UNIVERSITE PARIS 13

UFR LETTRES, SCIENCES DE L'HOMME ET DES SOCIETES

DOCTORAT ETHOLOGIE

Organisation du travail chez la souris glaneuse *Mus spicilegus* lors de la construction du tumulus

Par

Maria José Hurtado Herrera

Laboratoire d'Ethologie Expérimentale et Comparée, LEEC.

Membres du jury

Vilmos Altbäcker (Eötvös Lorand University)

Claire Detrain (Université Libre de Bruxelles)

Patrick Gouat (Université Paris 13)

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Résumé

Les souris glaneuses juvéniles, *Mus spicilegus,* édifient un imposant tumulus pour passer l'hiver. Cette structure est essentielle pour la survie de ces animaux et seuls les individus pouvant s'abriter sous le tumulus survivront jusqu'à la saison de reproduction suivante. Des études antérieurs menées an condition de laboratoire, ont révélé l'existence d'une différentiation comportementale entre les membres d'un groupe de six individus. En règle générale deux individus transportent jusqu'à 80% du matériau proposé pour la construction du tumulus. Dans notre étude, et grâce à la technique RFID, nous mettons en évidence une division du travail dans des tâches de transport de deux matériaux différents lors de la construction. Ces matériaux correspondent à deux étapes différentes de la construction en milieu naturel. Certains individus se spécialisent dans le transport d'un matériau et alors que d'autres individus se spécialisent dans le transport de l'autre matériau. Cette division du travail lors de la construction a rarement été décrite chez les mammifères. Dans chaque groupe on observe trois catégories d'animaux : des transporteurs, des transporteurs occasionnels et des non-transporteurs. L'affinité pour le matériau de construction semble jouer un rôle dans la mise en place de la spécialisation. Les souris qui présentent une plus haute affinité avec le matériau ont une probabilité plus forte de devenir des transporteurs. Ce résultat suggère que la détermination des spécialistes dépendrait d'un système basé sur un seuil de réponse. D'autres caractéristiques individuelles (i.e. anxiété, néophilie et niveau général d'activité) ont été testées mais ne semblent pas jouer de rôle important dans la mise en place de la spécialisation. Le retrait de certains membres du groupe entraîne une modification des rôles des individus. Ce résultat est obtenu que les individus retirés soient des transporteurs ou des non-transporteurs. Malgré ce bouleversement l'organisation du travail avec les trois catégories d'individus est maintenue et cela même au sein de groupes de quatre individus. Ces résultats suggèrent que si un mécanisme du type seuil de réponse agit lors de la mise en place de l'organisation du travail dans cette espèce, un processus d'auto-organisation est présent et permet de maintenir la stabilité de la division du travail en cas de perturbation de la structure. Des nouvelles études sur ce sujet sont nécessaires pour déterminer les mécanismes impliqués dans l'organisation du travail de la souris glaneuse et comprendre comment ces mécanismes agissent.

Mots-clés : *Mus spicilegus*, division du travail, organisation du travail, tâches de construction

Abstract

The juveniles of the mound-building mouse, *Mus spicilegus*, build an imposing mound to overwinter. This structure is vital for the species, since only the animals that enter the mound will survive to the next reproductive season. The impressive work accomplished by these tiny animals is the result of a communal effort. Previous work had shown that, inside a group of six juveniles, there was a behavioural differentiation in a transport task of the building process, with animals transporting up to 80% of the proposed material. In the present work, by using the RFID technique, we determined the existence of a division of labour during the transport task with different individuals transporting two different materials. These two materials correspond to two different steps of the building process in the field. This division of labour in building tasks has rarely been described in mammals before. Three categories of animals were observed: carriers, occasional carriers and non-carriers. We did find that the affinity for the building material played a role in the determination of the specialists, with mice showing a higher level of affinity having a higher probability to become a specialist, pointing to a response threshold. Other individual characteristics (i.e. levels of anxiety, neophilia and locomotion) were tested but do not seem to play an important role during the setting up of the specialization. In the other hand, when removing individuals from the group the identity of carriers changed, even if the removed individuals did not perform any transport. However, the work organization with a structure of carriers, occasional carriers and non-carriers was extremely robust and resisted the perturbations even at a low initial group size. So, the mechanism behind the work organization in this species seems to be modulated by a response threshold as first instance, followed by self-organized processes that override the existing response thresholds and ensure the stability of the system when the group is disturbed. More work is necessary in order to determine which are the mechanisms involved in the work organization of work of *M. spicilegus* and understand how they interact.

Key words : *Mus spicilegus*, division of labour, work organization, building task

Preface

This thesis is organized in six chapters and a general conclusion, each one with its partial bibliography. The complete bibliography is added as an appendix at the end of this work.

The first chapters are a general introduction and background to the totality of my work. Then, each following chapter follows a structure of an introduction and a question to be answered. The results are written following the pattern of one or more articles, with their own bibliographies, layout and figure numbers.

Aknowledgements

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Et merci Thomas, pour m'avoir attendue.

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The construction among animals

The third little pig met a man with a load of bricks, and said:

"Please, man, give me those bricks to build a house with."

So the man gave him the bricks, and he built his house with them. So the wolf came, as he did to the other little pigs, and said:

"Little pig, little pig, let me come in."

"No, no, by the hair of my chiny chin chin."

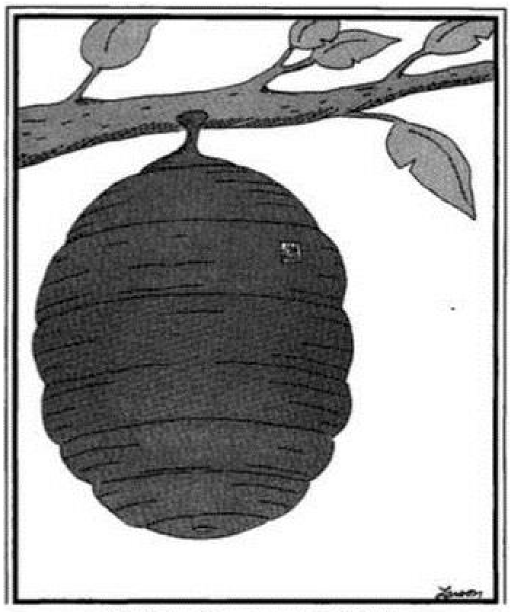
"Then I'll huff, and I'll puff, and I'll blow your house in."

Well, he huffed, and he puffed, and he huffed and he puffed, and he puffed and huffed; but he could not get the house down.

Joseph Jacobs, "The story of the three little pigs"

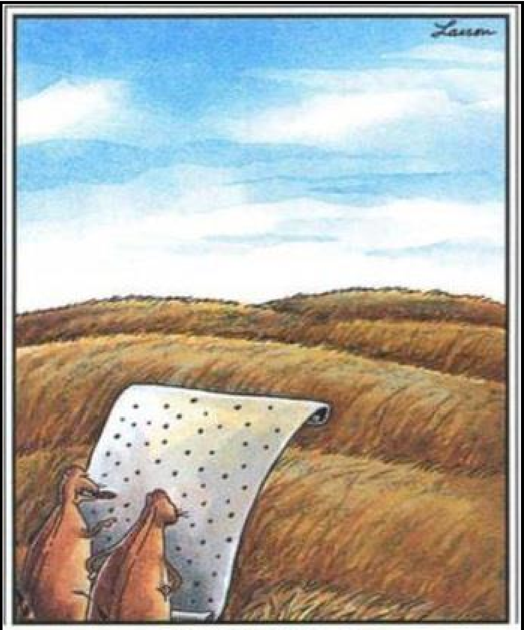
A spider conducts operations that resemble those of a weaver, and a bee puts to shame many an architect in the construction of her cells. But what distinguishes the worst architect from the best of bees is this, that the architect raises his structure in imagination before he erects it in reality

Karl Marx, Das Kapital



Iggy knew he was extremely lucky
to get a room with a view.

When looking at the definition of the verb “to build”, we find that this verb means “to form by ordering and uniting materials by gradual means into a composite whole” (Merriam-Webster online dictionary). Among living creatures, only a few show a building behaviour. For instance, many arthropod species are very good at building. In vertebrates, birds are very sophisticated builders (Collias & Collias 1978). In mammals, some building can be found in carnivores (Stewart et al. 1999), bats (Baker & Clark 1987) and primates (Goodall 1962). Among the last, the more remarkable builders are humans. But the majority of the mammalian building species are rodents. There are some examples of sophisticated building among rodents, e.g. the beaver (*Castor canadensis*) and its complex dam and lodge. However, the overwhelming majority of rodents dig simple burrows as nests (Hansell 2007).



Prairie dog developers

1. What and why do animals build?

When we think about building, we immediately think in the construction of a house or a nest. But there are other examples in nature, although rare, where the built structure do not serves the purpose of a simple shelter.

1.1. Traps

The purpose of a trap is to catch prey. The ability to build traps is absent in vertebrates, exception made of humans. In the other hand, there are many species of arthropods that are able of building a trap. Most spiders build traps that can be astonishing in their delicate pattern using a self-produced silk. Indeed, most examples of trap construction are based on self-produced materials. The exceptions are the conical pit traps of ant lions (Myrmeleontidae, Fig. 1) and "worm lions" (Rhagionidae) (Hansell 2005).

Figure 1: Sand pit trap of an ant lion (Myrmeleontidae)

1.2. Communication structures

To communicate with other members of the species using architecture can seem a bit costly, since other communication means are certainly available.

However, we find some great examples of this feature in the context of territoriality and courtship. Male bowerbirds build a bower (a structure made with plant material) and then “decorate” it with vividly coloured objects such as fruits, flowers, feathers, stones, shells and even plastic and glass detritus (Fig. 2). This structure is not a nest, but a courtship arena. Females will prefer males with higher quality bowers as mates (Barber et al. 2001; Hansell 2007). In other species such as the pufflefish (*Torquigener sp.*), the male build a large circular structure which both serves as an ornament to attract females and as a nest for their eggs (Barber et al. 2001; Kawase et al. 2013).

Figure 2: Bower of the Vogelkop bowerbird (*Amblyornis inornata*)

1.3. Nests and shelters

The vast majority of builder species are actually home builders. The materials used to build a structure can be self-secreted by the animal (e.g. the cocoons of the silk worms), collected from the environment (e.g. bird’s nests), and a mix from these both categories (e.g. the nests of paper wasps and the portable cases of the larvae of caddis flies). The caddis fly larvae (*Trichoptera* *sp.*) build a portable case soon after hatching, using materials from the environment and silk (Merrill 1965). This feature was used by the artist Hubert Duprat, in the project "The wonderful Caddis worm: sculptural work in collaboration with *Trichoptera*". By giving gold, pearls and gemstones as building materials to the larvae, he obtained beautiful jewels made by insects (Fig. 3).

Figure 3: Larva of *Trichoptera sp*. (caddis fly) with a case build using gold flakes and pearls, as part of the project "The wonderful Caddis worm", by Hubert Duprat.

The use of a structure as shelter or nest is advantageous in many ways. A nest is a protection against predators and harsh environmental conditions such as extreme heat or cold. It is also a place to storage food and to rear youngs (Kinlaw 1999; Ebensperger & Bozinovic 2000). But the construction of a nest can be extremely costly, both in terms of spent energy and time consumed (Lovegrove 1989; Hildner & Soulé 2004). For example, in the pocket gopher *Thomomys bottae*, the energy spent on burrowing from one point to another is increased in 360 - 3400 times when compared to the energy needed to move the same distance on the surface (Vleck 1981). When building a nest, animals are also exposed to a risk of predation (Soler et al. 1998; Ebensperger &

Bozinovic 2000). The need to find strategies to cope with these costs, or at least minimize them is important.

2. Collective or solitary building?

Living in groups has costs that sometimes can be very high. The costs are the increased transmission of diseases and parasites, competition over the existent resources, increased aggression and even infanticide and cuckoldry (Ebensperger 2001). Evolutionary explanations to group-living assume that there might be a fitness advantage to individuals, or that environmental constraints might force individuals to live together in spite of the associated costs (Ebensperger & Cofré 2001). One of the hypotheses is that the need for expandable, long-lasting nests is linked to the evolution of group-living, since collective building diminishes the time allocated to this activity and the energy expenditure *per capita* by sharing the effort of building and maintaining the structures among the members of the group (Ebensperger 1998). Sometimes the benefit of communal building may not be immediate. This is the case for the semi-fossorial rodent *Octodon degus*. While in group, the time spent burrowing is not different from the time spent by solitary degus. However, grouped degus dig mostly in the same sites, forming digging chains. In this way, they remove more soil than solitary individuals and can therefore obtain a more extended system of burrows (Ebensperger & Bozinovic 2000).

3. Complexity of the nest structure

Even inside the same taxon, building can go from simple burrowing to the erection of very sophisticated structures using different materials. However, the complexity of the built structure does not depend on the phylogenetic level or cognitive complexity of the builder. For instance, a very delicate example of a complex structure build by a simple organism is the work of the amoeba *Difflugia coronate*. This unicellular organism builds a little portable shell using sand grains (Figure 4). The result is astonishing, especially for a single-cell organism, with no nervous system (Hansell 2007).

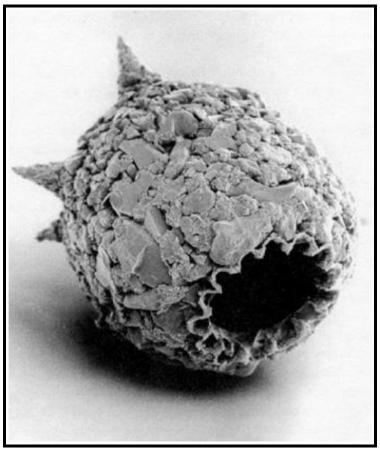
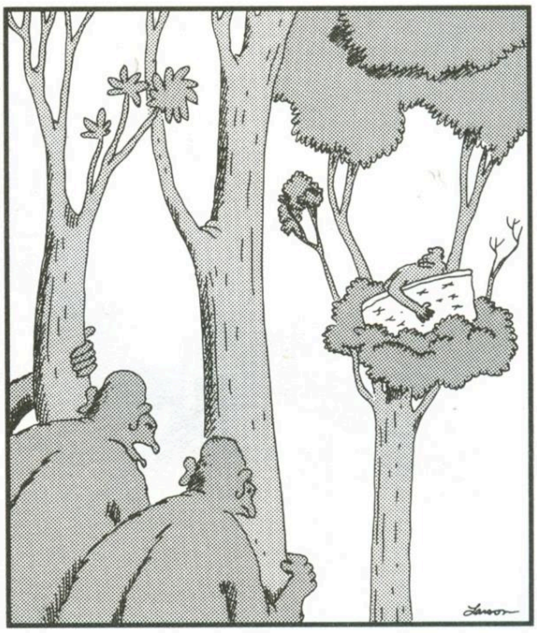


Figure 4: Shell build with sand grains by the amoeba *Difflugia coronate*.

Humans are in the other extreme of the cognitive complexity spectrum. We are very complex animals, and we build very complex shelters. Our capacity to build is one of the keys of the success of our species. As proposed by Coolidge and Wynn (2009), the cognitive shift between the *Australopithecus* and the early *Homo* might be explained by the adaptation to the life on the ground. One part of this was the construction of nests or beds. The quality of sleep on these ground nests would be increased since the fear of falling would be avoided, and this would result in an enhancement of waking survival skills and in the consolidation of procedural memories (Coolidge & Wynn 2009).

But when we look at our closer cousins, the great apes, the ability to build is much less spectacular than ours at the present day. Gorillas, orang-utans, bonobos and chimpanzees build nests to spend the night. These structures are built each nightfall and often used only once. The gorilla builds a nest on the ground by bending some leafy vegetation, while the orang-utan, the bonobo and the chimpanzee build nests on trees by using a fork in the branches and then bending and weaving some nearby branches and covering the resulting platform with leaves (Goodall 1962; Coolidge & Wynn 2011). Therefore, neither the phylogenetic level nor the cognitive complexity of the builder is necessarily linked with the complexity of the build structures.



“He’s making his nest now. ... There! See it? That son-of-a- ... he’s got himself a futon!”

Social insects are individually simple animals, at least when looking at their cognitive abilities and comparing them to big apes. However, they are able to build very complex structures. For example, termite mounds can go up to 6 meters (Fig.5). They have a royal chamber, nurseries and food storage. When compared with the size of an individual, these mounds are comparatively higher than any human construction to scale (Hansell 2007). These huge structures have architectural characteristics to deal with some issues inherent to the concentration of insects, such as thermoregulation, ventilation and humidity control. In human engineering, the example of these mounds can be used to find solutions to improve the homeostasis of intelligent buildings (Worall 2011).

Figure 5: Cathedral termite mound of the species *Nasutitermes triodiae.*

What do social insects and humans do differently and what do we share when building a complex structure? One difference is that in humans, the organization of work is centralized. The architect communicates verbally or via a blueprint with the builders, and the builders follow the given instructions. For us, the idea that a group of builders, each one doing a set of tasks independently, without knowing the expected result and without being guided by someone that has the global vision of the house in mind could finally achieve a building such as the Versailles palace seems impossible. But this is exactly how most structures are built by social insects. They organize their work in a decentralized way, i.e. they do not have a leader that guides them or any kind of group that makes the decisions while others follow, and they do not have a blueprint of the finished structure. At an individual level, while human workers engage in complex behaviour in order to create a complex structure, the complexity of the nests of social insects emerges from very simple individual behaviours.

However, we do share a crucial feature for the construction of complex structures. Both social insects and humans organize their work by dividing the available tasks among members of the group. The division of labour is then a shared trait between humans and social insects. This feature will be discussed in the next chapter.

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The division of labour

"Este dedito compró un huevito,
Éste lo echó a cocer,
Éste lo peló,
Éste le echó la sal,
¡Y este perro cochino se lo comió!"

("This little finger bought a little egg,
This one cooked it,
This one peeled it,
This one salted it,
And this filthy dog ate it!")

Chilean child rhyme

"In small towns, the same man makes couches, doors, plows and tables, and often even build houses, and still he is thankful if only he can find enough work to support himself. And it is impossible for a man of many trades to do all of them well. In larger cities, (…) one man makes shoes for men, another for women, there are places even where one man earns a living just by mending shoes, another for cutting them out, another just by sewing the uppers together (…). Of necessity, he who pursues a very specialized task will do it best. "

Xenophon, Cyropaedia

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The division of labour is a fascinating feature, present in human and other animal societies, that arouses the interest of many disciplines. It is a complex phenomenon that involves both the individual level and the group level. Individual behaviours modulate the group output, and this group output modulates the behaviour of the group members. It has been defined as a stable pattern of variation of work made among members in a colony or group, where each member of the colony or group specializes in a subset of all the available tasks and this subset varies among individuals (Beshers & Fewell 2001). Embedded in the concept of division of labour we find the concept of task specialization (Fewell et al. 2009). An individual is considered specialized when it engages in certain behaviour far more often than expected compared to other similar members of its group (Robson & Traniello 1999b). The division of labour can be considered as high if two conditions are filled: the individuals are biased toward a subset of task from all the tasks available, and the individuals are distributed among tasks so different individuals perform different tasks. In contrast, the division of labour is low or inexistent if simply some individuals are more active than others, or if every individual specializes in the same task (Fewell et al. 2009).

1. Division of labour in humans

Division of labour is a very familiar concept for humans. For example, you are now reading this text inside a building that was not built by you. In fact, this building needed the combined work of many people to be built: bricklayers, electricians, plumbers, etc., and you probably would not be able to do their work in an optimal way. We live in a society where tasks are divided among individuals. And every one of us is aware of that. So if you are feeling sick, you will call the doctor but if one of your bathroom pipes is leaking you will call a plumber.

All around us, there are many now indispensable items such as a computer or a phone, and also less complex objects such as a pen, a piece of clothing, etc. But if one day we find ourselves deprived of such items, we probably would not be able to make any of them from scratch. We need the work of others to live as we do, and this can be stretched to the point to say most of us need the work of others to merely survive. One interesting project called "The Toaster Project" (Thwaites 2011) illustrates this reality (Fig. 6). He set out to make from scratch the cheapest electric toaster he could find, in this case one found in the retail commerce at £3.99. He started mining and processing the raw materials to end up with the finished product, a very imperfect version of the original appliance. He spent nine months to make one sole toaster, and its final cost was £ 1187.54, almost 300 times more than the store-bought version. Thwaites means to make a statement about how our consumption habits have a profound impact on environment; however, it also shows how dependent we are from industrial processes and how as a society we achieve much more than as an isolated individual.

Figure 6: Cover of the book “The Toaster Project”, by Thomas Thwaites. This cover shows a picture of the final toaster made from scratch.

The division of labour is at the foundations of our economic system of trade. Individuals could be specialized in the production of a certain good and then be able to trade such product for other products they were not as able to produce. This specialization could also work as a community specialization, where a whole community engages in the production of a certain good in order to trade it for something else. For example, the Silk Route allowed the exchange between East and West and went all the way from China to the Mediterranean Sea. China owned the production for silk and extremely valued porcelain, and traded them for Western goods like domestic animals such as horses, camels and dogs, or vegetable products such as grapes and grape wine (Schafer 1963). In order to achieve a trade, both parties needed to establish the inherent value of such goods, comparing the cost and time used to produce the products, as well as its rarity or the technical ability required to make them.

The specialization in humans is often referred as the origin of an improvement in individual performances, and in the general output of society. For Adam Smith, the division of labour and specialization is the main cause for the increase of production in modern societies. He took the pin manufacture industry as an example of how the division of labour could dramatically increase productivity (Fig. 7). For him, this diversification is responsible for the "universal opulence" of more industrialized countries.

Figure 7: Specimen of an English twenty pounds note, featuring Adam Smith and the division of labour in pin manufacturing. Highlighted in red, the phrase: "The division of labour (and the great increase in the quantity of work that results)".

The division of labour greatly enhances the productivity by lowering the costs of production and the time spent making a certain good. The Industrial Revolution produced the extreme division of labour we find in assembly lines

(Fig.8). Before Henry Ford, each car was crafted by a team of very skilled men that mastered the whole process of building a car. The assembly lines created by Henry Ford made the craftwork unnecessary, since each part of the process was performed by specialized men that could have never seen a car in their lives. Skill was no longer necessary, not at least at the previous levels, where the craftsmen needed to know about everything concerning the car building. This process was by far more efficient, cheaper and faster than the old method (Price 2004).

Figure 8: A group of workers of a Ford assembly line, 1913.

2. Division of labour in other animals

Division of labour is present in many taxa, with different degrees of specialization. Most of the work made on this topic has been based on social insects as model. The division of labour is a major component of the undeniable ecological success of social insects by increasing the overall efficiency of the colony (Jeanne 1986; Robinson 1992; Sendova-Franks & Franks 1999). As an example, ants are present in almost every landmass on Earth (exception made of Antarctica and few very remote or inhospitable islands) and may represent between 15 and 20% of the terrestrial biomass and up to 25% in tropical ecosystems (Schultz 2000).

This organization of work improves the colony's efficiency. By having specialized individuals, the colony makes an economy of resources by matching the number of workers engaged on a certain task with the need for that task (Beshers et al. 1999). Benefits can occur due to an enhancement of individual performance (e.g. utilizing the different abilities of the individuals, improved by experience or suited morphology). They can also be the consequence of an enhancement of the overall system by eliminating a constraint that affects the performance of a certain task (e.g. when a collector retrieve enough building material for many builders, therefore eliminating the more modest capacity of builders to handle material as a constraint for the retrieval of material) (Ratnieks & Anderson 1999). Time and energy are also saved by a spatial partitioning of work, which avoids for an individual the need to move from one point to another in order to perform concurrent tasks (Seeley 1982; Bonabeau et al. 1998).

In the social wasp *Polybia occidentalis,* there are specialized individuals that collect water while other individuals collect pulp and others build the nest. Work is organized in series-parallel. Both collectors can retrieve more material that what is needed by builders, thus limiting the number of retrieval trips and as a consequence saving energy and limiting the exposure of foragers to predators (Jeanne 1986).

Solitary individuals perform tasks in series, meaning that they need to follow a sequence and perform the totality of required tasks in order to achieve a result. For example, nest building is a complex activity where at least two different tasks need to be coordinated. The female of solitary mud-dauber wasps (*Sceliphron formosum*) begin by collecting water to moisture the soil, then making a mud pellet that will be incorporated to the nest. This sequence cannot be changed, and the quantity of mud obtained depends on how much water the wasps collected. This example of difference of efficiency between solitary and collective building in wasps is similar to the example of the car building previously exposed in the previous section of this chapter, where assembly lines of highly specialized workers minimized costs and time when compared to single craftsmen.

3. Categories of division of labour

3.1. Division of labour in reproduction

This specialization happens among reproductive and non-reproductive members of the group. It is observed in social insects such as honeybees, ants and termites. Hymenoptera colonies are often composed of one or a few reproductive individuals (breeding females or “queens” and males) and many sterile or pseudo-sterile females whom ensure the work necessary for the survival of the colony. The case of termites is slightly different, with a reproductive pair (the “queen” and the “king”) and workers of both sexes. Even if it has been mainly studied in social insects, this specialization into reproductive and non-reproductive individuals is not specific to these animals. Many examples are found in vertebrates, such as fishes, birds and mammals.

This feature is present in a small proportion of social mammals, more specifically in four groups: tamarins and marmosets (Callitrichidae), terrestrial carnivores such as wolves, coyotes and African wild dogs (Canidae), mongooses and meerkats (Herpestidae) and African naked mole-rats (Bathyergidae) (Clutton-Brock 2009). Only the dominant couple will reproduce, while the other members of the group act as helpers. Dominant females suppress subordinate reproduction at different levels in those species, either by evicting potential competitors, by killing subordinate pups or even by inducing hormonal changes in subordinate females (Clutton-Brock 1998). For example, in naked mole-rat *Heterocephalus glaber*, only the dominant female will reproduce while the other females will accomplish the rest of the work, digging galleries and taking care of pups. The dominant female even shares some of the features of social insect's queens, such as enhanced body size and unusually long lifespan (Clutton-Brock 2009). If the dominant female is removed from the colony (but is kept in olfactive contact with the colony), other females will become fertile (showing perforated vaginas and well developed teats). Once one of them is pregnant, the other females regress to their non-reproductive state (Jarvis 1981). If the queen dies, the new dominant female will evict or kill her competitors in the year following the disappearance of the former dominant (Clutton-Brock 2009)

"OK, time for lunch. … And Dwayne here will be dismissing you by row number, since he's alpha wolf today."

3.2. Division of labour in ergonomic tasks

This division of labour happens among non-reproductive members of the group, which engage in diverse ergonomic tasks such as building the nest, collecting food and taking care of brood. The task allocation among the non-reproductive individuals allows the existence of impressive performances, especially in construction tasks in social insects. There are some magnificent examples in nature, such as the mounds of termites and the nests of leaf-cutter ants. But how does the allocation of tasks work, and how is it determined which and how many individuals would perform each task?

Classically the division of labour among non-reproductives is attributed to three general patterns, namely age polyethism, morphological polyethism and genetic polyethism (Oster & Wilson 1978; Robinson 1992). These patterns are based on internal factors, based on an attribute of the individual often considered to be fixed, like age range, size or shape and genotype. A worker of a behavioural caste was thought to be more suited to certain tasks, so it would perform it more or less exclusively (Gordon 1996).

3.3. Patterns of division of labour for ergonomic tasks

Age polyethism is a mechanism where workers specialize on different tasks along their lives, instead of performing all the tasks concurrently (Hurd et al. 2007). In general terms, younger individuals stay inside the nest and take care of the brood and nest-related tasks, while older individuals go out and forage. Age-related division of labour is found in most species of social Hymenoptera (Hölldobler & Wilson 1990; Robinson 1992). In the honey bee *Apis mellifera*, four age subcastes could be identified: cell cleaning caste, broodnest caste, food storaging caste and foraging caste (Seeley 1982). However, a change in the demography of the colony might affect such pattern. If many foragers are lost due to predators, young individuals can replace them. Conversely, in a reproductive swarm, after establishing the colony in a new nest, nurses can be overaged, since the new adults will not emerge before three weeks (Robinson 1992).

Morphological polyethism, in which the size and/or shape of workers are related to the tasks they perform, only occurs in most of the species of termites and in a few species of ants (Robinson 1992). In general terms, in polymorphic species of ants larger workers guard the nest entrance, mill seeds and preys and can even serve as storage for food like in the honeypot ant, while the smaller ants will forage or perform nest-related tasks (Hölldobler & Wilson 1990). Leaf-cutters ants from the genus *Atta* are extremely polymorphic (Fig. 9), with larger workers (majors) that forage fresh leaves and flowers that are cut in small pieces and enzymatically processed in an assembly line of workers that decrease in size. At the end of the line, the smallest workers (minors) place the substrate in the fungal garden and cultivate the hyphaes of the fungus that serves as food for the larvae (Beshers & Traniello 1996). As in age polyethism, changes in the conditions of the colony can elicit a shift of tasks between the members of the colony. When the ratio minor to major drops in some species of *Pheidole* ants, majors could perform tasks usually performed by minors (Wilson 1984). Minors would not perform the tasks of majors, even if the conditions change (Robinson 1992). In vertebrates, the morphological polyethism is very rare. However, in the naked mole-rat, small individuals take care of pups and build galleries while larger individuals are less active and seem to be in charge of the defence of the nest (Jarvis 1981).

Figure 9: Major and minor workers of *Atta cephalotes* demonstrating the size extremes among workers in a single leaf-cutter ant colony.

Genetic polyethism has been less widely demonstrated, but two examples stand for this pattern of division of labour. In the honey bee, the queen can have several mates, creating different patrilines. These different subfamilies show differences on their likelihood to guard the nest entrance, to remove corpses from the hive (Robinson & Page 1995) and to groom nestmates (Frumhoff & Baker 1988). They also show differences in their way to forage (different amounts of pollen or nectar collected) and in the amount of scouting for new sites they perform (Robinson & Page 1989). In the polyandric leaf-cutter ant *Acromyrmex echinatior*, the different patrilines show significant differences in their behaviour, with some patrilines being more prone to forage while others engaged more frequently in waste disposal (Waddington et al. 2010).

