The effect of animal personality on Morogoro virus infection status of multimammate mice

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Master Thesis submitted to obtain:
the degree of Master in Biology,
Specialisation: Evolution and Behaviour,
2013 - 2014

Universiteit Antwerpen

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Abstract 1

Inter-individual variance in behaviour has long been neglected and was considered as noise around a behavioural optimum. More evidence has shown that individual variation in behaviours can be consistent over time and contexts and are called personality traits. These personality traits could have important ecological and evolutionary consequences. To our knowledge, however, very little empirical research has been done in the field concerning personality and virus transmission.

This study aims at unraveling the interaction between exploration, a personality trait, an individual's weight and the exposure and susceptibility of wild *Mastomys natalensis* to infection with Morogoro virus (MORV)**.** Blood samples and behavioural measurements were taken in Morogoro, Tanzania for three months. We hypothesized that personality could either be directly responsible for the individual variation in MORV antibody presence or indirectly via a larger space use. Alternatively, the individual's weight, which is a proxy for age, may be responsible for the variation in MORV antibodies and the individual's personality.

Our results provided evidence for the existence of personality in *Masotomys natalensis*, more specifically in the exploration-avoidance continuum, with a repeatability of 28%. Contrary to our expectations, we did not find any relationship (direct or indirectly via a larger space use) between exploration and MORV specific antibodies presence. Exploration was negatively correlated with weight, where juveniles (i.e. individuals with a low weight) are more explorative than older individuals. We suspect that exploration acts as a behaviour to gather information about the environment and is a life-stage specific personality trait in *M. natalensis*. Secondly, our results showed that the individuals activity was positively correlated with weight, we suggest that this behaviour can be interpreted as an investment in reproduction. Lastly, our results indicate that older individuals are more likely to have MORV antibodies present in their blood, although this might be biased due to accumulation over time.

Abstract 2

Individuals may differ in their behaviour from one another. Animals have different personalities if these behavioural differences, in exploration for example, persist through time or contexts, where one individual is always more explorative than another. These consistent individual differences might influence their susceptibility of getting infected with viruses. Such as in humans, where more social persons are more likely to get the flu. This study tries to unravel the interaction between the natal multimammate mice (*Mastomis natalensis*) and Morogoro virus transmission. This virus is a safe alternative to study a closely related virus species: Lassa virus which causes a deadly disease in humans in West Africa and has the same host.

We hypothesize that individuals who are more exploratory might have a higher probability of coming into contact with infected materials (e.g., individuals) by being either more active or more explorative. Secondly, we propose that the individuals weight, which is an indication for their age (i.e., older individuals are bigger and hence weigh more), influences the susceptibility of infection or it might influence their personality. We took blood Samples and behavioural measurements in Morogoro, Tanzania for three months.

Our results provided evidence for the existence of personality in *Masotomys natalensis*, namely exploration. We showed that younger individuals were more explorative than older ones. We suspect that this is needed to gather information about the environment, which is necessary for younger individuals and not older ones, who are experienced. Contrary to our expectations, we did not find any relationship between exploration and MORV antibodies (a remnant of infection) presence. Secondly, older individuals were more active in their habitat. We suspect that this behaviour increases their chance of finding a mate and hence reproduce. Lastly, our results indicates that older individuals were more likely to have antibodies against the Morogoro virus, although this might be incorrect because once antibodies are made, they stay present in the individuals body.

Samenvatting

Inter-individuele variatie in gedrag werd genegeerd voor een lange tijd en werd bezien als ruis rond een gedragsoptimum. Toenemend bewijs toont aan dat deze individuele variatie in gedrag constant is over tijd en contexten en wordt gelabeld als persoonlijkheidskenmerken. Deze kenmerken kunnen belangrijke ecologische en evolutionaire gevolgen hebben. Al is, tot zover we weten, weinig empirisch onderzoek gedaan naar de link tussen persoonlijkheid en virus transmissie.

Deze studie focust zich op de interactie tussen nieuwsgierigheid (i.e., exploratie), gewicht en de blootstelling en gevoeligheid van wilde *Mastomys natalensis* aan infectie met het Morogoro virus (MORV). Bloed stalen en gedragsobservaties werden genomen in Morogoro, Tanzania gedurende drie maanden. We veronderstelde dat persoonlijkheden direct verantwoordelijk konden zijn voor de variatie in MORV antilichamen ofwel indirect door een hogere activiteit. Anderzijds is er de mogelijkheid dat het gewicht van het individu, wat een benadering is voor de leeftijd, verantwoordelijk is voor de variatie in MORV antilichamen en persoonlijkheid tussen de individuen.

Onze resultaten tonen het bestaan aan van persoonlijkheden in *Mastomys natalensis*, specifiek in het exploratie - vermijding continuüm, met een herhaalbaarheid van 28%. In tegenstelling to onze verwachtingen vonden we geen relatie (direct nog indirect) tussen exploratie en aanwezigheid van MORV antilichamen. Exploratie was negatief gecorreleerd met gewicht, wat wilt zegen dat juveniele (i.e., individuen met een laag gewicht) nieuwsgieriger zijn dan adulte. We veronderstellen dat exploratiegedrag gebruikt wordt om informatie te verwerven over de omgeving en dat het specifiek is aan de levensfase van *M. natalensis*. Hiernaast toonde onze resultaten dat activiteit in het veld positief gecorreleerd is met gewicht. We suggereren dat dit gedrag geïnterpreteerd kan worden als een investering in reproductie. Ten laatste hebben we indicaties dat oudere individuen een hogere kans hebben om MORV antilichamen te vertonen, al is dit mogelijks vertekend door een accumulatie over tijd.

1) Introduction

1.1) General

Inter-individual variation has long been a topic of interest for evolutionary and behavioural ecologists (Mather & Anderson 1993; Carter et al. 2013). Starting with the publication of Darwin's (1859) *On the Origin of Species,* many researchers regarded genetically based phenotypic variation as the raw material on which selection acts (Bock 2003). In contrast, behavioural variation was long neglected and was considered instead as noise around a behavioural optimum. Traditionally, behavioural ecologists considered behaviour as a very plastic phenotype, such that there was no restriction on behavioural expression within an individual (Sih et al. 2004a; 2004b; Réale et al. 2007). This was a source of behaviour-related sampling bias in many studies (Réale et al. 2007; Biro & Dingemanse 2009; Stuber et al. 2013). Fast exploring great tits (*Parus major*) are more likely to approach novel objects, such as cameras within their nest boxes, for example, which means that fast explorers are over-represented in camera-sampled behavioural studies (Stuber et al. 2013). Similarly, a study on Namibian rock agama (*Agama planiceps*) showed that bolder individuals entered traps sooner than shy individuals and hence trapping success was higher for bolder individuals (Carter et al. 2012).

1.2) What is a personality?

During the last decade, however, more evidence has shown that individual variation in aggression, activity, and many other behaviours may be adaptive and consistent within individuals (Réale et al. 2007; Réale et al. 2010). Such within-individual behavioural consistency is commonly referred to as "**personality**" and is the phenomenon that individual differences are consistent over time (e.g., animals that are highly explorative at one point will tend to be explorative at a later point in time) and/or across situations (e.g., individuals that tend to be aggressive towards conspecifics also tend to be bolder in novel environments) (Réale et al. 2007). This consistency does not mean that trait values cannot change with age or environmental conditions, but differences between individuals should be maintained. For instance, depending on the situations, all individuals shift their aggression levels up or down, although some remain consistently more aggressive than others (Réale et al. 2007; Sih et al. 2004). Personality has been documented in a wide range of animals including mammals, birds, reptiles, crustaceans and even insects (Gosling 2001; Bell 2007; Briffa & Weiss 2010).

A personality type consists of several **personality traits**, which are a quantifiable repertoire of behaviours that show between-individual variation, such as aggression or activity (Carter et al. 2013). One important characteristic of personality traits is the repeatability of specific behaviours within an individual. Such consistency could be advantageous if information about the environment is uncertain or if plastic behaviours could lead to costly mistakes (Bell 2007). Repeatability is estimated through replicated measurements of a specific behaviour or set of behaviours over time, and is the proportion of phenotypic variation explained by the between individual variation (Réale et al. 2007; Martin & Réale 2008; Formula in material and methods). A high repeatability score occurs when total phenotypic variance is high (between-individual variance) and within individual variance is low. Some factors, however, may influence the repeatability of measurements, such as short-term micro-environmental differences between tests and habituation to novelty (Martin & Réale 2008). In the longer-term, behaviours may be more flexible depending on an individual's life stage (Herde & Eccard 2013). Bold rainbow trout (*Onchorhyncus mykiss*) alter their response to a novel object (boldness) based on their prior experience, for example, resulting in a low repeatability for novel object tests (Frost et al. 2007). A meta-analysis across a range of taxa showed that the average repeatability of behaviours was around 35 percent (Bell et al. 2009).

In principle, animal personalities can be measured along any behavioural axis, but Réale et al. (2007) divided personality traits into five general categories according to the ecological situation in which it is measured: (1) The shyness-boldness continuum: an individual's reaction to a risky situation, for example a predator, in a non-novel environment; (2) The exploration-avoidance continuum: defines an individual's reaction to a new, non-risky, situation, including food, habitat or objects; (3) Activity: the general activity level of the animal, measured in a non-risky and non-novel environment; (4) Aggressiveness: an individual's agonistic reaction towards conspecifics; (5) Sociability: individual's reaction to a conspecific's presence or absence, excluding aggressive behaviour.

These personality traits may be correlated with other behaviours within a population or correlated with itself if the same trait is expressed in different contexts (e.g. high aggressiveness both when foraging and in anti-predator contexts). These correlations are properties of populations not individuals and are referred to as **behavioural syndromes**. It is within this syndrome that individuals possess a behavioural type (Sih et al. 2004; Bell 2007). Correlation between personality traits are widespread and found in a variety of different taxa. A common positive correlation is found between boldness, aggressiveness and exploration in several species. A behavioural syndrome that involves a correlation between

aggressiveness and exploration means that more exploratory individuals are also more aggressive towards conspecifics, for example (Sih et al. 2004; Boon et al. 2008; Stuber et al. 2013). Other correlations commonly found are between boldness, activity and dispersal (Boyer et al. 2010; Kekäläinen et al. 2014), or between sociability and metabolic rate (Careau et al. 2011; Cote et al. 2013), although the magnitude of these associations depends on their repeatability (Garamszegi et al. 2012). Such associations between behaviours suggests that they evolve in tandem, as a suite of traits, instead as separate evolutionary pathways (Sih et al. 2004; box 1). These behavioural syndromes can often be misinterpreted as bimodal variables (e.g., bold or shy), when instead individuals vary along a continuum between two extremes (Réale et al. 2007). Importantly, the absence of a syndrome does not mean an absence of personality (Carter et al. 2013).

Box 1. Evolution of behavioral syndromes

A behavioral syndrome is a correlation between two or more personality traits through time or across contexts (Sih, A. Bell, et al. 2004; Carter et al. 2013). A common behavioral syndrome in several species is the correlation between boldness and activity (Boyer et al. 2010; Kekäläinen et al. 2014). But why are they correlated? A possible mechanism is that these personality traits share a common neuroendocrine pathway, such as the hypothalamic-pituitary-adrenal axis. Behavioural traits are not directly influenced by genes, they result from a complex network of neurophysiological (e.g., hormones, neurotransmitters) and structural traits (e.g., neuronal structures, muscle characteristics) which are then influenced by several genes (Réale et al. 2007). Therefore the possibility arises that one gene affects the production of several hormones which influences diverse behaviors. Individual variance could arise from fundamental differences in these organization between individuals (Réale et al. 2007; Coppens et al. 2010). This correlation makes it difficult to undergo a personality transformation. To decouple one trait, one has to rewire the whole neural machinery. This is very difficult, costly and maintaining an intermediate strategy in a changing environment could be advantageous (Sih et al. 2004; Bell 2007). The main evolutionary force that contributes to the maintenance of variation in personality in a population is fluctuating selection and tradeoffs. Behavioral syndromes are very important in the persistence of this selection, where one personality trait is advantageous in a certain context but costly in another. Aggressive individuals might be more competitive and therefore acquire more resources but have a higher chance of being predated due to their higher activity (Barber & Dingemanse 2010; Quinn et al. 2009). A meta-analysis of Smith & Blumstein (2007) across species found that boldness relates to a higher reproductive success, but to a lower survival rate. Proactive animals take higher risks and pay the price, reactive animals are often unnecessarily cautious and miss some opportunities. Another trade off is found in male eastern chipmunk (*Tamias striatus*) where bolder individuals have a higher reproductive success but they exhibit a higher endoparasite load, either due to depleted energy reserve or the immunosuppressive effects of testosterone (Patterson & Schulte-Hostedde 2011).

Not all three-spined stickleback (*Gasterosteus aculeatus*) populations, for example, exhibit a well-documented correlation between aggressiveness, activity and exploration. The association between these traits depends largely on predator presence. These traits are strongly associated with one another in large ponds where predators are present, but are only weakly associated in small ponds where predators are absent (Dingemanse et al. 2007). There is a growing body of evidence showing that personalities are heritable and certain syndromes may be a reflection of genetic correlation (Dingemanse et al. 2002; Ariyomo et al. 2013). These constraints by behavioural syndromes lead to a reduction in behavioural plasticity and seemingly maladaptive behaviour in certain environments (Sih et al. 2004; Korpela et al. 2011). A reduction in one specific behavioural trait (e.g. aggression) can also reduce the presence of another trait (e.g. exploration), if they are clustered in a behavioural syndrome (box 1). An example can be found in environments with predators where prey species exhibit a low activity and a shyer personality. The same prey species however, are more active and bolder when the predator is absent because they outcompete shyer individuals (Sih et al. 2012).

