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Thesis

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- Spatial dynamics of Mopeia virus occurrence in contrasting habitats -

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Abstract

Mopeia virus is a rodent-borne arenavirus that occurs in East-Africa. It is closely related to the West-African Lassa virus, a highly pathogenic arenavirus that causes severe haemorrhagic fevers in humans, and responsible for around 5000 deaths annually. Mopeia virus and Lassa virus share the multimammate mouse (*Mastomys natalensis*) as their reservoir host, and despite its close genetic resemblance to Lassa virus, Mopeia virus has not been shown to elicit pathogenic symptoms in humans. It therefore provides a safe alternative for studying arenavirus population ecology.

This study investigated the abiotic and biotic drivers behind the spatial distribution of Mopeia virus in three very different habitats around Morogoro (Tanzania): woodland, vegetable garden and fallow field. Anti-Mopeia virus IgG antibody presence was investigated using immunofluorescence assay. We trapped 557 animals in 3300 trap nights using 2-night capture-mark-removal to estimate multimammate mouse densities. Host characteristics (body length, weight, age estimated from lens weight, gender, sexual maturity) were measured and related to antibody presence.

There was no significant difference in Mopeia virus antibody prevalence between the three ecotopes, and we found no evidence for a role of abiotic factors such as humidity, temperature and sunlight in determining Mopeia virus occurrence around Morogoro in the three sampled habitats. No significant correlation between antibody presence and other factors (age, sexual maturity, gender, body condition) was found. Antibody-positive animals were found in habitats with both very low (3.5/ha) and high (582/ha) estimated host densities. Given the high dispersal rates of *M. natalensis*, a plausible explanation for this distribution pattern is the movement of seropositive animals from "source" areas of Mopeia virus infection (where densities are high enough to maintain virus transmission) into "sink" habitats where densities would otherwise be too low or abiotic conditions unsuitable for virus transmission. Our results show that future studies must take into account the large spatial scale on which transmission seems to operate, and highlights the importance of long-term longitudinal observations.

Samenvatting

Mopeia virus is een door knaagdieren overgedragen arenavirus dat voorkomt in Oost-Afrika. Het is nauw verwant aan het West-Afrikaanse Lassa virus, een zeer pathogeen arenavirus dat ernstige hemorragische koorts kan veroorzaken bij mensen en verantwoordelijk is voor ongeveer 5000 jaarlijkse sterfgevallen. Mopeia virus en Lassa virus hebben beiden de veeltepelmuis *Mastomys natalensis* als hun hoofdgastheer, en desondanks de nauwe verwantschap aan Lassa virus is Mopeia virus totnogtoe ongevaarlijk gebleken voor de mens. Daardoor is Mopeia virus een goed alternatief voor de studie van de populatie ecologie van Afrikaanse arenavirussen.

Deze studie onderzocht de abiotische en biotische oorzaken van de ruimtelijke distributie van Mopeia virus in drie contrasterende habitats in de buurt van Morogoro (Tanzania): jong bos, groentetuin en braakliggend veld. De aanwezigheid van anti-Mopeia virus IgG antilichamen werd onderzocht met behulp van immunofluorescentie technieken. In totaal werden 557 dieren gevangen gedurende 3300 val-nachten. Dit gebeurde d.m.v. vangst-hervangst tijdens 2 nachten om zo gastheerdensiteiten te schatten. Gastheereigenschappen (lichaamslengte, gewicht, leeftijd geschat op basis van het gewicht van gedroogde ooglenzen, geslacht, seksuele activiteit) werden gemeten en gerelateerd aan de aanwezigheid van antilichamen.

We vonden geen significant verschil in antilichaam-aanwezigheid tussen de verschillende habitats, en er werd geen significant verband gevonden tussen de kans op antilichaam-aanwezigheid en abiotische factoren zoals vochtigheid, temperatuur en zonlicht. Ook werd geen significant verband gevonden met gastheerfactoren (leeftijd, seksuele activiteit, geslacht, lichaamsconditie). Seropositieve muizen werden zowel in habitats met een lage gastheerdensiteit (3,5/ha) als in habitats met een hoge gastheerdensiteit (582/ha) gevonden. Een geloofwaardige verklaring hiervoor, rekening houdend met de grote dispersieafstanden van *M. natalensis,* zou de dispersie zijn van seropositieve dieren vanuit “source” gebieden van Mopeia virus infectie (waar densiteiten hoog genoeg zijn voor transmissie) naar “sink” habitats waar de densiteiten te laag zouden zijn, of de abiotische condities ongeschikt voor Mopeia virus transmissie. Onze resultaten tonen aan dat toekomstige studies rekening moeten houden met de grote ruimtelijke schaal waarop transmissie lijkt plaats te vinden, en benadrukken het belang van longitudinale observaties over een langere tijdspanne.

