner) depending on the presence, the familiarity and their connection with their social companion. Groups of sparrows comprising one individual faster than the others to approach a limited food source outcompeted the opposing group that they were facing. Social position in sparrow dyads are context-dependent, with individuals behaving according to their behavioural traits and assuming different roles according to the context.

To conclude, this thesis produced novel findings on the interaction between individuals and the social environment, underlining the need to study social groups with a comprehensive framework that takes into account diversity within and among groups as a potential force influencing individual performances, survival and fitness.

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