1.3) Pace of life syndrome

Evidence suggests that genetic polymorphism in the Drd4 gene region may be associated with variation in similar personality traits among taxonomically diverse vertebrates (e.g. passerines, horses, monkeys, etc.) (Fidler et al. 2007). Even so, only 20 to 50 percent of the total phenotypic variation in animal personality has a genetic basis (Oers et al. 2005). Equally important in the evolution and sustainability of animal personality is the ecology of an individual, and how this influences their fitness. An interesting recent theoretical development is the incorporation of animal personalities into the "pace-of-life syndrome" (POLS) hypothesis, which combines personality types, life history and physiology (Réale et al. 2010). The POLS hypothesis predicts that life history traits associated either with a "fast" (i.e., r-strategy) or a "slow" (i.e., K-strategy) phenotype in a population are in turn associated with a personality type. Thus, individuals that adopt the r-strategy, associated with early maturation, high reproductive effort and fecundity and thus high population growth, should also tend to be bolder, more aggressive and more active. According to the POLS hypothesis, the high resource needs observed in such r-strategists are linked to their fast growth rates and fast metabolism, which leads to a lower investment in antipredator behaviours and immune function (Careau et al. 2008; 2011) – traits that are also commonly observed in bolder individuals. In contrast, individuals that adopt the K-strategy are slower-growing, have delayed maturation and lower fecundity and are often associated with more stable environments. The POLS

hypothesis predicts that these individuals have high future expectations, and should therefore be more risk-averse, shyer, and less active than r-strategists (Wolf et al. 2007; Réale et al. 2010). To maintain their longevity, resources are allocated to their immune system and they exhibit better-developed antipredator behaviours. A recent study of Eccard & Herde (2013) tried to use POLS to explain the behavioural differences in the common vole (*Microtus arvalis*) which are short lived and iteroparous animals that experience seasonal variation in density. Eccard and Herde predicted that individuals that breed in the breeding season of their birth (fast-types) should have a bolder personality than individuals that overwinter. The latter are born late in the breeding season and live through the winter as subadults. In order to increase survival during this long period, these individuals should profit from a shy and risk-averse personality (slow-types). Eccard and Herde found that this hypothesis was true, but only if the overwintering individuals were sub adults. The boldest measurements were observed in overwintering adults, which were supposed to express a shy personality. They suggested that these adults became bold in the beginning of the next reproductive season to increase their reproductive success.

1.4) Parasites and personality

Personality has important evolutionary and ecological consequences, mainly due to consistent behaviour over time, behavioural syndromes and their effects on mortality and fecundity (Sih et al. 2004; 2012; Wolf & Weissing 2012). One particularly interesting consequence of personality is its contribution to an individual's load and heterogeneity of parasites and pathogens (hereafter referred to collectively as "parasites"). There are generally three ways to describe the influence of personality on parasites. Firstly, an individual's personality has implications for the level of parasite exposure it experiences. For this reason, animals that are highly active, explorative and aggressive are more likely to come into contact with novel parasites (Sih et al. 2004; Barber & Dingemanse 2010; Hawley et al. 2011). For example, Boyer et al. (2010) found a positive correlation, although indirectly, between tick load and boldness in Siberian chipmunks (*Tamias sibricus*): bolder individuals covered more space in their habitat than shyer individuals, and also had higher tick loads. Similarly, a study on eastern chipmunks (*Tamias striatus*) found that bolder males had higher endoparasite loads, caused either by depleted energy reserves or a reduction in immunity (Patterson & Schulte-Hostedde 2011).

Secondly, different behavioural types may vary in their resistance to invading parasites (Barber & Dingemanse 2010; Boyer et al. 2010; Patterson & Schulte-Hostedde 2011), although it is not clear whether proactive animals are more susceptible or more resistant to parasites. Within the POLS framework, it is expected that proactive animals allocate their energy away from their immune system and towards other metabolic functions, increasing their susceptibility to parasite infection (Réale et al. 2010). This is supported by Patterson & Schulte-Hostedde (2011) who suggested that bolder males have a higher testosterone level which has an immunosuppressive effect. In contrast, other researchers have suggested that proactive animals, who have a higher exposure probability to parasites, may benefit more than reactive animals from investing in a potential expensive immune system (Hulthén et al. 2014). Immunologically competent individuals may behave more boldly and active because the cost of acquiring parasites is smaller than less immunocompetent individuals (Kortet et al. 2010). A study on house finches (*Haemorhous mexicanus*), for example, found a positive relationship between innate immune function and individuals that express high-risk behaviours in a novel environment (Zylberberg et al. 2014).

Thirdly, some parasites can induce behavioural changes in their host, which can be explained by three alternative hypotheses. First via the "sickness" effect, where the behavioural change is a consequence of the infection and is not-beneficial neither for the host nor the parasite. Secondly, parasites may directly manipulate their host's behaviour to increase their transmission. *Toxoplasma gondii* infection in rats and mice, for example, reduces their overall activity and are less responsive to novel stimuli, which increases the chance that these infected rodents are predated by the pathogen's definitive host, a cat (Piekarski 1981). Another example is Seoul virus, where males that engaged in aggressive behaviours for a longer duration than others, had more virus present in their body. This elevated aggression duration may increase the viruses transmission via open wounds, although the relationship between Seoul virus transmission and aggression is largely unknown (Klein et al. 2004). Lastly, the host may express an adaptive behaviour to eliminate or mitigate the harmful consequences of the parasite, such as grooming behaviours (Barber & Dingemanse 2010; Kekäläinen et al. 2014). Although these manipulations could be subtle and remain undetected, parasites could play an important evolutionary role in shaping personalities (Barber & Dingemanse 2010; Kortet et al. 2010). Simply by altering the levels of certain neuromodulator molecules, parasites could alter or decouple behavioural syndromes (Poulin 2013) or change the repeatability of certain behaviours. If an active individual has a greater probability of acquiring an infection, for example, the infection may in turn further increase the individual's activity levels to enable it to acquire more resources (to support the host and the parasite) or to promote the parasite's transmission (Barber & Dingemanse 2010; Kekäläinen et al. 2014; but see Coats et al. 2010).

There is a growing body of evidence to supports the hypothesis that personality affects parasite load and parasite heterogeneity, but there are very few studies that have looked at the effect of animal personalities on virus transmission. The first and only empirical study was done by Dizney & Dearing (2013) who showed that the personality of deer mice has an important effect on the Sin Nombre virus transmission: bolder individuals were more likely to be infected with the virus and also engaged more frequently in behaviours that increased the probability of virus transmission. Hence, Dizney and Dearing suggested that a small percentage of individuals are responsible for a majority of the virus transmission. Importantly, however, they could not make conclusions about whether boldness was responsible for a higher virus exposure, or if animals behaved more boldly as a consequence of infection. These effects are understudied and could eventually play an important role in virus transmission (Lloyd-Smith et al. 2005). The multimammate mouse (*Mastomys natalensis*) is an interesting species to test this interaction because they are the reservoir host of Lassa virus in western Africa and Mopeia and Morogoro virus in eastern Africa (de Bellocq et al. 2010) and secondly because its ecology has been studied extensively in the fields of Morogoro, Tanzania (Leirs et al. 1994), although no personality studies were conducted on this species before. In this study, we'll investigate if *Mastomys natalensis* populations in Morogoro express consistent inter-individual differences in behaviour and if these personality traits influence the transmission of Morogoro virus.

1.5) Mastomys natalensis

The Natal multimammate mouse (*Mastomys natalensis,* figure 1) is the most common indigenous rodent in sub-Saharan Africa. Although it probably originated in southern savannahs, it now exists commensally in houses and farmlands (Leirs et al. 1994) and it can be found in cultivated habitats, human settlements, natural grasslands and bushy habitats. It is known as an important pest for agriculture and a host for diseases such as bubonic plague, leishmaniasis, Lassa fever and the Morogoro virus (Leirs 1994a). Their reproductive cycle is strongly related to rainfall patterns in Tanzania (Leirs 1994b; Leirs et al. 1997). The first rainfall peak (March to May) offsets the main reproductive season, which lasts until the end of the dry season (September-October). During this reproductive season, juveniles grow quickly but do not reach sexual maturity and even stop growing near the end of the season. Their growth resumes when enough rain has fallen. Abundant rainfall in the beginning of the rainy season can allow for an extra breeding season in December. Litters produced in this period exhibit a rapid growth to maturity. As a

consequence of this reproduction cycle, populations vary a lot throughout the year (Leirs 1994b; Leirs et al. 1997). *Mastomys* individuals seldom survive beyond 12 months (Monadjem 1998).

Mastomys populations reach high densities in habitats where food is abundantly available. van Hooft et al. (2008) found evidence for negative density dependent dispersal, which may arise as a strategy to avoid inbreeding in low density, and hence low quality, habitats. Dispersal distances are usually between 20-100m but sometimes over 400m and dispersal is not sex-biased (Leirs 1994a; van Hooft et al. 2008). Daily movements generally do not exceed 20m (Leirs 1994a). In high quality habitats, with plenty of food available, home ranges for males and females decrease, which may indicate a low level of territoriality and a reduction in activity (Borremans et al. 2014). During the breeding season home ranges of males and females are the same size (Borremans et al. 2014). These findings are in contrast with previous suggestions of Kennis et al. (2008) that the Natal multimmamate mice uses a 'scramble competition' mating strategy, in which males generally have a larger home range in the breeding season compared to the non-breeding season. There is a positive correlation between body weight and reproductive success (Leirs et al. 1994; Kennis et al. 2008) which might indicate that males stay in their normal home range but use a dominance hierarchy as mating strategy instead, although both hypothesis are not mutually exclusive and both might be used as a mating strategy (Borremans et al. 2014). Leirs et al. (1997) found a negative correlation between survival and population density (i.e., predation pressure increases when population density increases).

Figure 1: multimammate mouse (*Mastomys natalensis*) in the field. Picture taken by Bram Vanden Broecke

1.6) Morogoro virus

Lassa virus is the etiological agent of Lassa fever in humans which can occurs after the inhalation of aerosolized viruses or by consuming contaminated food (Mills & Childs 1998). A severe human disease with more than 100,000 patients annually and a mortality rate of 5-10%, Lassa virus is classified as

biosafety level 4 because there is no vaccine against this disease at the moment (Yun & Walker 2012). The virulence of Lassa virus in humans also hinders research into its ecology and treatment, however there has been recent interest in the use of Morogoro virus, a closely related species (Charrel et al. 2008), as an alternative. There are two important groups of rodent-borne parasites: Arenaviruses (*Arenaviridae*) and Hantaviruses (*Bunyaviridae*). Every virus, with a few exceptions, in these two groups is primarily associated with a single species of rodent host within the *Muridae* family. In the specific host, the virus may establishes a prolonged infection which rarely causes diseases in the animal (Mills & Childs 1998). The Lassa and Morogoro viruses are both arenaviruses and have the same reservoir host: *Mastomys natalensis* (Lecompte & Fichet-Calvet 2006). Only a small percentage of *M. natalensis* mice carry Morogoro virus, but a large proportion shows specific antibodies which indicates that most animals clear the virus during their life (Günther et al. 2009). Although the pathogenicity of Morogoro virus for humans is not known, the absence of hemorrhagic fever in the area suggests that it does not cause severe diseases in humans. This makes it a promising model for studying virus-host dynamics for highly pathogenic arena viruses, such as Lassa virus (Günther et al. 2009; de Bellocq et al. 2010; Borremans et al. 2011).

Transmission of the Morogoro virus between rodent hosts does not happen via a vector but is rather thought to occur during direct interactions (e.g. in saliva or blood during aggressive fighting) or indirectly (environmentally) between hosts. Environmental transmission occurs because infected hosts shed their virus into the environment as aerosols or released in urine and faeces. This is a 'short' free living stage outside the host (Ramsden et al. 2009; Charrel et al. 2008; Borremans et al. 2011). Borremans et al. (2011) found a higher RNA prevalence in juveniles and suggested that vertical transmission might be important, or that horizontal transmission is increased in this age group, due to lack of immunity, higher susceptibility to infection and/or higher contact rate with other juveniles. High antibody and viral RNA prevalence has been found in low quality habitats, with low densities, which indicates density independent transmission (Borremans et al. 2011).

1.7) Path model and research questions

We hypothesized that the weight, which is a proxy for age in *Mastomys* (Leirs et al. 1990) and the individual's personality could be directly responsible for infection with Morogoro virus, due to differences in their immune system for example (Barber & Dingemanse 2010), or indirectly by being

more active and hence cover a larger area, which may increase the chance of coming into contact with infected faeces (Charrel et al. 2008; figure 2). Alternatively, infection with the Morogoro virus might influence the individual's weight, personality or space use (Barber & Dingemanse 2010; figure 3). To test our hypothesis, we repeatedly sampled individuals from six different fields over a three-month period. Multiple sample populations were used to account for specific environmental variations, such as predation pressure, food abundance and other variations known to influence weight (Leirs 1994b) and personality (Sih et al. 2012). Personalities were measured by recording *M. natalensis* behaviour during repeated exposures to an Open field (OF) and Novel object (NO) test. Activity had to be measured in a non-novel environment (Réale et al. 2007), we therefore used all the trapping information (see material and methods). Blood samples were collected from animals each time they completed an OF/NO test, and were checked for the presence of MORV-specific antibodies. An animal was assumed to be or have been infected with MORV when it had MORV-specific antibodies in its blood. We used the individual's weight, their exploratory behaviour, activity and space use to test the two hypotheses and their predictions (Figure 2 and 3). These *a priori* biologically arrangements are organized in a path model that allows testing for direct or indirect relationships in the cause-effect linkage between several variables, which is called a path analysis (Scheiner et al. 2000; Shipley 2002). A path analysis allows us to specify a model and relationship between variables, by correcting for certain correlations and may eventually tease apart correlation and causation. It differs from a regression analysis because the variables in a path analysis can be dependent and independent, whereas variables in a regression analysis are either independent or dependent. It may also show indirect pathways which are invisible in a regression analysis (Shipley 2002).

1.7.1) Model A

We hypothesize that animal personalities can contribute to individual variation in susceptibility to pathogen infection, which may be influenced by differences in immune system (Barber & Dingemanse 2010; figure 2, arrow 5). For example, the POLS hypothesis suggests that bolder animals, with a high metabolic rate, use a lot of their resources for fast growth and maturation, to the detriment of their immune system (Réale et al. 2010; Careau et al. 2011). This is a direct effect of personality on MORV infection. As boldness and activity are commonly linked, we therefore hypothesize that more exploratory individuals in the OF and NO tests should be trapped more often (figure 2, arrow 1) and should visit a larger variety of traps compared with less explorative and less active individuals (Boyer et al. 2010; Kekäläinen et al. 2014; figure 2, arrow 2). Animals that are more active and exhibit a larger space use also have an increased chance of coming into contact with infected faeces or individuals, and are hence

more likely to get infected (figure 2, arrow 4). This would be a positive, but indirect pathway between personality and MORV infection.