4. Mechanisms that produce a division of labour

In recent years, the theorization about division of labour has become an attempt to explain the "how" behind the organization of work. The question is how tasks are allocated among individuals in a group, in a way that remains efficient even if conditions change. In recent years many models have addressed this issue, helping us to understand which mechanisms are behind the division of labour and how all of these non-mutually exclusive mechanisms and patterns can be unified in comprehensive explanations at both levels of biological organization.

4.1. Self-organization

Self-organization models refer to a wide number of pattern-formation processes in both physical and biological systems, where the formation of organized arrangements in time and space is the result of numerous interactions among the lower components of the system. The rules that specify such interactions are based solely on local information and do not take into account the global pattern. In that way, self-organized models have no need for individual complexity (Camazine et al. 2001).

The self-organization goes from the extremely tiny like the formation of snowflakes, to the extremely enormous such as the formation of planets and galaxies. The ripples on sand dunes (Fig. 10) are a fine example of a self-organized system. They are formed by the action of wind, gravity and friction on sand. The result of simple interactions between these elements is an easily observable complex pattern.

Figure 10: Self-organized pattern of wind-blown ripples on the surface of a sand dune.

Inanimate objects such as water molecules and sand grains only obey to the laws of physics to form more complex patterns. But in biological systems, the subunits are more complex and the nature of interactions is not only physical but also physiological and behavioural. However, even with a more sophisticated subunit, the complexity can emerge based just on a few simple rules of thumb that determine the animal’s response.

Based on how the organisms gather and process information, we can divide self-organization in two big clusters: information gathered from one's neighbour or from work in progress (Camazine et al. 2001).

One animal can act based on the information gathered from its neighbour, as in the case of many collective movements. As an example, starlings form complex patterns by reacting to the behaviour of their closest neighbour, with no information of the rest of the murmuration (Fig. 11). There is an attraction-repulsion dynamic that results from "being attracted by a large number of conspecifics" and "take distances when too close to another bird".

Figure 11: Self-organized patter formed by a murmuration of starlings in flight.

In the other hand, the gathering of information can be independent from other members of the group and be based on the work in progress. In a populous colony, where a high number of individuals contribute to the communal effort, some clues coming from the emerging structure and the local environment can be used to achieve work. This is the case in termite colonies, where the building of their complex nests is based on the information conveyed by the advancement of the work. This type of recursive building is called "stigmergy", and was studied by Grassé in the termite *Bellicositermis natalensis* (Grassé 1959). When building a mound, termites deposit pellets of material randomly at first because each location is equally attractive. Pellets are impregnated with a pheromone that elicits the deposit of more pellets. As the termites randomly deposit or move pellets, by chance there are aggregations that are bigger than the rest. Those aggregations will be even more attractive, so termites will continue to deposit the material on top of them and pillars emerge. Pillars will lean towards one another by physical constraints, creating arches. A complex structure emerges, with no need for the termites to make a lot of decisions or to understand the global project (Hansell 2007).

4.2. Response threshold model

The response threshold model explains the division of labour as a result of different individual internal response thresholds for every task available. The threshold model was at first described with fixed thresholds. Variation in response threshold can explain, at least in part, many features of individual behaviour in a context of division of labour, such as: task specialization, activity levels, elitism (unusually high frequency of task performance by a worker), idiosyncrasy (variation in task performance) and behavioural flexibility (Beshers et al. 1999) . However, models with fixed thresholds could not account for the existence of task allocation because fixed thresholds were predetermined (Theraulaz et al. 1998b). New integrative models accept the variation of thresholds (Beshers et al. 1999). When a specific stimulus reaches the internal threshold of a worker, it triggers a response of this worker but not the response of workers which have higher thresholds for this task. If a certain task stimulus is low, only workers with the lowest thresholds will perform it. In the other hand, if the stimulus for a task is high, it will exceed the internal response thresholds of more individuals, resulting in a recruitment of a larger number of workers (Beshers & Fewell 2001). From the perspective of the individual, an individual with a low internal threshold for a certain task will be most likely to perform such task and become a specialist, and an individual with a very high threshold for the same task will rarely or never perform such task (Fig. 12) (Barron & Robinson 2009).

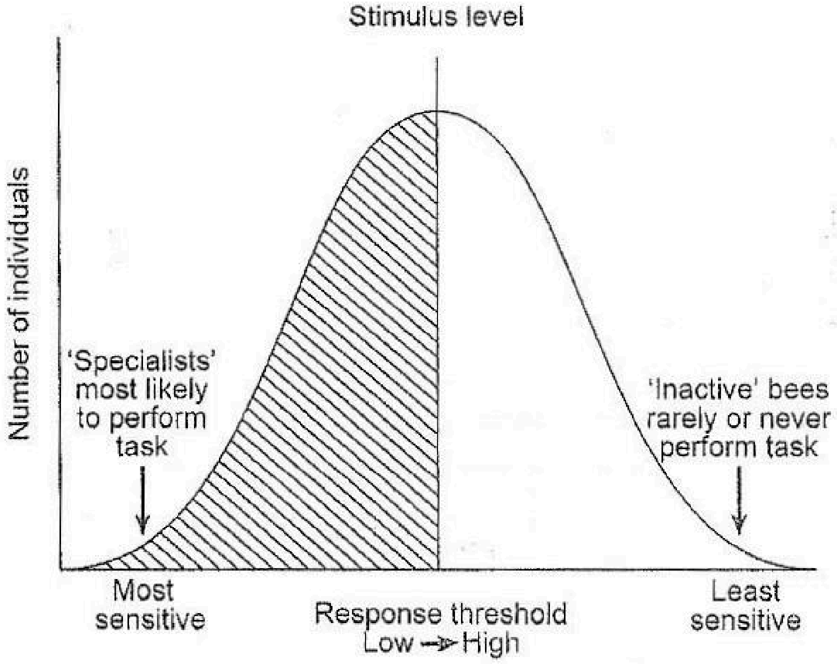

Figure 12: Hypothetical response threshold model to explain inter-individual differences in task specialization in a bee colony (obtained from Barron & Robinson 2009).

Variation in response thresholds can explain the existence of the previously discussed patterns of division of labour. Age polyethism can be explained by changes in the response thresholds caused by the maturation of the individual (Beshers et al. 1999). As for morphological polyethism, differential recruitment of majors can be explained with higher thresholds to recruitment. For example, in the ant *Pheidole pallidula*, only minors are recruited to retrieve small preys. However, if the prey is too big and needs to be dissected by majors before being retrieved to the nest, the recruitment displays are more vigorous. In that way, a stronger stimulus can reach the higher threshold of majors and elicit a recruitment response (Detrain & Pasteels 1991). Genetic polyethism can be explained by a difference of response thresholds among different subfamilies. The workers that perform a certain task belong to the subfamily with the lower threshold for that task (Beshers et al. 1999).

4.3. Self-reinforcement model

Division of labour could be the result of experience. The self-reinforcement model postulates that the successful performance of a task increases the probability of performing such task, while an unsuccessful attempt or the lack of opportunities decreases the probability of performance (Beshers & Fewell 2001)..

In the ant *Ceparachys biroi*, when the ants were experimentally successful at every foraging attempt they showed a high propensity to explore for food. In the other hand, the individuals that were always unsuccessful progressively decreased their foraging behaviour. When tested up to one month later, individuals that were successful presented higher exploratory behaviour compared with unsuccessful individuals. The later ones performed most of the brood care and stood closer to the brood than successful ants (Ravary et al. 2007). In this case, learning was at the origin of a division of labour, since other factors like age, morphology and genetics were controlled.

The effects of self-reinforcement on response thresholds by the amplification of the performance of a successful task can result in task specialization (Deneubourg et al. 1987). Individuals with a low threshold for a task will perform it before the individuals with a higher threshold. By that means they lower the threshold of the task, making even more unlikely for the individuals with higher thresholds to step in. This phenomenon is amplified by learning (Theraulaz et al. 1998b).

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The mound-building mouse as a model for the study of the division of labour

I think mice

Are rather nice.

Their tails are long,

Their faces small,

They haven't any

Chins at all.

Their ears are pink,

Their teeth are white.

They run about the house at night

They nibble things they shouldn't touch,

And no one seems to like them much

But I think mice are nice.

Rose Amy Fyleman

Fig. 13: Group of juvenile mound-building mice

The mound-building mouse (*Mus spicilegus* Petenyi, 1882*)* is our model of study. It is very closely related with the house mouse, *Mus musculus musculus* and *Mus musculus domesticus*. (Guénet & Bonhomme 2003).They are very similar, and sometimes it is difficult to set them apart in the field. They are often sympatric, and can share the same habitat. However, their habitats differ drastically during winter, since the domestic mouse lives in human constructions or very nearby, while the mound-building mouse lives in agricultural areas (Hölzl et al. 2011b). But the main behavioural character specific to *M. spicilegus* that can easily set both species apart is the ability of *M. spicilegus* to build mounds (Sokolov et al. 1998). Our model is a species that builds collectively, which makes it a species worth studying to widen the understanding of the mechanisms of the work organization in mammals. The rarity of suitable mammalian models for the study of division of labour in building tasks makes the study of this species even more interesting and appealing.

1. Distribution and life cycle of *M. spicilegus*

The mound-building mouse (*Mus spicilegus*) is a wild rodent mainly found in agricultural fields of Central and Eastern Europe. The limits of its distribution are Austria on the west, Slovakia on the north, Ukraine on the east and Bulgaria on the south (Fig. 14) (Sokolov et al. 1998).

Figure 14: Distribution of *Mus spicilegus*, according to Sokolov et al. 1998. The approximate distribution area is highlighted in light purple.

The life cycle of mound-building mice is far from being simple. In autumn, young individuals often from different litters and born in late summer and early autumn (the "autumn cohort") build imposing mounds out of plants and soil. They overwinter in this mound without reproducing. No adult enters the mound, with the exception of a few occasional females, mothers of some of the juveniles in the mound, that die early in the winter season. Mice from the same mound are more genetically related with each other than with mice from other mounds (Garza et al. 1997; Poteaux et al. 2008). In early spring individuals from the autumn cohort, now aged six months, leave the mound, disperse and reproduce. This species shows a monogamous mating system (a unique trait in this genus) (Patris & Baudoin 1998; Gouat & Féron 2005; Féron & Gouat 2007). The autumn cohort will produce the "spring cohort", which will reproduce during summer. The young produced by the spring cohort will build the mound and will form the autumn cohort (Fig. 15) (Garza et al. 1997; Poteaux et al. 2008) .

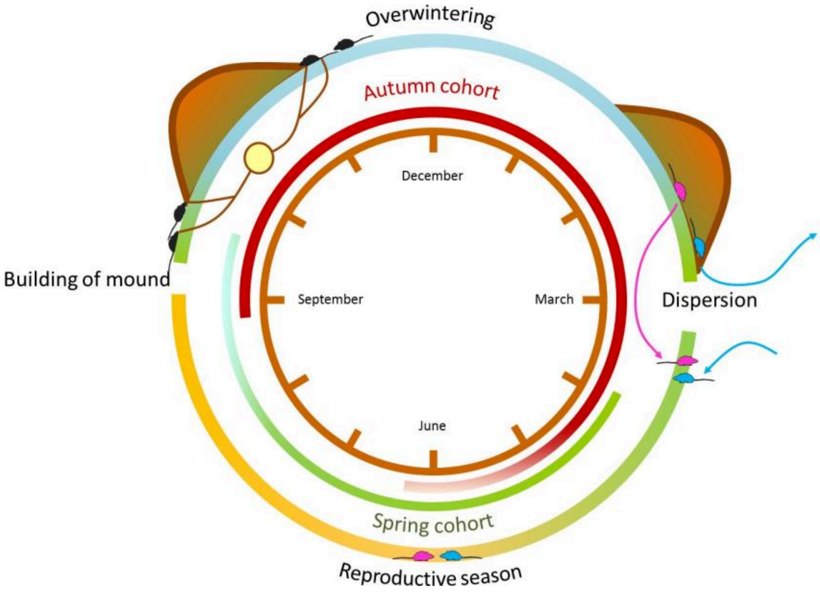

Figure 15: Scheme of the annual cycle of *Mus spicilegus*.

Since no adult survives the harsh winter, the reproduction is ensured only by the members of the autumn cohort that overwinter. The construction of the mound that keeps the autumn cohort alive is one of the most striking and exclusive features of this species. Indeed, its Latin specific name stands for “gathering together spikes of grain”, while its name in Magyar is *güzüegér,* which means something like “hardworking mouse”, and there’s a very well-known saying that goes “Dolgozik, mint a gúzú” (work like a mound-building mouse) that means work hard (Kriztian Katona, personal communication).

2. Structure of the mound

The mounds can be very imposing, reaching up to 2m long for 60cm high and mean volume of 165 l, but it can go as high as 700 l. Their size and volume seems to vary according with the number of individuals in the mounds (Szenczi et al. 2011), but the results of Hölzl et al. 2009 differ, linking the size of the mound with the availability of plant species used for construction in the vicinity of the mound.

The construction of the mound follows precise steps. First, mice gather a fair amount of plant material (Hölzl et al. 2011a). Then, they cover the pile with soil. The animals also make clumps of clay (called “tiles”) by digging shallowly and by arching their bodies. This activity leaves traces around the mound called “mines”. Once the tiles are made, mice transport on their mouth to the top of the mound by climbing backwards (Zoltan Bihari, personal communication) (Fig. 16). Finally they dig galleries and one or two nest chambers beneath the mound. The nest can be 30 to 80cm deep on the ground (Hölzl et al. 2009).

Figure 16: Photography of a mound from the Gyöngyös region in Hungary, taken in October 2011. The trails on the sides of the mound are made by mice when climbing the mound to lay the clay tiles in top of it. Tile mines are visible around the mound.

3. Role of the mound

The role of the mound in the survival of the mice follows three non-exclusive hypotheses: 1) the mound is used as food storage for winter, 2) the mound works as a thermal and moisture insulator and 3) the mound offers protection against predators.

The plant material used to build the mound was first thought to be at least in some extent a storage of food to survive the winter (Muntyanu 1990; Sokolov et al. 1998). However, there is evidence that indicates that mice do not eat the plants they use to build their mound, even during the winter. Mice use only a small part of the plant species available in the fields in the construction of the mound, showing a strong preference for ears or infructescences of *Amaranthus spp*., *Chenopodium spp*. and *Setaria spp*. The abundance of these plants in the mound is much higher than the abundance in the surroundings of the mound (Hölzl et al. 2009). However, when looking at the actual diet of mice during winter by analysing faecal pellets, the plant species found were not coincident with the species found in the mound. In fact, most of the plants used as building material are monocots, while plants found in faecal pellets are dicots. Also, there are no tunnels going from the nest to the body of the mound (Szenczi et al. 2011).

During winter, temperatures can be very low in the distribution range of *M. spicilegus*. The mound has proved to have thermal insulation properties. Thermal insulation increased with the size of the mound, and mounds of vegetal material and soil showed higher thermal insulation properties than mounds made uniquely from soil (Hölzl et al. 2011a; Szenczi et al. 2012). The nature of the plant filling also had an effect on the insulation capacities of the mound. Artificial mounds made of the plant species most used for mound building were heated and then allowed to cool in order to test their insulation abilities. Results showed that *Setaria spp*. mounds absorbed significantly more heat than *Amaranthus spp*. mounds (Hölzl et al. 2011a).

The ground beneath the mound was not only warmer than the ground on the vicinity of the mound, but it was also drier. The conic shape of the mound diverts part of the water, while the plant layer absorbs the moisture, keeping the galleries and nests dry (Szenczi et al. 2012).

Even when stated as a hypothesis for the mound's function, its role as protection against predators remains speculative. There are anecdotal accounts of only a few mounds being partially dug by ground predators (Szenczi et al. 2011), but the exact role of the mound in the reduction of predation risk remains to be studied.

4. Organization of the building work in *M. spicilegus*

Since bigger mounds are more effective in terms of thermal and moisture isolation and since the size of a fully built mound can go up to 40 000 times the volume of a mouse (Hölzl et al. 2009), mice need to be organized and performant in order to achieve the completion of the collective building of the mound. Previous work demonstrated the existence of a behavioural differentiation for a transportation task in this rodent species (Serra et al. 2012)(the published paper is under the "Appendix" section of this thesis). Groups of six juvenile individuals from three different litters were followed using the Radio-Frequency Identification Device (RFID) while building a mound in laboratory conditions. A classic RFID system is formed by three main components: a glass-encapsulated tag (also called "transponder") (Fig. 17), a reception antenna and a data processing system. This technology is extremely useful for tracking several animals in conditions where the visual individual identification might be hard (for instance, during dark cycle). In our studies, the glass tags were injected under the dorsal skin of the mice. Cotton balls tagged with a RFID transponder were used as building material since it replicated the nature of the plant material used in the field to build mounds. Both materials are light and bulky, but cotton balls are a standardized material that was also easy to tag with the RFID transponder.

Figure 17: RFID glass-encapsulated tag

Mice needed to go through a tunnel surrounded by two antennas in order to collect the material and retrieve it to the building site (Fig. 18). Thanks to the anti-collision system, several tags could be detected simultaneously. The obtained data allowed determining the spatio-temporal distribution of animals and cotton balls when passing through the antennas. The identity of the animals that transported cotton balls and the temporal dynamic of transport could then be established.

Figure 18: Experimental device with a central experimental cage, two tunnels surrounded by RFID antennas and two cotton dispensers.

Two behavioural profiles emerged. In each group, two individuals (called “carriers”) performed up to 80% of the total transport of materials, while the rest only transported occasionally or did not transport at all. This behavioural differentiation was not affected by gender or parental origin, and the identity of the carriers did not change during the four days of test. These results were highly innovative, since a behavioural differentiation during collective building is extremely rare in mammals and it was only been described in humans and naked mole-rats (Serra et al. 2012).

This work was at the origin of my doctoral project. Having demonstrated that a behavioural differentiation existed during the construction of the mound in *M. spicilegus*, three main questions arise:

1) Is this behavioural differentiation a real division of labour?

When we talk about division of labour, it is implied that there is more than one task to perform. Since in Serra et al. 2012 we only tested one subtask of the mound-building (the transport of cotton balls), we needed to really establish that the behavioural differentiation was task-dependent in order to call it a task division.

2) Is there a link between individual characteristics and the specialization?

Mus spicilegus showed a specialization in building tasks. But the mechanism that could explain the emergence of such specialization was yet to be known. Threshold model predicts that individual differences could result in a behavioural differentiation. Temperamental or personality traits have proved to have an ecological importance and may have fitness consequences by influencing reactions facing predators, dispersal home exploration, etc. (Réale et al. 2000; Réale & Festa-Bianchet 2003). It is then likely to think that social regulation of task performance could be influenced by individual characteristics.

3) What happens when the structure of the group is modified?

In natural conditions, the structure of a group can be modified by the death of some individuals due to predation or other causes. The group must be flexible enough to counterpart such losses. In the case of our species, where there are specialists for building tasks, the loss of such specialists (more exposed to predation since they must go collect the building material) could have a devastating effect on the group. When a specialist disappears, is there another individual that takes the place of the missing mouse? And what if a non-specialist disappears?

I will try to answer to these three questions in the following chapters.

QUESTION 1: Is the behavioural differentiation a real individual specialization, as an evidence of a division of labour?

The division of labour for non-reproductive tasks has been poorly studied in mammals. There are few examples of evidence of specialization and task repartition, and those examples are mainly related with hunting strategies and foraging. Female lions (*Panthera leo*) hunt in groups. Some of the lionesses circle the prey and drive it towards other lionesses that stay hidden in the centre, waiting for the prey. Each individual repeatedly fulfils its role, and the efficiency of the hunting task is higher when each lioness of the pride occupies its preferred position (Stander 1992). Similarly, bottle-nose dolphins (*Tursiops truncatus*) show a behavioural differentiation during group hunting. One individual acts as a “driver”, herding the fishes toward the other members of the group that act as barriers. This specialization is stable, the same individual acting as the “driver” in multiple occasions (Gazda et al. 2005).

Another example of specialization is found in rats (*Rattus norvegicus*). Groups of six Wistar rats were tested in a diving-for-food paradigm. This paradigm consists in a progressive increase of the difficulty to obtain food. Rats need to go through a tunnel in order to get food pellets. This tunnel is then progressively flooded with water. At final stages of such paradigm, rats need to dive to get the food. The interesting thing is that a specialization arises. Two of the six individuals (the “divers”) will dive and collect the pellets. They come back to the cage, where the other members of the group (the “nondivers”) are waiting to steal the food. This specialization remained stable during the duration of the experiment (Colin & Desor 1986). Similar results were obtained when testing house mice (*Mus musculus*) in the same paradigm (Nejdi et al. 1996).

This specialization seems to be independent of any constraint. In a slight modification of the diving-for-food paradigm, rats could chose to return to the main cage, where nondivers were waiting to steal their food, or to go to another cage where they would be alone. The authors expected that specialization was going to disappear, since there would be a lack of suppliers. Unexpectedly, the specialization was maintained, with divers returning to the main cage (Grasmuck & Desor 2002). But again, this specialization happens in a foraging context.

The task specialization on building tasks has been described in naked mole-rats (*Heterocephalus glaber*). In this eusocial rodent the “workers” are behaviourally differentiated by their level of activity and are described as “frequent workers”, “infrequent workers” and “nonworkers” (Jarvis 1981). Small individuals tend to dig and transport soil, while larger individuals are less active but defend the nest against predators, thus performing the “soldier” tasks (Dowkins 1976).

In the mound-building mouse *Mus spicilegus*, a behavioural differentiation has been described during a transportation task which is a part of the collective construction of the mound (Serra et al. 2012). But since only one task (the transport of cotton balls) was studied, we could not actually say that this behavioural differentiation was related to a task specialization that could be part of a division of labour. Indeed, in division of labour, each member of the group or colony specializes in part of all the available tasks, and different individuals will specialize in different tasks. The results obtained by Serra et al. 2012 can be explained without a task specialization. For example, all the available building tasks could be performed by the same individuals, and the rest of the group will not show any specialization. Or maybe there could be a behavioural differentiation for this particular transport task, but all the other tasks are performed equally by all the members of the group.

In order to establish the existence of a task specialization, we proposed two different transport tasks to mice. By doing so, we expected to see if there were different specialists for both tasks. This protocol is based on the tasks that are naturally performed by wild mice. In the field, mice need to transport different materials at different stages of the building process. The different steps of building are: 1) selecting a suitable site and cleaning the soil by removing stones and plants, 2) gathering plant material and piling it on the selected site, 3) covering the mound with soil, 4) placing clay tiles on top of the mound and 5) digging galleries and nest chambers underneath the mound. The transport tasks are different by the nature of the transported materials and by the locomotor sequences involved. The plant material is bulky and very light, while the clay tiles are compact and heavy. In the laboratory we used cotton balls and plaster tiles for the study of the transport tasks. The cotton balls replaced the plant material and plaster tiles were made to resemble the clay tiles found in the field (Fig. 19).

Figure 18: Building material, from left to right: cotton balls, plant material (mostly *Setaria spp.* and *Hibiscus trionum* infructescences), plaster tiles and clay tiles.

In the field, the transport of plant material and clay tiles is separated by another task that does not involve transport (the covering of the mound with soil). We choose to reproduce these two transport tasks because there were both associated with transport and the technique to record the mice's behaviour using RFID is more precise, even if they were not directly consecutive in the field.

In our first approach, we presented both building materials sequentially, starting with the cotton balls in order to respect the sequence observed in the field. The detail of methods and results of this experiment is showed in Hurtado et al. 2013, further in the text.

The existence of specialized individuals in different tasks of the mound-building process and the necessary sequence of the different steps of the construction can be evidence of a task partitioning. Indeed, a task is called partitioned if it is split in sequential subtasks performed by different individuals and the material is passed from one individual to another, either directly (the material is handed to another individual) or indirectly (the material is gathered in piles or “caches”) (Anderson et al. 2001). However, we don’t know if the building task is partitioned or not. We have no evidence for the passage of material between individuals, neither direct nor indirect. The cutting and gathering of plant material could be a partitioned task, with individuals that cut the plants and carriers that retrieve this material to the construction site. The production and transport of clay tiles can also be a partitioned task, with individuals that make the tiles and other mice that carry the tiles to the top of the mound. In order to establish the existence of such task partitioning, the role of each individual for each subtask needs to be determined. This is certainly an interesting continuation of this work that could extensively improve the understanding we have of the organization of work in *M. spicilegus*.

The existence of specialists leads us to another question: what determines the individual specialization? This question is treated in the next chapter of this work.

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Specialization in building tasks in the mound-building mouse, *Mus spicilegus*

Maria José Hurtado*, Renée Fénéron, Patrick Gouat

Université Paris 13, Sorbonne Paris Cité, Laboratoire d'Éthologie Expérimentale et Comparée, Villetaneuse, France

Specialization in building tasks in the mound-building mouse, *Mus spicilegus*

Specialization can be defined as when specific individuals perform a specific task for a relatively long period of time. The mound-building mouse is a suitable species to study specialization during a collective construction task, as juveniles build imposing mounds to overwinter. The process includes several successive phases involving the transportation and piling of different kinds of materials along with covering up with dirt and digging of galleries. Laboratory studies revealed that inside a group of six individuals, two individuals transported most of the proposed building material. We tested whether this behavioural differentiation corresponded to a real specialization. Mice were proposed two different transportation tasks corresponding to different phases of the building process. Experimental groups received two different building materials in two consecutive periods while control groups received the same building material for both periods. As expected, in experimental groups, carriers for one material were not the same individuals as the carriers for the second material. This shift in the identity of the carrier according to the proposed material indicates a specialization for a different transportation task. By contrast, in control groups, mice tended to keep their carrier status during the two periods. We concluded that, at least under control laboratory conditions, a task-related specialization occurred during the collective construction of the mound. This specialization could be explained as part of a division of labour in the mound-building mouse.

Keywords: building task, division of labour, mound-building mouse, *Mus spicilegus*, specialization

Inside a social group, the specialization occurs when specific workers are engaged in specific tasks during a relatively long period of time (Gordon 1996; Ratnieks & Anderson 1999; Beshers & Fewell 2001). In social insects, the primary specialization concerns reproduction, whereas non-reproductive individuals are also dedicated to specific ergonomic tasks such as offspring care, foraging or nest construction (Robinson 1992). Usually, the specialization among non-reproductives is attributed to three general factors: maturation, differences in size or shape and genotype (reviewed in Robinson 1992; Ratnieks & Anderson 1999; Page et al. 1998). For example, in most species of highly eusocial insects, younger workers perform tasks inside the nest and older workers perform outside tasks such as defence of the nest and foraging (Manning 1975; Robinson 1992; Beshers & Fewell 2001; O'Donnell 2001). In mammals, there are only few examples of specialization with respect to non-reproductive tasks, and these examples are mostly associated with hunting and foraging. For example, lionesses, *Panthera leo*, specialize in different positions and roles during hunting (Stander 1992). Another example has been described in the bottle-nosed dolphin (*Tursiops truncatus*), where “drivers” consistently lead banks of fishes towards “barrier” dolphins (Gazda et al. 2005).

Collective building has been widely documented in social insects (Franks & Deneubourg 1997), whereas it is scarcely present among mammals (Hansell

1984). Moreover, in only few examples such as humans and naked mole rats a behavioural differentiation has been observed during collective building. The eusocial naked mole-rat, *Heterocephalus glaber*, has a social system structured around one breeding female and pseudosterile female “workers” (Sherman et al. 1992; Burda et al. 2000). In these rodents, the “workers” within a group are behaviourally differentiated in three categories depending on their level of activity: “frequent workers” (frequently performing tasks associated with nest building and foraging), “infrequent workers” (performing the same tasks but at less than half of the rate than frequent workers) and “nonworkers” (rarely performing any digging or foraging task) (Jarvis 1981).

Another exception refers to the mound-building mouse, which constitutes a fine model to study task specialization. Unlike eusocial insects and naked mole rats, this species does not engage in reproductive division of labour, being mainly monogamous (Patris & Baudoin 1998; Gouat et al. 2003). Juveniles of this wild species collectively build complex mounds of imposing dimensions that can go up to 2 m long and 60 cm high (Hölzl et al. 2009) for an animal of only 10 g of average body weight. The first step of the building of the mound is the collection of vegetal material (mostly infructescences or ears of *Amaranthus spp.*, *Chenopodium spp, Echinochloa sp,* and *Setaria spp.*, as described in Hölzl et al. 2011a and Unterholzner et al. 2000). Then mice cover the vegetable pile by projecting dirt on it (Gouat et al. 2003; Hölzl et al. 2009). They also make “tiles” with clumped clay and transport them climbing backwards to the top of the mound while holding a tile in the mouth (Zoltan Bihari, personal communication). As a last step, they dig galleries underneath and one or two nest chambers, where they will overwinter without reproducing (Gouat et al. 2003). Construction of the entire mound can be completed in less than 15 days, depending on weather conditions (Zoltan Bihari, personal communication). The mound ensures the abiotic conditions of temperature and moisture levels necessary for the survival of mice (Szenczi et al. 2011). Since only juveniles survive winter, the mound building process reveals its vital importance.

It is possible to stimulate the construction of a mound structure under laboratory conditions in mound-building mice. When given natural materials such as plants (*Amaranthus sp.*), the animals cut and gather the material (unpublished data). This material is relatively bulky and light. It is possible to replace natural materials for more suitable items such as cotton balls easier to standardize and manage in an experimental protocol. Serra et al. (2012) proved the existence of a behavioural differentiation in the transportation of such material. In a group of six juveniles behavioural differentiation was set up rapidly, with two individuals carrying most of the proposed material to the nest. This work organization was stable in time over a four-day period and the proportion of carriers *versus* non-carriers remained constant between groups. No influence of gender or parental origin was observed. This species gave us the opportunity to test for the existence of a task specialization, where the behavioural differentiation among individuals will depend on different tasks. In the field, mice transport different kinds of materials at different phases of the mound-building process (i.e. collection of vegetal material and transportation of clay tiles). The transportation of these two materials constitutes two distinct tasks differing in both the nature of transported material and in the locomotor patterns involved. Moreover these two tasks are separated by a phase where mice cover the mound by projection of dirt.