I. Introduction

This study aims to tackle unresolved questions about Lassa virus ecology, in order to provide a way to better control Lassa fever, an infectious disease caused by this virus. Because research on this highly pathogenic Arenavirus is dangerous, we try to answer questions by studying Mopeia virus, a very closely related Arenavirus that has the same reservoir host (*Mastomys natalensis*) and has not shown any pathological symptoms in humans, which makes handling the rodent host much easier and safer. We try to elucidate the driving factors behind virus distribution by comparing Mopeia virus prevalence in contrasting ecotopes.
This introduction will start by addressing emerging infectious diseases in general and rodent-borne haemorrhagic diseases in particular, including a description of Arenaviruses. We will then go into the population ecology of *Mastomys natalensis* and the dynamics of virus transmission.

1. Emerging infectious diseases

1.1. General

Infectious diseases are responsible for 15 million annual deaths (26% of all deaths) worldwide (WHO 2007). As reported by the World Health Organization, the last 30 years alone have seen the emergence of about 30 highly infectious human diseases like HIV, Hepatitis C and Ebola (WHO 2007). Emerging infectious diseases (EIDs) can be defined in several ways but can generally be categorized into one of 2 groups: (1) those arising from previously unknown pathogens (e.g. HIV, hantavirus cardiopulmonary syndrome, SARS), and (2) those that result from the re-emergence of previously known pathogens, such as yellow fever, malaria and bubonic plague (Morens et al. 2008, Chomel 1998). In some instances the 'deliberately emerging diseases', e.g. anthrax bioterrorism, are considered a third group (Morens et al. 2004).
Many factors can be involved in the (re-)emergence of infectious diseases: the development of antibiotic resistance; an increase in global human movement and transportation resulting in a more rapid dispersal of parasitic organisms; an increase of the human population which amongst others results in the encroachment of humans into more remote areas, allowing

previously unknown EIDs to arise from sylvatic cycles; deforestation; climate change; microbial adaptation to new hosts; animal migration and movement; changing human susceptibility to infection; bioterrorism; ... (Daszak et al. 2000, Morens et al. 2008, Cleaveland et al. 2001). Aside from the negative impact infectious diseases have on humans and domestic animals, they also pose a serious threat to wildlife biodiversity (reviewed by Daszak et al. 2000).

Usually however EIDs arise from changes in the ecology of host and/or pathogen (Schrag and Wiener 1995), and often they are *zoonoses* (pathogens with an animal reservoir host). A literature study by Taylor and coauthors (2001) showed that over 60% of the known human diseases are zoonotic, and that zoonoses are twice as likely to become an EID as non-zoonotic pathogens, with many of these pathogens involving free-ranging wildlife (Cleaveland et al. 2001). Two-thirds of the zoonotic pathogens are able to infect multiple hosts and have a broad host range (Cleaveland et al. 2001); ungulates, carnivores and rodents are the most important hosts of zoonoses, at least partly due to the close contact that exists between humans and their domestic animals and livestock (Cleaveland et al. 2001, Ashford 1997). For a zoonotic disease to emerge as a human infectious disease, two necessities must be fulfilled: it must be able to come into contact and infect a human host, and it must be able to spread between humans (Morse 1995).

1.2. Rodent-borne haemorrhagic fevers

Rodent-borne haemorrhagic fevers are among the most dramatic examples of emerging zoonotic diseases (Mills and Childs 1998), of which the rate of discovery has been rising during the last decades. They are characterized by high body temperatures, bleeding, organ failure and shock. For most viral haemorrhagic fevers there are currently no vaccines or drugs available (Marty et al. 2006).The number of known hantaviruses endemic to the Americas for example rose from 1 in 1993 to 21 in 1998 (Mills and Childs 1998). A more recent example is the 2008 outbreak of a new pathogenic arenavirus in Zambia and South Africa, where several people died before the outbreak was contained (Zeller 2008, Briese et al. 2009). There are two groups of rodent-borne parasites that cause haemorrhagic fevers: Arenaviruses (*Arenaviridae*) and Hantaviruses (*Bunyaviridae*). Most viruses in these groups are primarily associated with one rodent host species of the Muridae family (Mills and Childs 1998).