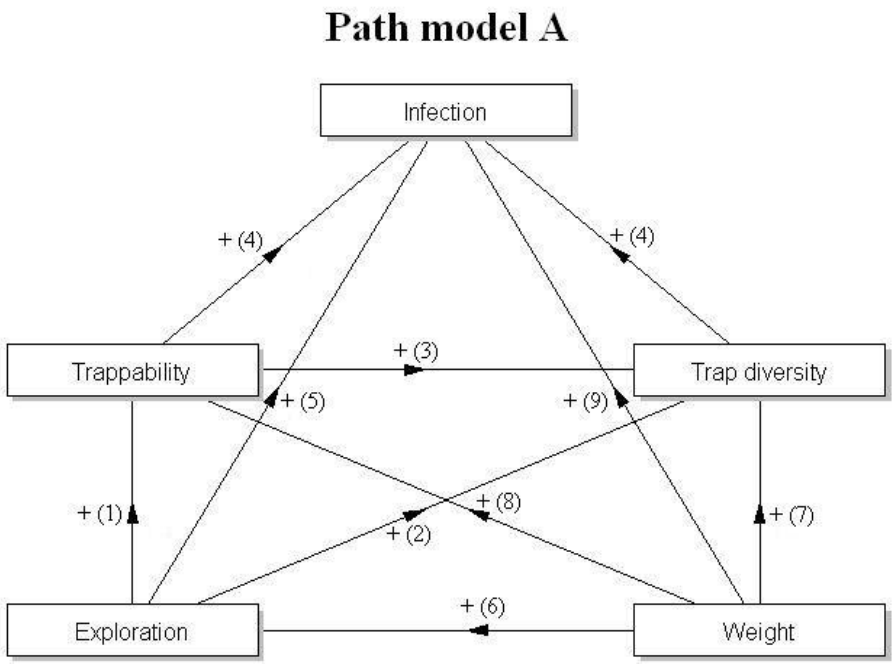

Figure 2: Path analysis diagram of model A linking exploration (i.e. personality) and weight with Morogoro virus infection based on *a priori* hypothesized links. Each line indicates a relationship between two boxes, the arrow indicates the direction of causality, while the sign (+ or -) indicates the direction of the correlation. The numbers normally represent the model coefficients, but in this figure they identify each of the possible relationships: (1) Explorative individuals, measured via OF and NO tests, are hypothesized to be trapped more often (i.e. increase trappability) and are (2) more active (visit a greater range (i.e. diversity) of traps) based on the commonly found behavioural syndrome correlating exploration with activity. (3) Animals that are trapped more often should visit more unique traps and have a higher probability (4) to come into contact with infected faeces and/or individuals. These are indirect effects of personality on MORV infection, (5) is a direct effect where explorative individuals have a lower immune system, which increases their chance of becoming infected (see text for more information). (6) Heavier individuals could be more explorative and thus influence the exploration measurements and/or (7,8) cover more space in the field and hence are trapped more often in a wider variety of traps. (9) weight could also be positively associated with infection status because bigger individuals might have reduced their immune function to reproduce more.

In common voles, risk taking and activity are interpreted as investments into behaviours that enable the animal to reproduce (Eccard & Herde 2013). This may also be the case in *Mastomys* ,where there are two hypotheses about their mating strategy (i.e. scramble and dominance) although they are not mutually. Risk taking and activity may increase an individual's chance of finding mates (i.e. scramble competition) or securing their rank (i.e. dominance hierarchy). Weight is a good indicator for fitness in multimammate, with mice because larger males having a higher reproductive success (Leirs et al. 1990; Kennis et al. 2008). We therefore hypothesize that larger individuals are bolder (Figure 2, arrow 6) and more active (figure 2, arrow 7 and 8) than smaller animals, and hence have a higher probability of coming into contact with infected faeces or animals (figure 2, arrow 4). This is an indirect way in which weight influences MORV infection. Alternatively, working in the POLS theory, weight may be directly responsible for MORV infection and personality variation (Réale et al. 2010; figure 2, arrow 9). Individuals that adopt

the “fast” (i.e. r-strategy) phenotype are associated with an early maturation and high growth rate. This means that these individuals weigh more, relative to their age, than individuals whom express the “slow” (i.e. K-strategy) phenotype. The high resource needs observed in these r-strategists are linked to their fast growth rates and fast metabolism, and thus to their lower investment in antipredator behaviour and immune function (Careau et al. 2008; 2011) which would eventually lead to a higher chance of infection. Another possibility is that bigger animals, which use the r-strategy, are also older than lighter animals (Leirs et al. 1990; Borremans et al. 2011). We predict that these individuals should behave more boldly and have a lower Immune system (POLS) because they have nothing to lose since *Mastomys* seldom survive beyond 12 months (Monadjem 1998) hence surviving to next reproduction event is not likely (Eccard & Herde 2013).

1.7.2) Model B

This model is fairly similar as the previous model, but there is still a possibility that MORV infection could directly or indirectly induce behavioural changes in the infected mice. MORV infection could directly alter the host’s behaviour if it increases their transmission to the next host. This can be done by altering the hosts response to novel stimuli (figure 3, arrow 5), activity (figure 3, arrow 4) or aggression, for example (Piekarski 1981; Klein et al. 2004; Dizney & Dearing 2013). Secondly, MORV infection could directly alter the individual’s weight (figure 3, arrow 9). Infection with the Sin Nombre virus in deer mouse, for example, has a negative impact on their health, where infected animals gained less weight over one month following the seroconversion (Douglass et al. 2007).

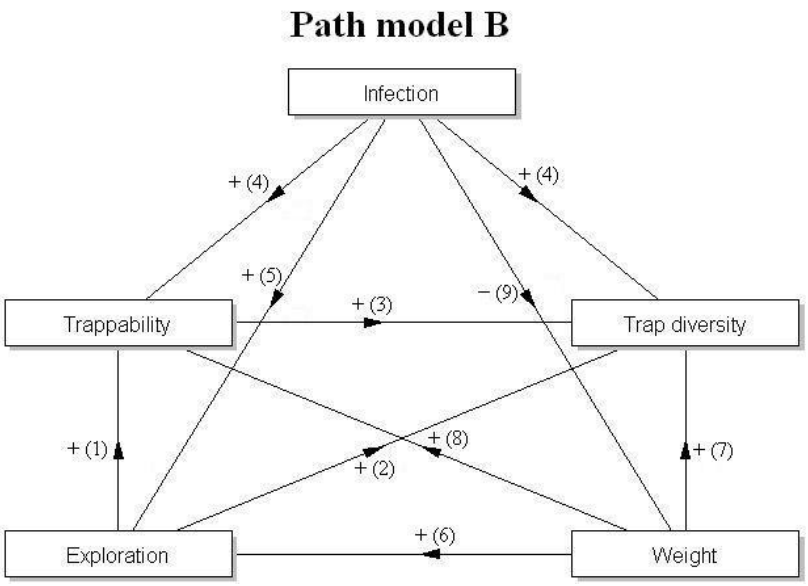

Figure 3: Path analysis diagram of model B where MORV infection could influence activity, exploration and weight. Each line indicates a relationship between two boxes, the arrow indicates the direction of causality, while the sign (+ or -) indicates the direction of the correlation. The numbers normally represent the model coefficients, but in this figure they identify each of the possible relationships. Arrows 1, 2, 3, 6, 7 and 8 are similar to those from model A (Figure 2) and will not be described here. (4) MORV infection could increase the animals’ activity in the field to increase its transmission or could directly influence the animals’ personality (5) by reducing their reaction to novel stimuli. (7) It could also directly influence weight due to the physically harmful effects of MORV infection.

1.8) Importance of this study

This study aims at unraveling the interaction between animal personality (and thus behaviour) and the exposure and susceptibility of wild *M. natalensis* to infection with Morogoro virus. To our knowledge, very little empirical research has been done in the field concerning personality and virus transmission. The sole study we are aware of, that of Dizney & Dearing (2013), found that bolder deer mice were three times more likely to be infected with Sin Nombre virus than shy individuals. This study could not determine whether this infection was the cause or consequence of risky behaviour, a weakness that is common to most studies using naturally infected hosts instead of experimentally infected ones (Poulin 2013). This study could be the first which bring to light this problem, using the path model, proposed by Boyer et al. (2010). If bolder animals are indeed more likely to get infected with Morogoro virus, then these individuals might be responsible for a large proportion of the transmission events, and are called "superspreaders" (Meyers et al. 2005; Modlmeier et al. 2014). This would affect current epidemic models and disease control as Lloyd-Smith et al. (2005) showed that individual-specific control measures for outbreaks are far better than population wide measurements.

2) Material & methods

2.1) Study area and trapping

The fieldwork was conducted on the campus of the Sokoine University of Agriculture (Morogoro, Tanzania; hereafter referred to as the SUA) between 29 July and 18 October 2013. This period between June and September-October is the dry season which is followed by a bimodal rainy season starting in October, peaking around December (*vuli*-rains) and then again more strongly around March-April (*masika*-rains) (Leirs 1994b). Animals were trapped on six 1 ha grids (100 traps in a 10 x 10 arrangement, with 10 m between traps) in agricultural fields which were bare clay/loam used to grow maize. Grids were spaced at least 700m apart for spatial independence, more than twice the average dispersal distance (300m) for *M. natalensis* (Leirs 1994b). Capture-mark-recapture trapping was conducted for three consecutive nights every two weeks using Sherman LFA live traps (Sherman Live trap Co., Tallahassee, FL, USA). A mix of peanut butter and maize flour was used as bait. Traps were checked in the morning and captured rodents were transported to the SUA Pest Management Center by car. They were

released in the evening on the same spot as we caught them and all traps were rebaited. A total of 6 trapping sessions (three nights each) were conducted on each field, except field 2 with only 4 sessions.

At first capture, animals were individually marked using toe clipping. Each time an individual was trapped, independent of the trap session, they were weighed and sexed using a standard protocol to determine their reproductive status (following Leirs 1994). Mice were considered to be subadults until a sign of sexual activity could be observed (scrotal tested in males; perforated vagina or pregnancy in females) after which they were considered to be adult. Blood samples were taken when the animal was caught for the first time in a certain trap session. Blood samples were taken from the retro-orbital sinus and preserved on pre-punched filter papers (± 15 µL/punch; Serobuvard, LDA 22, Zoopole, France). To minimize any potential effects of stress on behaviour, toe clipping and blood sampling occurred after the behavioural tests.

2.2) Behavioural tests

Behavioural tests were conducted in the SUA Pest Management Center. Tests were performed once every trapping session in which an individual was trapped. Two different tests were conducted: first an open-field test (OF), which measures the behavioural reaction of an animal in a novel environment from which escape is prevented. OF test are used to quantify activity and exploration (Archer 1973; Martin & Réale 2008). Secondly, we used a novel object (NO) test to assess exploration and boldness (Réale et al. 2007). The two tests were performed in the same testing session, with the OF test also serving as habituating time before NO test. The testing arena for OF and NO tests was a 75 x 55 x 44 cm semi-translucent box. The walls of the box were covered with red plastic (figure 4), which nocturnal species perceive as dark and 16 squares (19x13) were drawn on box floor. Behavioural observations were made with a digital video camera (Panasonic, Lumix DMC-SZ1, USA) that was installed above each box, which allow us to record the whole area.

At the start of each trial, an animal within a trap was placed inside the box with the trap opening facing away from the door of the room. The OF test started after manually opening the trap. After five minutes the NO test started with the introduction of a novel object, a dark blue plastic microscope slide storage box, on the opposite side of the box to the trap. The NO test also lasted 5 minutes. The observer left the room during each test to avoid potential disturbance. Natural daylight was used as lighting (but due to the red plastic sheets around the arena, the arena was perceived as "dark" by the rats. The arena and

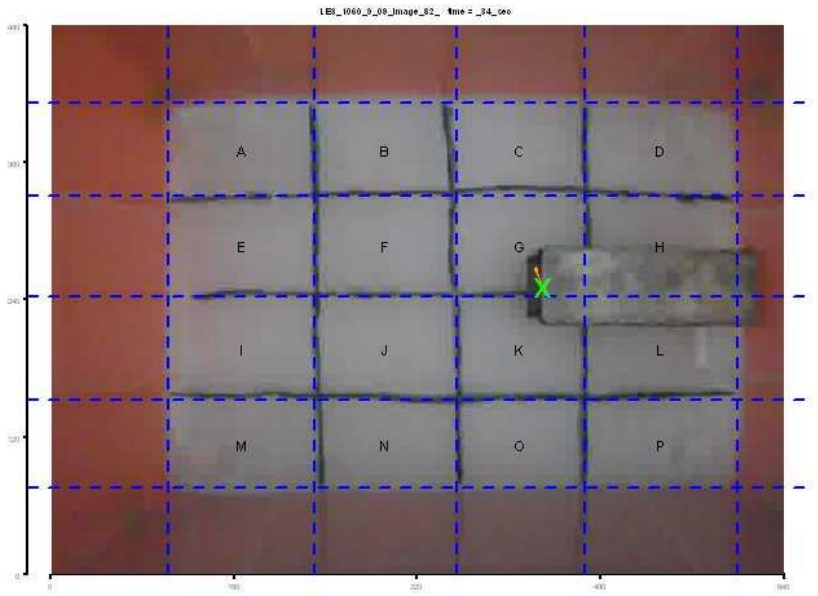

Figure 4: Experimental setup used during the behavioural tests, with lines added from the analysis. The floor of the testing arena is divided into 16 squares (4x4) of approximately equal size and a specific letter for Identification. The green cross represents the position of the mouse, which is not visible in this case and therefore takes the trap entrance. The red surrounding the ground floor are the walls of the box covered with a dark red plastic.

the novel object were cleaned after every trial using 70% ethanol Video recordings were later analyzed using R statistical software 3.0.2 (R Development Core Team 2013). We measured the following behaviours. (i) Activity: measured as the total number of times the animal crossed the lines of the squares, assessed in OF and NO (ii) Latency to leave the trap (in seconds). If an animal did not leave the trap after 5 minutes we noted 300 seconds as being the maximum value. Animals were not forced to leave the trap, as this would be a measurement of fear and/or anxiety instead of exploration from the free OP/NO tests (Misslin & Cigrang 1986). Latency was measured in both tests. (iii) Latency to approach the novel object. This defined as reaching one of the 4 squares on the opposite side of the box (squares A, E, I and M; figure 4).

2.3) Morogoro Virus

Blood samples were analyzed for Morogoro virus (MORV) NP protein specific IgG antibodies using Immunofluorescence assay (Borremans et al. 2011) at the University of Antwerp (Belgium). Dried blood on filter paper was dissolved in 100µL phosphate-buffer saline, 10µL was presented to MORV-infected Vero cells on a microscopy glass (Bernhard Nocht Institute for Tropical Medicine – Hamburg, Germany). After one hour of incubation, 10µL of Fluoreceïne-isothiocyanaat- (fitch)- conjugated secondary mice Ab were added to every sample. Samples were analyzed with a fluorescence microscope and were classified as antibody negative, antibody positive, or uncertain. All uncertain samples were analyzed again until a conclusive result was obtained.

2.4) Statistical analysis

2.4.1) Density estimation

The abundance of the rodent population during each 3-night trapping session (in which the population was assumed to be closed) was estimated with DENSITY software (version 5.0.3), a Windows application for the analysis of capture-recapture data from different arrays of passive detectors (Efford et al. 2004). Estimations were done by using the heterogeneity estimator M(h) which allows variability in individual capture probabilities and has been widely used to evaluate *M. natalensis* densities from field data (Leirs et al. 1997).