The goal of our work was to establish the existence of a task-related specialization. This specialization should be revealed by a change in the identity of the carriers between the two tasks following the change in the proposed material. In order to replicate the timing of the natural process, we chose to present the two different construction materials in two consecutive phases: light cotton balls and then heavy plaster tiles. We expected to find that the animals that transported the cotton balls were not the same animals that transported the plaster tiles. The stability of the behavioural differentiation over time was evaluated in a control situation where only cotton balls were provided during both phases. Since body size can be a main factor to determine a behavioural differentiation (Clutton-Brock et al. 2003) we controlled for animal's body weight. Moreover, due to the weight of the tiles we expected to see a link between mouse body mass and transportation of tiles.

MATERIAL AND METHODS

Animals and housing conditions

Experimental animals derived from a stock of 80 wild mound-building mice caught in the Gyöngyös region in Hungary in October 1999 and completed with new animals caught in 2004 in this same region. Mice were bred to the F12 generation under laboratory conditions (20 ± 1 °C) with a 14:10 h reverse light:dark cycle with red light between 12:00 and 22:00 hour. They were housed in standard polycarbonate cage (28 × 41.5 cm and 15 cm high) with sawdust (Special Diet Services, Witham, Essex, UK) and cotton was provided for nesting material. Food (type M20, Special Diet Services, Witham, Essex, UK) and water were supplied *ad libitum*. Mice were weaned at 28 days. As the behavioural differentiation is known to be poorly influenced by parental origin (Serra et al. 2012) and since this species shows a high level of social intolerance (Patris et al. 2002; Suchomelová et al. 1998, both studies in adults), we chose to work with siblings. Twenty groups of six sibling individuals were formed, maintaining an equilibrated sex-ratio as far as possible. Each animal was marked by hair clipping to allow visual identification. They were housed in standard polycarbonate cages, with clean sawdust and twelve cotton balls for nesting material, and transferred to an experimental room one week before the beginning of tests.

At least 96 h before the experiments, mice were weighted and identified with a RFID anti-collision glass tag developed by Spacecode (Verrières-le-Buisson, France). The anti-collision technology ensures that several tags can be detected simultaneously by an antenna (Serra et al. 2012). The tag (length: 12 mm; diameter: 1.5 mm) was injected under the dorsal skin.

Material used for the transportation task

In order to confirm the suitability of cotton balls as a substitute for vegetal material and to determine the weight of plaster tiles for the experiment, we collected and measured a diversity of construction material from mounds in

Hungary. The infructescences of two species of plants (*Hibiscus trionum* and *Amaranthus sp*.) were collected from three different mounds from a site in the Gyöngyös region. We also collected the ears of *Setaria sp*. in a site in Tokaj, from the top of three different mounds. Vegetal material is relatively bulky and very light (mean ± SEM: 0.11 ± 0.06 g for *Hibiscus trionum, N* = 70, 0.11 ± 0.07 g for *Amaranthus sp, N* = 29 and 0.03 ± 0.01 g for *Setaria sp, N* = 549). Clay tiles were collected from two different sites in the Gyöngyos region, directly from the top of three different mounds in each site. The mean weight and standard error of clay tiles was respectively 2.2 ± 1.5 g and 2.1 ± 1.9 g for both sites (*N* = 77 and *N* = 53 respectively). Clay tiles show great variability in weight, going from 0.3 g to 7 g.

Under laboratory conditions, cotton balls are a good substitute for vegetal material, since they are easy to standardize and manage in an experimental protocol. Their relatively higher mass (0.6 ± 0.1 g) compared with the mass of the collected vegetal material is still within the ranges of what a mouse can easily transport. We used coloured cotton balls to differentiate them from the white cotton balls used for nesting and to ease the retrieval of the tags once the test was over. Glass tags (the same as used to identify animals) were fixed inside cotton balls using hot glue at least 24 hours before the experiments.

The tiles were made of little plaster chunks with an inserted glass tag. They were homogenized to 2.0±0.1 g (about the average weight of collected clay tiles) to avoid any bias. This weight represents one fifth of the average body mass (10.27±1.05 g) for this species at this period (Poteaux et al. 2008). Plaster tiles were allowed to dry at least 24 hours before the experiments.

Experimental protocol

In order to test the hypothesis of the existence of specialization during the transportation task during the mound-building process, mice were confronted with two different kinds of materials at two different times. The experimental group received cotton balls during the first four-day phase (P1) immediately followed by a second four-day phase (P2), where mice were given small plaster tiles. The control group received cotton balls during the two phases (P1 and P2).

Experimental devices

During P1, the experimental cage was connected to one tunnel (diameter 4 cm, length 50 cm) leading to a transparent box made of Plexiglass (15 x 15 x 40 cm; figure 1a). Inside this box, there was a vertical hanger device made of a standard floral foam (height 30 cm), covered with metallic grid. In order to imitate the natural conditions, mice had to climb to get the construction material. Cotton balls were attached to the hanger device with cotton threads at three levels: ground level, 15 cm high and 30 cm high. For each of the two higher heights, two branch-like hangers made of metallic wire were inserted in the floral foam. Cotton balls at the bottom were directly attached to the metallic grid. The tunnel was encircled with two RFID antennas, one next to the cage and the other next to the cotton ball dispenser. A double antenna was used to accurately assess the exact time of entry into the tunnels, the direction taken by mice and construction materials, and the time spent both in the cage and in the material dispenser.

During P2 and for the experimental groups only, the experimental cage was connected to a plastic box (10x10x9 cm) as tile dispenser (figure 1b). The tunnel was set on an angle of 56° in order to imitate the slope of a mound. The cotton dispenser was kept for the control groups.

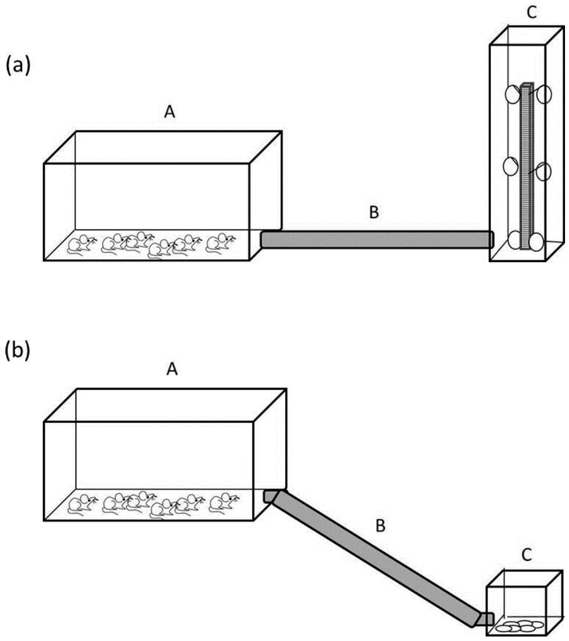

Figure 1: The experimental device was composed of a polycarbonate cage (A) connected with a tunnel encircled with two circular antennas (B). This tunnel led either to a cotton dispenser (C, fig. 1a) or to a box containing plaster tiles (C, fig.1b). In this later case, the tunnel presented a slope of 56° to imitate the slope of the mound.

Testing procedure

Groups of six sibling mice were housed in experimental polycarbonate cages (28 x 41.5 x 15 cm). We formed ten control groups (*N* = 60, 28 females and 32 males, 11.3 ± 0.6 g, 36.8 ± 5.1 days old) and ten experimental groups (*N* = 60, 26 females and 34 males, 10.9 ± 0.2 g, 37.5 ± 4.6 days old). Each cage had a circular opening that remained closed until the beginning of the experiments. The cages contained sawdust, water and food *ad libitum* and twelve white cotton balls as nesting material. Mice were allowed a four-day period to construct their nest.

During P1, six cotton balls were attached daily with cotton threads to the hanger device, at three different heights. This protocol was followed during P1 in experimental groups and during both P1 and P2 in control groups. Cotton balls not collected during a 24 hour-period were removed and replaced with new ones. Since the apparatus was closed, the items remaining in the device were heavily impregnated with mice odour. Difference in the odour of the cotton balls could have biased the probability of being picked. Collected cotton balls were left in the nest, undisturbed until the end of the phase.

During P2 and only in experimental groups, six plaster tiles were placed daily in the box. If a tile was not transported after 24 hours, it was replaced with a fresh one, since the remaining tiles were impregnated with the mice odours. To avoid any bias in transportation the tile dispenser was cleaned with mild soap and warm water each day before the fresh tiles were placed. Transported tiles were left undisturbed until the end of the experiment.

Four groups were tested at the same time (two control and two experimental groups). Mice activity was continually measured during both phases of test using the RFID technique. Transport of a construction item was attributed to a mouse when their tags were detected moving synchronically inside the tunnel from the dispenser to the cage. When two mice were detected at the same time with a construction item, they were each considered as transporting 0.5 item.

Data analysis

In a first step we tested the effect of the position of the cotton ball in the hanging device. We compared the observed distribution of the collected cotton balls to a theoretical distribution based on equiprobability (i.e. a third of the cotton balls were collected in each of the three levels of the hanging device). We then compared the observed distribution of the collected cotton balls with the distribution of the first cotton ball collected. In both comparisons we used a Pearsons's chi-square test with the exact procedure. Partial chi-squares with a sequential Bonferroni's procedure (Holm 1979) were used to assess the significance between the positions. The corrected *P*-values are given.

The transportation performance of a mouse corresponded to the number of material items transported by the animal. The number of transported items could vary between days and groups, and it happened that for undetermined reasons mice of a group did not transport any item in a given day. For these reasons we used the relative value over the complete four-day phase (i.e. the number of transported items by a given individual divided by the total number of items transported by the group) to assess the individual contribution. If all the six animals of a group transported an equal amount of material then their individual contribution should be of 0.17 (1/6). To determine the level of behavioural differentiation of a given individual we calculated the ratio between its individual contribution and this equiprobality value. This ratio is thereafter referred as the specialization index (SI). This index is equal to 0 when the mouse did not transport any material during a phase and is equal to 1 when the individual transported the mean number of transported items. A carrier, a specialist in the transportation task, is defined when his SI was equal to or higher than 1.45. The cut value of 1.45 was assessed by plotting the obtained data. There was a clear separation between carriers and occasional carriers.

We performed binomial tests to compare the number of carriers on the two phases (P1 and P2) for each transportation category, in control and experimental groups. To test the phase x group interaction on the number of carriers, we compared the change in the number of carriers between P1 and P2 between the two groups using a permutation test for independent samples.

To check the stability of the behavioural differentiation over time for both experimental and control groups, we used a multivariate generalized linear mixed-effect model with Poisson error distribution. Group was set as random factor. As predictor we used the specialization index obtained by each individual during P1 (covariate), sex (factor with 2 levels) and control/experimental group (factor with 2 levels). The response variable was the specialization index for each animal on P2. Analyses were done with the R software version 2.14 (R Development Core Team 2012) using the package lme4 (Bates et al. 2011). Post-hoc comparisons were made using only the corresponding subset of data for each control and experimental groups. *P*-values were calculated by likelihood-ratio tests based on changes in deviance when each term was dropped from the full model. We checked homogeneity of variances and goodness of fit by plotting residuals *versus* fitted values (Faraway 2006). For significant mixed-effects models, we calculated Nagelkerke's Pseudo-R^2, which can be used as a measure of explained variance for this kind of analysis (Nagelkerke 1991).

To complete this analysis we focused on the evolution of carriers between P1 and P2. We compared the SI of carriers between the two phases using a permutation test for paired samples. We expected SI of carrier to remain stable in control groups whereas a decrease was expected in experimental groups. To test the difference of evolution of SI between the two groups we used the absolute value of the difference of SI between P1 and P2 as a numeric variable. We expected this difference to be smaller in control groups than in experimental groups. The comparison was made using a permutation test for independent data. For repeated comparisons we used a sequential Bonferroni procedure and corrected P-values are given (Holm 1979).

The effect of body mass on the transportation performance for the experimental group was tested using a permutation test for independent data. Juvenile males tend to be heavier than same age females (e.g. Poteaux et al. 2008) and we used group and sex as a stratum to compare body-mass between carriers and other mice. A similar analysis was made in the control group during the second period. We expected carriers to be the heaviest individuals in experimental groups because mice had to carry plaster tiles, whereas no such differences were expected in control groups because material to be transported consisted of bulky cotton balls.

Chi-squared, binomial and permutation tests were performed using StatXact-8 (Cytel Inc., Cambridge, MA 02139 USA). Data are given as mean ± SEM.

Ethical note

Animal care and experimental procedures were approved by the Regional Ethics Committee in Animal Experiment N°3 of Ile-de-France (Ce5/2010/074). The number of mice used was restricted to the minimum needed for statistical analysis. Whenever it was possible, we used animals from litters produced by experiments on reproduction, where pups were not used for any other experiment. Subcutaneous injection of the tag was performed by a specifically trained animal keeper. The behaviour of mice was systematically observed 24 hours after the procedure. Young mice behaved normally and did not seem to be affected by the injection. As the mice were not used in other experiments and could not return to our breeding stock for sanitary safety, they were killed at the end of the experiment. They were anesthetized with isoflurane and then put into a rising concentration of carbon dioxide.

RESULTS

Transported material

During P1, from a total of 480 delivered cotton balls, 384 (80%) cotton balls were actually transported and detected. During P2, from a total of 240 delivered materials, 173 (72%) cotton balls, and 201 (84%) tiles, respectively for the control groups and the experimental groups, were successfully transported and detected.

During the first phase, cotton balls were not collected independently from their position in the hanging device (Pearson $X^2_2 = 21.98$, $P < 0.001$; Figure 2). Mice tended to collect more cotton balls situated in the bottom position than balls situated in higher positions (Pearson $X^2_1 = 16.92$, corrected $P < 0.001$). Moreover, among all the balls collected, the first ball collected was more frequently in a bottom position than in the other two positions (Pearson $X^2_1 = 24.19$, corrected $P < 0.001$).

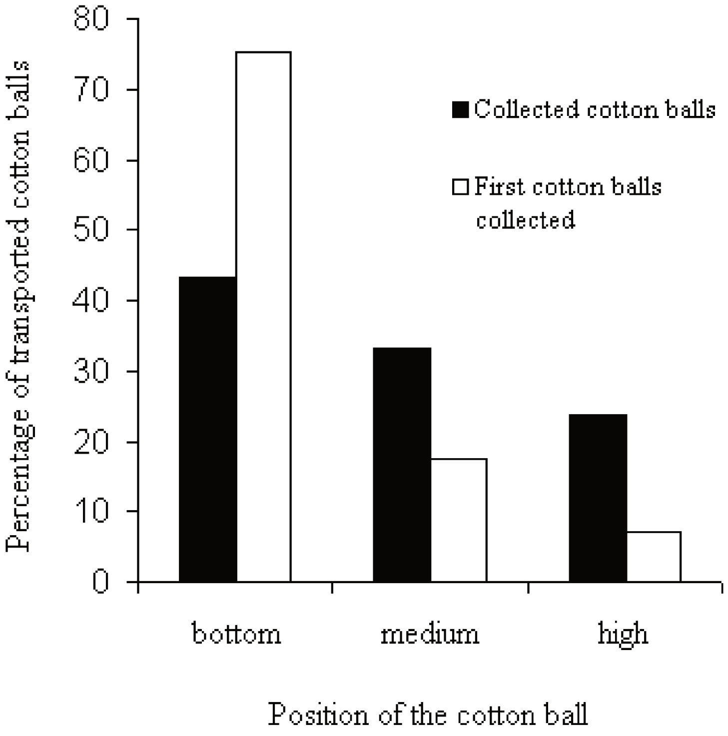

Figure 2: Percentage of the collected cotton balls (N = 384, black bars) and of the first cotton balls collected (N = 69, open bars) during the first phase of the experiment for each of the positions on the hanging device.

Specialization for the transportation of materials

The number of carriers (i.e. mice with a SI > 1.45) per group varied from 0 to 3. The number of carriers was significantly higher in P1 than in P2 in control groups ($P = 0.031$), but did not vary significantly in experimental groups despite the change of transported material ($P = 1$; Figure 3). The number of carriers, nevertheless, did not differ significantly between control and experimental groups whatever the phase (P1: $P = 0.066$; P2: $P = 0.195$). The change in the number of carriers between P1 and P2 was significantly higher in experimental than in control groups ($P = 0.04$).

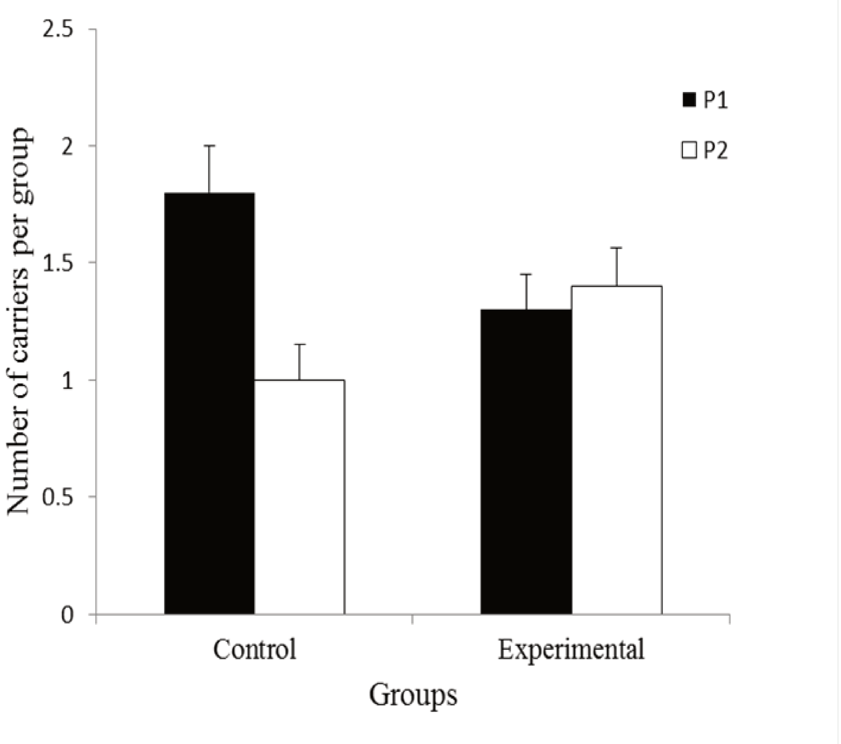

Figure 3: Mean number + SEM of carriers per group in P1 (black bars, N = 10) and P2 (open bars, N = 10).

Stability of the specialization for different phases and tasks

In control groups, mice tended to keep the same specialization level in both phases (X^2 = 20.92, $R^2_{Nagelkerke}$ = 0.388, $P < 0.001$; Figure 4a). No such correlation was found for experimental groups, and the SI in the first phase was not a good predictor of the SI in the second phase (X^2 = 1.58, P = 0.20; Figure 4b). Note that, in control groups, we repeated the analysis excluding all data of the individuals with a specialization index which exceeded values of 5 during P1; these two respective values causing strong heterogeneity of model variances; and we obtained the same statistical results.

The main analysis revealed that control groups and experimental groups differed significantly (interaction treatment x specialization index: $X^2 = 9.17$, $P = 0.002$). There were no significant differences between males and females ($X^2 = 3.09$, $P = 0.08$).

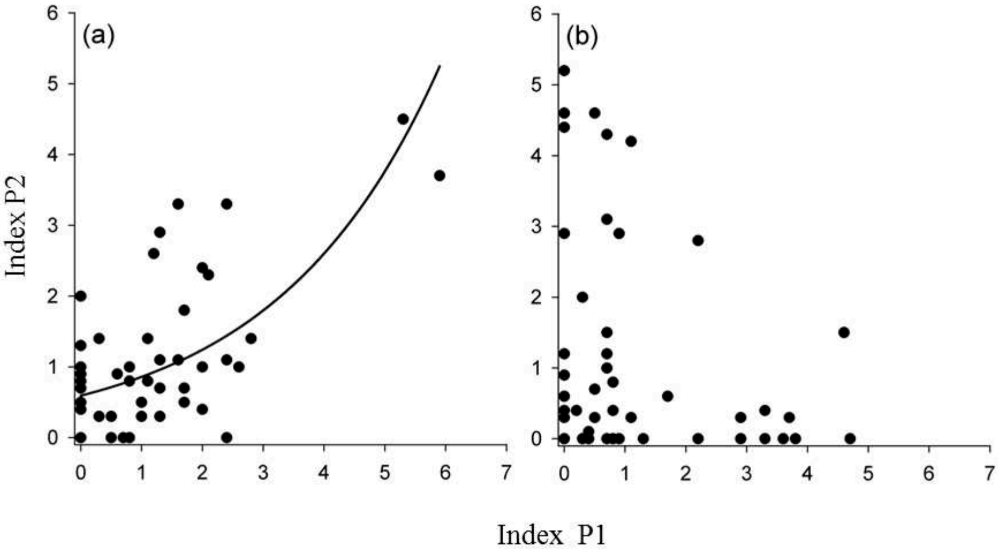

Figure 4: Stability of specialization. Correlation between the Specialization Index obtained during P1 and during P2 for control groups (a) and experimental groups (b). Regression model based on a Poisson regression is given for control groups (a). N for both control and experimental groups = 60.

This general tendency can be also observed when focusing on the evolution of carriers (Figure 5). In control groups the specialization index of P1 carriers decreased slightly and significantly in P2 (permutation test for paired data, $P = 0.034$, $N = 17$). Nevertheless, the mean SI remains above the 1.45 limit. In experimental groups the specialization index of P1 carriers decreased strongly in P2 ($P < 0.001$, $N = 13$). The mean SI of carriers was clearly below the 1.45 limit and the SI differed significantly between groups in P2 ($P < 0.001$, $N_1 = 13$, $N_2 = 17$), whereas the difference was in the other way and not significant in P1 ($P = 0.073$, $N_1 = 13$, $N_2 = 17$). The change of the SI between the two periods was significantly higher in experimental groups than in control groups ($P < 0.001$, $N_1 = 13$, $N_2 = 17$).

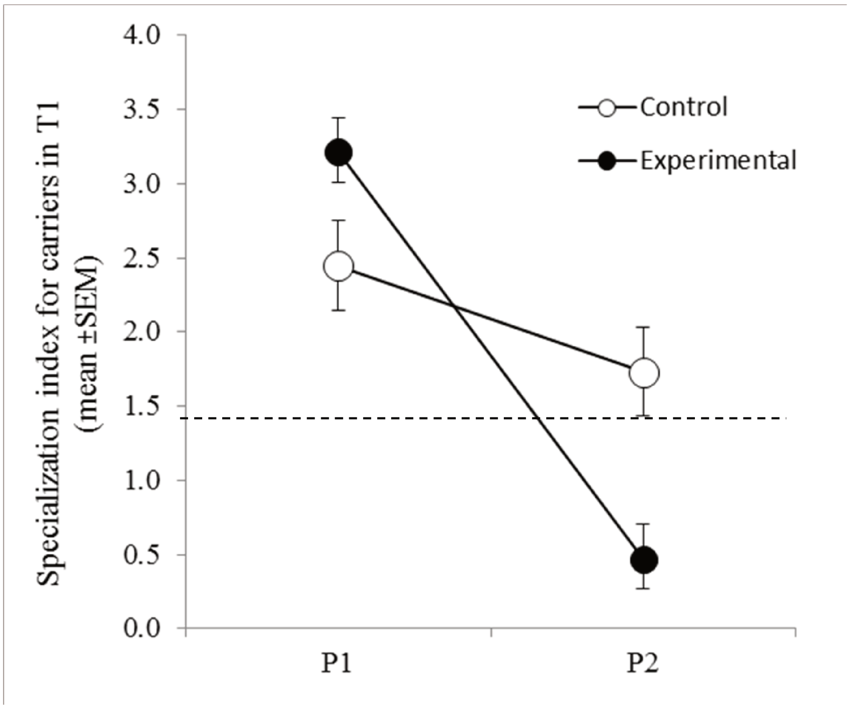

Figure 5: Difference between the specialization index for carriers in P1 and their specialization index in P2, for both control (N1 = 17, open circles) and experimental groups (N1 = 13, black circles). Data are given as mean ± SEM. The dotted line shows the cutting value of specialization index between carriers and occasional carriers.

Effects of body weight on specialization

Males were significantly heavier than females (mean ± SEM; males: $N = 65$, 11.38 ± 0.21 g, females: $N = 55$, 10.70 ± 0.18 g, $P = 0.001$). In experimental groups during the second period when mice had to carry plaster tiles, carriers did not differ in body mass from the other mice (carriers: 10.59 ± 0.42 g, $N = 14$, other mice: 10.88 ± 0.42 g, $N = 46$; $P = 0.20$). The same result was obtained in control groups during P2 (carriers: 10.94 ± 0.44 g, $N = 10$, other mice: 11.41 ± 0.23 g, $N = 50$; $P > 0.83$).

DISCUSSION

Our results provide strong evidence for the existence of an individual task-related specialization during the mound-building process. Our expectations were fulfilled: in control groups the mice performing the transportation task during the second period differed from those performing the transportation task during the first period, whereas in control groups carriers during the second period were carriers during the first period. In the experimental groups, the individual specialization level changed when a new material was proposed, sometimes very drastically, with the cotton carriers for the first phase becoming occasional carriers or non–carriers, and new carriers appearing during the second phase. In the control groups, the level of specialization tended to remain stable, even if the number of carriers decreased in the second period. Despite this difference, the carriers during the first period maintained a high level of contribution to transportation of cotton balls throughout the experiment. This strongly suggests that a change in the identity of the carrier as observed in the experimental groups could not be attributed to a temporal factor, since the specialization showed a high stability in control groups during the eight-day period of the test. Eight days of collection of vegetal material is rather long for a task that is only the first step of a process not exceeding two weeks in the field

(Zoltan Bihari, personal communication). But even with this long period of time, no new specialists appeared, and there was just a reduction in the number of the specialists that were already present. Experiments performed in rats (*Rattus norvegicus*) also showed the existence of a specialization, this time in a foraging task in a diving-for-food paradigm (Colin & Desor 1986). In groups of six Wistar rats, two individuals (the “divers”) dive to reach the food and bring it back to the home cage, where the other four that never dived (the “non-divers”) stole their food. The specialization between divers and non-divers remained constant during the experimental period.

Under laboratory conditions, we presented a fixed succession of tasks by presenting the cotton balls before the plaster tiles, since in natural conditions the transportation of vegetal material and clay tiles was performed at distinct phases of the building process (Unterholzner et al. 2000; Hölzl et al. 2009), with nature vegetal material being transported prior to clay tiles. In the field the two transportation tasks are separated by an intermediate task consisting in the projection of soil over the mound with the hind legs. This sequence can be frequently observed in the wild. The vegetal material is covered by a layer of soil, and the tiles are always on top. The tracks made by the mice to climb on top of the mound with the tiles are highly conspicuous (personal observation). This sequence of tasks is not modulated by the presence of the different materials, since vegetal material and clay tiles are present simultaneously in natural conditions. The change in the identity of the carrier could be part of a task partitioning, where the whole transportation task is divided among individuals. For example, in the honey bee (*Apis mellifera*) foragers transfer their nectar to workers inside the nest, making the collection of nectar a partitioned task (Ratnieks & Anderson 1999). The task of transporting the construction material would be the same, but different individuals would perform the sub tasks of transportation of the different items (cotton balls and tiles). The transportation of different materials requires different motor sequences. These motor sequences were determined under laboratory conditions, by observing mice in a 1 m^2 enclosure enriched with soil and plants available (unpublished data) and by observing mice during tests. Vegetal material is carried facing forward and does not need a big amount of strength to be transported. In the other hand, clay tiles are transported backwards, and as their weight is considerable for mice, the amount of strength needed is higher than for vegetal material. We observed that under laboratory conditions, cotton balls were transported using the paws and mouth and plaster tiles were transported in the mouth and never with the paws. In our experiment the intermediate task is missing so we cannot confirm or rule out the existence of a task partitioning where subtasks are performed sequentially to complete the task (Ratnieks & Anderson 1999 on social Hymenoptera), but it allows us to determine the existence of a task-dependent specialization. Therefore these results suggest the existence of a division of labour in the mound-building mouse.

Among collective tasks, construction of nests can be energetically demanding (Lovegrove 1989). For example, studies in fossorial rodents revealed that the cost of burrowing nests might be extremely high (360 to 3400 times higher than the costs for transporting material over the same distance across the surface, measured in the pocket gopher *Thomomys bottae* (Vleck

1979). When building collectively, the energetic costs of building are shared among several animals, making the individual cost to decrease (Ebensperger & Bozinovic 2000; Taraborelli 2009). This is supported by studies in the semi-fossorial rodent *Octodon degus*, which can dig burrows communally and may form burrowing chains. Although the time spent digging was the same as for solitary diggers, the amount of removed soil per capita was higher, suggesting a long-term energetic benefit of communal burrowing in this species (Ebensperger & Bozinovic 2000). However, such a group effort might not involve any specialization, as it has been shown in the plains vizcachas *Lagostomus maximus*, where each member of the group will equally engage in burrowing activities (Branch 1993). Burrows and nests are of vital importance for many small mammals, since they provide protection from harsh weather and predation, along with food storage and nesting (Ebensperger & Cofré 2001; Hölzl et al. 2009; Szenczi et al. 2011). In the case of the mound-building mouse, mounds appear to have thermoregulatory and water proofing properties (Szenczi et al. 2011; Szenczi et al. 2012). These insulating properties are mainly provided by the vegetal layer of the mound. The collection of this material is time consuming (Szenczi et al. 2012) and the size of the mound is important to keep suitable environmental conditions for the mice (Hölzl et al. 2011b), making the collective work crucial for the survival of this species. The specialization on building tasks could improve the efficiency of the whole building process. As theorized for work organization in social insects (Oster & Wilson 1978) and vertebrates (Anderson & Franks 2001), behavioural specialization implies that some individuals are more suitable to perform a task than others and then perform it more efficiently (but see Chittka & Muller 2009 for discussion).