2.4.2) Personality

We conducted a total of 994 behavioural tests during the study period. Only individuals that were recorded at least twice were analyzed. Animals that escaped during the behavioural tests were deleted from the dataset. The final dataset therefore consisted of 299 behavioural tests on 122 unique individuals (including 78 females and 44 males) 77 individuals were recorded twice, 32 three times, 12 individuals were recorded four times and one individual five times (figure 5). These replicated measurements allowed us to estimate the repeatability of the behavioural responses in the behavioural tests (Réale et al. 2007). For the same individual two consecutive tests were separated by a minimum of 11 days (mean 21 ± 10 days).

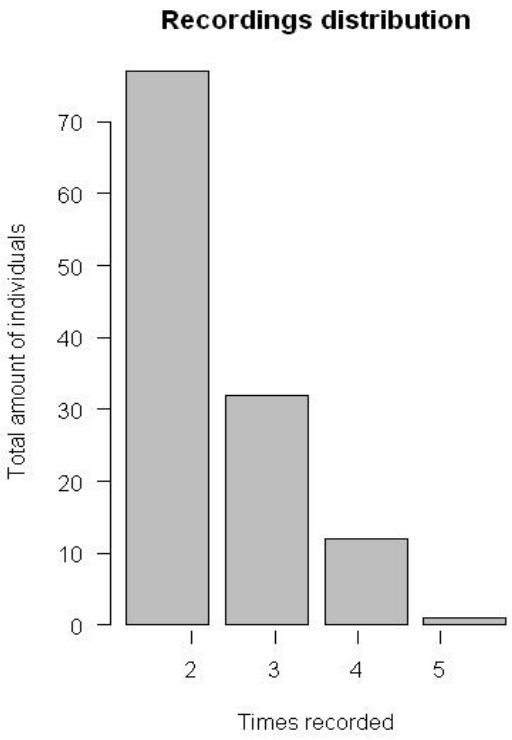

Figure 5: Graphical representation of the recordings that were used. 122 unique individuals were recorded: (*N*=77) were only recorded twice, (*N*=32) were recorded three times and represents the second largest group, (*N*=12) were recorded four times and (*N*=1) five times.

A principal component analysis (PCA) was executed on the behavioural variables from the OF and NO to reduce the number of behavioural variables. The construction of the PCA does not require that the variables show a multivariate normal distribution and hence data can be used untransformed (Martin & Réale 2008). The Kaiser-Guttman criterion was used to select the number of principal components to retain. Components with an eigenvalue > 1 (i.e., squared standard deviation of each component) summarize more information than any single

original variable and are therefore retained (Kaiser 1991; Jackson 1993; Peres-Neto et al. 2005). Behaviours with a loading of at least 0.4 were considered to contribute to a component.

For each component we first ran a linear-mixed model (LMM) (Pinheiro & Bates 2000; Crawley 2007) with maximum likelihood. We used sex, weight, density within the trapping session and a binomial variable describing whether it was the first time this individual had been caught and recorded (1 or 2, further referred to as first recording) as fixed effects, with a three-factor interaction and *M. natalensis* identity (hereafter ID) as a random effect. Significant fixed effects ($P < 0.05$) were selected by a stepwise backward procedure. This was done by the package lmertest (version 2.0 Kuznetsova et al. 2014). The significance of the random effect (i.e. between-individual variance in behaviour) was tested by comparing the final LMM (with restricted likelihood) with a linear model (LM) without ID as a random effect. A likelihood ratio test (LRT) was carried out between the two models. This was calculated as **2 [(log-likelihood LM)-(log-likelihood LMM)]** which results in a chi-squared distribution with one degree of freedom (Pinheiro & Bates 2000; Martin & Réale 2008). A *P* - value lower than 0.05 indicates that a significant amount of the variance can be contributed to between-individual variance. The use of LMMs allowed us to control for pseudoreplication of the data that occurs with repeated measurements on the same individual (Crawley 2007).

Repeatability, as described above, can be defined as the proportion of the total variance described by differences among individuals:

$$R = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_\varepsilon^2}$$

where σ_α^2 is the between-individual variance and σ_ε^2 the within-individual variance. The sum of σ_α^2 and σ_ε^2 represents the total phenotypic variance (Nakagawa & Schielzeth 2010; Wolak et al. 2012). R is a value between 0 and 1 but is often notated as a percentage. A repeatability of 1 (i.e. 100%) would mean that there is no behavioural variation within an animal and the only measured variance would be due to between-individual differences. The repeatability drops when individuals vary in their behavioural responses. LMMs allow us to directly calculate the repeatability, because they provide the between-individual variance estimate (σ_α^2) and residual variance (σ_ε^2) (Nakagawa & Schielzeth 2010). Another possibility is using the rptR package which also provides 95% confidence intervals (Nakagawa &

Schielzeth 2010). Finally, we calculated the average Component score of the PCA for each individual to estimate the individual behavioural profiles. These average values were used as indices of personality for each *M. natalensis*.

2.4.3) Trappability, trap diversity and minimum distance

We used the live-trapping data for each animal with a personality index to estimate their movements and space use. We made two different datasets: the first "explorative" dataset consisted of 124 individuals, 78 females and 44 males, each with a personality index (average PC1 score per individual, see results). A second "comparative" dataset was constructed to compare the results of the explorative dataset to a dataset with more individuals. The individual's in this dataset do not have a personality index because not every individuals behaviour has been analyzed. This comparative dataset consisted of 255 individuals (148 females and 107 males) that were caught twice or more during the study. We calculated for each individual in the two dataset a single weight value by taking the average of all weight measurements during the study. We calculated an individual's "trappability" as the total number of times they were trapped. Trap diversity was calculated as the total number of unique traps an individual was trapped in. We also calculated the straight-line distance between an animal's successive trapping locations; a distance of 0 was recorded for animals that were only caught at one trap location. The sum of these inter-trap distances gave us an index of the minimum distance covered by an individual during the study.

We ran LMs for trappability, trap diversity, minimum distance travelled, weight and personality index as a function of sex, length of capture period (i.e. the number of trapping nights between the first and last date of capture), infection status (see below), average population density, trappability, trap diversity minimum distance travelled, weight and personality, with an interaction between sex and weight. All models were run for both the explorative and comparative datasets, allowing us to examine whether any correlations in the explorative dataset occurred due to a small sample size. We compared the slope values from the tests on both datasets to see if they were significantly different from each other (Crawley 2007).

2.4.4) Infection status

As all the animals in the dataset were caught at least twice, multiple measurements of MORV antibody presence were available for each individual. For further analysis, it was necessary to provide each individual with a single infection status. Animals that were negative during the whole study were classified as negative, while individuals that tested positive throughout the study, or which changed from

negative to positive during the study, were classified as positive. The explorative dataset therefore contained 15 and 10 infected females and males respectively, while the comparative contained 28 and 21 infected females and males respectively. 30 individuals were infected with MORV prior to the study, while 19 individuals changed from antibody negative to antibody positive during the study.

We tested if sex, weight, trappability, trap diversity, minimum distance travelled, personality index, length of capture period and density had an effect on infection status using a generalized linear model (GLM). This was necessary because infection state was noted as a binary response variable, which ceated a non-normal distribution of errors. This was corrected by using a binomial error distribution (Crawley 2007) and the model ran with 1000 iterations. A proportion test of infected animals between males and females was conducted to control for differences by sex and a Tukey test with weight, sex and first order interaction was conducted to decide if an interaction between weight and sex was necessary for the GLM.

2.4.5) Path analysis

A path model is organized of *a priori* logical and biological based relations that allows testing for direct or indirect relationships in the cause-effect linkage between the measured variables (Shipley 2000; 2002). For instance if you have a model where A,B and C influence the dependend variable Y, it might be possible that A positively influences B which influences Y independently from C. In this case, A has an indirect effect on Y. A path analysis allows us to distinct between direct and indirect effects. Secondly, it allows us to make a distinction between several proposed models. A proper example of the use of path models can be found in (Shipley 2000; Thomas et al. 2007; Bonser et al. 2010; Boyer et al. 2010).We constructed two models (figure 2 and 3) and each hypothetical link is explained in the introduction. The first model (figures 2, model A) hypothesized that exploration or an individual's weight could be either directly responsible for the infection heterogeneity between *M. natalensis* or indirectly via variations in space use (Boyer et al. 2010). The second model considered the probability that infection with MORV has an effect on weight, exploration or space use (Barber & Dingemanse 2010).

All the variables were linked together in both models. We calculated all the path coefficients separately with a LM or GLM (depending on the error distribution) to identify the causal pathway that connects variables and best fits the data (Boyer et al. 2010). These path coefficients correspond to the standardized partial regression coefficients, and describe the strength and the direction of the linear association. These coefficients are a measurement of the unique association between the dependent and independent factors by controlling (i.e., partialling) any association with other predictors (Crawley

2007). The standardization means that values range from -1 to 1 and tells us how strong the relation is (McDonald 2009). To identify the path model (A or B) that best fits the data, we obtained the predicted conditional independence constraints that must apply if the hypothesized causal relationships are true (Shipley 2002). We therefore estimated the basis set, which is al list of the smallest set of 'direct-separation' or 'd-separation' of variables that are not directly related with each other (i.e. they do not have an arrow between them) and are predicted to be probabilistically independent of each other. We then list the 'causal parents' of all the pairs (i.e. the variables that directly cause either variable in the pair). Each d-separation relation in the basis set states that the two variables forming the pair are probabilistically independent of each other, conditional on the causal parents of the pair (Shipley 2004; Thomas et al. 2007). We use the d-separation test to evaluate if the other chains of causality could be rejected. We tested each independence claim against the data using Pearson's partial correlation coefficient and *P*-value, which can be extracted from a LM. For example (Shipley 2004), if trait X and Y were predicted to be independent of each other (i.e. no direct arrow between them), conditional on a set of variables Q={A,B,C, …} which are their causal parents (i.e. directly correlated), we regress X and Y separately on the variables of Q and estimated the correlation between the residuals of X and Y by using a LM (Bonser et al. 2010; Boyer et al. 2010). The final step of the path analysis is to combine these separate tests of independence into a combined test of the entire model. This is done by using the Fisher's C statistic: $C = -2 * \sum \ln (p_i)$, where (P_i) are the probabilities of the pairs that were tested for independence. A large value of C indicates a significant difference between the observed and predicted patters of conditional independence and is evidence against a certain path model. C follows a chi-square distribution with 2*k* degrees of freedom, where *k* is the total number of pairs tested for independence in the model. A probability below the significance level of 0.05 leads to the rejection of the causal and proposed model (Shipley 2000; 2002).

Statistical analyses were executed with R software 3.0.2 (R Development Core Team 2013) and graphs were constructed in R, except figure 1 in the appendix in excel.

3) Results

3.1) Density

The rodent abundance in grid 1, 3, 4 and 5 were fairly similar throughout the study period (figure 6), with values between 25 and 100 individuals (Min = 26.5 ± 5.1 (field 1), Max = 99.7 ± 9.3 (field5)). Field 2 had the lowest densities (Min = 2.7 ± 1.1; Max = 15.3 ± 3.7) and field 6 the highest of all the grids (Min = 106.9 ± 10.1, Max = 149.4 ± 11.3). Due to a low recapture rate, it was not possible to estimate the density of some trap sessions in some grids, and these were replaced by "NA" in the dataset.

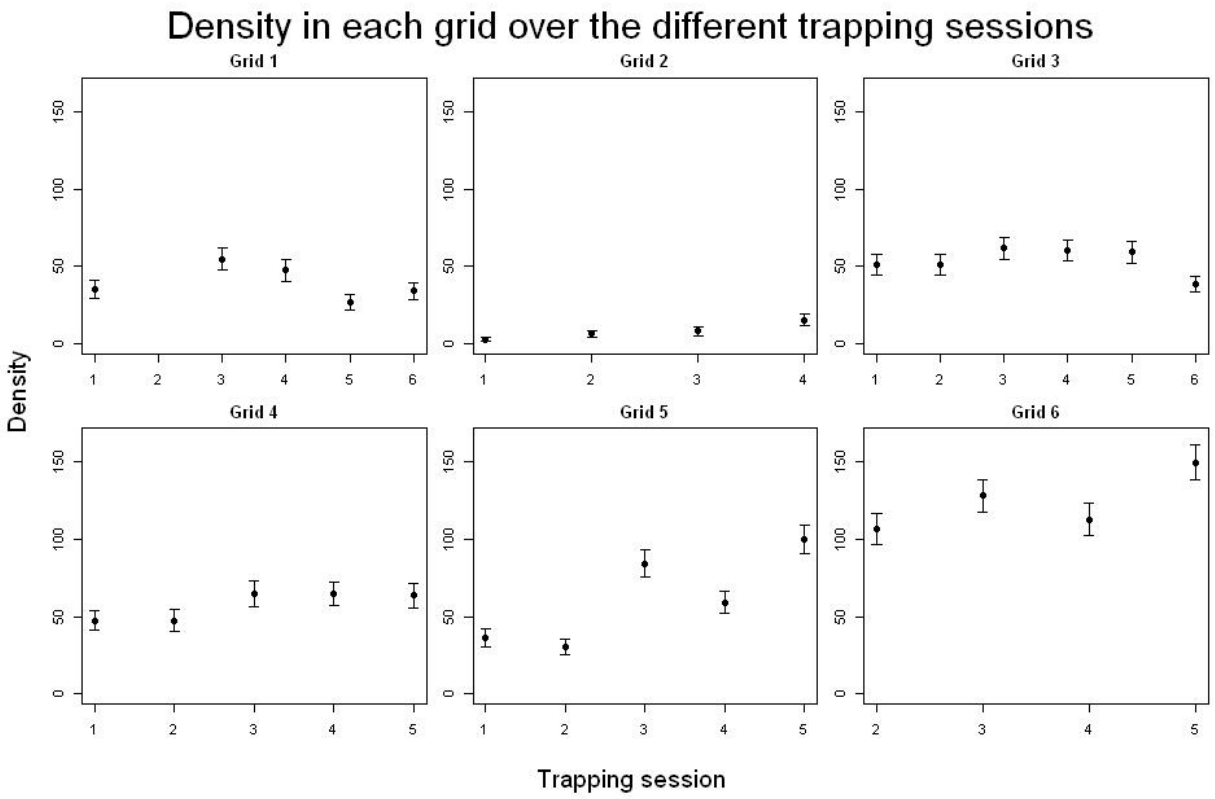

Figure 6: Graphical representation of the fluctuations in rodent density over trapping sessions divided for each grid separately. Each density estimation is represented as a point with standard error bars.

3.2) Individual behavioural profiles

The principal component analysis (PCA) reduced the number of variables to only two components with an eigenvalue larger than 1.0 (table 1), which means that they contain more information than any single original variable and were therefore retained (Kaiser 1991; Jackson 1993; Peres-Neto et al. 2005). The two components combined explained 85.72% of the total variance. All behavioural variables, except the latency to leave the trap in the OF, were associated with the first component (PC1). PC1 explained

58.84% of the total variance and was positively correlated with activity measurements (Squares crossed) and negatively with the latency measurements from the NO (Leaving trap and Contact with object) which means that individuals with a high PC1 value were faster to enter the arena in the NO and moved around more than those with a low PC1 value. We will refer to this component as exploration hereafter. The second component (PC2) explained 26.88% of the total variance and was strongly correlated with measurements of the OF test, where individuals with a high PC2 value were very active in the OF test and it took them less time to leave the trap in comparison with animals that exhibit a low PC2 value.