In our study, the mice collected the bottom cotton balls first. In natural conditions, mice climb on top of plants to collect the upper portion (Hölzl et al. 2009). This fact could lead us to think that we are confusing the collection of nesting material with an actual collection of mound-building material. However, in natural conditions they can also gather harvested material, which would be energetically more efficient (Hölzl et al. 2009). The bottom cotton balls can be assimilated with harvested material, since it is readily available and the cost of fetching it is lower than climbing on the device to fetch the upper cotton balls. The fact that they also climb to gather these less accessible items should comfort us on the use as building material. However, our experimental setting was not successfully adapted to reproduce the natural conditions. But the results obtained were robust since the observed specialization was similar to the specialization reported in a previous study on this species using a simpler cotton dispenser (Serra et al. 2012).

The nature of the proposed material might also explain the differentiation in the transportation tasks among the members of a group. In natural conditions, the gathered vegetal material is carefully selected from all the available plant species (Hölzl et al. 2009). Moreover, the plant parts collected for building are not used as food and vice-versa (Szenczi et al 2011). The origin of this choice can be found in a precocious experience with the nest materials that can effectively modulate adult preferences. For example, the mice would learn to choose these plants by being exposed to them as pups, if their parents used these plants as nesting material. The nest is a rich environment where the exposure to an olfactory environment can modulate the preferences of the pups when they reach adulthood. This modulation of preferences is observed, e.g. in the European starling *Sturnus vulgaris*, where the preference for milfoil-scented nest material over vanilla-scented nest material is present in naïve individuals, but young raised in a vanilla scented nest will not show any preference between vanilla and milfoil-scented building material (Gwinner & Berger 2008). Other examples of the influence of the early environment on adult behaviour can be found in mammals. In rats, pups raised in a lemon-scented nest modulate their adult behaviour by taking more care of lemon-scented pups compared to non-scented pups (Shah et al. 2002). In rabbits (*Oryctolagus cuniculus*), pups express a food preference for their mother's diet at weaning. This preference is equally transmitted by prenatal exposition *in utero*, contact with the mother during lactation and maternal fecal pellets in the nest (Bilkó et al. 1994). However, in natural conditions, mice building the mounds originate from a reduced number of litters (Poteaux et al. 2008) and inside each litter have shared the same experience. The differences of precocious environment could explain inter-litter differences but would be less conclusive about intra-litter differences. In the same way, in our experimental settings, all mice were raised in the same environment, so they were equally familiar with the nest material (cotton balls). They were also all naïve with regard to tiles. These mice have been kept in laboratory, away from natural conditions, for twelve generations. Thus, no influence of precocious learning on the environment could explain the specialization observed inside the groups when transporting cotton balls or plaster tiles.

The threshold model assumes that individual differences in behavioural response thresholds to a certain stimulus are at the origin of a specialization inside a group (Robinson 1992; Beshers & Fewell 2001). As an example, the recruitment of majors in the polymorphic ant *Pheidole pallidula* depends on the response to the prey weight and the high response threshold to recruitment stimuli of majors. With these factors, majors are specialized in the transportation of larger preys such as cockroaches (Detrain & Pasteels 1991). However, in our study mice had very similar body weights inside each group, so there was only little variability associated with morphology. Even when we found that females were slightly but significantly lighter than males, no relationship was found between body mass and transportation performance of light cotton ball and heavy tiles as well.

The different thresholds can be determined by certain factors such as differences on the maturity level or other factors that causes variability among individuals. These factors were controlled in our experimental design, since we used only groups issued from same litters. As a consequence animals were the same age and had grown under the same precocious environment. In Serra et al. (2012), parental origin did not affect the transportation performance, so the effect of the genotype on specialization for this species could not be determined. We controlled for this factor by using litters, and we obtained a differentiation anyway. Individual traits associated with temperament are another source of individual variation (Gosling, 2001) that could be at the origin of differential responses in communal building. Experiments performed in mice, *Mus musculus*, in a diving-for-food paradigm with specialization in a foraging task, showed that less anxious individuals were more likely to become the "divers", suggesting that the specialization was modelled by individual differences (Nejdi et al. 1986). However, whatever its cause; the individual specialization can be interpreted as being a part of a more wide-ranging feature, a division of labour. The specialization would then be observed in consecutive tasks but also in concurrent tasks. Future studies will help to enlighten to what extend the differences in personality types are associated with the setup of the observed specialization for transportation tasks and if a real division of labour exists during the collective mound-building process in mound-building mice.

CONCLUSIONS

Mound-building mice showed a real specialization on the transportation of building material during the collective construction of the mound. Carriers for cotton balls were not the same than carriers for plaster tiles. This change in the identity of the carriers when different materials were consecutively presented confirmed the existence of a task-related specialization. In the same time, when mice were confronted to a single task (transportation of cotton balls) during the entire duration of the experiment, the identity of carriers remained stable. Consequently we conclude that the change in the identity of carriers was associated with the different tasks presented and not with the duration of the test. This specialization could be explained as being a part of a division of labour.

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Sequential organization of work in the mound-building mouse, *Mus spicilegus*

Maria José Hurtado*(corresponding author), mj.hurtado.herrera@gmail.com

Mathilde Crowet*, mathilde.crowet@hotmail.fr

Renée Fénéron*, Renee.Feneron@leec.univ-paris13.fr

Patrick Gouat*, Patrick.Gouat@leec.univ-paris13.fr

*Laboratoire d'Éthologie Expérimentale et Comparée, EA 4443,

Université Paris 13, Sorbonne Paris Cité, 93430 Villetaneuse, France.

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Abstract

For many animals the nest is essential for survival by providing a shelter against harsh climatic conditions and predators. Building in group reduces the cost of this task for the individuals. Collective building can be organized in different ways. For instance, humans show a centralized work, while social insects have a decentralized organization. To set both hypotheses apart, we studied a mammalian collective builder. In *Mus spicilegus*, the communal construction of the mound allows the survival of juveniles over winter. In order to obtain a functional structure mound construction has to follow sequential steps. Under laboratory conditions mice displayed an individual specialization for the transport of the proposed materials and the transport of different materials was organized sequentially. This sequence was also present at an individual level. Nevertheless mice tended to transport only one material and our results indicate that the sequential organization of transport inside a group was an emergent property. The mound-building mouse organized the building work in a similar way to social insects.

Introduction

The construction of a nest is a major activity for many species (Ebensperger & Bozinovic 2000). The nest provides shelter against harsh environmental conditions and predators, and a place for food storage (Hansell 1984). The cost of building a nest, however, sometimes exceeds the capacity of a single individual. Not only the physical effort is costly (Lovegrove 1989), but also the construction behavior may imply a higher risk of predation for the animals involved in the building process (Jarvis & Bennett 1990). In social species, when the effort of building is high, animals may cooperate to minimize individual costs (Ebensperger & Bozinovic 2000). In humans, the system of control of collective work is centralized (e.g. an architect), specialized (i.e. trades involved in building) and requires a representation of the global structure (Theraulaz et al. 1998a; Théraulaz et al. 2003). Furthermore, the behavioral complexity is directly related with the complexity of the construction (Bonabeau et al. 1997). In social insects where collective nest building patterns have been largely theorized (Theraulaz et al. 1998a), individuals are also specialized but individual actions are simple and the system is decentralized (Théraulaz et al. 2003; Hansell 2005). This difference in the organization of work could be explained by specific characteristics of humans that make them different from other building animals such as cognitive abilities and levels of social organization, or it can originate from differences between arthropods and mammals. A simple way to set those hypotheses apart is to determine the organization of work in another collective building mammal. *Mus spicilegus* is one of the rare mammalian species with a collective building of the mound and an individual specialization in building tasks (Serra et al. 2012; Hurtado et al. 2013). It is a valuable model to determine whether a non-human mammalian species could display a centralized organization during collective building or if its work is decentralized like in the social insects.

In *Mus spicilegus* groups of juveniles build mounds to survive across the winter (Garza et al. 1997; Poteaux et al. 2008). At the beginning of autumn, the mice build mounds collectively following four successive steps. First, mice collect and gather plant material. Second, they cover the pile by projecting soil. Then they produce clay tiles by digging “mines” and then pushing upwards. These clay tiles are transported on the top of the mound. Finally, mice dig galleries underneath the mound to overwinter (Hölzl et al. 2009; Szenczi et al. 2012). When achieved, the mound is a complex and large structure, reaching up to 2 m long and 60 cm high for an animal of 10 g of average body weight (Hölzl et al. 2009). As a consequence, the mound presents a conic shape that diverts most of the water. The plant material as a thermal insulator and absorbent prevents the water to infiltrate into the galleries and the nest (Szenczi et al. 2012). The entire mound can be built in less than 15 days, depending on weather conditions (Zoltan Bihari, personal communication). Previous laboratory experiments have shown the existence of a specialization for the transport of two different building materials (cotton balls used as plant material and plaster tiles used as clay tiles) when available separately (Hurtado et al. 2013). Both tasks correspond with two completely separate steps of the building process. However, building materials are present simultaneously in the field and should be used successively for the completion of the mound. The study of the temporal dynamic of the transport when mice are confronted with two types of material at the same time (cotton balls as plant material and plaster tiles as clay tiles) gave us the opportunity to observe the sequential organization of the transport tasks in order to understand what the underlying mechanisms of this organizations are.

Methods

Animals and housing conditions

Experimental animals derived from a stock of 80 wild mound-building mice caught in the Gyöngyös region in Hungary in October 1999. Mice were bred to the F13 generation under laboratory conditions. They were weaned at 28 days and were 42.8 ± 0.3 SEM days old at the beginning of the experiment. Eight groups, each consisting of six litter siblings were formed, maintaining an equilibrated sex-ratio as far as possible. Mice were housed in standard polycarbonate cages (28 × 41.5 cm and 15 cm high) with wood shavings as bedding (Special Diet Services, Witham, Essex, UK) and twelve cotton balls as nesting material. Food (type M20, Special Diet Services, Witham, Essex, UK) and water were supplied *ad libitum*. Mice were transferred to an experimental room (T = 16.3 ± 0.4°C, light:dark cycle = 14:10 h, beginning of dark cycle at 13:00) one week before the beginning of the tests. At least 96 h before the beginning of the tests, mice were weighted and identified with a RFID (Radio Frequency Identification Device) anti-collision glass tag (Spacecode, Verrières-le-Buisson, France) which was injected under the dorsal skin. Cotton balls were marked with glued glass tags and plaster tiles were made by inserting a glass tag in a plaster chunk (Serra et al. 2012; Hurtado et al. 2013).

Experimental device and testing procedure

Before the test, the experimental cage (28 × 41.5 cm and 15 cm high) was connected by a tunnel (PVC tube; length: 50 cm; diameter: 4 cm) to a vertical cotton dispenser on one side and to a tile dispenser consisting of a transparent box made of Plexiglas on the opposite side. The tunnel connecting the cage and the tile dispenser was set on an angle of 56° to emulate the slope of a mound (Hurtado et al. 2013). Both tunnels were encircled with two RFID antennas, one next to the cage and the other next to the material dispenser. A double antenna was used to accurately assess the exact time of entry into the tunnels and the direction of movements of mice and construction materials (Fig. 1).

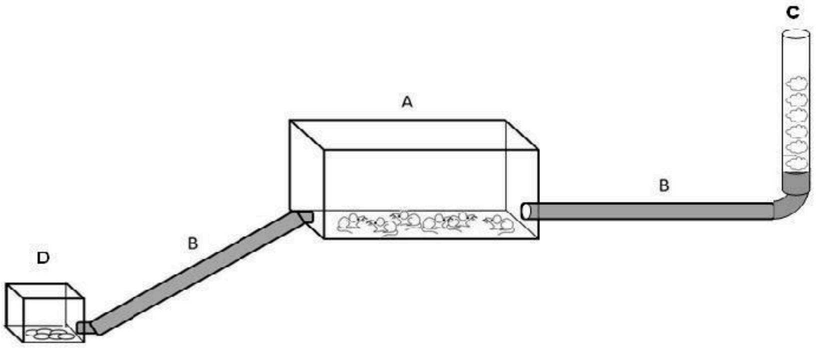

Figure 1. Elevation of the experimental device consisting in A) an experimental cage, B) tunnels surrounded by two RFID antennas, C) a vertical cotton dispenser and D) a tile dispenser. The tunnel leading to the tile dispenser was set with a slope of 56° in order to reproduce the slope of the mound. Diagram not to scale.

Six cotton balls and six plaster tiles were placed simultaneously in their respective dispensers two hours after the beginning of the dark phase of the four days of test. This period of time corresponds to the time spent by mice on each phase of the construction under natural conditions (Zoltan Bihari, personal communication). RFID monitoring was done continuously in order to determine the identity of transporters and the time of transport for each transported item.

All items not transported in a 24-hour period were removed from the dispenser and replaced with fresh ones.

Statistical Analysis

We examined the dynamic of transport of cotton balls and tiles by comparing the mean latency of transport per type of material and per day using a permutation test for two independent samples using group as stratum. The latency of transport was defined as the time elapsed between the beginning of the test and the transport of a given item. To complete this analysis, binomial tests were conducted to determine if animals tended to collect cotton balls before tiles when both materials were still available.

To determine the level of specialization of a given animal in the transport of a type of material we calculated a specialization index (SI) consisting of the ratio between its individual contribution and the equiprobability value (Hurtado et al. 2013). Since the total number of transported items could vary between groups, we calculated the individual contribution for each material type by using the number of transported items by a given individual divided by the total number of the items collected by the group over the four-day period. Mice with a SI higher than 1.45 are thereafter referred to as “specialists” (Hurtado et al. 2013). To check the reliability of the specialization, we calculated a Spearman’s correlation coefficient between the Sis for both transported materials. Since the range of SIs differed between each group, we the used the ranks instead of the raw data. By doing so, we corrected for the heterogeneity between groups that could have concealed the correlation. All statistical analyses were conducted using StatXact-8, Cytel Inc., Cambridge, MA, U.S.A.

Ethical note

Animal care and experimental procedures were approved by the Regional Ethics Committee in Animal Experiment N°3 of Ile-de-France (Ce5/2010/074). The number of mice used was restricted to the minimum needed for statistical analysis. Whenever it was possible, we used animals from litters produced by experiments on reproduction, where pups were not used for any other experiment. Subcutaneous injection of the tag was performed by a specifically trained animal keeper. The behaviour of mice was systematically observed 24 hours after the procedure. Young mice behaved normally and did not seem to be affected by the injection. As the mice were not used in other experiments and could not return to our breeding stock for sanitary safety, they were killed at the end of the experiment. They were anesthetized with isoflurane and then put into a rising concentration of carbon dioxide.

Results

In 22 out of the 32 observations (i.e. 8 groups and 4 days), the transport was initiated with a cotton ball and ended with a tile. For all days of the experiment the latency of transport of tiles was significantly higher than the latency of transport of cotton balls ($p < 0.001$, Fig. 2) even if both transporting tasks overlapped partially in half of the cases.

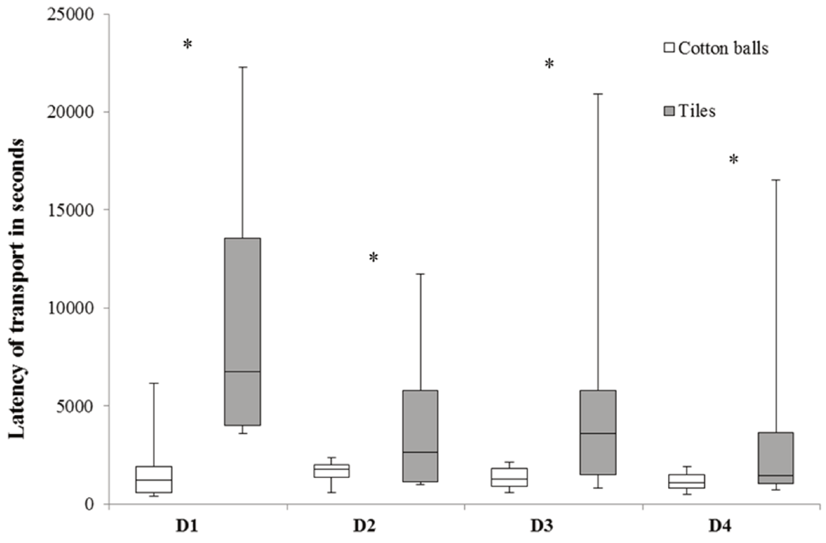

Figure 2. Boxes represent the median and 1st and 3rd quartiles of the latency of transport of cotton balls and tiles for each day of test. Whiskers represent the percentiles 10 and 90. Comparison using permutation test for independent samples, with group as stratum (***= $p < 0.001$). The number of cotton balls transported on D1 to D4 was 36, 35, 34 and 36 respectively. The number of tiles transported on D1 to D4 was 42, 32, 33 and 24.

Out of the total number of 48 tested animals, 44 mice transported at least one item but only 18 mice transported both materials in the same sequence (i.e. in a given day) at least once. When a same individual collected both materials in a given day, it generally started to collect a cotton ball (21 out the 27 sequences recorded: $p = 0.006$, $\alpha = 0.5$). In the sequences where both materials were transported, a total of 70 cotton balls were transported while both materials were available. Out of these 70 cotton balls, 57 were collected before the first collection of a tile. This proportion was highly significant ($p < 0.0001$, $\alpha = 0.5$).

Twenty-three mice were specialists in the transport of at least one type of material, carrying 77% of the cotton balls and 69% of the tiles. Within each group there were one to three specialists for each type of transported material. In four groups one individual was specialist for both materials (Fig. 3). There was no significant correlation between the SI for cotton balls and the SI for tiles (r_s = -0.14; p = 0.38).

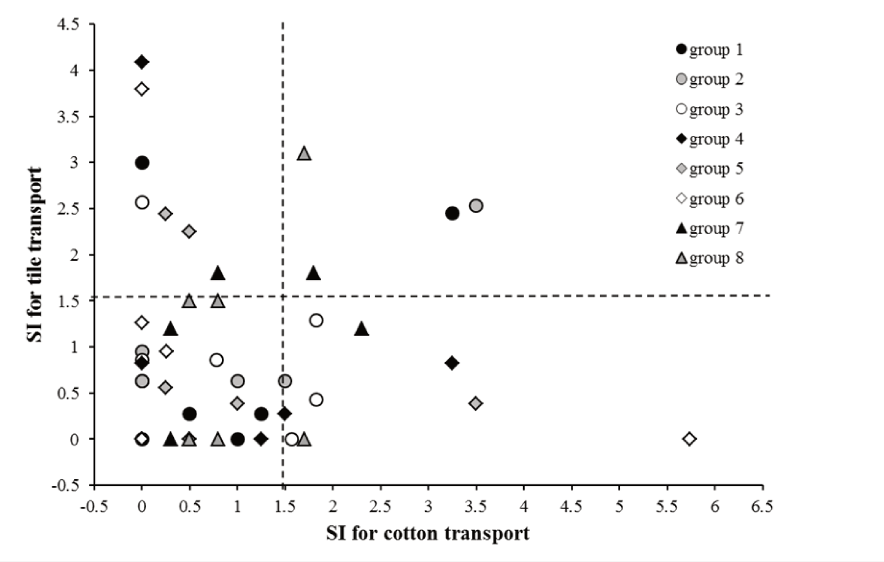

Figure 3. Individual Specialization Indices (SI) for transport of cotton balls (X axis) and tiles (Y axis) in eight groups of mound building mice. Each point represents an individual (n = 48) and the mice of a given group are represented by a common symbol. Carriers for tiles are above the horizontal dotted line and at the left of the vertical dotted line (n = 11) and carriers for cotton balls are below the horizontal dotted line and at the right of the vertical dotted line (n = 12). Specialists for both cotton balls and tiles are situated above the horizontal dotted line and at the right of the vertical dotted line (n = 4).

Discussion

The transport tasks observed in this study were temporally organized in a sequence. Every day animals within a group tended to transport the cotton balls first and then the tiles, even if some overlapping occurred between the two tasks. Under laboratory conditions, mice had experienced cotton balls since birth, whereas they discovered the tiles only in the first day of the experiment. The novelty of the tiles could have affected the temporal dynamic of transport. However, over the four days of test the difference between the mean of the latencies of transport for each material was always significant, with cotton balls being transported before tiles. This result suggested that the novelty of the tiles was not the main cause of the sequential work organization. In natural conditions, plant material is transported before tiles. The observed dynamic of transport in our experiment followed the same pattern. In the field, nevertheless, the transportation of plant material lasts for several days and it is the same for the transportation of tiles. In our experiment the sequence was repeated each day of test. This match between patterns allows us to explore the mechanisms underlying the organization of work in *M. spicilegus*.

Our results confirmed the presence of mice specialized in the transport of a given material, as previously reported (Hurtado et al. 2013). Moreover, our data revealed that the individual specialization was robust and occurred even when both materials were available simultaneously. This behavioral specialization can be explained by differences in individual response thresholds for each material. The response threshold models propose that individuals have internal thresholds for responding to task-related stimuli and that the variation in task thresholds between individuals can generate various behavioral patterns at the basis of the division of labor (Gordon 1996; Beshers & Fewell 2001).

Individuals that transported both materials in a given day tended to transport cotton balls first and tiles after. This result suggests that those individuals followed a simple rule: “transport cotton balls and then transport tiles”. The sequence of transport observed at the group level could have resulted from this simple rule if all the mice transported both materials. However, only a few mice transported both materials in a sequence, and most of the specialists transported only one material. The scarcity of events where both materials were transported by the same animal in the same day suggested that the organization of work was regulated at an upper level, where the individual behavior of mice is coordinated through local external cues, giving rise to a collective emergent pattern. The switch between the transport of cotton balls and the transport of tiles might have been modulated by the result of the transportation task itself. This mechanism is usually referred as stigmergy, defined as the “stimulation of workers by the performance they have achieved” (Grassé 1959). The external cues could have been the accumulation of cotton balls in the pile or the exhaustion of the available material, since a fixed and limited number of items was provided each day. Once the building mound would reach a certain point or the material would be no longer available, those who were working stopped, and those who were not active for this task began to transport tiles.

Addressing organization of collective work, mound-building mice, although a mammalian species, act like social insects. As a consequence the centralized organization of collective work does not appear as a general mammalian characteristic, but as a specificity of the human species instead. This human organization rests on a hierarchical structure with organizers giving orders to subordinates performers within a pyramid organizational system (Pennings 1973). In social insects, a form of centralized organization is observed in some ant species. For certain tasks, there are “key individuals” that can act as catalysers of the task by eliciting the work in others, as performers by doing most of the work or as organisers, not taking place directly in the action but being part of the organization of the workers (Robson & Traniello 1999b). However, examples are very rare and there is no firm evidence of this kind of organization in construction tasks. In *M. spicilegus*, there is no leadership inside the group of juveniles during the mound building. There is also no competition to access to the building material (Serra et al. 2012) and even if adult mice are aggressive against any unfamiliar individuals (Patris et al. 2002), tolerance and cooperation between mice in overwintering groups are the rules (Gouat et al. 2003a).

Without this pyramidal organization, mound-building mice succeed in constructing an imposing an efficient overwintering structure (Hölzl et al. 2009; Szenczi et al. 2012). Our results suggest that a plausible model for both laboratory and natural conditions could be a temporal sequence of work modulated by individual specialization paired with simple individual decision rules such as “transport the plant material until the structure is advanced enough and/or the material is over". However, in natural conditions, other factors could be added to this model. For instance, the collect of plants will have an increasing cost as mice will have to go further in order to collect plants when the ones around the mound are already collected. Not only the energetic expenditure will increase, but also the risk of predation will be more important. On the other hand, the risk of tile harvesting is constant, since the tiles are built and collected in the immediate surroundings of the mound. We do not know how this difference between the collect of both materials will impact in our suggested model. This is why a more extensive study in natural conditions is needed to confirm our hypothesis and fully understand the mechanisms of the organization of work during the communal building of the mound by *M. spicilegus*.

Acknowledgements

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Individuality and specialization

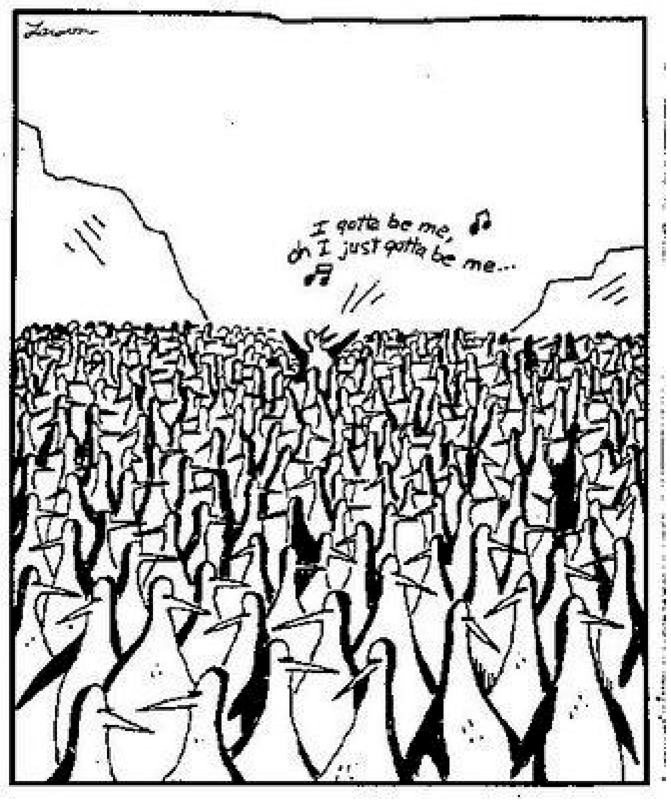
One of these things is not like the others,

One of these things just doesn't belong,

Can you tell which thing is not like the others

By the time I finish my song?

Sesame Street



Mus spicilegus has showed a specialization when facing different transport tasks (Hurtado et al. 2013). The carriers for two different materials (cotton balls and plaster tiles) were different, whatever the materials were presented sequentially or simultaneously, and they maintained their carrier status during several consecutive days. This specialization is set from the very beginning of the experiment, suggesting that specialization can be the result of pre-existing individual characteristics. The mechanism involved could be a threshold model, where individuals with different response thresholds for certain stimuli respond differently when facing the same stimulus (Beshers & Fewell 2001). Consequently, animals with a low threshold for a given task-related stimulus will become specialists for this task, while those with a higher threshold will not be specialists for this task but might be specialists for another task or remain inactive. The features that are source of individual variability include, but are not limited to, morphology, genotype, development, physiology and learning and experience (Duarte et al. 2011). In our work, the task-related stimulus is the presence of the building material. In order to explain specialization by differences between individual thresholds, we needed to individually characterize the mice before they are confronted with the transport task and for doing so we focused on behavioural traits, since the effects of body weight, age and parental origin have already been studied and ruled out as factors influencing the specialization (Serra et al. 2012; Hurtado et al. 2013). These behavioural traits are expected to be at the origin of the differences of threshold among individuals.

The affinity for the building material might influence the onset of the specialization. The non-carrier individuals for a given material are often carriers or occasional carriers for the other material. This suggests that the difference on the response threshold due to the affinity for a given material can determine the level of specialization of an individual, with animals with higher levels of affinity for the building material being more likely carriers than those with lower affinity levels.

However, the specialization can be the product of intrinsic individual characteristics, independently of the transported material. In the work of Nejdi et al. (1996), a specialization was observed in a diving-for-food paradigm. Some mice in a group dived to get food and retrieved it to the rest of the group. There were also individuals that only dived sporadically, and some individuals that never dived. The animals that carried the food to the nest had a distinctive behavioural profile, showing less anxiety than the other members of their group. In *M. spicilegus* we also found that some individuals carried most of the material, while others only transported sporadically or never transported any material. According to the work of Nejdi et al. (1996) we expected to see a similar pattern, with carriers being less anxious, more active and bolder. Since retrieving materials to the mound can be hazardous due to predation risks, a less anxious individual might be more fitted to this task. In the same way high levels of locomotion and neophilia might be needed to go away from the mound to retrieve the building material.

It is tempting to only look at those animals that are the most involved in a certain task. It is indeed fascinating to wonder why they work more than the others. But those animals that do not intervene at all in a certain task deserve our attention as well. They are the animals that differ the most from the rest of the individuals of the group, since the individuals that perform a certain task but are not specialists share with the specialists the drive to perform such task. In our research, non-carrier individuals could be more anxious, less neophilic, less active and with lower levels of affinity for the building material than the rest of the group. Both approaches need to be evaluated.

1. Study of behavioural traits

When observing a group of animals, an untrained observer could think that all animals of a group are the same. But if the observer just looks a little deeper, he will see that even in a seemingly uniform group of animals, individuals have features that make them unique. For example, individuals do not react in the same way when facing certain situations such as contact with novelty, predation or social interactions (Wilson et al. 1994), and this difference of reaction is a great source of variability inside a group. This is why even at a simple level of observation it is relatively easy to establish differences in behaviour between individuals. Despite this common acknowledgement of behavioural differences among animals, the study of behavioural individuality in animals is relatively recent. The idea of the existence of individual characteristics in animals was often rejected in science as simple anthropomorphism (de Waal 2002). But during the last decades, the study of animal behavioural individuality has blossomed. There are numerous studies on behavioural individual differences in animals ranging from ants to gorillas (e.g.Retana & Cerda 1991; Gold & Maple 1994; Gosling 2001).

In vertebrates, the core traits that appear to be common to most of the studied species are emotionality/fearfulness, exploration/novelty seeking, activity, aggression and sociality (Gosling 2001) . They form a relatively consistent multidimensional construct that underlies and modulates behavioural responses (Martin & Réale 2006; McDougall et al. 2006).

In rodents, factorial studies on behaviour during a standardized test showed the presence of two consistent traits:

- An anxiety trait (inhibitory behaviour when facing a naturally aversive stimulus, also defined as the emotional anticipation of an aversive situation difficult to predict and control and which is likely to occur (Ramos & Mormède 1998)).
- An activity trait (horizontal and vertical movements, i.e. ambulation and rearings).