Table 1: Principal component analysis loadings for each behaviour observed in the Open Field test (OF) and Novel Object test (NO). Bold typing indicates behaviours that had a strong contribution to that component. The standard deviation of PC1 is the square root of the eigenvalue of that component. Each component is represented with its mean repeatability and standard error. The *P* - value of each component indicates the ID effect.

Behavioural variables	Component 1 (PC1)	Component 2 (PC2)
OF: Squares crossed	**0.405**	**0.556**
OF: Latency leaving trap	- 0.347	**- 0.640**
NO: Squares crossed	**0.457**	- 0.265
NO: Latency leaving trap	**- 0.507**	0.313
NO: Latency contact with object	**- 0.500**	0.336
Standard deviation	1.7153	1.1594
Total variance explained	58.84%	26.88%
Repeatability	28.1 ± 7 % ; $P < 0.001$	7.8 ± 6.3 % ; $P > 0.05$

The only important variable that explained a significantly amount of variation of PC1 after a stepwise reduction of fixed effects in the LMM was the individual's weight (R = - 0.0182 ± 0.0075, t = - 2.43, P = 0.0171; figure 7) all other variables were not significant ($P > 0.05$; table 1 appendix), *M. natalensis* ID explained a significant proportion of the variance for PC1, and the behaviours of this axis were repeatable for the same individual (repeatability = 28.1 ± 7.0 %, CI=[0.136, 0.411], LRT = 11.74, P = 0.0002) which is moderately high. The recording order (first recording) was the only significant effect for PC2 (R = 0.4644 ± 0.1281, t = 3.63, P = 0.0004) suggesting that individuals that were recorded for the first time in their life were less active in the OF. ID had no significant effect on PC2 and the repeatability of this component was very low (repeatability = 7.8 ± 6.3 %, CI=[0.000, 0.227], LRT = -230.52, $P > 0.05$). As PC1 was the only component showing significant behavioural differences between individuals, behavioural profiles or "exploration" scores were calculated from this component.

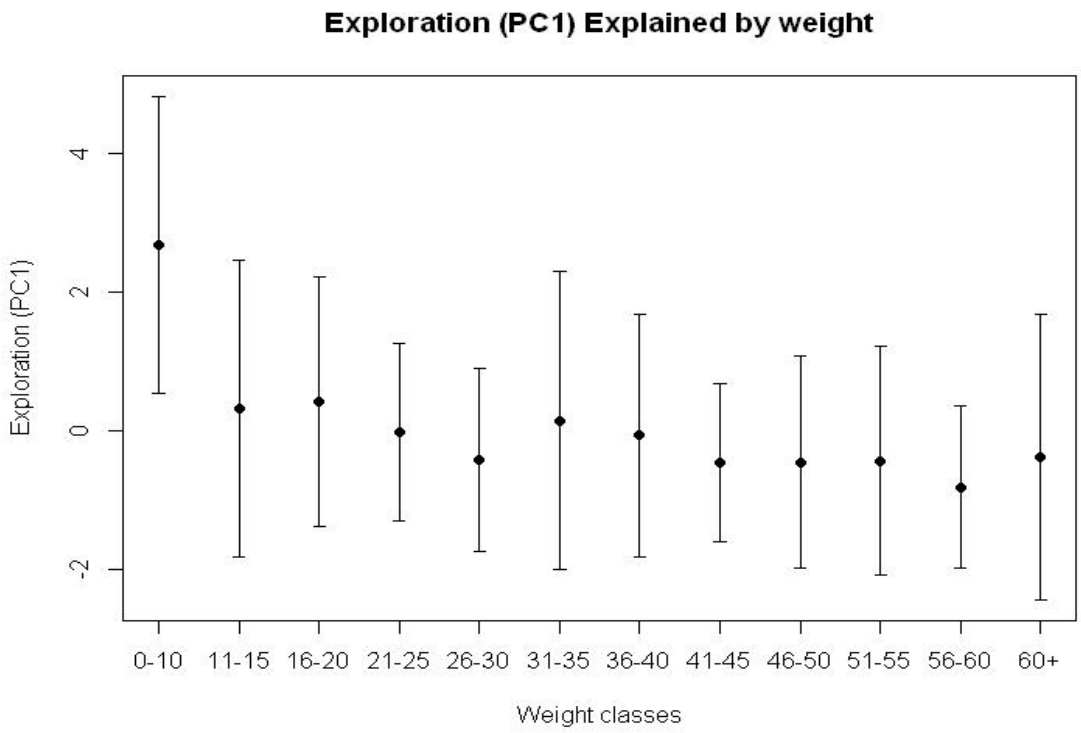

Figure 7: Exploration (PC1 score) reduces with increasing weight. Weight was divided into different classes. PC1 score was averaged for each class (dots) separately with standard error bars. Each individual provides more than one PC1 value, depending on the total recording for that specific individual.

3.3) Trappability, trap diversity and minimum distance

Using the small explorative dataset we found several significant factors that influence trappability, trap diversity and the minimum distance they travelled. These variables were all highly correlated with one another. Trappability (i.e., the total number of times we trapped an individual) was significantly positively correlated with capture length (i.e., the time between first and last capture; $R = 0.220 \pm 0.038$, $t = 3.77$, $P < 0.001$; figure 8), and also with trap diversity (i.e. the sum of unique traps a certain animal was trapped in; $R = 0.123 \pm 0.033$, $t = 3.77$, $P = 0.0003$; figure 9a,). Thus, the greater the length of time between an animal's first and last capture, the more often they were trapped, and the greater the diversity of traps in which they were caught. The mean weight of an animal was also significantly positively related with trap diversity ($R = 0.012 \pm 0.004$, $t = 2.76$, $P = 0.0067$, figure 9b), as was the minimum distance travelled ($R = 28.245 \pm 2.260$, $t = 12.50$, $P < 0.0001$; figure 9c). All other results were non-significant (table 1 found in the appendix).

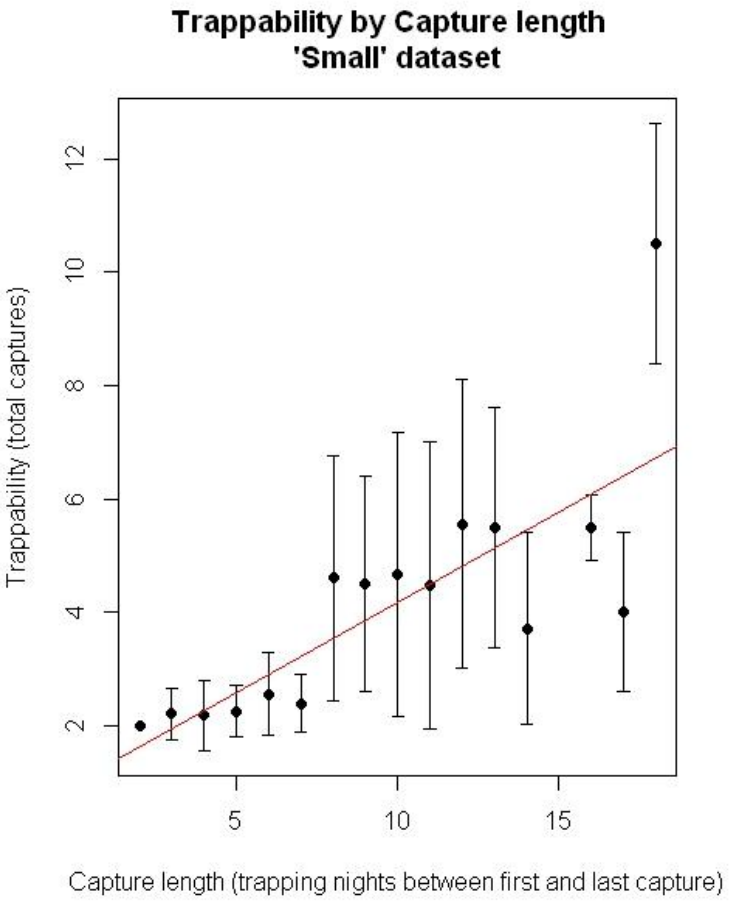

Figure 8: Correlation between the total number of times we caught an individual (i.e. trappability) and the number of trapping nights between their first and last capture (R = 0.318 ± 0.041, t = 7.70, P < 0.0001, R^2 = 0.32) The probability to trap an individual increases with an increasing trapping effort. Each point is the average "trappability value" for each capture length period, accompanied with standard error bars. The red line is the regression line.

Similar LMs were constructed with data from the larger comparative dataset. These LM yielded similar results (Table 2 appendix) as those described above for the explorative dataset. Trap diversity was strongly correlated with trappability (R = 0.197 ± 0.024, t = 8.24, P <0.0001; figure 9a); when an animal was trapped more often it was also more likely that they were trapped in a greater number of unique traps. Similar to the results from the smaller dataset, heavier animals visited a higher variety of traps than lighter individuals (R = 0.010 ± 0.003, t = 2.87, P = 0.004; figure 9b). Trap diversity did explain on its turn a significant amount of variation in the minimum distance travelled by a specific individual (R = 23.735 ± 1.768, t = 1.43, P <0.0001; figure 9c). There was no significant difference between the regression coefficients of both datasets (table 2), suggesting that there was no bias due to a smaller sample size in the explorative dataset and that it holds sufficient information. Density, sex nor MORV infection status explained a significant proportion of the variance in either datasets (table 1 and 2, appendix).

Table 2: Comparison of the slopes of the 2 different regression lines by the different dataset by looking at the interaction. P - values greater than 0.05 indicate no differences in slopes.

Dependent Independent	**Coefficient ± SE**	***t* - Value**	***P* - Value**
Trap Diversity			
Trappability : Dataset	0.033 ± 0.043	0.75	0.453
Weight : Dataset	- 0.004 ± 0.008	- 0.48	0.635
Activity			
Trap Diversity : Dataset	- 1.672 ± 2.051	- 0.82	0.416

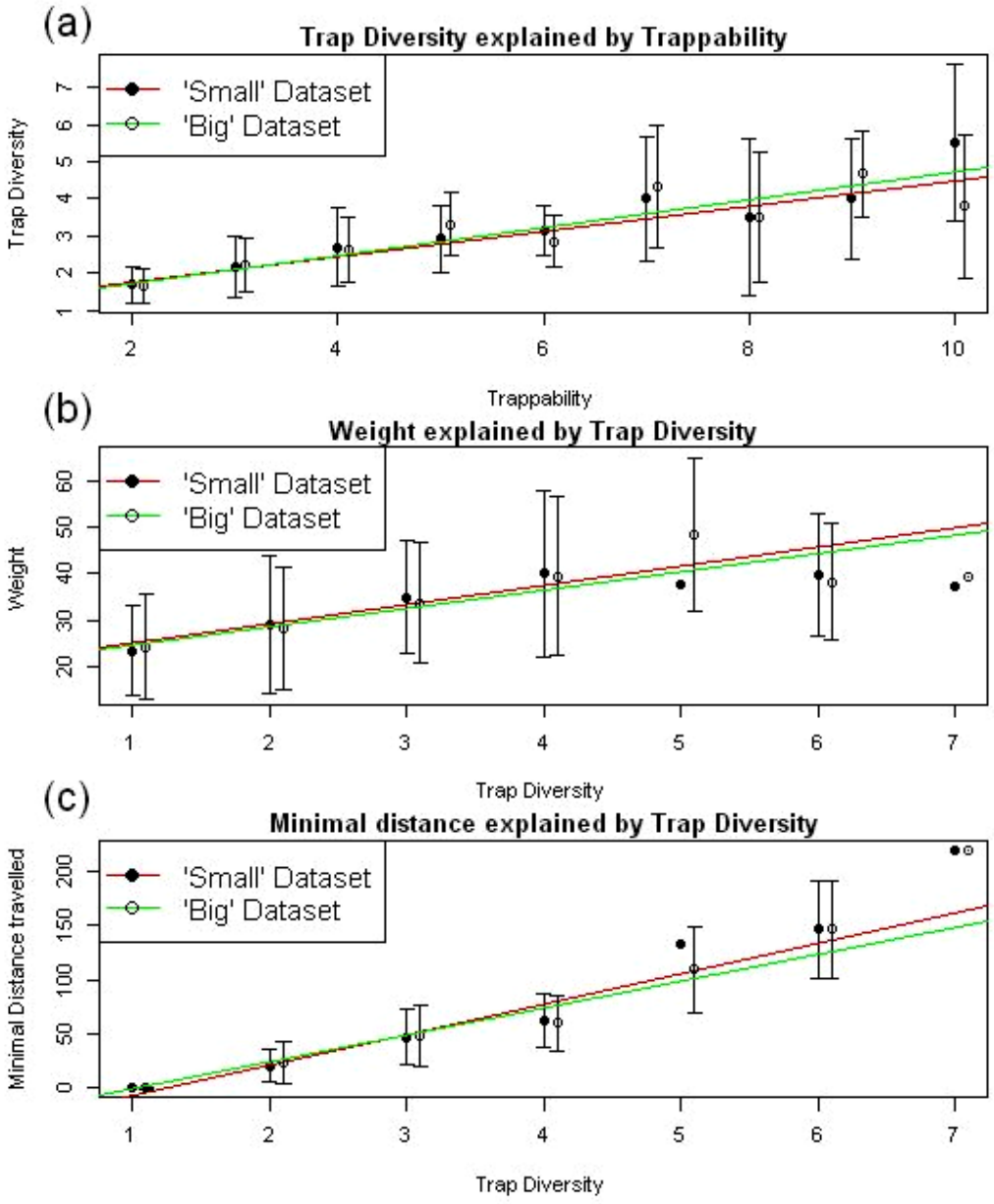

Figure 9: Comparing the two different datasets with each other. In every plot, the red regression line is derived from the smaller, explorative dataset, while the larger comparative dataset is represented by the green regression line. The full dots are the average values, derived from the explorative dataset and the open dots are the average values from the comparative dataset. Each point is accompanied with standard error bars. (a) the positive correlation between trap diversity and trappability (Small dataset: $R = 0.338 \pm 0.037$, $t = 9.01$, $P < 0.0001$, $R^2 = 0.395$; Big dataset: $R = 0.377 \pm 0.028$, $t = 13.58$, $P < 0.0001$, $R^2 = 0.419$). Individuals that were trapped more often were trapped in more unique traps. (b) this graph was pivoted for the sake of graphical representation, where the use of more unique traps increases with an increasing weight (Small dataset: $R = 4.115 \pm 1.039$, $t = 3.96$, $P < 0.001$, $R^2 = 0.107$; Big dataset: $R = 3.939 \pm 0.780$, $t = 5.05$, $P < 0.0001$, $R^2 = 0.088$). (c) the individuals activity, which was calculated by summing the minimum distance an individual must have travelled between each trapping against the total unique traps used (Small dataset: $R = 27.872 \pm 1.569$, $t = 17.76$, $P < 0.0001$, $R^2 = 0.719$; Big dataset: $R = 24.714 \pm 1.272$, $t = 19.44$, $P < 0.0001$, $R^2 = 0.597$).

3.4) Exploration and Weight

We constructed a LM with exploration (the average PC1 score per individual) as the dependent variable. The only significant factor explaining the variance in exploration was weight (R = - 0.023 ± 0.011, t = - 2.13, P = 0.035; Table 2 appendix; figure 10) which was negatively correlated with exploration, which means that heavier animals are less explorative. Both datasets showed that females weighed more than males (explorative dataset: R = - 6.715 ± 2.325, t = - 2.89, P = 0.0046; comparative dataset: R = - 4.358 ± 1.597, t = - 2.73, P = 0.007; figure 11b) and that *M. natalensis* with antibodies for MORV weighed more than those that were antibody negative (explorative dataset: R = 7.672 ± 2.757, t = 2.78, P = 0.0063; comparative dataset: R = 8.149 ± 2.009, t = 4.06, P <0.0001; figure 11a).

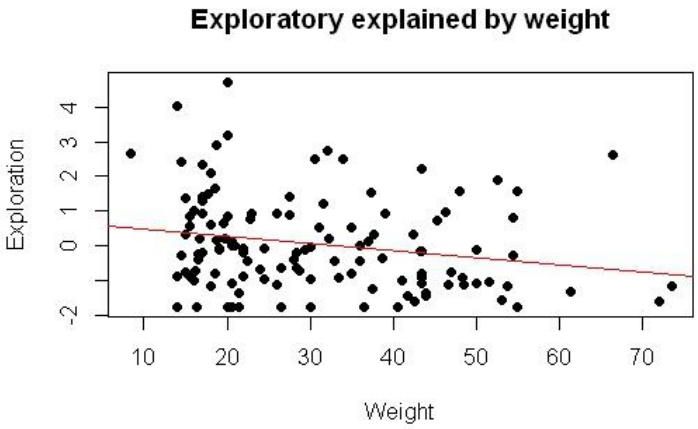

Figure 10: The average exploration score, which is an indication of the individuals personality, plotted against weight. There is a negative correlation between these two variables (R = - 0.021 ± 0.009, t = - 2.44, P = 0.0161, R^2 = 0.039 which means that the exploration score decreases with an increase in weight. Each point is a single individual and the red line is the regression line.

3.5) Infection with MORV

Individuals with MORV antibodies present throughout the whole study weighed significantly more than animals that were not infected with MORV during or before the study (Tukey test: Diff = 11.95, P <0.0001) but were not heavier than those that became infected with MORV during the study (Tukey test: Diff= -7.61 grams, P = 0.12). This latter group was not significantly heavier than those that were negative throughout the whole study (Tukey test: Diff= 4.34 grams, P = 0.35; Figure 11a). 18.92% of females and 19.63% of males were positive for antibodies against MORV (N = 28 and 21 respectively). These two proportions were not significantly different from each other (Proportion test: df = 1, P = 1). Infected males were on average significantly lighter than uninfected males (Tukey test: diff= 13.79 grams, P <0.0001), and significantly lighter than the two infection groups (prior and during) in females (Tukey test: $Diff_{neg♀}$ = -6.87 grams, P = 0.001; $Diff_{pos♀}$ = -12.41 grams, P <0.0001). Males that had antibodies present in their blood were not significantly heavier than females with MORV specific antibodies ($Diff_{pos♂\text{-}pos♀}$ =

1.37 grams, *P* = 0.98). We therefore did not use the interaction between weight and sex in the GLM. The GLM with data from both sets stated that weight was the only significant factor (table 1 and 2 appendix) that explained the presence of MORV antibodies in their blood (explorative dataset: *R* = 0.056 ± 0.021, *t* = 2.73, *P* = 0.006; comparative dataset: *R* = 0.048 ± 0.013, *t* = 3.81, *P* = 0.0001). Although individuals start showing MORV antibodies in their blood when they weigh more than 20 gram (figure14)

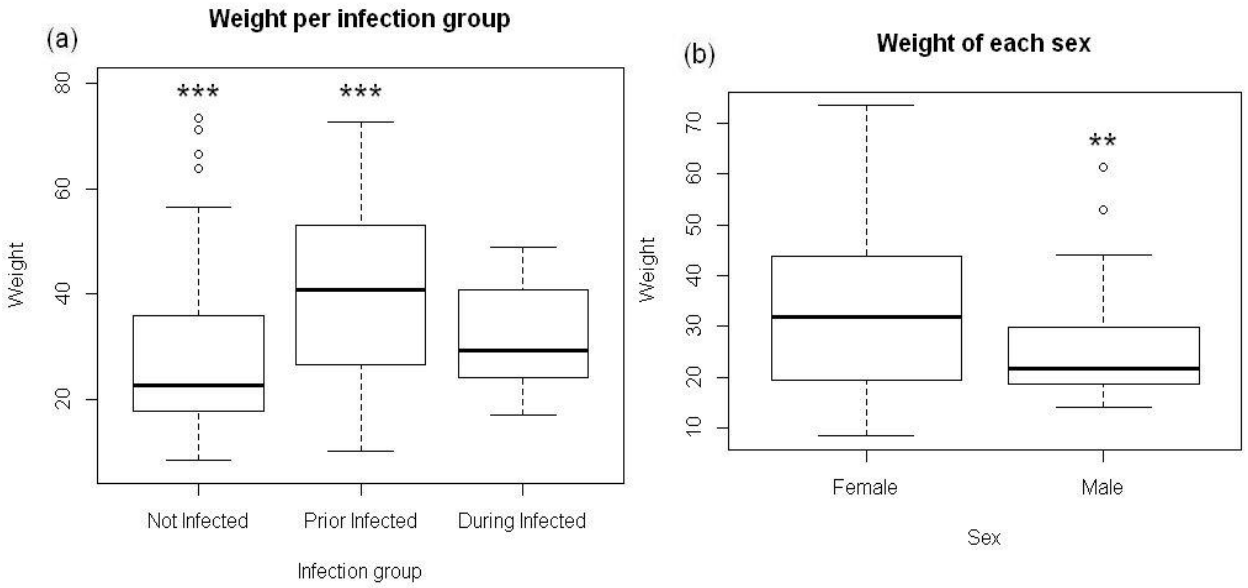

Figure 11: Boxplot representation of: (a) the weight of each infection group. The "Not Infected" groups represents all the individuals that were negative for MORV antibody presence before and during the whole study. "Prior Infected" are all the individuals that were antibody positive from the first day of capture, which indicates that they were infected before the study started. "During Infected" represents all the individuals that turned positive for MORV antibodies during the study. Individuals that were infected before the study began were significantly bigger that individuals that were negative during before and during the whole study (* *P* < 0.001). (b) Weight differences between the two sexes, where males are significantly smaller than females (** *P* < 0.01)**

3.6) Path analysis

3.6.1) Model A

The significant structural equations, with path coefficients and standard errors, derived from the causal path model (figure 12) based on the data from the explorative dataset were:

Exploration = - 0.216 ± 0.089 Weight (*t* = -2.44, *P* = 0.016)

Trap Diversity = 0.389 ± 0.083 Weight (*t* = 4.66, *P* <0.0001) **+** 0.663 ± 0.068 Trappability (*t* = 9.78, *P*<0.0001)

Lifetime Infection = 0.210 ± 0.089 Weight (*t* = 2.37, *P* = 0.0194)

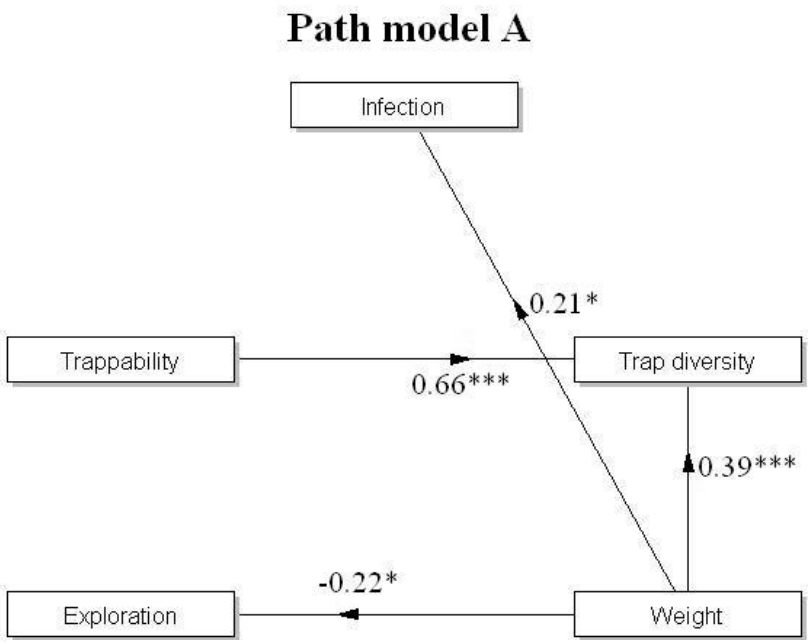

Figure 12: Resulting path analysis diagram of Model A with their associated standardized path coefficients. Weight has a negative influence on exploration and a positive on trap diversity and presence of MORV antibodies, all independently from each other. Trappability is not influenced by any other factor but has a positive influence of trap diversity, besides the positive influence of weight. Arrows indicate the direction of causality. (* *P* < 0.05, ** *P* < 0.01, ****P* < 0.001)

Intercepts approximated zero and had a *P*-value of 1 and were therefore ignored and are irrelevant for this study. Weight had an influence on exploration independently from other correlations, where exploration decreases when weight increases. Exploration had no effect on any other individual traits. Trap diversity was directly positively affected by trappability and by weight. These correlations suggest that heavier animals visit a greater number of traps, independent of the total time an individual was trapped. Trap diversity and trappability had no effect on infection rate, but were indirectly connected with exploration through weight. MORV infection status was directly affected by weight, with heavier animals having a higher probability of showing MORV specific antibodies in their blood. This model is graphically represented in figure 12. Considering the basis set of independence, this path model was not rejected ($P > 0.05$; table 3).

3.6.2) Model B

The structural equations derived from the second alternative path model, where MORV infection influences weight (figure 13) were:

Exploration = - 0.196 ± 0.089 Weight (t = -2.21, P = 0.029)

Trap Diversity = 0.389 ± 0.083 Weight (t = 4.66, P <0.0001) **+** 0.663 ± 0.068 Trappability (t = 9.78, P<0.0001)

Weight = 0.249 ± 0.088 infection (t = - 2.21, P = 0.0292)

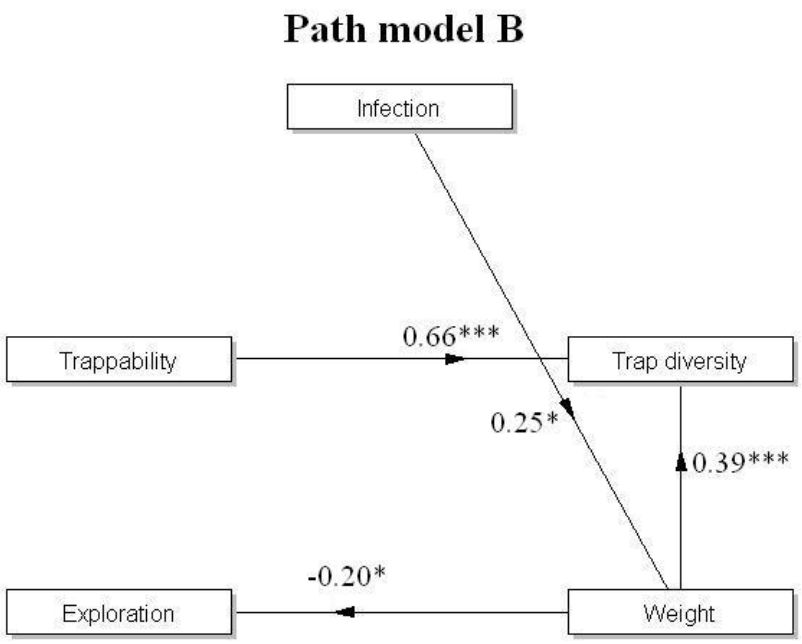

Figure 6: Resulting path analysis diagram of Model B with their associated standardized path coefficients. This model is fairly similar as model A, except the direction of causality between weight and MORV antibody presence. Here, antibody presence has a positive influence on the individuals weight. Arrows indicate the direction of causality. (* *P* < 0.05, ** *P* < 0.01, ***P* < 0.001)

All Intercepts had a *P*-value of 1 and were left out of the equations. We found similar results in modelA, where weight had a negative effect on exploration via a direct correlation. Trap diversity was influenced, independently, by weight and trappability. Trap diversity was indirectly linked to MORV infection through a pathway between weight and infection status. In this model, infection status had a direct positive and significant effect on animal weight. This model was not rejected, based on the basis set of independence (*P* > 0.05; table 3). Both models fitted the data very well and they both had a very low C value which meant that both model A as model B are fairly similar, although the C value of model B is slightly lower than model A (table 3).

Table 3: Basis sets, tests of conditional independence and the Fisher's C statistics associated with the 2 path model A and B from figures 12 and 13. "(X , Y) |{ Z_i , Z_j, … }" means that X and Y are d-separated and hypothesized to be independent, conditional on the set of variables { Z_i , Z_j, … } and "ϕ" represents a null (empty) set. The model is rejected if the C probability <0.05. Basis set numbers refer to: 1 (Exploration), 2 (weight), 3 (Trap Diversity), 4 (Trappability), 5 (Infection).

Model A				Model B			
Basis set	*t* - Value	Partial r	*P* - Value	Basis set	*t* - Value	Partial r	*P* - Value
(1,3)|{2,4}	- 0.60	- 0.054	0.551	(1,3)|{2,4}	- 0.60	- 0.054	0.551
(1,4)|{2}	- 0.06	- 0.005	0.952	(1,4)|{2}	- 0.06	- 0.005	0.952
(1,5)|{2}	- 0.64	- 0.058	0.521	(1,5)|{2}	- 0.63	- 0.057	0.531
(3,5)|{2,4}	- 0.43	- 0.039	0.668	(3,5)|{2,4}	- 0.36	- 0.033	0.716
(4,5)|{2}	0.97	0.087	0.336	(4,5)|{ϕ}	0.96	0.086	0.340
(2,4)|{ϕ}	0.28	0.025	0.779	(2,4)|{5}	0.05	0.004	0.964
d.f.	10			d.f.	10		
Fisher's C	6.082		0.912	Fisher's C	5.456		0.941

4) Discussion

4.1) General findings

The main objective of this thesis was to study whether weight and/or animal personality of *Mastomys natalensis* could be directly or indirectly (via space use) related to infection with Morogoro virus. We did not find a direct link between the individual's personality and MORV infection, nor did we find an indirect pathway between personality and MORV antibody presence by a larger space use, which has been proposed by Boyer et al. (2010). We did find evidence that the individual's weight, which is a proxy for age, had an important effect on their personality and space use, independent of each other. Our results also suggests that there is a positive correlation between weight and MORV infection, although the direction of causality is not clear, as neither proposed models could be rejected. Model B (figure 13) did fit the data slightly better than Model A (figure 12), however, and its Fisher's C value was also slightly lower, potentially indicating that MORV infection has an influence on the individuals weight.