When exploration of novel objects or environments is included, a third independent factor has been found and named exploration or neophilia, defined as the propensity to approach novel situations (Ibáñez et al. 2007). Anxiety and neophilia represent the responsiveness to two opposed stimuli: anxiety is the reaction facing a potential punishment that results in harm avoidance behaviour, while neophilia is the reaction towards a potential reward that elicits the novelty seeking behaviour (Ray & Hansen 2004).

2. Behavioural tests

Studying the interindividual differences among animals implies that we must be able to detect and reveal such differences. The vast majority of the behavioural tests used to study such differences have been developed by the pharmaceutical industry to be used on rodents to study the effects of anxiolytic drugs. Basically, they confront the subject to anxiogenic stimuli that can be physical (e.g. extreme temperatures, food deprivation, electric shock) or more psychological (e.g. novel environments, strongly illuminated areas, open spaces, heights) (Ramos & Mormède 1998).

A test must meet four quality requirements in order to be appropriate to study behaviour. The administration and notation of the test must be standardised, with the animal as the only changing variable. The test also needs to be reliable and the scoring of the test has to be sensitive, with individual behavioural differences translated to an appropriate behavioural scale. At last, the test needs to be valid, measuring accurately what the researchers want to measure (Diederich & Giffroy 2006).

The most common standardized tests used on rodents to measure anxiety are the open field, the elevated plus maze and the light/dark box (Ramos & Mormède 1998; Chapillon et al.1999; Kazlauckas et al. 2005)(Fig. 20). For these tests, the subject is confronted to an unfamiliar stimulus and an environment that presents a gradient of anxiogenic factors, i.e. central zone/peripheral zone, open arms/closed arms and light/darkness respectively. More anxious animals will tend to spend more time in the less anxiogenic zones. Choosing the appropriate test can sometimes be tricky. For instance, the dark/light box paradigm has been extensively used with laboratory rats and mice. But its effectivity is compromised when using non-albinos individuals, since they do not experience the same level of anxiety when standing in the lightened area (Ramos & Mormède 1998). Parameters measured to determine anxiety levels are numerous and of different nature, such as defecation, urination, rearings, attempts to escape, hesitations, freezing time. It is imperative to determine which of these variables are relevant and linked to the searched behavioural traits.

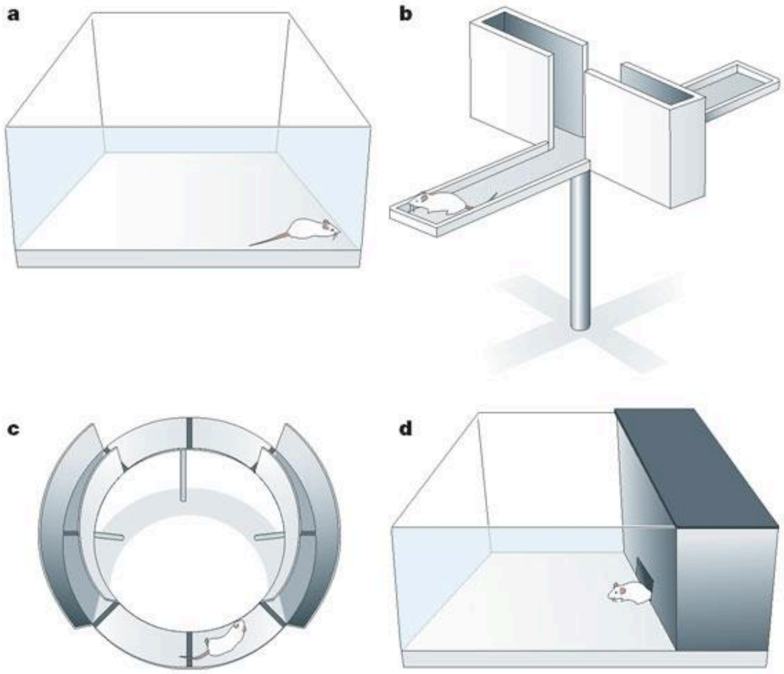


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Figure 20: Tests for assessing anxiety in mice. a) open field, b) elevated plus maze, c) elevated zero maze, d) light/dark box.

Activity is often measured in the same apparatus used to obtain data from anxiety or exploratory behaviours. The distance covered is the variable that most commonly indicates activity.(Ramos & Mormède 1998; Ibáñez et al. 2007).

Neophilia is often measured with the novel object test and free exploration tests such as the hole board test or novel location preference (Ray & Hansen 2004). Variables measured to determine neophilia are number of stretching towards the object, sniffing, direct contact in the case of the novel object, frequency and proportion of head-dipping (i.e. when the animal puts its head in a hole) in the hole board test and time spent on the novel environment, latency to get out of the familiar cage and escape for novel location test.

QUESTION 2: Is there a link between the individual profile and the performance at a transport task?

Manuscript in preparation for submission

Introduction

The juveniles of the mound-building mouse (*Mus spicilegus*) build a mound communally to overwinter (Szenczi et al. 2011). The building process can be divided in four successive steps. First, the animals collect and gather plant material. Then, they cover the pile of plants with soil. After the pile is covered, they make and transport clay tiles to the top of the structure. Finally, they dig galleries under the mound (Hölzl et al. 2009). Studies made under laboratory conditions have revealed that this species shows a real specialization on transport of material while building the mound. In previous works held under laboratory conditions, mice were confronted to two building materials: cotton balls and plaster tiles, in order to replace plant material and clay tiles found in the field. Mice can fit in one of three categories based on their transport performance. The individuals that specialize in this task are called carriers, while those which transport some items but less than carriers are occasional carriers. Finally, there are individuals that do not transport any item, the non-carriers. This categorization was only valid for a given material; mice could transport one of the materials and not the other, and there are different specialists for different materials. This specialization appeared from the moment the material became available to mice (Serra et al. 2012; Hurtado et al. 2013; Hurtado et al. 2014). This early onset of specialization suggests that there might be pre-existent interindividual differences that could be at the origin of this specialization.

The model that could take into account the specialization of carriers following individual differences is the response threshold model. This model assumes that every individual has internal thresholds to respond to task-related stimuli, and the variation of such thresholds among the members of the group generates division of labour. Individual with the lowest threshold for a certain task-related stimuli are the specialists for that task (Beshers & Fewell 2001).

A study in the house mouse (*Mus musculus)*, a close species of *M. spicilegus* (Guénet & Bonhomme 2003) showed that when facing a diving-for-food paradigm similar to the one used in Colin & Desor (1996) with rats, a pattern of behavioural differentiation occurred. Mice were confronted to a social interaction test where they needed to dive in order to reach the food dispenser and then carry the food to the cage. Based on their behaviour when facing this paradigm, mice could belong to one of three categories: major carriers, sporadic carriers and non-carriers. The animals were also tested to establish their anxiety levels, half of them before the social interaction test and the rest after the social interaction test. Carrier mice showed less anxiety than non-carriers and showed a higher level of exploration of the "unsafe" parts of the dive-for-food experimental device when compared to non-carriers and occasional carriers. These results remain constant whatever the time the elevated-plus maze was performed, i.e. before or after the social interaction test (Nejdi et al. 1996).

Following this example, we could then think that individual differences in behavioural traits such as anxiety levels or activity could be at the origin of the specialization observed in *M. spicilegus*, even when our paradigms were different and did not imply a physical constraint and relied on the building behaviour and not on a foraging situation.

The aim of our study was to determine if the specialization observed in this species could be originated by individual differences. Since morphological, age and sex differences were already ruled out as a source for specialization (Serra et al. 2012; Hurtado et al. 2013), we chose to focus on behavioural differences as the factor that could explain the specialization in this species.

The question of the influence of the behavioural profile on specialization can be answered with two different approaches. There might be a specific profile for carriers that make them different from the rest of the group, as seen in Nejdi et al. 1996. We expected the carriers to be less anxious than the other mice, since retrieving the building material implies that the mouse would face a higher predation risk in the field. We also expected the carriers to show more neophilia and activity in order to get away from the mound to get materials.

But we could also look at the question from another angle. The carriers might not have a particular behavioural profile, but the non-carriers might. Indeed, carriers and occasional carriers perform the same work, only at different levels, while non-carriers show a totally different behaviour by not performing any transport. This differentiation could indicate that non-carriers show a different behavioural profile when comparing them with the rest of the mice. For example, they could be far more anxious than the rest of the group, or they could be less neophilic or active. In order to set apart both hypotheses, we compared the individual profile of carriers versus the rest of the group (occasional and non-carriers together) and consequently we did the same between non-carriers *versus* occasional carriers and carriers. We performed a battery of tests composed of the classic open field and novel location preference test on each individual to assess the traits associated with behavioural individuality such as locomotion, anxiety and neophilia and then we compared the results with their transport performance.

Another main characteristic of the specialization during the building of the mound is that the category of each animal regarding their transport performance for a given material is not a predictor of the category individuals will belong to when facing a different material. The performance showed by each animal for cotton balls is not a predictor for what their category will be when transporting plaster tiles (Hurtado et al. 2013). This result suggests that the existence of each category is likely to be associated with a difference in the affinity with a specific building material, i.e. some mice could show a preference for the building material and be more willing to touch it, handle it and carry it, being more likely to retrieve the material to the mound, and vice-versa. We measured the affinity with the building material in individual tests where the animals were given a unit of building material and we observed how the animal reacted. We then compared the obtained results with the individual transport performance, expecting that carriers will show higher levels of affinity with the material.

We expected to see a link between the behavioural profile (including the affinity for the building material) and the transportation performance of the individuals.

Materials and methods

Animals and housing conditions

Experimental animals derived from a stock of 80 wild mound-building mice caught in Hungary in October 1999 and bred to the F12 generation under laboratory conditions (20 ± 1 ◦C) with a 14:10 h light:dark cycle. The mice were housed in standard polycarbonate cages (28 × 41.5 cm and 15 cm high) with sawdust (Special Diet Services, Witham, Essex, UK) and cotton was provided for nesting material. Food (type M20, Special Diet Services, Witham, Essex, UK) and water were supplied *ad libitum*. Mice were weaned at 28 days. Twelve groups of six sibling individuals were formed, maintaining a 1:1 sex-ratio as far as possible, with two exceptions: one group of four males and two females and another group of two males and four females. For each set of tests, two groups were tested at the same time. They were housed in standard polycarbonate cages, with clean sawdust and 18 cotton balls for nesting material, and transferred to an experimental room. Thirty-six females and thirty-five males were tested. One male died before the beginning of the experiments.

a) Individual characterization

Mice were 37.18 ± 1.5 days old when experiments started. During the behavioural testing period (four days) mice were placed in individual polycarbonate cages (26 x 14 x 16 cm high) with sawdust, standard food and water *ad libitum*. These test cages were perforated in order to facilitate the free exploration test, and the perforation (4 cm) was closed with a plastic cap. The original nest of the litter was dispatched in the individual test cages in order to provide nest material for isolated mice. The isolation was performed during the daylight phase of the first day of experiments. All experiments were observed through a video-monitor situated in a contiguous room. For a given animal, tests were always performed in consecutive days, beginning with the open field test, followed by the cotton manipulation test and ending with the free exploration test. All tests were performed during the dark phase under dimmed red light. At the end of the testing period, mice were returned to their original cage with their siblings.

Open field test

The open field test in its low frightening version, i.e. low levels of light and sound (Ibáñez et al. 2007), was chosen because it allowed us to measure both the behavioural variables of anxiety and activity. It is more suitable than other tests such as elevated plus maze for young *M. spicilegus* which are not afraid of jumping out of the experimental device.

The test was performed on the nightlight phase following isolation. The open field consisted on a circular white plastic recipient of 42cm of diameter and 100cm of height to avoid any escape. Each mouse was gently placed in the centre of the surface and let free to explore during 5min after the animal started moving. Latency to make the first move after being placed in the open field was considered as freezing time. Movements were recorded with a Sony DCR-SR90E Handycam digital video recorder for later analysis. After each trial mice were returned to their individual cages, faecal pellets were counted and the recipient was cleaned with a solution of Cleansinald™. The videos obtained were processed with EthoVision 3.1 (Noldus Information Technology). The area of the open field was divided in two concentric zones of equal surface (693 cm^2), the peripheral zone and the central zone. Parameters recorded were time of freezing, distance travelled in centimetres, time spent in the central zone and time spent in peripheral zone, measured in seconds.

Cotton manipulation test

A coloured cotton ball, in order to distinguish it easily from the white cotton balls used in the nest, was presented to each animal in the test cage. Direct observations were carried. Time in contact with the cotton ball was measured with a chronometer during a 5-min period. Contacts were counted when the animal touched the cotton ball with the mouth or the forepaws. For statistical analysis, only time in contact with the cotton ball was considered, since other measurements showed to be redundant with this variable (data not shown).

Novel location preference test

The novel location preference test is based on the exploration of a novel place from a familiar place. We chose this test because it allows measuring novelty-seeking behaviour independently from any anxiety bias, since it provides an anxiety-free environment for the tested mouse (Roy & Chapillon 2004).

The apparatus was composed of the isolation cage linked to a larger polycarbonate cage (28 × 41.5 cm and 15 cm high) by a tunnel (PVC pipe, diameter 4 cm, 10 cm length). The isolation cage (familiar environment) contained sawdust bedding, nest and food and water *ad libitum*. In the second compartment (novel environment) there was no sawdust on the floor. The test started when both compartments were connected and the entry to the tunnel was opened. Latency to first entrance into the new compartment was measured when the mouse has the four paws inside the compartment. Once the mouse entered the new compartment, time in seconds in the new compartment was measured for a 5min period. If the mouse did not enter the new compartment in 10min the time of latency was set at 600s and the time in the new compartment was 0s. The novel environment was cleaned using a solution of Cleansinald ™ between each individual.

b) Specialization in transport tasks

Performance on transportation task

After behavioural tests and at least 96h before the beginning of the transport tests, mice were weighted and identified with a glass tag (length: 120 mm; diameter: 15mm) injected under the dorsal skin. Each group of six mice was placed in a large polycarbonate cage (28 × 41,5 cm and 15 cm high) connected to two tunnels (diameter 4cm, length 50cm) leading to a vertical cotton balls dispenser each (fig1). The cage contained sawdust bedding, water and food *ad libitum* and twelve white cotton balls as nest construction material. The entry to tunnels was closed until the beginning of the test. Each tunnel was encircled with two RFID antennas, one next to the cage and the other next to the cotton ball dispenser. Double antennas were used to accurately assess the exact time of entry to the tunnels, the direction of movements of mice and cotton balls and the time spent both in the cage and in the cotton dispenser (Serra et al. 2012).

At least 48h before the beginning of the transport tests, sixty-four coloured cotton balls were tagged with glass tags, the same as used to identify animals. Tags were glued to the cotton ball using hot glue.

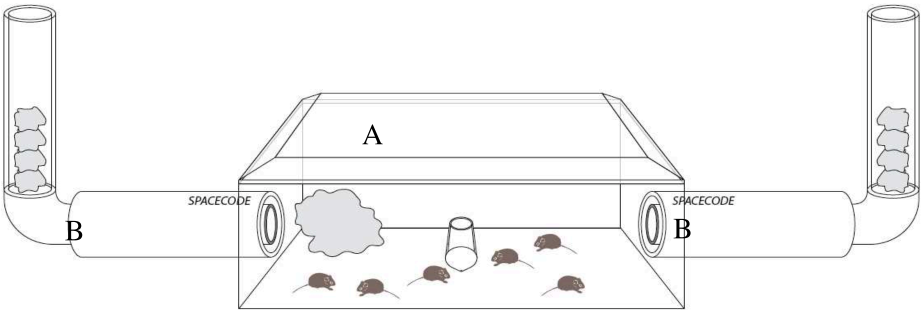

Figure 1: Mound-building device and cotton dispensers. A) Polycarbonate cage; B): Circular antennas (two for each tunnel); C) Cotton dispensers.

After four days of acclimation during which mice started a mound with the provided white cotton balls, the entry to tunnels was opened. On the first phase of the test, four coloured tagged cotton balls were delivered in each dispenser. This procedure was repeated the next day. Each passage of mice or transported cottons was detected by the antennas encircling the tunnels. When a cotton ball was seen in synchrony with a mouse during the passage between the two antennas of each tunnel, it was assigned as being transported by this mouse.

Statistical analysis

a) Individual characterization

In order to reduce the number of variables and to determine a typology of the mice, we used a standard principal component analysis (PCA) on the correlation matrix of behaviour variables in the open field test (defecation, freezing time, time spent in central zone) and free-exploration test (latency to exit the nest cage, time spent out the nest cage). Time spent in periphery on the open field was left out of the analysis for being complementary with time spent in the centre of the open field. The factorial coordinates of each individual for the main axes were used as data in following statistical analyses.

Activity was estimated by measuring the locomotion inside the open field, i.e. the amount of centimetres each mouse moved inside the arena.

b) Specialization on transport task

As previous studies revealed that the specialists were constant through four days of experiments (Serra et al. 2012; Hurtado et al. 2013), specialization was based on a two-day experiment. The individual contribution of each mouse was obtained by dividing the number of cotton balls they transported during two days of RFID test by the total number of cotton balls transported by the group. If all the six animals of a group transported an equal amount of material then their individual contribution should be 0.167 (1/6). To determine the level of behavioural differentiation of a given individual we calculated a specialization index (SI), consisting of the ratio between its individual contribution and this equiprobability value. A mouse was defined as a carrier when its SI was equal to or higher than 1.45 and a non-carrier when its SI was equal to 0. Animals with intermediate SI were defined as occasional carriers (Hurtado et al. 2013).

C) Influence of the individual behavioural profile on specialization

In order to establish if carriers have a specific behavioural profile that differentiates them from the rest of the group, we compared behavioural data from carriers *versus* occasional carriers and non-carriers. Then, we compared the behavioural profiles of non-carriers *versus* carriers and occasional carriers to determine if non-carriers have a different behavioural profile than those animals that transport material. For both comparisons we used a permutation test for independent samples using group as stratum.

We also wanted to see if there were differences between the two most similar groups, so we compared the behavioural profiles of carriers *versus* occasional carriers using a permutation test for independent samples using group as stratum.

Data were analyzed with Statistica 8 (StatSoft Inc.) for the PCA and StatXact-3 (Cytel Software, Cambridge, MA, USA) for permutation tests.

Ethical note

Animal care and experimental procedures were approved by the Regional Ethics Committee in Animal Experiment N°3 of Ile-de-France (P3/2007/023). The number of mice used was restricted to the minimum needed for statistical analysis. Subcutaneous injection of the tag was performed by a specifically trained animal keeper. The behaviour of mice was systematically observed 24 hours after the procedure. Young mice behaved normally and did not seem to be affected by the injection. As the mice were not used in other experiments and could not return to our breeding stock for sanitary safety, they were killed at the end of the experiment. They were anesthetized with isoflurane and then put into a rising concentration of carbon dioxide.

Results

Specialization

The total number of carriers (i.e. SI > 1.45) was 18, ranging from 1 to 3 carriers per group. There were also 35 occasional carriers (1.45 > SI > 0) and 18 non-carriers (SI = 0) (Fig. 2).

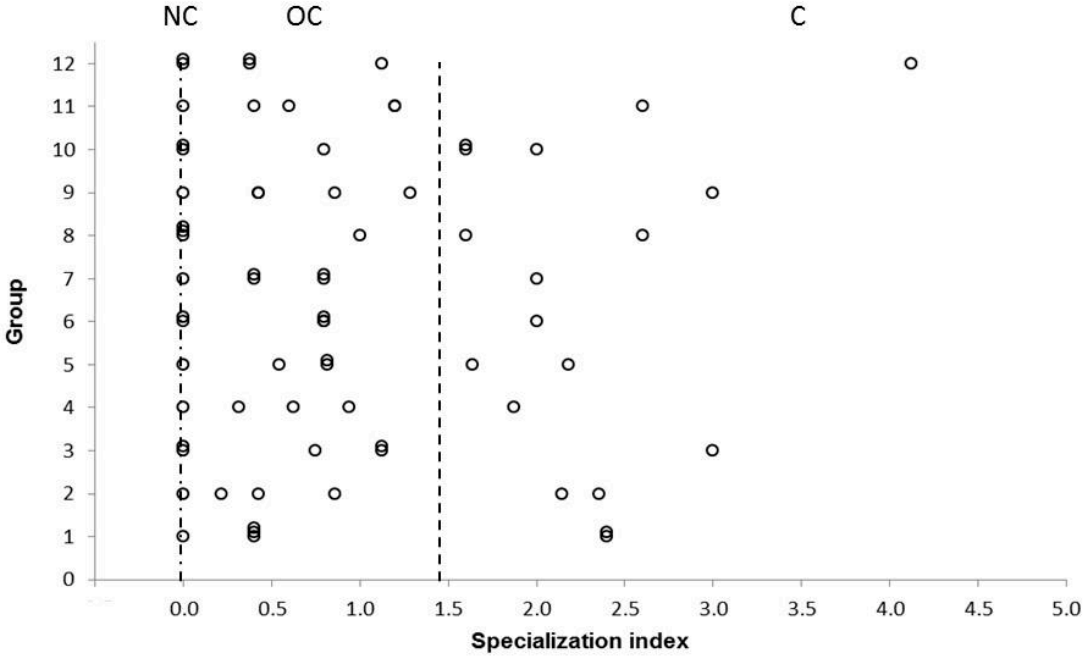

Figure 2: Plot of the transport performance of each individual, expressed on specialization index. Each horizontal line of dots corresponds to the animals of a given group (numbered 1 to 12). Non-carriers appear in the column of SI = 0. The vertical dotted lines indicates the threshold of specialization: individuals that are situated to the right of the right dotted line are considered as specialists (carriers, C), and the occasional carriers (OC, individuals which transported at least one item, SI > 0) are situated between the right and the left dotted line. Non-carriers (NC) are situated over the left dotted line.

Evaluation of behavioural traits in mice

Behavioural data obtained from the 71 mice was incorporated in the analysis. In the standard PCA, the two first components explained 52.3% of the total variance (C1= 27.1%, C2= 25.2%). The results of each test were mostly represented by a single axis. Data from the open field determine an “anxiety” axis. The first component opposed defecation in the open field and freezing time with time spent in the central zone of the open field. Data from the novel location preference test determines a “neophilia” axis. The second component opposed latency to exit the nest cage to time spent out of the nest during the free exploration test (Table 1, Fig 3).

	Factor 1	Factor 2
Latency	-0.255	**0.739**
Outside nest	0.173	**-0.777**
Open field defecation	**-0.783**	-0.112
Freezing time	**-0.533**	-0.269
Time Central	**0.602**	0.153

Table 1: Summary of the principal component analysis for open field and novel location preference test data. Each value represents the contribution of each variable to the factors 1 and 2. The highest contribution of each variable is in bold.

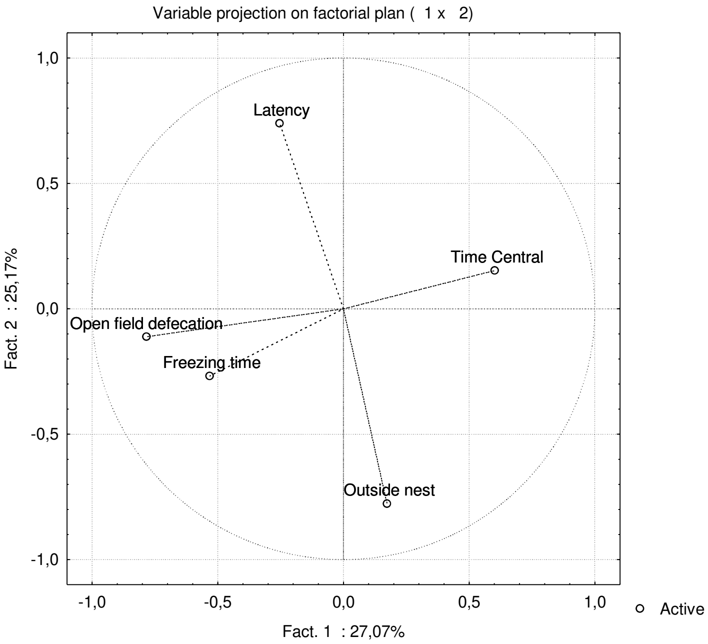

Figure 3: Variable projection on the factorial plan for factors 1 and 2.

Influence of the behavioural profile on specialization

The values obtained for each tested factor and for each category of carriers are shown in Fig. 4.

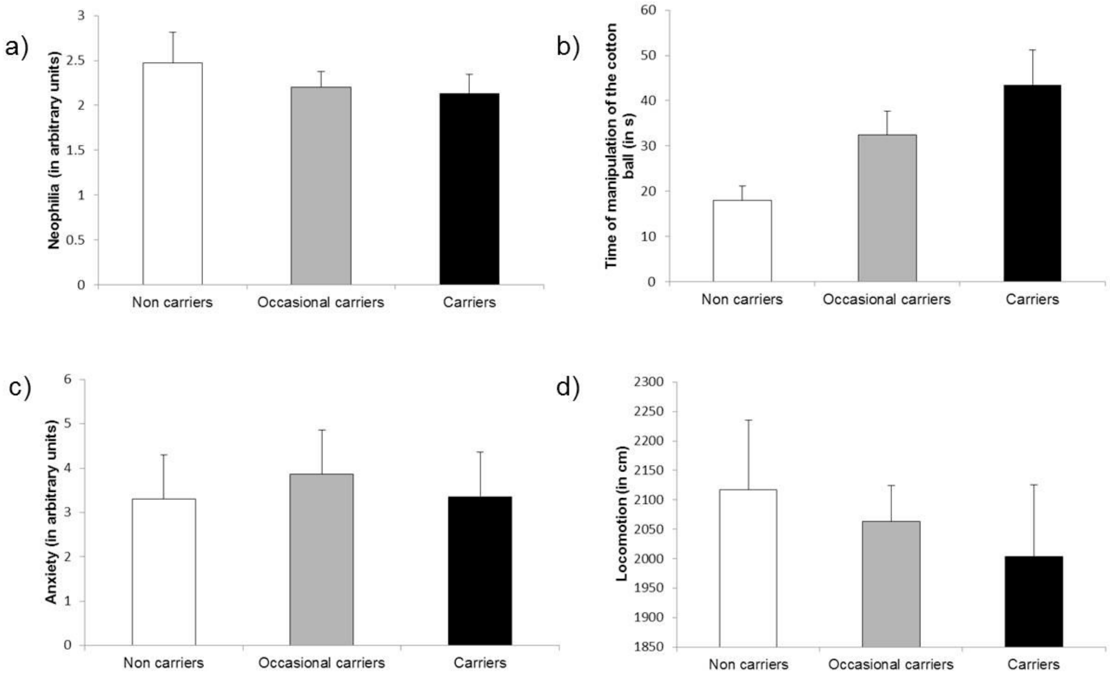

Figure 4: Comparisons of the behavioural traits of the three categories of carriers (white bars: non- carriers; grey bars: occasional carriers and white bars: non-carriers): a) neophilia (arbitrary units obtained in the PCA); b) affinity for the cotton ball (time spent with the cotton ball in seconds); c) anxiety (arbitrary units obtained in the PCA); d) locomotion in the open field in centimetres. Data are presented as mean ± SEM.

The first comparison was performed between carriers (n = 18) and the rest of the group (occasional carriers + non-carriers, n = 53). Carriers showed a higher affinity with the building material, spending more time in contact with the cotton ball than the rest of the group (p = 0.005). No significant difference was observed for locomotion: p = 0.22; anxiety: p = 0.39 and neophilia: p = 0.64.

The second analysis compared non-carriers (n = 25) and the rest of the group (carriers + occasional carriers, n = 46). Non-carriers clearly differ from individuals that carry at least one item for their affinity for the building material: they spent less time in contact with the cotton ball than the other mice of their group (p = 0.007). The rest of the comparisons did not show any significant differences (locomotion: p = 0.34; anxiety: p = 0.12; neophilia: p = 0.27).

According to the previous results, we search for differences between carriers and non-carriers. When comparing carriers with non-carriers, carriers spent more time with the cotton ball (p < 0.01), while there was no significant difference for the other factors (locomotion: p = 0.32; anxiety: p = 0.74; neophilia: p = 0.40).

No significant differences were observed when comparing occasional carriers to carriers (locomotion: p = 0.25; time spent in contact with the cotton ball: p = 0.25; anxiety: p = 0.08, neophilia: p = 1).

No significant differences were observed when comparing occasional carriers to non-carriers, although a tendency is noticeable in the affinity for the cotton ball (locomotion: p = 0.35; time spent in contact with the cotton ball: p = 0.06; anxiety: p = 0.09, neophilia: p = 0.28).

Discussion

We expected that the specialists for the transport task would differ from the other animals by their behavioural traits. Indeed, carriers do have a specific behavioural profile when compared with the other individuals of the group. They show higher levels of affinity for the cotton balls. But no other specific trait could be identified as being characteristic to carriers.

In *M. spicilegus*, individuals that do not perform any transport of material show lower levels of affinity with the building material when compared with other members of the group. Previous works have shown that the specialization index (S.I.) for one material was not a predictor of the S.I for the other material (Hurtado et al. 2013; Hurtado et al. 2014).

Non-carriers for cotton balls can stay as non-carriers or become carriers when facing with the plaster tiles change their category. We observed in some cases that non-carriers for cotton balls became carriers for plaster tiles (Hurtado et al. 2013; Hurtado et al. 2014). This indicates that non-carriers most probably perform other tasks inside the group. They might engage in other ergonomic tasks related with the building process such as digging galleries or transport of other material. Since the individuals that are non-carriers for one material are different from the non-carriers for the other material, the affinity for the building material might play an important role on the determination of non-carriers. A study on the relationship between the affinity for plaster tiles and the performance on transport of the material should be performed in order to assert the underlying mechanisms of specialization.