4.2) Personality

We repeatedly measured several behaviours per individual in a new, non-risky environment via an Open field (OF) and a Novel object (NO) test, which are used to measure and quantify exploration (Archer 1973; Réale et al. 2007; Carter et al. 2013). The behavioural measurements correlated strongly with each other in the first component of the PCA. We named this first component exploration and it had a repeatability of 28%, which is the common range of repeatability and indicates that *M. natalensis* behave consistently over time in their exploration behaviour (Bell et al. 2009). This within-individual consistency and consistent difference between individuals are necessary to form a personality trait (Réale et al. 2007; Carter et al. 2013). This is the first study that defines personality in *M. natalensis* and hence the first one that quantifies personality on the exploration-avoidance continuum.

There was no influence of population density or different grids on exploration scores. This corresponds to the results of Korpela et al. (2011) on bank voles, where population density had no effect on novelty seeking nor extroversion. This might suggest that exploration is a stable personality trait and should not be viewed as a flexible strategy depending on the rodent abundance (Bell 2007). The OF and NO tests quantified the individual's willingness to react to a novel stimuli (Denenberg 1969) and were repeatable over time and contexts, which means that the individual differences in exploration are also present in

their natural environment. Interestingly, we found a negative association between weight and exploration, where heavier individuals were less explorative. We suspect that exploration is part of the animal's way to process information and reduce uncertainty about the external environment through information gathering (Renner 1990). Since older individuals are heavier than younger ones (Leirs et al. 1990), we propose that exploration is a life-stage specific personality trait in *Mastomys natalensis*. Juveniles may need to gather more information about their environment to optimize their foraging behaviour, adults on the other hand already have this "prior-experience" because they lived longer. The juveniles that are born later in the reproductive season and need to grow more quickly and hence might be more explorative to increase their likelihood of finding resources (Leirs et al. 1994; Biondi et al. 2013).

Secondly, we hypothesized that more explorative individuals have a higher metabolic rate and therefore need a lot of energy, possibly to the detriment of their immune system (Réale et al. 2010; Careau et al. 2011). However, we did not find a direct nor indirect (via a larger space use) relationship between exploration and MORV infection status. Borremans et al. (2011) found a high RNA prevalence in juveniles, which is a direct indication for infection of MORV in this species. They suspected that juveniles are more susceptible to infection due to behavioural differences or a lack of immunity. Our data might confirm the hypothesis that juveniles are more susceptible to infection due to behavioural differences, as we found that smaller animals were more explorative than larger ones. We suggest that small animals like juveniles might therefore have a higher probability of coming into contact with infected faeces or infected individuals. It would be very interesting to test this hypothesis by looking directly at the correlation between virus RNA prevalence (which is a direct measurement of MORV infection) and the between individual variation in exploration. This could not be included in this study due to a limitation in time. Still, we did not find a direct link between exploration and MORV antibody presence, which might suggest that there is no small percentage of individuals, who are bolder or more explorative than others, responsible for a majority of the MORV transmission events. This contrasts with the situation for deer mice and Sin Nombre virus transmission, where bolder individuals might be responsible for a high percentage of the transmissions between individuals (Dizney & Dearing 2013). Exploration does not increase the individual's susceptibility to MORV infection nor does the antibody absence/infection alter the hosts personality in exploration. Possibly because *Mastomys natalensis* is the only natural host for MORV (Charrel et al. 2008; Günther et al. 2009) and transmission is not via vectors but rather indirectly via a 'short' free living stage outside the host (Ramsden et al. 2009; Borremans et al. 2011) and therefore might not need to alter the hosts behaviour to increase their transmission. Although there is evidence that some viruses might alter their host behaviour to increase their transmission via aggressive

interactions for example (Klein et al. 2004; Dizney & Dearing 2013). Our results indicate that exploration does not increase nor decrease the probability of the presence of MORV specific antibodies. Even so, the absence of an effect does not mean we can reject our hypothesis that explorative individuals have an increased probability of getting infected with MORV, especially because we looked at MORV antibodies, which are remnants of a previous infection. Juveniles have a higher RNA prevalence (Borremans et al. 2011) and are more explorative, which might indicate a correlation.

The PCA has two components with an eigenvalue larger than one. The first component represented exploration, and a significant amount of the variance in this first component was due differences between individuals. Individual variation on the other hand, had no significant effect on the second principal component, which means that the variance in this component is not due to individual differences, but due to another factor. PC2 is positively correlated with activity in the OF test. We suspect that this effect is just a consequence of the novel object introduction during the experimental setup, which varies independently between every trial. Our results showed that trial order explained a significant amount of the variance, where individuals were more active in their later recordings. We argue that the second component may reflect the habituation to the setup (Martin & Réale 2008), but is equal for each individual which is reflected in the low repeatability and the absence of the individual effect.

The repeatability of exploration was significantly different from zero, but the 28.1% was slightly lower than the 35% behavioural repeatability found in a recent meta-analysis (Bell et al. 2009). A low repeatability occurs when the repeated measurements of a certain behaviour shows a relative high within-individual variance compared to a lower among-individual variance (Nakagawa & Schielzeth 2010; Wolak et al. 2012). The first explanation is that the within-individual variance is much higher relative to the between-individual variance. There are several factors that could affect the inter-individual variation. First, males have been found to be more consistent in their behaviour than females because of the female oestrus cycle phase which might affect their behaviour, which has been recorded in house sparrows (*Passer domesticus*; Nakagawa et al. 2007) and other species (Bell et al. 2009; Schuett & Dall 2009). Although we did not find any sex effects on any variable (except weight), the use of both sexes in our study (to fully represent the population) might reduce the repeatability by increasing the within-individual variance. The same effect could be present depending on the life stage of a certain individual (adult-juvenile), both life stages are represented in our study. In common voles, for example, behavioural consistency in exploration decreases over time and depended on the different life stages (Herde &

Eccard 2013). Although Bell et al. (2009) found no difference in the repeatability of behaviours in juveniles or adults in their meta-analysis. Secondly, repeatability of a personality trait does not necessarily mean that all of the individuals within the populations behave equally consistently: some might be more consistent while others might be more flexible (Dingemanse et al. 2010). Sih et al. (2004) argued that individual plasticity is beneficial to cope in rapidly changing environments because individuals with a limited plasticity might express inappropriate behaviours. That may be the case in this study, because the populations we sampled were from agriculture fields used to grow maize, and hence changed constantly. Another common effect is that bolder individuals are reportedly more rigid (i.e. reduced behavioural plasticity) compared to shyer individuals (Bell et al. 2009; Coppens et al. 2010; Hulthén et al. 2014; but see Frost et al. 2007). Lastly, micro-environmental differences, such as light intensity or the condition of transfer, for example, between the tests could potential influence the within-individual variation and hence repeatability (Martin & Réale 2008). Alternatively to a higher within-individual variance, a decrease in the between individual variance could eventually reduce repeatability as well. The first explanation for a reduced between-individual variance originated from the choice of using the free OF and NO tests, in which we did not force the individual to enter the testing arena (Misslin & Cigrang 1986). As a consequence of this method, several individuals did not enter the arena during the whole behavioural test which may reduce the between-individual variance. Secondly, it has been suggested that stabilizing selection to a certain optima decreases the between individual variance as well (Schuett & Dall 2009). Since explorations by *Mastomys* have not been studied before, it is very difficult to argue about this latter effect, but it should not be neglected.

4.3) Activity

We did not find a correlation between exploration and trappability (i.e. the total number of times an animal was trapped) or exploration and trap diversity. This indicates that there is no behavioural syndrome between activity and exploration. The presence of a behavioural syndrome might depend on certain environmental factors, for example predation pressure in three-spined sticklebacks (Dingemanse et al. 2007) and certain studies have found this bahavioural syndrome between activity and exploration (Boyer et al. 2010; Kekäläinen et al. 2014) while others found no correlation between these two personality traits (Patterson & Schulte-Hostedde 2011; Carter et al. 2013). A meta-analysis has shown that the average strength of phenotypic correlations between behaviours like exploration and activity were weak (Garamszegi et al. 2012). Still, the lack of correlation does not necessarily imply a lack of a

behavioural type (Réale et al. 2007). A possibility is that exploration could be correlated with personality traits we did not measure during this study such as boldness, aggression or socialization (Sih et al. 2004; Réale et al. 2007). There are two possible explanations for the absence of a correlation between activity and exploration. Firstly the different phenotypical traits may be controlled by different hormones and genes that interact separately in the complex network of neurophysiological and structural traits (Réale et al. 2007). The second explanation is a possible age-dependent tradeoff between exploration and activity. We argue that older individuals are more active in the field but are less explorative compared to juveniles. Younger individuals at the end of the reproductive season might not yet reach sexual maturity and hence do not profit from a higher activity (Leirs 1994a; Kennis et al. 2008) but need to gather more information about their environment to improve their chances of survival until the next reproductive season.

The trappability of an individual depended solely on the capture duration, where the trapping probability increases as trapping effort (i.e. time spent trapping) increases. These results indicate that trappability is not affected by certain individual traits (i.e. weight and exploration) and only by trapping effort. This suggests that trappability does not reflect the individuals exploratory behaviour, as was suggested by (Boon et al. 2008; Boyer et al. 2010). This means that current and previous studies on *Mastomys natalensis* in Morogoro were unlikely to be affected by an individual based sampling bias due to personality differences in exploration. Although other personality traits, such as boldness could potentially influence the trappability, as has been recorded in great tits (Stuber et al. 2013) and Namibian rock agama (Carter et al. 2012) but were not accounted for in this study. The minimum distance a certain individual travelled (i.e. the individuals activity) during the study depended solely on the trap diversity (i.e. total unique traps) the individual used. Trap diversity is therefore a good indicator for the individual's activity during the study. The trap diversity of a certain individual was strongly correlated with trappability, which indicates that the activity level of the individuals that were repeatable trapped were better determined by increasing trapping effort. Taking into account this effect, trap diversity was also positively correlated with individual weight. This indicates that heavier, and therefore older individuals are more active than younger and lighter ones, which corresponds to our hypothesis that older individuals are more active in the field to increase their reproductive success. In common voles, risk taking and activity are interpreted as investments into behaviours that enable the animal to reproduce (Eccard & Herde 2013), which might also be the case in *M. natalensis* considering the two proposed hypotheses for their mating strategy: scramble competition (Kennis et al. 2008) and dominance hierarchy, or both (Borremans et al. 2014). Weight is correlated with reproductive success in

M. natalensis (Leirs et al. 1994; Kennis et al. 2008). Our results suggest that heavier individuals are more active, and may hence find more mates. This supports the hypothesis that *Mastomys natalensis* uses scramble competition as mating strategy. Secondly, these results are quite similar to those from the common voles, where Eccard & Herde (2013) found that adults were bolder and invested more into reproductive effort because reproductive events become scarce. This may be a possible explanation for our results as well. We took measurements from July until October, which is near the end of the reproductive season (Leirs 1994a). Juveniles grow quickly but do not reach sexual maturity at this time, but they resume growth in the following rainy season (Leirs 1994a). Surviving until the next reproductive season might be impossible for the adults since *M. natalensis* individuals seldom survive beyond 12 months (Monadjem 1998). Therefore, adults have nothing to lose and hence express a bolder behaviour and are more active to find more mates to increase their reproductive success. Furthermore, our results showed that density had no effect on trap diversity, which might indicate that the reduction in home ranges in high density populations (Borremans et al. 2014) does not result from a reduction in activity.

4.4) MORV infection State

Our results showed that the presence of MORV specific antibodies in *Mastomys* were not sex-dependent, which indicates that males and females have a similar probability of getting infected with MORV. There was no effect of density which hints at density independent transmission, proposed by Borremans et al. (2011). This same study by Borremans and colleagues showed that antibody presence increases with age. This corresponds with our findings that weight, which is an indication of age, is positively correlated with antibody presence. Both path models show that antibody presence is correlated with weight but differed in their causation: either weight influences the probability of showing MORV antibodies (Model A; figure 12) or the presence of MORV antibodies affect the hosts weight (Model B; figure 13). Model B fitted the data slightly better than model A, which indicates that MORV antibody presence positively influences the individual's weight. This model seems unlikely, however, because we studied MORV antibodies, which are a consequence of infection and not infection itself. Model A does not differ that much from the model B and is biologically more plausible. In this model, heavier and hence older individuals are more likely to show MORV-specific antibodies in their blood than younger ones. There is a strong possibility that these results are biased, since antibody presence is a consequence of infection at an earlier age and stay present in the host even after the virus is cleared and thus accumulates over time (Mills et al. 2007). This effect of accumulation can be seen in

figure 11a where individuals that had MORV antibodies in their blood before the start of our study were significantly heavier than individuals that were not infected prior to or during the study. Our data supports the hypothesis about the accumulation through time since there are individuals with MORV antibodies in their blood when they weighed only 20 grams (figure 1 appendix), individuals from this weight class are still considered juveniles (Leirs et al. 1990). The positive correlation between weight and MORV antibody presence might be just due to the absence of uninfected large animals and the accumulation through time of antibodies which elevates the mean and hence is responsible for the positive correlation.

Interestingly, we found several individuals that turned seropositive during the study, which may indicate that these individuals got infected during the study. These individuals weighed slightly more, although not significantly, than individuals that stayed negative during the whole study. It would be interesting in future studies, with a larger sample size, to see if heavier individuals are indeed more likely to get infected during the end of the reproductive season or if they transmit the virus through the population. If this would be the case, it would have consequences for current epidemiological models, such as those by Goyens et al. (2013). Studies have shown that Individual-specific control measures for disease spreading are far better than population based measures (Lloyd-Smith et al. 2005).