One possible mechanism that could explain the differentiation between carriers and occasional carriers could be that carriers and occasional carriers are in fact very similar, but it is their success at performing the transport the factor that determines the later differentiation (Bonabeau et al. 1996; Theraulaz et al. 1998c). It has been demonstrated in ants that previous experience can determine their later behavior. Ants that were successful in their first foraging attempts showed a high propensity to perform such task and one month later they were specialized as foragers, while ants that were repeatedly unsuccessful decreased their foraging activity and one month later specialized in brood care (Ravary et al. 2007). In our case, we can hypothesize that successful retrieval of material could elicit the specialization as a carrier. However, the success in the transport of cotton balls did not affect the later performance of mice while transporting plaster tiles (Hurtado et al. 2013). This could mean that each transport task is considered by mice as a totally different task depending on the material. This would be the case even if both materials were present simultaneously (Hurtado et al. 2014). Indeed, both tasks differ not only on the nature of the transported material, but also in the motor sequences needed to perform the task. Cotton balls are often pushed with the forepaws or pulled with the mouth while tiles are retrieved on the mouth, being heavier than cotton balls (Hurtado et al. 2013). Also, in nature both tasks are separated by the second step on the building process (i.e. covering the plant pile with soil). This could explain why the experience obtained by transporting cotton balls has no effect on the performance of the transport of plaster tiles. However, further studies modifying the success or failure to accomplish the task could be useful in order to validate this hypothesis.

In natural conditions the building of the mound is essential for the survival of juveniles through winter. However, the building process can be risky by exposing the animals to predators while working. Since so much is at stake, the system of partition of the tasks needs to remain flexible to cope with the possibility of one or several individuals disappearing. In this work, the level of affinity for the building material was associated with the level of specialization. However, those levels of affinity varied between groups. It was not the absolute value of affinity that determined the differentiation; it was the differences of affinity between the animals inside a given group. As a consequence, if a carrier disappears another animal with the highest level of affinity with the transported material should take its place. The dynamic of specialization when the composition of the group is altered needs to be studied in order to comprehend all the different factors that can intervene in the organization of building work in *M. spicilegus*.

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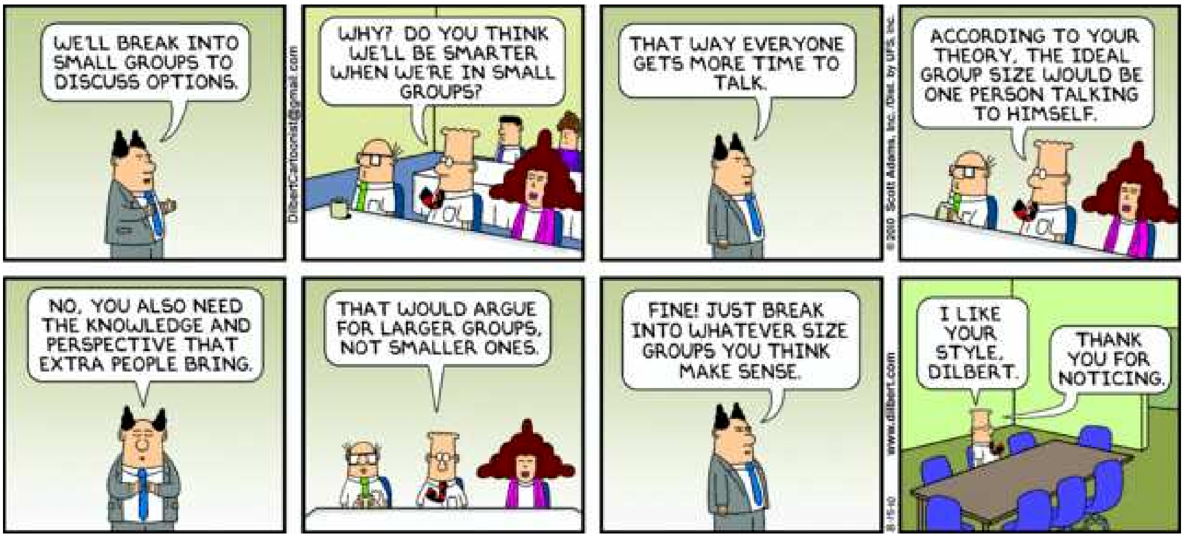
Social affinity, group size and organization of work

"Plus on est des fous, plus on rit"

« Éramos pocos y parió la abuela »

("We were few and the grandmother gave birth", Spanish saying, used when there are unexpected and undesired visitors).



In previous works, we have demonstrated the existence of a specialization during the building task in *Mus spicilegus*. We have also searched for the individual characteristics that could be at the origin of such specialization, and we found that the affinity for the building material plays a role in the behavioural differentiation inside the group. However, the effect of the social regulations inside the group was not addressed before. Indeed, an individual's behaviour depends on two balancing forces: its assessment of the environmental clues and its interactions with other members of the group (Pacala et al. 1996). One of the clues available is the size and composition of the group. This factor can change over time, since in nature the animals are exposed to variable conditions so the group size is likely to suffer perturbations due to disappearing of some of the components of the group, e.g. due to predation or other causes of death. The construction of the mound is crucial for the survival of the animals during the winter; consequently the group needs to be flexible, since it is likely that some loss of individuals will occur.

But the scenario can be different depending on the role of the missing individual. Inside a homogeneous group in terms of age and morphology, there is still a great amount of variability among individuals. The organization of the group labour can be modulated by some individuals that are crucial for the good proceedings of a certain task, that sometimes are called "key individuals" (Robson & Traniello 1999a). For example, some individuals can perform way more of the task that the other members of the group, even if some others might as well take part in the labour. The role played by these performers is capital, since the removal of such individuals will result in a considerable loss of efficiency, even when a replacement is set up. In nest moving ants *Formica* *sanguinea* and *Camponotus sericeus*, if those that replace the missing individuals are also removed, the nest emigration stops. In the other hand, the remaining individuals can sometimes replace the missing specialist with success. In the ant *Neoponera apicalis*, a part of the colony engages more frequently in brood care while the rest engages more often in external tasks such as foraging. If the original colony is splitted in two, with one group formed by nurses and the other formed with the rest of the original colony, both sub-colonies tend to re-establish the former collective activity profile, with nurses, foragers and inactives (Lachaud & Fresneau 1987).

The difference in group size can change the social network inside the group. In a study using the “diving-for-food” paradigm in groups of six and three rats, researchers obtained two different profiles: “carriers” (individuals that dive and retrieved the food to the nest) and “noncarriers” who stayed in their home cage and stole the food retrieved by the carriers. All groups of six presented both types of rat, whereas in three rat groups, half of the groups were constituted by carriers only (Thullier et al. 1992). In social insects division of labour has been positively correlated with number of individuals in the colony. (Jeanson et al. 2007). However, there is evidence of cases where group size might not have any consequence on the task allocation inside the group. The ants *Tapinoma erraticum* transport their brood into a different nest location when their original nest is disturbed. Workers show different levels of performance in the transport task, with very active individuals, individuals that do not engage at all in brood transport, and an intermediate level with very variable level of transport. The proportion of active transporting individuals remained stable even at different group sizes, going from three to twenty individuals, with a number of transportable items (nymphs) that matched the number of individuals. Also, in artificially formed groups of with only “active” or “inactive” individuals, there is a change in the behavioural response of individuals that makes the organization of work in these groups to be similar to the organization of the original colony (Meudec 1977). In the social wasp *Polybia occidentalis* the proportion of specialists remained constant across different sizes of colonies, even when in small colonies generalists switches tasks more often than in large colonies (Jeanne 1986).

QUESTION 3: What happens when the structure of the group is modified?

1. Social affinity and specialization

In our previous work we demonstrated that carriers show a higher affinity with the cotton balls than occasional and non-carriers. This result is consistent with a threshold model, where differences on internal thresholds on affinity with the cotton balls modulate the specialization. However, this is not an absolute rule. Even if carriers show higher levels of affinity with the cotton balls, some individuals can actually have the same levels of affinity than occasional carriers or even non-carriers and still be carriers, showing that other factors than the affinity with the cotton balls are influencing the specialization. For example, the affinity for the cotton balls can be modulated by another individual trait such as the social affinity. Individuals with low social affinity are expected to be more likely carriers than those individuals with a strong social affinity that might not want to stray from the other members of the group. Also, a bigger group could be more attractive to social animals and they would prefer to stay in the group instead of going to pick up the construction material. In the other hand, a smaller group would be less attractive and then the material would be attractive enough to every individual in the group, making the heterogeneity disappear.

To determine the influence of the individual social affinity in the transport performance of each mouse, we measured this feature and we compared it to the level of specialisation. We expected the non-carriers to have a high level of social affinity, while specialists should show lower social affinity than non-specialists. Also, the levels of social affinity should be higher in larger groups.

Materials and methods

Animals and housing conditions

Since the experimental conditions, the housing of mice and the use of RFID technique have already been described in this work, only the particularities of the following experiments will be described here. For more detail on animal housing and the manipulations concerning the RFID technique used to monitoring the animals, please see Hurtado et al.2014 or previous work in this thesis.

We tested 80 animals in 8 groups of 4 siblings (32 animals, thereafter referred as G4) and 8 groups of 6 siblings (48 animals, thereafter referred as G6), with a balanced sex ratio. In total, forty-one females and thirty-nine males were tested. Each group was housed in a standard large polycarbonate cage, with clean sawdust and 18 cotton balls for nesting material, and transferred to an experimental room. Each animal was tagged with a glass transponder following the procedure described in Serra et al. (2012).

Experimental procedure

In order to test the social affinity, mice were isolated in a small cage (26 x 14 x 16 cm high) containing part of the nest material from the home cage. This small cage was linked with the home cage (28 × 41.5 cm and 15 cm high). Their siblings were inside the home cage behind a transparent fence with holes and the isolated individual could see and smell its mates (Fig.1). Each test was video-recorded and observations were made using the recordings. We measured the latency to leave the small cage (“Latency”). After the mice left the small cage, data was collected during five minutes. The measured parameters were the time spent in the small cage (“Smallcage”), the time spent in the large cage but away from the fence (“Middle”), the time spent in contact with the fence near its siblings (“Fence”) and the number of contacts through the holes of the fence (“Nb contact”).

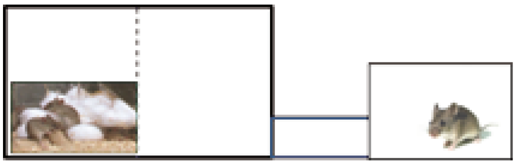


Figure 1: Social affinity testing device. The mouse is placed in the small cage and is separated from the rest of the group by a transparent perforated fence.

After the social affinity test, we tested the animals on the transport task for two consecutive days, providing 2 cotton balls per animal and per day (8 cotton balls per day for groups of four, 12 cotton balls per day for groups of six). We determined the specialization index (SI) for each individual (see Annexes for detailed methods).

Statistical analysis

In order to reduce the number of variables and to determine a typology of the mice, we used a standard principal component analysis (PCA) on the correlation matrix of behaviour variables in the social affinity test. The factorial coordinates of each individual for the main axes were used as data in following statistical analyses. The two first components explained 79% of the total variance (C1= 56.9%, C2= 22.1%). The first axis mostly represented social affinity, with high social affinity values placed in the positive extreme (table 1, figure 2). Contrary to the other variables Latency has its major contribution on the second axis.

	Fact. 1	Fact. 2	Fact. 3	Fact. 4
Latency	0.21	-0.85	-0.48	-0.08
Smallcage	-0.98	0.02	-0.18	0.11
Middle	0.73	-0.42	0.47	0.26
Fence	0.90	0.26	-0.08	-0.34
Nb contact	0.71	0.38	-0.49	0.34

Table 1: Summary of the principal component analysis for the social affinity test data. Each value represents the contribution of each variable to the factors 1 and 2. The highest contribution of each variable is highlighted in yellow.

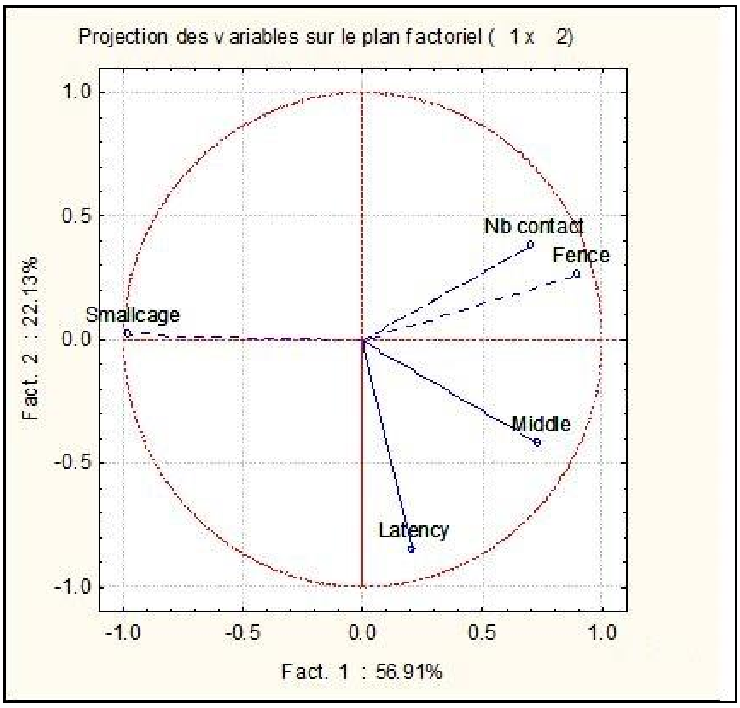


Figure 2: Variable projection on the factorial plan for factors 1 and 2.

We first compared between the levels of social affinity between groups of 4 individuals and groups of 6 individuals using a permutation test for independent samples to see whether larger groups were associated with higher levels of social affinity.

We then compared the level of social affinity with the performance in the transport task, using a permutation test for independent samples and using group as stratum. As we did repeated comparisons we used the Bonferoni-Holm's sequential procedure and the corrected p-value (P') is given (Holm 1979).

To check if there was a relationship between the levels of social affinity and the individual performance in the transport of cotton balls we used a Pearson's correlation using the Montecarlo procedure for both G6 and G4. Data on social affinity was normalised by subtracting the group mean to each value inside a group, in order to minimize the effect of variability among groups.

Results

The animals of groups of 4 individuals showed higher values of social affinity than the animals of group of 6 individuals ($P = 0.05$; fig. 3).

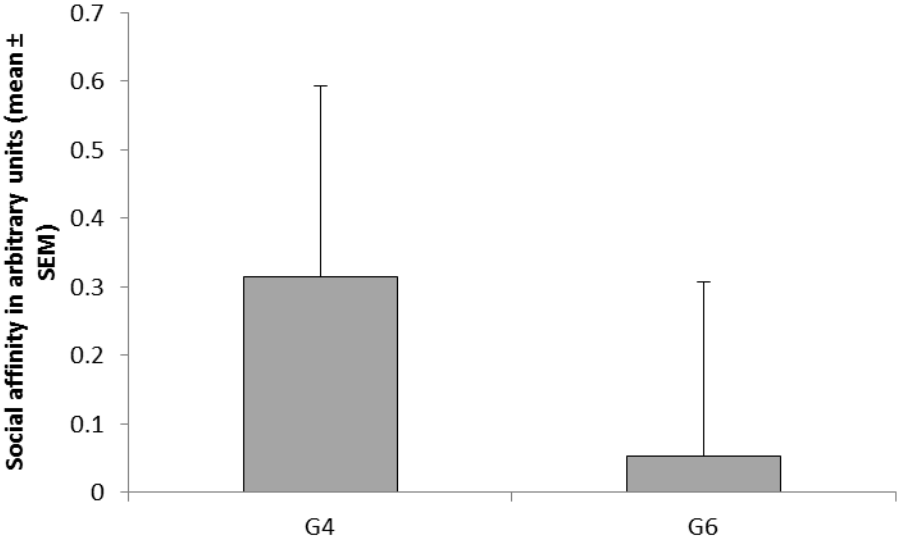
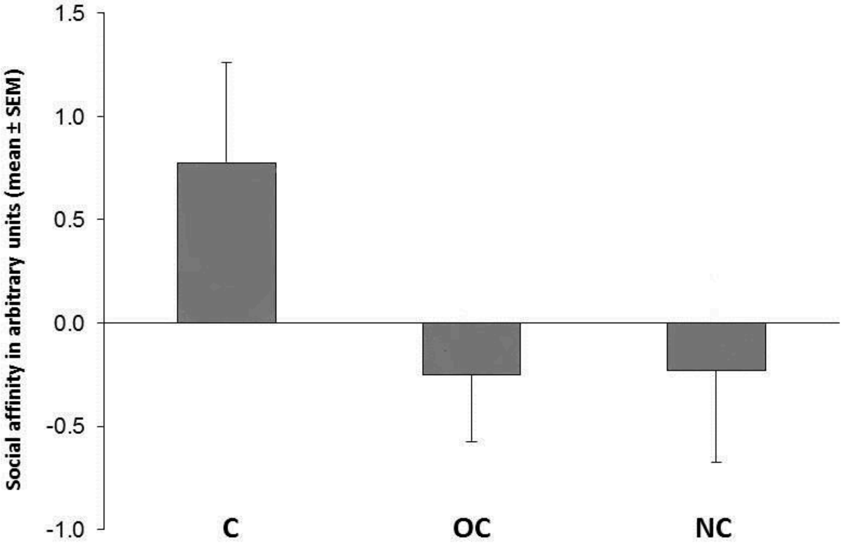


Figure 3: Values of social affinity in arbitrary units for both group sizes: G4 (four individuals) and G6 (six individuals). Data are presented as mean ± SEM.

In G6 we found 7 carriers (C), 26 occasional carriers (OC) and 15 non-carriers (NC). We did not find any significant differences when comparing carriers *versus* occasional and non-carriers (P = 0.54), carriers *versus* occasional carriers (P = 1), carriers *versus* non-carriers (P' = 0.40), occasional carriers *versus* non-carriers (P' = 0.45) and non-carriers with the rest of the group (P' = 0.24) (Fig. 4a).

In G4 we found 10 carriers, 10 occasional carriers and 12 non-carriers. When performing the same comparisons stated above, we did not obtain any significant result (P' = 0.88 for the highest difference) (Fig. 4b).

a)



b)

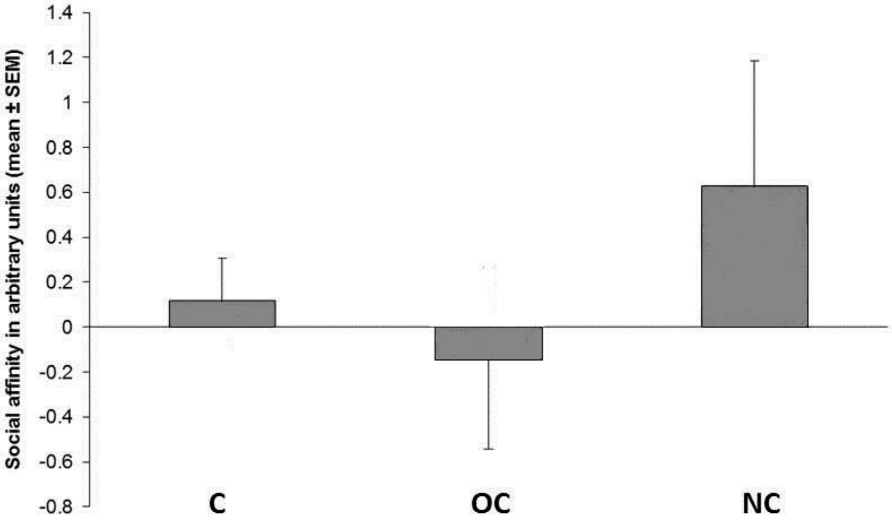


Figure 4: Comparison of levels of social affinity (arbitrary units from the PCA) of the three categories of carriers (C = open bars, OC = diagonal striped bars, NC = fishnet striped bars), in a) groups of 6 individuals and b) groups of 4 individuals. Data are presented as mean ± SEM.

Our hypothesis was that larger groups would be more attractive for the isolated individuals, but there is no evidence of a relationship between social affinity and the performance in the transport task. When looking at group sizes in the wild, mounds are been reported of being inhabited by groups ranging from 2 to 23 individuals. Our groups are not especially large, but they have a number of individuals that is consistent with observations in the wild (Garza et al. 1997; Poteaux et al. 2008; Hölzl et al. 2009; Szenczi et al. 2011)

Social affinity does not play a major role on the determination of the specialization in M. *spicilegus*. No marked effects were observed when comparing the social affinity values of different categories of mice.

2. Effects of the removal of specialists and non-carriers in the work organization

In *M. spicilegus* we observed a pattern of specialisation in the transport task, with the occurrence of carriers, occasional carriers and non-carriers in groups of six individuals. This specialisation was linked with different levels of affinity for the building material, with animals that showed a strong affinity for the material becoming the specialists for the transport. In the context of a threshold model, animals with a lower threshold for the contact with the material would be the more willing to transport it and therefore will be specialists.

Following the threshold model, the removal of the individuals with lower thresholds for the transport task would allow the animals with the lowest thresholds among the remaining mice to become specialists. In the other hand, if the animals that do not perform any of the transport (therefore those with higher thresholds for the transport task) are removed, the dynamic of specialization should stay the same since carriers with lower thresholds would continue to be the carriers. When removing the carriers, we expected that the removal of specialists would allow the specialization of new individuals. We also expected that the removal of inactive individuals would not have any strong impact on the work organization and carriers would continue to be carriers.

Materials and methods

Animals and housing conditions

The animals for this experiment were used in a previous experiment (see chapter “Individuality and specialization, p.108). Twelve groups of six sibling individuals were formed, maintaining a 1:1 sex-ratio as far as possible. They were housed in standard polycarbonate cages, with clean sawdust and 18 cotton balls for nesting material, and transferred to an experimental room. Thirty-six females and thirty-five males were tested. One male died before the beginning of the experiments.

Experimental procedure

Every animal received a RFID glass transponder following the procedure described in the annexes. Each group of six mice was placed inside the experimental cage with water and food *ad libitum* and 12 cotton balls to start their nest. The access of the tunnels was closed. After four days of acclimation during which mice started a mound with the provided white cotton balls, the entry to tunnels was opened.

On the first phase of the test, four tagged cotton balls were delivered in each dispenser each day for two days. The SI was calculated for each individual.

On the second phase of the test two mice were removed from each group. In six groups the two removed mice were the two specialists, while in the other six groups the two removed mice were two non-specialists, i.e. mice with the lowest contribution to transportation of cotton balls, which can be non-carriers and/or occasional carriers. The remaining four mice were tested for two more days. As during the first phase, four cotton balls were delivered in each dispenser for each day. At the end of the test, a new SI was calculated from data from the phase 2 for each animal. We included only four groups where non-carriers were removed in the analysis, since one animal died in one group and in other group one mouse managed to escape after the first phase and before the second phase.

Statistical analysis

In the six groups where carriers were removed, we compared the SI of categories occasional and non-carriers for each phase of the test, using a permutation test for independent samples, with group as stratum. In the four groups where non-carriers were removed, we first compared the SI of carriers for both phases and then we compared the SI of carriers and occasional carriers for each phase using a permutation test for paired samples.

Results

Removal of carriers (6 groups)

In the first phase there were 8 carriers, 21 occasional carriers and 7 non-carriers (n = 36). After the removal of carriers, there were 6 carriers, 17 occasional carriers and 1 non-carrier (n = 24). The three categories are preserved after the removal..

The SI of occasional carriers and non-carriers was significantly higher during the second phase of the test (P = 0.004 and P = 0.03 respectively). The SI of occasional carriers was higher than the SI of non-carriers during the first phase (P < 0.001), while the SI of the occasional carriers was not different from the SI of the non-carriers on the second phase (P = 0.57; fig. 5).

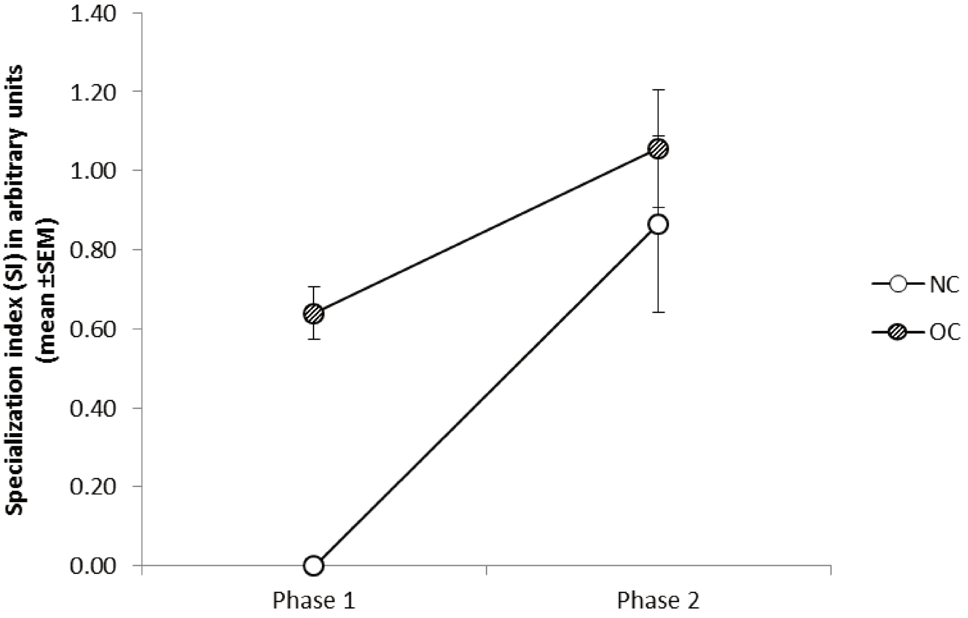


Figure 5: Specialization indexes for occasional carriers (striped dots) and non-carriers (white dots), for both experimental phases. Data is presented in mean ± SEM.

Removal of non-carriers (4 groups)

In the other 4 groups used for analysis there were 8 carriers, 8 occasional carriers and 8 non-carriers (n = 24). After the removal of the non-carriers we observed 5 carriers, 10 occasional carriers and 1 non-carrier (n = 16). The three-category structure is maintained.

The SI of carriers decreases during phase 2 (P = 0.03). In the other hand, the same comparison for occasional carriers did not show any difference (P = 0.19). During phase 1, there were significant differences between carriers and occasional carriers (P = 0.003), but during phase 2 those differences disappear (P = 0.93; fig. 6).

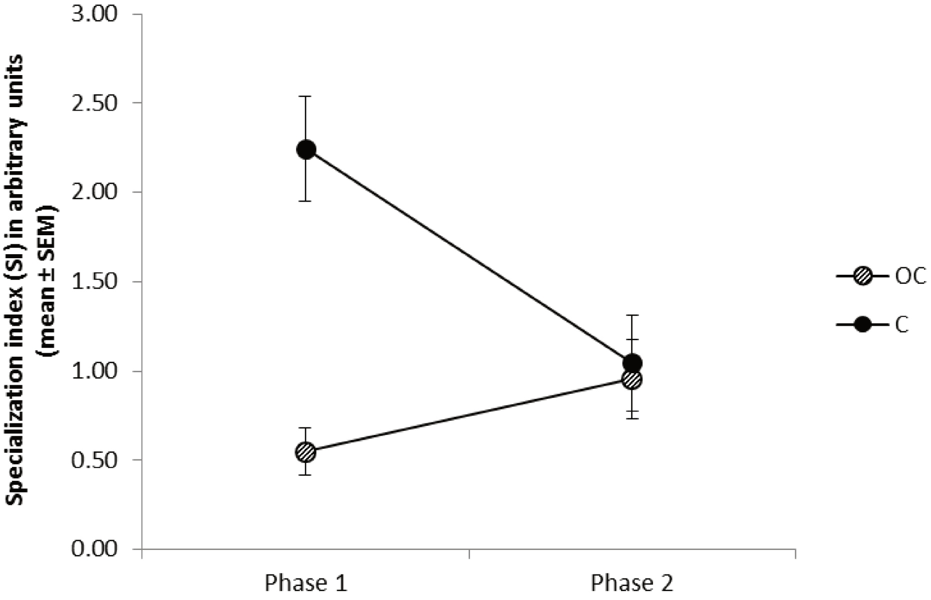


Figure 6: Specialization indexes for occasional carriers (striped dots) and carriers (black dots), for both experimental phases. Data is presented in mean ± SEM.

As we expected, when removing the specialists in a group other individuals become specialists and the work organization remains stable, with an overall increase on transport activity and a maintenance of the three categories. However, the removal of the individuals with the lowest SI, even when the work organization remained stable, caused a disturbance and the identity of carriers changed. The individuals that were carriers in phase 1 decreased their transport activity while occasional carriers increased theirs. However, the three categories were still present.

The removal of carriers or non-carriers caused a disturbance in the group, with a shuffling of individuals between the different categories.

3. Effect of the initial group size on the work organization

Removing individuals might lower the difference between individuals, causing a homogenization of the performances among the remaining animals. In a group, it might be individuals with very different thresholds that would result in strong differences among individuals’ performances. If we imagine a normal distribution for this threshold, individuals with extreme thresholds (specialists and inactives) would be less abundant than middle thresholds. In smaller groups the probability of having those middle values would be higher, resulting in more homogeneous groups.

We tested two different sizes of groups to see if the initial group size influences the work organization in this species.

Materials and methods

Experimental procedure

We used the animals of the experiment of social affinity (see p.120 for details on animals and housing conditions). After the test of the transport task and the obtainment of a first SI, the mouse with the lowest individual contribution of the group was removed from each group. By doing this, we obtained eight groups of three individuals and eight groups of five individuals each. Measurements continued with the remaining animals for two more days, delivering 6 cotton balls per day for groups of 3 individuals and 10 cotton balls per day for groups of 5 individuals. The SI for the second phase of the test was calculated for each animal.

Statistical analysis

First we analysed the permanence of the general structure for each group size. We compared the SIs of each category before and after the removal of one individual using a permutation test for paired samples.

We compared the SI obtained on each phase by animals that were carriers in the first phase. We also compared the SI of those animals that were occasional or non-carriers. We used a permutation test for paired samples.