We conducted our fieldwork from August till the end of October, which is the end of the reproductive season for *Mastomys natalensis* (Leirs et al. 1994). Although we did not find any correlation between exploration and MORV infection state, the study period might be important, although this is purely hypothetical. For future research, we recommend to follow behavioural and personality changes through the whole year. We hypothesize, based on the POLS hypothesis and the findings of Eccard & Herde (2013) that adults should behave more boldly throughout the whole year. Juveniles on the other hand may change their behaviour throughout the whole season because individuals born in the start of the reproductive season might adopt a “fast”-strategy to grow and mature very quickly, at the expense of their immune system (Réale et al. 2010; Eccard & Herde 2013), while juveniles that are born later might have a “slower”-strategy to survive until enough rain has fallen and become sexual mature (Leirs et al. 1994). At this point, they might change their strategy to a bolder one, to contribute very early to the growing population (Eccard & Herde 2013). Secondly, we did not look at other personality traits, such as boldness, sociability or aggression, which might influences MORV transmission (Klein et al. 2004; Barber & Dingemanse 2010; Dizney & Dearing 2013).

4.5) Conclusion

This is the first study that provides evidence for the existence of personality in *Mastomys natalensis*, more specifically in the exploration-avoidance continuum. The repeatability of exploration as a personality trait was 28%, which is slightly lower than the average 35% of behaviours (Bell et al. 2009) either due to a high within-individual variance or a low between-individual variance. The latter could be a consequence of the experimental setup, the former of individual plasticity which might be beneficial in a changing environment (Sih et al. 2004). Contrary to our expectations, we did not find any relationship (direct or indirectly via a larger space use) between personality and the presence of MORV antibodies, although the absence of this relationship does not necessarily mean than our hypotheses are incorrect. There is a possibility that exploration might increase the individuals susceptibility to infection, by increasing the likelihood of coming into contact with infected faeces or individual, since juveniles were more explorative in this study and are found to have a higher RNA prevalence than adults (Borremans et al. 2011). We found no behavioural syndrome between exploration and activity and might even suspect that there is a tradeoff between these two traits. Exploration is negatively correlated with weight, where juveniles (i.e. individuals with a low weight) are more explorative than older and thus heavier individuals. We suspect that exploration acts as a behaviour to gather information about the environment which is important for young individuals but less so for older ones, since these latter individuals already lived longer and thus are more experienced. We suggest that exploration is a life-stage specific personality trait in *Mastomys natalensis* populations in Morogoro, Tanzania. Activity on the other hand is positively correlated with weight and thus age. Since weight is a good indicator for fitness (Leirs et al. 1994; Kennis et al. 2008) and this study was conducted near the end of the reproductive season (Leirs et al. 1994). We believe that activity can be interpreted as an investment that enables the individual to reproduce (Eccard & Herde 2013). This supports previous studies which have suggested that *Mastomys natalensis* uses scramble competition as mating strategy (Kennis et al. 2008). Our results indicate that older individuals are more likely to have MORV antibodies present in their blood, although this might be biased due to accumulation over time.

Although the results from this research are promising, more dedicated research is necessary to confirm or reject the proposed hypotheses in this thesis. It would be interesting to do this study for a longer period, to see how certain personality traits or strategies vary throughout the reproductive season. Besides measuring the MORV antibody presence, measuring the RNA virus prevalence (i.e. a direct measurement for infection) would increase the robustness of the results, since these do not accumulate through time, as is the case with antibodies. Secondly, we would like to know if *Mastomys natalensis* has

other personality traits and if activity is repeatable. The latter (repeatability of activity) can be done by increasing the repeated measurements per individual in the field by doing one weekly trap session (seven days) every month for multiple fields. By increasing the capture length, trap diversity per individual increases. The resulting activity measurement (minimum distance) corresponds more closely to "real activity" of the captured individual. It would be very interesting to know if aggression and sociability are repeatable and if they correlate together, or with other personality traits, into a behavioural syndrome and if these traits affect MORV prevalence. We tried to write the material and methods section to make it as accessible as possible so future researchers can use this thesis as a manual for their research.

5) Acknowledgment

I would like to thank my promoter Doctor Nelika Hughes who helped me through some tough periods and had a lot of patience even though time was a scarce resource for her. Secondly, Benny Borremans who helped us with our fieldwork in Tanzania and thaught us to love beans with rice. And I would like to thank him for his enormously great contribution of the video analysis. Without him, I would be manually analysis the video recordings till the end of this year. Then I would like to thank Joachim Mariën, who did all the blood sampling and analysis, helped me with the field work and is one hell of a driver! Asante sana to the hospitality of the Pest Management Center at the Sokoine University of Agriculture in Morogoro, Tanzania and especially Geoff and Shabani who made our stay unforgettable. I would like to thank Professor Doctor Herwig Leirs, the University of Antwerp and VLIR for providing us the opportunity to do research abroad. Lastly, I would like to specifically thank my girlfriend who supported me the whole way through even if though I spend our last summer holiday sitting behind my computer instead of being with her. Thank you so much for the support and the patience, you are an incredible woman!

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7) Appendix

Table 1: Summary outputs from all the LM that were performed using the explorative dataset. On the left are all explanatory values used to explain each variable written at the top of each table. Estimates, or coefficients are given with their standard errors. Significant P – values are noted with * p < 0.05, ** P < 0.01, *** P < 0.001.

Dependent	**Trappability**			**Trap Diversity**			**Minimum distance**		
Variable	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value
Trappability	-	-	-	0.123 ± 0.033	3.77	0.0003 ***	0.677 ± 1.286	0.53	0.600
# different traps	0.901 ± 0.239	3.77	0.0003 ***	-	-	-	28.245 ± 2.260	12.50	<0.0001 ***
Capture Length	0.220 ± 0.038	5.74	<0.0001 ***	0.008 ± 0.016	0.49	0.624	- 0.255 ±0.597	- 0.43	0.670
Minimum distance	0.004 ± 0.007	0.64	0.600	0.020 ± 0.002	12.50	<0.0001 ***	-	-	-
Density	0.006 ± 0.004	1.34	0.182	0.003 ± 0.002	1.54	0.126	- 0.088 ± 0.061	- 1.43	0.156
Exploration	0.050 ± 0.101	0.50	0.621	0.007 ± 0.038	0.20	0.845	- 1.190 ± 1.390	- 0.86	0.394
MORV infection (Infected)	0.388 ± 0.353	1.10	0.274	- 0.156 ± 0.130	- 1.19	0.235	4.807 ± 4.857	0.99	0.324
Weight	- 0.020 ± 0.012	- 1.70	0.092	0.012 ± 0.004	2.76	0.0067 **	- 0.117 ± 0.166	- 0.71	0.480
Sex (Male)	0.241 ± 0.726	0.33	0.741	- 0.438 ± 0.266	- 1.65	0.102	11.370 ± 9.931	1.145	0.255
Weight : Sex	- 0.018 ± 0.024	- 0.75	0.455	0.017 ± 0.009	1.86	0.0633	- 0.458 ± 0.333	- 1.38	0.171
Dependent	**Exploration**			**Weight**			**MORV Antibody presence**		
Variable	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value
Trappability	0.043 ± 0.086	0.50	0.621	- 1.618 ± 0.757	- 2.14	0.0348 *	0.195 ± 0.165	1.19	0.236
Different traps	0.046 ± 0.234	0.20	0.846	7.496 ± 1.939	3.87	0.0002 ***	- 0.442 ± 0.462	- 0.96	0.339
Capture length	- 0.013 ± 0.040	- 0.34	0.738	- 0.287 ± 0.358	- 0.80	0.424	- 0.016 ± 0.082	- 0.20	0.844
Minimum distance	- 0.005 ± 0.006	- 0.86	0.394	- 0.072 ± 0.055	- 1.30	0.195	0.009 ± 0.012	0.758	0.448
Density	0.002 ± 0.004	0.41	0.687	- 0.007 ± 0.037	- 0.19	0.849	0.003 ± 0.008	0.36	0.720
Exploration	-	-	-	- 1.688 ± 0.822	- 2.05	0.042 *	- 0.131 ± 0.197	- 0.67	0.506
MORV infection (Infected)	- 0.194 ± 0.327	- 0.59	0.555	7.672 ± 2.757	2.78	0.0063 **	-	-	-
Weight	- 0.023 ± 0.011	- 2.13	0.035 *	-	-	-	0.056 ± 0.021	2.73	0.0063 **
Sex (Male)	- 0.676 ± 0.668	- 1.01	0.314	- 6.715 ± 2.325	- 2.89	0.0046 **	0.790 ± 0.536	1.47	0.141
Weight : Sex	0.014 ± 0.023	0.61	0.547	-	-	-	-	-	-

Table 2: outputs from all the LM that were performed using the Comparative dataset. On the left are all explanatory values used to explain each variable written at the top of each table. Estimates, or coefficients are given with their standard errors. Significant P – values are noted with * p < 0.05, ** P < 0.01, *** P < 0.001.

Dependent	Trappability			Trap Diversity			Minimum distance		
Variable	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value
Trappability	-	-	-	0.197 ± 0.024	8.24	<0.0001 ***	0.641 ± 0.985	0.65	0.516
Different traps	1.095 ± 0.133	8.24	<0.0001 ***	-	-	-	23.735 ± 1.768	13.43	<0.0001 ***
Minimum distance	0.003 ± 0.004	0.65	0.516	0.018 ± 0.001	13.43	<0.0001 ***	-	-	-
Density	- 0.002 ± 0.003	- 0.75	0.452	0.001 ± 0.001	0.87	0.386	- 0.045 ± 0.039	- 1.15	0.250
MORV infection (Infected)	0.180 ± 0.232	0.78	0.439	- 0.068 ± 0.099	- 0.69	0.490	3.837 ± 3.597	1.07	0.287
Weight	- 0.011 ± 0.008	- 1.39	0.167	0.010 ± 0.003	2.87	0.004 **	- 0.051 ± 0.127	- 0.40	0.690
Sex (Male)	0.151 ± 0.427	0.35	0.724	- 0.060 ± 0.181	- 0.33	0.741	- 4.377 ± 6.608	- 0.66	0.508
Weight : Sex	- 0.009 ± 0.014	- 0.67	0.502	0.001 ± 0.006	0.169	0.866	0.145 ± 0.216	0.67	0.504

Dependent	Weight			MORV Antibody presence		
Variable	Coefficient ± SE	t - Value	P - Value	Coefficient ± SE	t - Value	P - Value
Trappability	- 1.168 ± 0.572	- 2.04	0.042 *	0.088 ± 0.118	0.74	0.457
Different traps	4.643 ± 1.328	3.50	0.0006 ***	- 0.207 ± 0.282	- 0.73	0.464
Minimum distance	- 0.002 ± 0.037	- 0.06	0.950	0.008 ± 0.007	1.16	0.246
Density	- 0.011 ± 0.023	- 0.50	0.620	0.008 ± 0.005	1.59	0.111
MORV infection (Infected)	8.149 ± 2.009	4.06	<0.0001 ***	-	-	-
Weight	-	-	-	0.048 ± 0.013	3.81	0.0001 ***
Sex (Male)	- 4.358 ± 1.597	- 2.73	0.007 **	0.381 ± 0.352	1.08	0.279
Weight : Sex	-	-	-	-	-	-

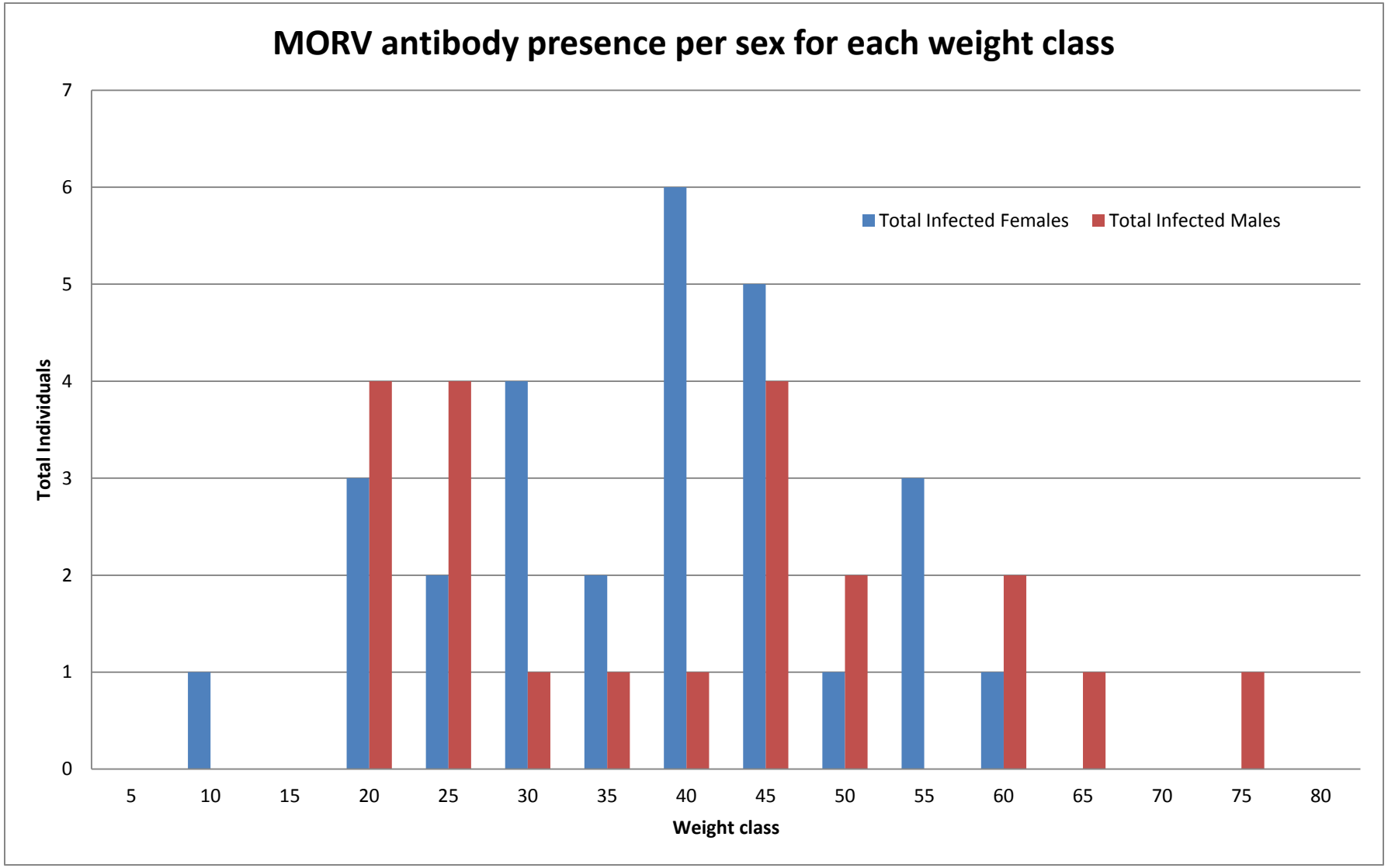

Figure 7: Histogram of individuals that were MORV antibody positive and hence were previously infected with MORV. Each weight class is sperated for the two sexes, where the blue bars represent females and brown represents males.