We also compared the SI of both categories (carriers *versus* occasional and non-carriers) for each phase of the test, using a permutation test for non-paired samples, using group as a stratum.

Results

Groups of four individuals

In the eight groups of four individuals, during the first phase there were ten carriers, eleven occasional carriers and eleven non-carriers (n = 32). After the removal of one individual per group, there were seven carriers, thirteen occasional carriers and four non-carriers (n = 24). The structure with the three types of role (carriers, occasional carriers and non-carriers) is present before and after the removal and do not suffer any significant change (P > 0.78 for the three categories, Fig. 7).

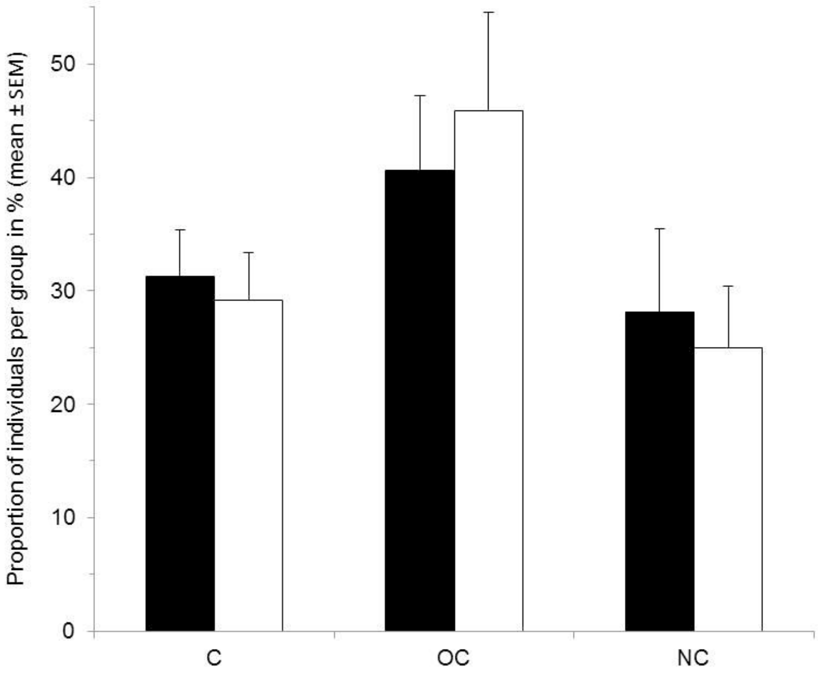


Figure 7: Proportion of individuals in each category per group of four individuals, before (black bars) and after (white bars) the removal of an individual, expressed as percentage (mean ± SEM).

The SI of carriers was significantly lower during the second phase of the test (P = 0.01), while the SI of the rest of the animals did not show any significant difference (P = 0.2). The SI of carriers was higher than the SI of occasional and non-carriers during the first phase, while the SI of the carriers was not different than the SI of the rest of the group on the second phase (P = 0.35; fig. 8).

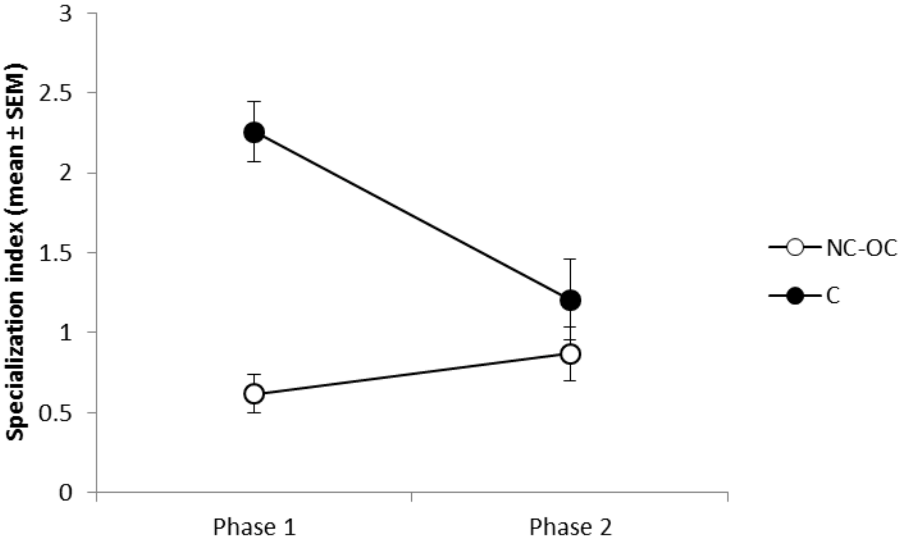


Figure 8: Specialization indexes of carriers (black dot) and occasional and non-carriers (white dot) of groups with 4 individuals for both phases of the test. Data is presented as mean ± SEM.

Groups of six individuals

In the eight groups of six individuals, we obtained seven carriers, twenty-five occasional carriers and sixteen non-carriers on phase 1 (N=48). After the removal of one individual, there were twelve carriers, seventeen occasional carriers and eleven non-carriers (N=40). The structure with the three types of role (carriers, occasional carriers and non-carriers) is present before and after the removal. The categories of occasional carriers and non-carriers did not suffer any significant change (P = 0.24 for occasional carriers and P = 0.438 for non-carriers) whereas the proportion of carriers increased significantly after the removal (P < 0.01; fig. 9).

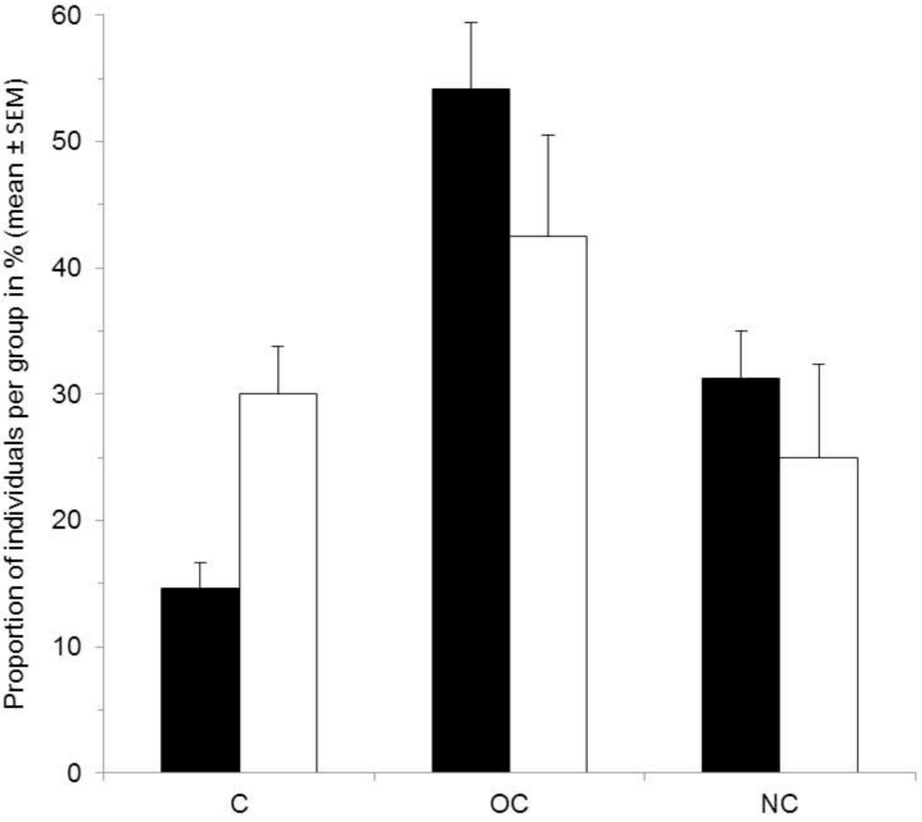


Figure 9: Proportion of individuals in each category per group of six individuals, before (black bars) and after (white bars) the removal of an individual, expressed as percentage (mean ± SEM).

There was no difference between the SI of carriers when comparing the first and the second phase (P = 0.59). The SI of occasional and non-carriers was higher during the second phase of test (P = 0.04). In phase 2, carriers maintained a higher level of SI than the other mice, but the variability in the groups increased and the difference between the groups did not reach the significant level (P = 0.051, fig. 10). However, we must remain cautious since the lack of significance can be attributed to the fact that in this particular case the number of carriers was lower than the number of individuals in the remaining categories (twelve carriers and twenty-eight occasional and non-carriers) .

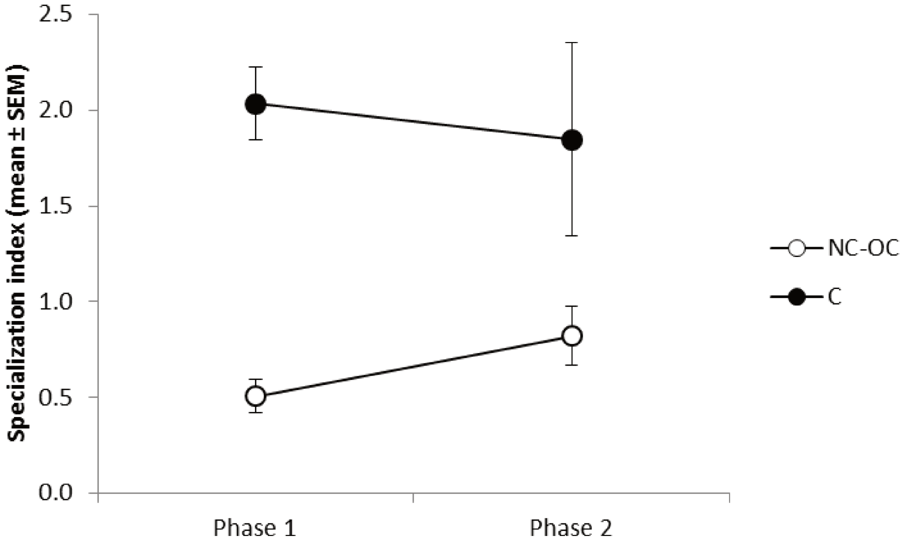


Figure 10: Specialization indexes of carriers (black dot) and occasional and non-carriers (white dot) of groups with six individuals for both phases of the test. Data is presented as mean ± SEM.

General discussion

In nature, group size can change for different reasons. In social insects it is common that colonies change its size over the course of their ontogeny (Jeanne & Bouwma 2002; Dornhaus & Franks 2006). In the case of *M. spicilegus* there is evidence of passages of mice between mounds, and it is thought that groups of mice can join other groups in early stages of the building process (Patrick Gouat, personal communication). Also, animals can disappear during the delicate process of building the mound due to predation, since working on the mound building increases the risk of being caught. All of these factors pointed to the direction that group size and group composition can vary all along the building of the mound. Animals should then be flexible enough to cope with these changes and to continue the work in the best possible way, even if the length of the building period is not very long (about fifteen days) so the risk of losing animals might be low.

The specialization in the transport of the building material is present even in small groups of four individuals. When removing individuals (carriers or non-carriers), there is a general reorganization and the roles of each animal change. However, the organization of work with animals in three categories (i.e. carriers, occasional carriers and non-carriers) is maintained. This organization of work is extremely stable, resisting the loss of specialized individuals. Mice are able to adapt their behaviour to the new social conditions (i.e. different group size and composition), and the disappearance of one or several members of the group is not likely to jeopardize the general outcome of the building process. Since the experimental number of individuals was within the natural ranges for this species (around six individuals in Szenczi et al. 2011, up to eleven individuals in Hölzl et al. 2009 and Garza et al. 1997), we can infer that this stability of the organization of work is also found in natural conditions even if sometimes the number of individuals in a mound can be several folds the number of individuals used in laboratory (up to twenty-three individuals in Poteaux et al. 2008).

We have established the robustness of the work organization in *M. spicilegus*. However, the mechanisms that are involved in the regulation of this organization are still to be fully understood. In previous work, we had determined that the affinity for the building material played an important role in the onset of the specialization for the transport task. The mechanism involved might be based on internal thresholds, but it surely is not the only mechanism involved in the stability of the work organization. The threshold model predicted that those with lower thresholds for task-related stimuli such as the presence and type of the building material would be the carriers. Following the same logic, if those individuals with low thresholds and specialized were removed, the next in line (thus, with the lowest thresholds of the remaining animals) would replace them in their task. Effectively, we observed that new specialists appeared when carriers were removed. These new carriers did not necessarily have the highest SI of their group (excepting the carriers). The SI obtained and the role played by an animal was not a predictor of its role during phase 2.

In the other hand, the removal of inactive members of the group should not affect the carriers since we are removing the individuals with higher thresholds for the task, while keeping the low threshold carriers. But our results show otherwise. The identity of carriers changed when the least performant members of the group were removed. This result shows that other powerful mechanisms are involved in the group's homeostasis by maintaining the general work organization after a disturbance, but the roles of individuals are easily shifted. The changes in the identities of the carriers differs from experiments where the group remained unaltered for four days Without any disturbance specialization is highly stable, animals keep their role during at least this length of time.

Removal of individuals clearly affects the role of each individual inside the group, while the general work organization remains stable. It is then likely to think that social interactions between the remaining members of the group are an important factor in the organization of the work. But our results show that social behaviour (measured as social affinity) does not play a major role in the onset of the specialization or in the reorganization of the roles after the removal of individuals.

With our work, we had ruled out several mechanisms that could have explained the onset and maintenance of work organization in *M. spicilegus*. However, our results show only a slight effect of those mechanisms in the maintenance of the work organization. It is even more intriguing, since all our results strengthen the evidence of a robust work organization that resists the loss of individuals.

Next steps could involve adding individuals instead of removing them. This is a situation that happens in nature, where groups can join other existing building groups, thus increasing the number of individuals in the system. What is has been observed in the field and in laboratory is that after the addition of individuals in a group, the social structure is disturbed for a while but at the end construction resumes (Malá 2003). But nothing is known about the repartition of the work after the addition, or about the stability of roles in such circumstance. Studies must continue to finally fully understand which are the mechanisms involved in all the process of onset and maintenance of work organization in this species.

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Conclusions

So here we are, at the end of this thesis that led us to look into the work organization of a small wild rodent, the always surprising mound-building mouse.

During the cold season, rodents can use different mechanisms to stay alive. For example, all species of the genus *Marmota* (with the exception of the woodchuck, *M. monax*) hibernate in groups. The winter mortality is reduced and the female fertility is increased in the next reproductive season when the hibernating groups are large, since the thermoregulation obtained from huddling and mutual warming reduces the energy required to warm the body and reach the state of euthermia (Arnold 1988; Arnold 1990a; Arnold 1990b). Other strategy is migration, like the changes in habitat of the house mouse *Mus musculus musculus*, a near cousin of our studied species (Boursot et al. 1993; Guénet & Bonhomme 2003). The house mice spend the summer in the cultivated fields, but when cold weather comes they migrate to the barns and stables and even to the farm houses (Carlsen 1993).

In the other hand, the mound-building mouse, *Mus spicilegus*, inhabits the agricultural fields of Eastern Europe, where winters can be very harsh. However, they never approach the human buildings looking for warmth, and they stay active during the whole winter season. The mechanism to overcome such challenging environment is to communally build an imposing mound that protects them from the cold and the moisture. Only juveniles build and enter the mound, with the exception of a few adult females. No adult survives the winter, making this species quite exceptional (Garza et al. 1997; Milishnikov et al. 1998; Poteaux et al. 2008; Szenczi et al. 2011; Szenczi et al. 2012).

The construction of this mound is performed collectively, and it is divided in a series of steps that need to be executed in the right order to ensure the correct structure of the mound. They first gather a big amount of plant material: they use some plant species over others that might also be present in the area (Hölzl et al. 2009). Next step is to cover the pile with soil. Third step is to cover it with clay tiles and finally mice dig galleries underneath the mound. The structure of the finished mound is crucial to ensure the survival of the mice overwintering below it. If the architecture of the mound is not respected, the insulating properties of the mound might be jeopardized (Szenczi et al. 2012).

Since the mound is crucial for the survival of the mice and its construction can be quite complex, it is very appealing to study how mice organize the work in order to achieve this huge piece of architecture. The dimensions and complexity of the mound clearly indicate that the construction needs to be the result of communal work. But how is the work distributed among the members of the group? Which are the mechanisms involved in the organization of work inside the group? Our main objective was to answer these questions in this thesis.

1. What do we know now?

The first evidence of an organization of work was obtained during the work of Jessica Serra (2012). Groups of mice were observed while transporting cotton balls to build a mound. Mice showed a strong behavioural differentiation, with some animals being responsible for over 80% of the transport task. This differentiation was not associated with gender, age or parental origin.

We thought that maybe we were facing a division of labour, where different individuals would perform different tasks inside the group. But since only one task was studied before, we could not really affirm that this was actually a division of labour or it was only an “elite” of animals that performed all the available tasks or at least that differentiate themselves on the transport task while for the rest of the tasks available all members of the group performed the same amount of work. There was our first question:

1) Is this behavioural differentiation a real division of labour?

In order to determine if there was a real division of labour inside the groups of *M. spicilegus*, we presented two successive tasks that involved different motor skills but were both parts of the transport tasks needed to build the mound. We first gave them cotton balls, and then we gave them plaster tiles that were heavier than cotton balls. We observed that individuals that specialized on the transport of cotton balls were not the same individuals as the ones carrying plaster tiles.

A control group where only cotton balls were delivered for the whole duration of the experiment showed that the specialization was stable for this amount of time (Hurtado et al. 2013). This is an acceptable length, since in the field the length of the mound-building process is around fifteen days in its totality.

In nature the different materials are present simultaneously. How the retrieval of such materials is organized so they are brought to the mound in the correct order? The architecture of the mound requires the plant material to be transported before the clay tiles; otherwise the mound would not be as performant to protect the mice beneath it. So we gave our mice simultaneous access to cotton balls and plaster tiles. They consistently transported the cotton balls before the plaster tiles, and the carriers for one material were not necessarily the carriers for the other material.

These two experiments showed the existence of a division of labour. Once the division of labour was established, we needed to understand which mechanisms were behind this division of labour, and how this organization would react if the social group was disturbed. That led us to two questions:

2) Is there a link between individual characteristics and task specialization?

We wanted to see which factor could explain the specialization in *M. spicilegus*. Our strongest hypothesis was that the differences of individual characteristics among the members of the group were at the origin of this specialization. We performed several tests in order to establish the influence of such differences on the onset of the specialization. However, our results were not as strong as we expected. Carriers and non-carriers did not show any differences in their level of anxiety, neophilia or locomotion. However, there was an effect of the affinity for the building material on the specialization. Mice with higher levels of affinity with the cotton ball had a higher probability of becoming the carriers later on.

Since our species is highly social we also checked for the role of social affinity in the specialization, but the social affinity does not play a major role on the onset of specialization.

3) What happens when the structure of the group is modified?

Modifications in group size might frequently occur in nature. But the disturbance caused by the loss or addition of individuals cannot completely change the work organization, since the disturbing factors might occur frequently and the construction of the mound should not be jeopardized or the mice might face catastrophic consequences. Also, the size of the group could affect the social bonding between the mice. Two hypotheses are possible: larger groups might be more attractive hence encouraging those with higher levels of social affinity to stay in the nest, or the absolute opposite where smaller groups stick together and individuals might be more reticent to leave the nest.

We formed groups of different sizes (six and four individuals), and in both cases we observed a specialization in the transport of the cotton balls. Individuals in smaller groups showed a higher level of social affinity than those in larger groups. Also, in groups of six the non-carriers showed a higher level of social affinity than the rest of the group.

But the more surprising result was obtained in another experiment, by removing two individuals from groups of six mice. In some of the groups we removed the specialists, while in the rest of the groups we removed those individuals with the lower SI of the group. As expected, when removing the specialists new specialists appeared. But what was somehow unexpected was that the removal of the less performant mice also led to the emergence of new specialists, and the former specialists lost their status. However, the general organization of the work (with carriers, occasional carriers and non-carriers) was maintained.

2. Insights and perspectives

One of the most notorious features of the organization of work in *M. spicilegus* is its stability. Even when the group was disturbed and the identity of specialized individuals changed, the organization based on different levels of involvement in the transport task was maintained. We are quite intrigued by the mechanisms that could be behind this organization of work and its maintenance across different situations. We have already ruled out the influence of “classic” factors such as age, sex, parental origin and body size. When looking at our results, individual characteristics (more specifically the affinity for the cotton balls) do play a role in the determination of the specialization, with individuals showing higher levels of affinity for the building materials being more likely to become carriers later on. This evidence led us to think that the mechanism involved was based on internal thresholds, where the animals organized their work according with those thresholds as described in the response threshold model (Beshers & Fewell 2001). Those with lower thresholds for the contact with the cotton ball would become carriers, while those with higher thresholds would not perform any transport. This hypothesis was challenged by the results obtained by removing two members of the group.

As expected, when the removed mice were the carriers, new carriers emerged. However, those who took the place of the carriers were not necessarily the next in line for the level of affinity with the cotton balls. But the most flagrant evidence of the existence of another mechanism determining the work organization in this species was obtained by removing two animals with the lowest performance for the transport task. If the mechanism was based solely on the internal thresholds of mice, removing two low-performance individuals should not have disturbed the identities of the carriers, who would still have the highest levels of affinity for the cotton balls. This was not the case: the removal of the two least performant animals caused the shifting of the role played by the mice inside the group. The organization of work (with carriers, occasional carriers and non-carriers) was maintained, but the identity of the animals fulfilling the different roles changed.

The role of experience does not seem to be central in the determination of the specialists, since the role of a mouse does not predict what is going to be its role in case of changes in the group or even in the presence of a new building material. The definition of the self-reinforced model is that a successful task increases the probability of performing that task again, while an unsuccessful task or a lack of opportunity to perform such task decreases the probability of performance (Beshers & Fewell 2001; Ravary et al. 2007). Following such premise, when both transport tasks (transport of cotton balls and plaster tiles) were available sequentially, the successful individuals in the first part of the experiment (hence, the carriers of the cotton balls) should have been the carriers for the next material. However, this could have been an effect of the different nature of the building material. Experience in transport of the cotton balls might not have an influence in a different task such as the transport of plaster tiles. But even if both tasks are too different to be considered as equivalent in terms of experience, again the removal of individuals of low performance allow us to discard the self-reinforcement hypothesis. Carriers are the most experienced individuals of the group since they perform the greatest number of successful transports. The removal of individuals with little or no participation in the transport task should not interfere with the acquired experience of carriers. But the fact is that the animals with the larger amount of successful transports lose their role when the group is modified, independently of their experience.

The stability of the social structure of the group is very important in this species. Juveniles that enter the mound stay in the group and not reproduce until the group disperse in the spring. Females disperse in the vicinity of the mound (Gouat et al. 2003b; Simeonovska-Nikolova 2012) whereas males disperse further (Poteaux et al. 2008). The arrival of these unfamiliar males from an adjacent population triggers the reproduction (Busquet et al. 2009). The avoidance of reproduction is the result of the social monotony and not of a lack of sexual maturity or an effect of seasonality (Gouat et al. 2003). This barrier disappears as soon as the individual is extracted from its social environment and placed with an unfamiliar conspecific (Féron & Gheusi 2003; Gouat et al. 2003a; Busquet et al. 2009). In a similar way, when the group is stable during the building process the roles played by each individual inside the group remain the same but a social disturbance such as the removal of some individuals triggers a reorganization of the work. In an undisturbed group, the determination of roles inside the group seems to follow a threshold-based mechanism with the affinity for the cotton ball as differentiating factor. But once the group is modified, another stronger mechanism overcomes the threshold-based mechanism and reorganizes the work, while keeping the structure of the organization of work intact. The stability of this structure seems to be an emergent phenomenon, where the social dynamics among mice modulate and shape the organization of work. The complex pattern of work organization would be the result of more than a mere addition of mice’s responses following simple behavioural rules based on local information, in a self-organized mechanism as defined in the literature (Camazine et al. 2001; Detrain & Deneubourg 2002; Detrain & Deneubourg 2006). These rules might be determined by the contact with other members of the group. Further work needs to be done in order to comprehend which simple rules might be at the origin of the decision-making process in *M. spicilegus*. The use of virtual models can be helpful to understand how the system organizes itself. It also would be noteworthy to see the effect of adding individuals to the group, since it is a situation that occurs in nature and might as well change the way a group is organized.

This thesis answers a few questions but clearly asks many more. We still do not fully understand all the mechanisms involved in the work organization in *M. spicilegus*, and how they intertwine to respond to external changes and other challenges faced by the group. But there is no doubt that the mound-building mouse still has many interesting features that will allow us to better understand how mammals work in groups, and not only from the point of mechanisms of organization of the work, but also from a functional approach.

In this work we were centred on transport tasks since the RFID technique allowed us to easily and precisely determine the distribution of the work among the mice. The experimental conditions were especially suited for this kind of study. The natural material was replaced by standardized materials such as cotton balls and plaster tiles and was accepted by the mice without any rejection problem. In fact, and just as an anecdote, while in Hungary during a field trip we placed three cotton balls near a mound in the evening, and the following morning the cotton balls had been transported to the mound just like the plant material (Fig. 21). This showed us that wild mice also could use the cotton balls as a building material, comforting us even more in our choice.

Figure 21: Picture of a mound where three cotton balls were used by wild mice as building material.

However, the rest of the tasks that are not associated with transport might be trickier to observe. The visual identification of individuals is particularly difficult in groups of this species, even in laboratory conditions. New experimental protocols need to be created to minimize ambiguity in the identity and performance of each mouse in order to assess the work organization in tasks other than transport.

Another aspect that needs to be explored is the behaviour of *M. spicilegus* in its natural habitat. At this point, our knowledge of this species in the field is rather weak and anecdotal. For example, we still do not know how the group chooses a location to start building the mound, or which is the rate of predation/disappearance of individuals during the phase of construction, and how do they manage with the effects of fusions or fissions of existent groups. These subjects are often mentioned but not documented enough.

The understanding of the constraints the mice experience in the field and the way they manage to cope with them, would surely revealed new leads about the underlying mechanisms of organization of the work. This work is just a small step in the understanding of how a social mammalian species works. Those who will come afterwards will still have a big territory to discover.

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APPENDIX N° 1

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Behavioral differentiation during collective building

in wild mice *Mus spicilegus*

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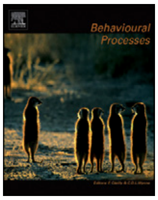

Behavioral differentiation during collective building in wild mice *Mus spicilegus*

J. Serra[a,*], M.J. Hurtado[a], A. Le Négrate[a], C. Féron[a], R. Nowak[b,c], P. Gouat[a]

[a] *Laboratoire d'Ethologie Expérimentale et Comparée, EA 4443, Université Paris 13, Sorbonne Paris Cité, Avenue JB Clément, 93430 Villetaneuse, France*
[b] *INRA/CNRS/Haras Nationaux, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France*
[c] *Université François Rabelais de Tours, F-37041 Tours, France*

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ABSTRACT

Although well documented in social insects, the possibility of behavioral differentiation during collective building has been poorly studied in mammals. In this context, the mound-building mouse *Mus spicilegus* is an interesting model. Under natural conditions, juveniles from different litters gather vegetal material and build a sophisticated structure, the mound, under which the mice will spend winter. The first steps of this complex building process may be elicited under laboratory conditions by offering cotton balls as building material. Spatio-temporal distribution of both animals and cotton balls was automatically recorded by RFID (Radio-Frequency Identification Device) technique. Our results revealed a behavioral differentiation during a collective building task. In a group of six individuals, only two mice (called carriers) transported 80% of the building material whereas the contribution of the remaining mice was weak or even non-existent. The proportion of carriers was constant in all of the six groups studied. This behavioral differentiation was implemented immediately after the building material was made available and remained stable during the 4 days of experiment. The high contribution level of carriers did not result from resource monopolization, nor did it depend on the gender or parental origin of the mice.

1. Introduction

Collective behaviors are one of the most fascinating areas of ethology, as they can explain a multitude of complex phenomenon such as dynamics of human crowds, pelagic fish schools or ungulate herds (Ballerini et al., 2008). Animal societies are characterized by numerous communal activities sometimes governed by a task allocation. Indeed, a task allocation with animals specialized in specific activities is considered to be more efficient than a sequential activity performed by unspecialized individuals (Gordon, 1989) since there is no need to reassign the tasks to the members of the community which may cost time and requires a complex communication network (but see Dornhaus, 2008 for discussion).

Various studies have illustrated the existence of such a phenomenon in social insects and this is thought to be determinant in their ecological success (reviewed in Robinson, 1992). Social animals are involved in diversified tasks such as foraging (Arnold et al., 2005), tunnel excavation (Ebensperger and Bozinovic, 2000), or nest guarding (Clutton-Brock et al., 2003). To explain this behavioral heterogeneity, different theoretical models have been developed (Beshers and Fewell, 2001; Traniello and Rosengaus, 1997). Among others, response threshold models postulate that individuals have internal threshold to respond specifically to stimulus associated with task (Robinson, 1992; Beshers and Fewell, 2001). The variation in response thresholds may be explained by differences in genetics and epigenetic factors such as morphology, age, developmental state and experience (Beshers et al., 1999; Jeanson et al., 2008; Robinson et al., 2009). This behavioral flexibility is a key feature to enable the colony to grow, develop and ultimately reproduce.

In mammals, only a few studies have investigated the possible role repartition during collective behavior. In the lion (*Panthera leo*), during collective hunting some females circle a prey toward others hidden in the centre waiting for the prey (Stander, 1992). Similarly in bottle-nosed dolphins (*Tursiops truncatus*), Gazda et al. (2005) illustrated a behavioral differentiation during group hunting: one individual was the “driver” herding the fishes in a circle toward the remaining “barrier” dolphins. In these two examples, animals show group behavior for achieving a common goal.

Under laboratory conditions, Colin and Desor (1986) demonstrated the existence of a behavioral differentiation in Wistar rats. They exposed groups of six animals to an experimental design in which access to the feeder was made difficult by progressively immersing the only way of access with water. In this foraging task, two rats dived and brought back food pellets to the cage (divers) while the four others (non-divers) stayed in the home cage and obtained food only by stealing it from the divers. Identically, Nejdi et al. (1996) demonstrated

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* Corresponding author.
E-mail address: serra@leec.univ-paris13.fr (J. Serra).

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the emergence of behavioral differentiation in groups of mice C57BL/6J exposed to the same water tank social interaction test. In contrast to what was observed in lions and dolphins, the behavioral differentiation in these rodents took place as a result of individual strategies.

Among the huge variety of collective behaviors, the ability to build sophisticated nests is fascinating for scientists. Nests provide protection against predators (Schradin, 2005; Ebensperger and Blumstein, 2006) and shelter against harsh environmental conditions (Hansell, 1993; Deacon, 2006; Hölzl et al., 2009); moreover nests are often a central place for the exchange of information between members of a social group. Collective building is found in various taxa but asymmetrically around the animal spectrum (Hansell, 1984). It is particularly well represented in social insects with extraordinary examples of architectural complexity found in termites, ants, wasps and bees (reviewed by Théraulaz et al., 1998a) and also birds (Hansell and Overhill, 2005). Commonly, social insects show a behavioral differentiation during the building of their sophisticated nests that can go from a simple difference in the level of participation (O'Donnell, 1998) to some more complex phenomena (Franks and Deneubourg, 1997).

In mammals, the ability to build nests occurs more sporadically and is limited to some orders like rodents (e.g. Jones, 1984; Ebensperger and Cofré, 2001; Layne, 1969; Rosell et al., 2005), lagomorphs (Deutsch, 1957), and carnivores (e.g. Neal and Cheeseman, 1996) which are often good burrowers, and to some primates with nest building by apes (Bolwig, 1959; Goodall, 1962; Casimir, 1966; Harrisson, 1969; Fossey, 1974). A particular exception concerns humans whose building capacities led to the beginning of sedentarization and played a remarkable role in the conquest of new territories (Finlayson et al., 2011). In rodents, one of the most documented examples of collective building is found in the atypical naked mole rat *Heterocephalus glaber* whose social system resembles those of eusocial insects. This mammal lives in large colonies structured around a unique breeding female. As described previously in other types of collective behaviors and in addition to their reproduction skew, the naked mole rat is also characterized by the existence of a behavioral differentiation (Sherman et al., 1992; Burda et al., 2000). During the collective building task, three categories of individuals have been characterized: "frequent workers" specialized in foraging and nest building, "infrequent workers" performing the same task as "frequent workers" but at less than half the rate, and the "non-workers" which very rarely dig or carry materials (Jarvis, 1981). As a eusocial species the naked mole rat seems to be rather unique and there is no other work mentioning behavioral specialization in mammalian collective building.

In this regard, the non-eusocial wild mouse *Mus spicilegus* is an extremely interesting model to explore the characteristics of collective building behavior. At the beginning of autumn, juveniles from several parental units gather and start building a voluminous and rather sophisticated mound in a very precise temporal sequence (Festetics, 1961; Orsini et al., 1983; Garza et al., 1997; Hölzl et al., 2009). First, they collect plant materials, pile them up in a selected place and cover the vegetal pile with soil. Hölzl et al. (2009) demonstrated that they select mainly *Amaranthus* spp., *Chenopodium* spp. and *Setaria* spp. as building materials, with a positive relationship between their availability within 3 m and mound size. Then they dig a set of tunnels below this mound and push the excavated soil out of on the mound. On top of the mound they deposit clay tiles which are extracted from so-called mines located in the surroundings (Bihari, 2004). Finally, the tunnel network is completed with one or two nest chambers. The mice remain inside the mound during winter without reproducing (Gouat et al., 2003a) and get out of it to disperse in spring (Gouat et al., 2003b). The mechanism by which individuals congregate to build mounds is kin-based since mothers of juveniles found inside the mounds are more related than if they had been chosen at random from the population (Garza et al., 1997; Poteaux et al., 2008). This collective behavior can be triggered under laboratory conditions by mimicking autumn conditions and by offering cotton balls as building materials to groups of juvenile mice from different parental origins. We used an original Radio-Frequency Identification system which records automatically in real time the localization of both animals and building materials.

Festetics (1961) suggested that only "two to six mice may be responsible of the construction" whereas up to 25 juveniles animals can be found inside a mound (Poteaux et al., 2008). The aim of our study was to test whether a behavioral differentiation occurred during the construction of the mound by the mouse *M. spicilegus*. We hypothesized that some individuals may specialize in carrying building materials whereas others would be poorly or not involved at all. This potential difference could reveal for the first time the existence of a task distribution during collective building work in a non eusocial mammalian species.

2. Material and methods

2.1. Animals and housing conditions

Thirty-six experimental animals were obtained from nine wild mound-building pairs derived from a stock caught in Hungary in October 1999 and reared for 11 generations under laboratory conditions (20 ± 1 °C) with a 14:10 h light: dark cycle. The young were housed with their mother and father until 35 days of age in standard polycarbonate cages (28 cm × 41.5 cm and 15 cm high) with sawdust (Special Diet Services, Witham, Essex, UK) and cotton balls as nest material. Food (type M20, Special Diet Services, Witham, Essex, UK) and water were supplied ad libitum. When 35 days old, mice were weaned and transferred into new cages in a climatic chamber with a 14 h:0 h light:dark cycle (dark phase begun at 11 am) and a room temperature progressively lowered to 14 °C to stimulate building activity and social cohesion. Indeed, the decrease in temperature allowed a more efficient social bonding within mixed litters groups, with a higher level of tolerance between animals as shown by unpublished experiments (Mala and Gouat, unpublished data). At 42 ± 2.2 days of age, the mice were allocated to six groups composed of six individuals. Each group was made of three pairs, i.e. one male and one female, originating from three different litters. They were placed in the experimental device 10 days before the beginning of the testing period.

2.2. RFID technique

Recording movements of animals in a complex environment, even under laboratory conditions, has always been a challenge in the study of behavior. Video recording is frequently used for this purpose but automation, even with modern techniques based on digital image analysis, remains difficult to implement when several animals are concerned or when conditions are not ideal for observations (during dark cycle for instance). By contrast, Radio-Frequency Identification (RFID) is a technique which allows recording automatically in real time and during a long period the localization of each animal or objecting. A typical RFID system consists of the following units: (i) a tag or transponder, attached to the item which will be tracked, (ii) a reception antenna, (iii) a data processing system to record the data. The RFID Anti-Collision System developed by SpaceCode (Verrières-le-Buisson, France) uses small passive glass tags (length: 12 mm; diameter: 1.5 mm), which have unlimited lifespan and contain no battery. A magnetic antenna detects the presence of each tag, and several tags may be detected at the same time thanks to the anti-collision system. This antenna is connected to a computer through an electronic device. The identification

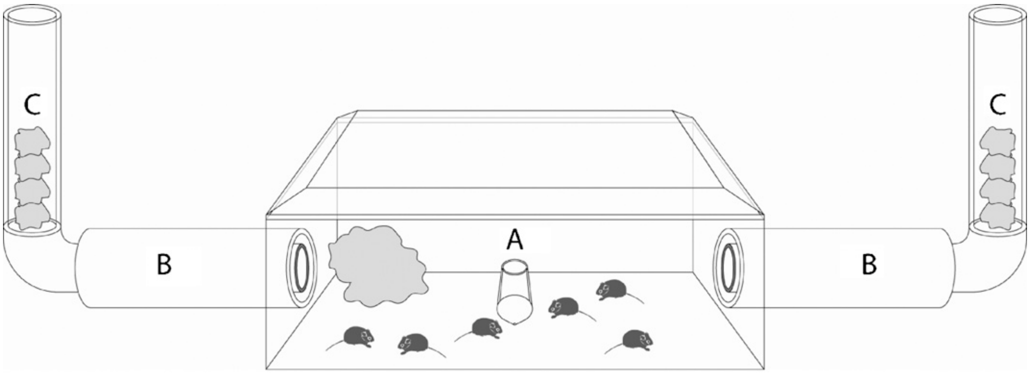

Fig. 1. Mound building device. A large cage (A) was connected to two dispensers containing tagged cotton balls (C) through tunnels (B) circled by RFID antennas (two for each tunnel).

number of the tag and the exact time of detection were recorded. There is no limitation in the number of tags (individuals or objects) or in the duration of the recording. The data are thus collected for the requested time span, pre-processed and saved in a database for further analysis. Kritzler et al. (2006) showed that the use of RFID technology does not alter social behavior and the outward appearance of laboratory mice.

For the experiment, each individual was identified by a small tag which was directly implanted by dorsal subcutaneous injection. Moreover, each cotton ball, used as building material, was identified by sticking a tag inside it. The processing system could thus record all the movements of both mice and building items.

2.3. *Testing apparatus*

The testing apparatus consisted of (A) a plastic cage (28 cm × 41.5 cm and 15 cm high) with sawdust and twelve cotton balls to initiate the building process (food and water were provided ad libitum) (B) two plastic tunnels (diameter 4 cm, length 50 cm) leading to (C) two vertical dispensers of cotton balls (Fig. 1). Each tunnel was encircled by a double RFID antenna: one near the cotton ball dispenser and one near the plastic cage. This made possible to detect the moving direction of the RFID tag.

2.4. *Testing procedure*

Ten days before the beginning of the testing period, the six groups of mice were placed in the experimental device but had no access to the tunnels. In field conditions, Szenczi et al. (2011) analyzed *M. spicilegus* feces which revealed that the plants used as building materials are not represented in their actual diet. In laboratory conditions, cotton balls were thus chosen because of their non-edible characteristics, their small weight and their easiness to be dilacerated. Moreover, cotton balls placed on the surroundings of mounds during the piling of vegetal material can be collected by mice in the field and inserted into the mound (Hurtado, Herraira and Gouat, unpublished data). Twelve cotton balls were placed inside their cage so that they could initiate the building process. By the end of this period of socialization with alien partners, all the groups had begun mound building. On day 1 of the testing period, three tagged cotton balls were delivered in each of the two dispensers (the six mice could thus carry six cotton balls). Access to the tunnels was allowed by opening the entries. On day 2, three new tagged cotton balls were delivered in each of the two dispensers. The same procedure was continued on day 3 and on day 4. The activity of the mice was recorded continuously from day 1 to day 4. Since there were constraints associated with the cotton dispensers themselves and the available space inside the central cage, the number of cotton balls provided was restricted.

Under laboratory observations, the maximum activity of mice is estimated at 1 h after the passage to red light and goes on average for a period of 2 h (Boucart and Gouat, unpublished data). In field conditions, above-ground activity is usually performed at dawn and throughout the night according to video monitoring (unpublished results in Hölzl et al., 2011). Tests were thus performed at 2.00 pm (3 h after the beginning of the dark cycle), corresponding to this maximal activity period. The test ended when all the cotton balls were collected, but the data recording was continuous during the whole experiment period.

Transport of a cotton ball was attributed to a mouse when the tag was detected moving inside a tunnel from the dispenser to the cage. When two mice were detected at the same time with a cotton ball, they were each considered as transporting 0.5 cotton ball.

2.5. *Behavioral observations and statistical analysis*

Due to the sample size, non parametric statistical analyses were performed using the exact procedure (Mundry and Fischer, 1998). All the statistical analyses were performed using StatXact-8 (Cytel Inc., Cambridge, MA 02139, USA). Data are given as mean ± SEM.

2.5.1. *Characteristics of cotton balls transport*

2.5.1.1. Time spent to collect the cotton balls. The time spent to collect the cotton balls was measured from the first to the last detection of a cotton ball in a tunnel for each day of test. A test of Friedman was used to determine if mice improved their performance during the 4 days of the experiment.

2.5.1.2. Individual contribution to the transport of cotton balls. To determine whether mice differed in their contribution to the transport of cotton balls, animals were ranked inside each group according to the number of cotton balls they transported during the 4-day period of the experiment. Rank #1 was attributed to the individual with the highest contribution to the transport. Mice tend to manipulate roughly the cotton balls during transport and some tags detached from the cotton balls and could not be detected. As a result the number of transported balls may slightly differ between groups (4–6 for each day; mean ± SEM: 5.5 ± 0.2). We then compared the performance of the mice, according to their ranks, to the average performance of each group using a permutation test for paired data. Average performance corresponded to the number of cotton balls detected divided by six, the number of mice within a group. Individuals with a contribution rate higher than the average value of the group were then called carriers.

To test the stability of individual relative contributions we analyzed the performance of the mice with a day-by-day analysis using a test of Friedman. Performances were also compared between carriers of different ranks and between carriers and non-carriers using permutation tests for independent samples using group as a stratum.

2.5.2. *Alternation between the two dispensers*

Mice collected cotton balls either by alternating between the two dispensers or by emptying one dispenser after the other or by a combination of these two strategies. To describe the strategy used we calculated the number of changes from one dispenser to the other. This value varied from 1 up to 5. To determine if a strategy was more common than others, we compared the observed frequency of each type of strategy to the theoretical probability obtained by chance (respectively, 1 or 5 changes $p = 1/10$; 2 and 4 changes $p = 2/10$; 3 changes $p = 4/10$). Because the mice were inexperienced at performing the task on the first day of the experiment we tested whether a global change of strategy occurred during the 4 days of experiment using a test of Friedman.

2.5.3. *Behavioral characteristics of the carrier and the non-carrier mice*

Mice movements were recorded continuously during the full 4 days of the experimental period since access to dispensers was possible. The time spent in each part of the apparatus by each animal could be deduced from our data. We then compared the time spent in each part of the apparatus between mice according to their rank using a permutation test for independent samples and group as a stratum. The analysis was performed for the complete period of observation, and then to the limited period when cotton balls were available from the dispensers.

2.6. *Ethical note*

Animal care and experimental procedures were approved by an Ethic committee (Regional Ethics Committee in Animal Experiment No. 3 of Ile-de-France: p3/2007/023). The number of mice used was strictly restricted to the minimum needed for statistical analysis. Subcutaneous injection of the tag was performed 3 days before constituting the groups of six mice by an animal keeper specifically trained in order to limit stress of individuals. Mice were taken from their cage very carefully and the injection which lasted only a few seconds was realized as gently as possible. Their behavior was systematically observed 24 h after the procedure. Young mice behaved normally and did not seem to be affected by the injection.

After the experimental period, mice were placed in familiar groups of the same sex to avoid aggression when reaching sexual maturity. Since these animals were already identified with RFID tags, they were kept and used to adjust subsequent protocols involving the RFID technique.

3. Results

3.1. *Characteristics of cotton balls transport*

3.1.1. *Time spent to collect the cotton balls*

On the first day of the test mice investigated the tunnels very quickly and adopted different strategies to cross the tunnels with their load. They could either push or pull the cotton balls using their mouth as well as their forelegs. Mice spent 15.8 ± 1.7 min to collect and transport six cotton balls (range: 4.2–36.2 min). The time spent for collecting six cotton balls varied day after day for each group, but no significant decrease was revealed (respectively from D1 to D4: 17.2 ± 4.9 min; 20.6 ± 3.6 min; 13.7 ± 2.0 min; 11.6 ± 1.7 min; Friedman ANOVA, $\chi^2 = 3.8$, $p = 0.32$).

3.1.2. *Individual contribution to the transport of cotton balls*

Inside each group, mice could be ranked according to the number of cotton balls they collected during the 4-day experiment. Mice ranking first and second transported 79.5 ± 5.4% (range: 67.3–98.1%) of the cotton balls. This observed contribution was significantly higher than the theoretical value for an equal contribution of each rank (Fig. 2); whereas contribution of mice from rank three to six was significantly lower than the theoretical value ($p = 0.031$ for each rank, permutation tests for paired samples; Fig. 2).

Over the 4 days of the experiment, in each group two mice transported clearly more cottons than the average (range for respectively rank # 1: 38.5–58.5%; rank # 2: 23.1–46.2%) and behaved as carriers whereas the overall contribution of the other four mice in each group was always lower than the average (range: 0–13.5%). Of the twelve carriers identified, five were females and seven were males, originating from eight of the nine reproductive pairs used in the experiment. In five of the six groups, carriers were originating from two different litters.

A day-by-day analysis confirmed these results (Fig. 3). In this analysis the rank was attributed to each mouse of a group according to its performance on the first day. Although the two carrier mice may shift rank from 1 day to the next, they did not differ from each other in their contribution to cotton ball collection (p respectively day 1: $p = 0.06$; day 2: $p = 0.\ 25$, and $p > 0.93$ for days 3 and 4) but they always differed significantly from the non-carrier mice ($p = 0.03$ for each day; Fig. 3). The contribution of each individual remained stable during the 4-day period and no significant change was observed over time (Friedman ANOVA, $\chi^2 = 1.032$, $p = 0.79$).

3.2. *Alternation between the two dispensers*

The most common strategy used by the mice was to empty a dispenser and then to collect cotton balls from the other dispenser (Fig. 4) even though it did not differ significantly from theoretical frequency (permutation test for paired data: $p = 0.063$, $N = 6$). Though being the most expected by chance, three changes of dispenser was far from common (permutation test for paired data: $p = 0.063$, $N = 6$). A systematic alternation between dispensers was

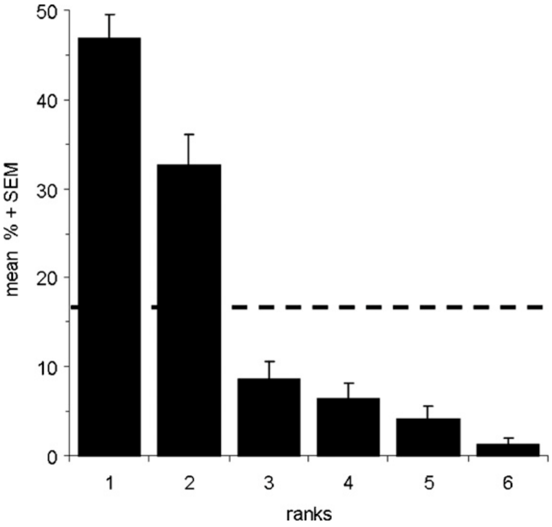

Fig. 2. Individual contribution to the global cotton ball transport. The observed contribution (in percentage of the total cotton balls) for each rank was compared to the expected equal contributions of each group represented by the black line.

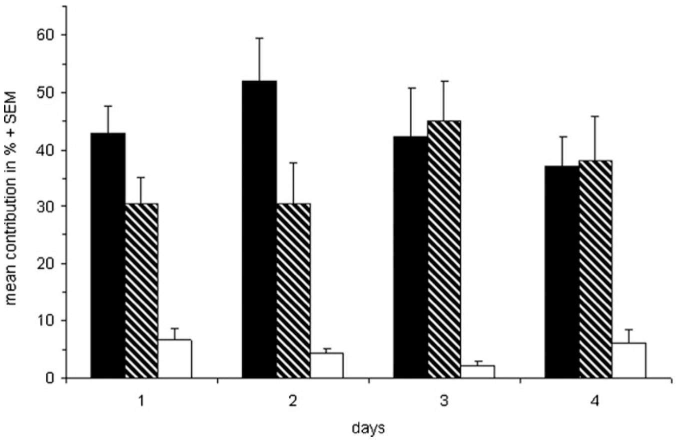

Fig. 3. Individual contribution to the cotton ball transport for each day of test. Black bars: carrier mice from rank # 1; hatched bars: carrier mice from rank # 2; white bars: non-carrier mice.

observed only once in a single group on 1 day and this result did not differ from random distribution ($p = 0.22$; Fig. 4).

A day-by-day analysis did not reveal any significant global changes (Friedman ANOVA, $\chi^2 = 4.46$, $p = 0.36$). Nevertheless each group could change strategy from 1 day to another. On day 3, four groups out of six had emptied one dispenser before fetching cotton balls in the other one (i.e. one change), and this proportion differed significantly from a random distribution ($\chi^2 = 21.41$, df = 5, $p = 0.0013$).

3.3. Behavioral characteristics of the carriers and the non-carriers mice

3.3.1. Time spent in the different parts of the device during the 4-day experiment

Dispensers were freely accessible during the 4 days of the experiment (mean duration of the full experiment ± SEM: 72 h 11 min ± 17 min). During this 4-day experiment, mice spent on average 9.1 ± 1.3% of their time inside the tunnels and the dispensers and never more than 20% except for a non-carrier female which spent 49% of its time in this part of the apparatus. The proportions of this time did not significantly differ between carriers and non-carriers (permutation test with group as stratum; $p = 0.71$; Fig. 5(a)).

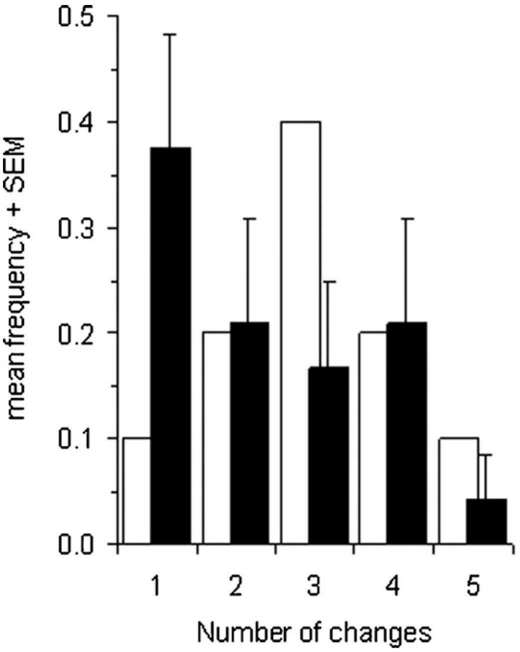

Fig. 4. Frequency of the number of changes (mean + SEM) of dispensers during collection of cotton balls for the 4 days of the experiment. White bars: theoretical frequencies; black bars: observed frequencies.

3.3.2. Time spent in the different parts of the device during the cotton balls' delivery

The mice spent clearly more time in the dispensers and the tunnels when cotton balls were available than during the full period of 4 days of experiment (mean ± SEM: 30.5 ± 2.0%; permutation test for paired samples: $p < 0.001$, $N = 36$). Carriers spent significantly more time than non-carriers inside the dispensers and tunnels when cotton balls were available (permutation test with group as a stratum: $p = 0.003$; Fig. 5 (b)).

4. Discussion

The main objective of our study was to examine whether behavioral differentiation could arise in the early stages of mound construction by juvenile mice. By providing cotton balls as building materials we succeeded in recreating appropriate conditions to initiate a collective building process in groups of six mice. During the

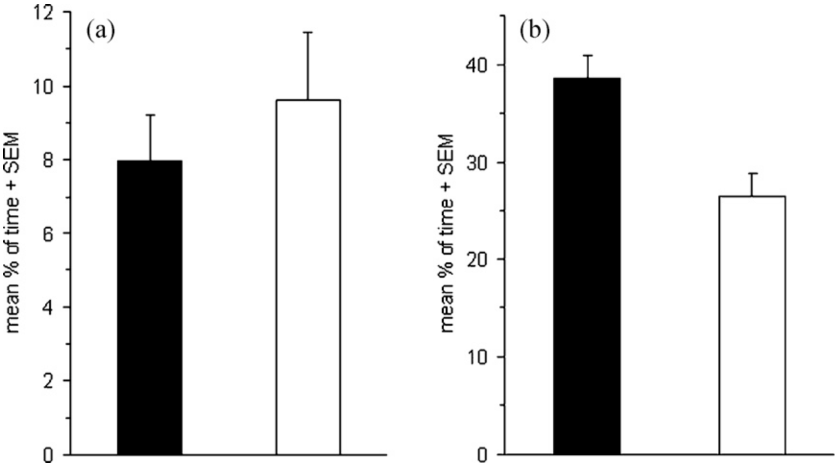

Fig. 5. Percentage of time spent (mean + SEM) by the carrier mice (black bar) and the non-carrier mice (white bar) of the six groups inside the tunnels and the dispensers during the 4 days of the experiment (a) and only during the limited period when cotton balls were available in the dispensers (b).

10-day period of socialization, mice began to build the mound as soon as they had free access to cotton balls put inside their cage in all the groups observed. When the experiment started, the task was more complicated since animals had to walk through tunnels to go to the dispensers delivering cotton balls. The mice had to transport the balls from the dispensers to their cage in order to incorporate them to the mound being built. In all cases, cotton ball transportation was performed with very short delays (on average 15 min) even on the very first day, demonstrating both the attractiveness of the task and the high level of adaptability of *M. spicilegus* challenged with a new situation. The totality of the transported material was shredded and used to build the communal nest.

When analysing the individual contribution to the transport of building materials, two behavioral profiles emerged. In each group and for each day of test, two out of the six mice achieved almost 80% of the transport whereas the contribution of the four other mice was limited or even nonexistent. This behavioral differentiation was not related to gender or parental origin, since carrier mice were both females and males originating from different litters. Moreover, the emergence of behavioral differentiation was implemented as soon as the first cotton ball was available, suggesting that such behavior can appear spontaneously in the social group. Interestingly, the identity of the carriers did not change over the 4 days showing that once behavioral profiles are set, they do not change. Our result corroborates the behavioral differentiation observed during collective tunnel building in the naked mole rat (Jarvis, 1981), during group hunting in dolphins and lions (dolphin: Gazda et al., 2005; lion: Stander, 1992) and during a conflicting foraging task in laboratory rodents (Colin and Desor, 1986; Nejdi et al., 1996).

In experiments where rats had to dive to access for food (Colin and Desor, 1986; Krafft et al., 1994), it is interesting to note that within groups of six animals, the number of divers was in the same proportion as ours (2/6) even though our conditions did not impose such drastic constraint. In the conflict diving-for-food situation, it has been shown that the occurrence of non-divers was strongly influenced by group size (Thullier et al., 1992). A similar result was described in insect colonies where changes in the division of labor could be artificially induced by altering colony size (e.g. Winston and Fergusson, 1985). In our experimental conditions, we can therefore wonder to what extent the number of carriers is influenced by group size.

Our data revealed that in each group, two mice transported most of the cotton balls suggesting that task distribution may have resulted from the monopolization of the resource by the two carriers. With two dispensers available, one may hypothesize that only two individuals could monopolize them, each mouse choosing a specific dispenser that they could empty and in which they would spend the majority of their time. In fact our results showed that this was not the case, the most common strategy being to empty one dispenser and then the other which precludes any resource monopolization by specific mice. Moreover, when we analyzed the behavioral characteristics of the carriers and the non-carriers, whatever their rank in the transport of cotton balls all the mice spent equivalent amounts of time in the different parts of the device (the two dispensers, the two tunnels and the cage), rejecting the resource monopolization hypothesis. Nevertheless, during cotton balls deliveries, carriers spent more time in the tunnels and the dispensers than non-carriers, indicating a renewed attractiveness for this part of the device only when building materials were available. These data corroborate the demonstration of a real behavioral differentiation illustrated for the first time during a collective building process in a non-eusocial mammal. The question of the potential role of non-carrier mice, which could be dedicated to other kind of activities in the building process, remains to be investigated by additional laboratory observations. Furthermore, future studies would be necessary to determine the possible costs and benefits of individual involvement in mound building. One may hypothesize that this task allocation achieves an energy saving since only carrier mice are exposed to the energetic costs of harvesting and transport. It also reduces the exposure of the non-carrier mice to predators in the field.

The emergence of such a behavioral differentiation, with a ratio of carriers being constant between groups and stable over the time, remains to be explained and is yet a subject of debate. The self-organization concept states that complex patterns can emerge spontaneously by means of simple and local interactions among the animals (Camazine et al., 2001). According to Bonabeau et al. (1997) highly structured collective behaviors do not rely on individual complexity. Based on this assumption, researchers have developed theoretical models for studying self-organizing systems. In insect societies, the phenomenon of task distribution has been modeled by a mechanism called response thresholds (Théraulaz et al., 1998b; Bonabeau et al., 1999) which explains the genesis of specialization in a group of homogeneous individuals. In mammals, only one mathematical model has been developed to simulate rat collective behaviors in a foraging situation (diving-for-food situation: Colin and Desor, 1986): the Hamelin theoretical model created by Thomas et al. (2004). By merging two models (adaptive response thresholds and dominance relationships), the Hamelin simulation system managed to reproduce the behavioral differentiation observed by biologists in groups of rats confronted to an increasing difficulty to reach food. This simulation (i) enables to explore the influence of numerous factors (number of animals, external conditions) on task performances and (ii) illustrates the way local rules (dominance hierarchies and hunger of the rats) can explain the emergence of the two behavioral profiles (the divers and non-divers rats) without requiring a high degree of complexity at the individual level. This theoretical model highlights the importance of local rules, responsible for the emergence of behavioral differentiation in rats considered like homogeneous agents without social recognition. Nevertheless, one cannot exclude that these local rules might interact with individual complexity to explain the task distribution observed in vivo. In collective mound building by *M. spicilegus*, could the emergence of carriers within a group have also resulted from an intrinsic predisposition of some individuals to explore new environments or novel objects (e.g. being less fearful or more curious) and carry materials? In rats, it has been reported that early behavioral features can predict behavioral profiles of adults in the difficult food supply situation (Deviterne et al., 1993). Similarly in mice placed in the same feeding experimental challenge, it has been found that major divers have a tendency to be less anxious than non-divers (Nejdi et al., 1996) which suggests a relationship between individual characteristics and task repartition. In this way, further research characterizing body weight, level of anxiety, degree of affinity with the building materials, general activity and novelty seeking behavior, should be undertaken in order to evaluate the possible connection between carriers' behavioral profiles and morphological traits. In addition, others studies investigating in what extent the size of the group and the availability of the building materials could have an influence on the ratio of carrier and non-carrier mice may be undertaken. Indeed, in insect societies, Gautrais et al. (2002) found that increasing the overall work demand while keeping the colony size constant causes an increase in the differentiation among individuals in their activity levels. In the same way, increasing colony size while keeping the demand proportional to the colony conducts to a similar result.

5. Conclusions

Life history traits of mice *M. spicilegus* gave us the opportunity to demonstrate that behavioral differentiation during collective building is not limited to eusocial species. The stability of the proportion observed during specialization (two carrier mice and four non-carriers) remains a fascinating area to explore in regards to intrinsic characteristics of animals and self-organization.

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