2. Arenaviruses

2.1. Morphology

Arenaviridae, the focus of this study, are a family of segmented negative-strand RNA viruses closely related to other negative-stranded RNA virus families such as the Hantaviruses and the *Orthomyxoviridae* (Günther and Lenz 2004). The family's single genus is *Arenavirus*, characterized by having a lipid envelope derived from host cell membrane and a single-stranded genome consisting of 2 RNA segments of unequal length, separated by a non-coding hairpin region. The large segment (L), ca. 7400 nucleotides, encodes a viral RNA-dependent RNA polymerase and a zinc-binding protein. The small segment (S), ca. 3400nt, encodes the nucleoprotein (NP) and the glycoprotein precursor (GPC) protein, both structural proteins. The glycoprotein precursor is posttranslationally cleaved into 2 envelope proteins. The glycoproteins are important for arenavirus entry into the host cell. They interact with cell receptors such as alpha-distroglycan and transferrin receptor 1, thereby inducing the cell to fuse with the virus (Flanagan 2008, Perez and de la Torre 2003, Charrel and de Lamballerie 2003). The termini of the segments, which are necessary for replication and transcription, are highly conserved among arenaviruses (Perez and de la Torre 2003).

2.2. Phylogeny and distribution

The arenaviruses are subdivided into 2 groups based on geographical distribution, sero-immunological properties and genetic phylogeny (Charrel et al. 2008): the Old World or Lassa-Lymphocytic Choriomeningitis serocomplex which includes the Africa-endemic viruses (Lassa, Mobala, Mopeia, Ippy) and the cosmopolitan lymphocytic choriomeningitis virus (LCMV), and the New World or Tacaríbe serocomplex which includes the viruses endemic to the Americas (e.g. Junín, Machupo). With the exception of one species (Tacaríbe virus) that occurs in fruit-eating *Artibeus spp.* bats (Downs et al. 1963), all arenaviruses can be found in a rodent host of the Muridae family. All Old World arenaviruses are associated with rodents of the Murinae subfamily, and all New World viruses can be found in rodents of the Sigmodontinae subfamily. Most viruses show an association with a single specific rodent species, probably as the result of long term codivergence although several host switching events have been suggested (Bowen et al. 1997, Childs and Peters 1993). Currently there are 23 known *Arenavirus* species, but new arenaviruses including highly pathogenic ones are still discovered (Charrel et al. 2008, Zeller et al. 2008, Briese et al. 2009).

2.3. Transmission

Rodent-to-rodent transmission can occur in horizontally through sexual contact, inhalation of aerosols or infected fomites (inanimate objects able to carry infectious organisms), contact with infected excreta/secreta (urine, faeces, saliva), allogrooming and fighting. Vertical transmission (mother to offspring) also occurs, and can result in the establishment of chronic infections (Jay et al. 2005).

Humans can become infected through contact with or consumption of infected rodents, consumption of infected food, inhalation of infected rodent excreta/secreta in aerosolized form or on fomites (Jay et al. 2005). Human-to-human transmission, although uncommon, is possible via sexual contact, direct contact with blood, tissue, excretions or secretions, or via inhalation of the virus in aerosolized form or on fomites. Intrauterine mother-to-child transmission also occurs (Bowen et al. 2000, McCormick 1987, Ter Meulen et al. 1996).

2.4. Lassa virus

More than 10 arenaviruses are capable of inducing more or less severe pathogenic effects in humans. Seven of those cause haemorrhagic fevers: Chapare, Junín, Machupo, Guanarito and Sabia in South America; Lujo and Lassa in Africa (Charrel et al. 2008, Delgado et al. 2008, Briese et al. 2009). Lassa fever is caused by the Old World arenavirus Lassa virus (LASV). It was discovered in 1969 (Frame et al. 1970) in the North of Nigeria, and is endemic to several West-African countries (Günther and Lenz 2004). Lassa virus is responsible for 300000 to 500000 annual cases of Lassa fever, with outbreak fatality rates of 30-70% and an overall case-fatality rate of 1% or 5000 deaths per year worldwide (WHO 2005). The main reservoir host of Lassa virus is the multimammate mouse *Mastomys natalensis* (Smith, 1834; O. Rodentia, F. Muridae), but the virus has also been found in *Mus spp.*, *Rattus spp.* and other *Mastomys spp.* (Lecompte et al. 2006, Wulff et al. 1975). Although there exists some evidence to the contrary (Demartini et al. 1975), the virus probably has no noticeable effects on the fitness of its main host (Walker et al. 1975). In West-Africa multimammate mice live in close contact with humans, often in or near houses, increasing chances of transmission via aerosols, excreta, and food contamination (McCormick and Fisher-Hoch 2002, Walker et al. 1975). Hunting and consumption of peridomestic rodents increases infection risks even more (Ter Meulen et al. 1996).

Pathology manifests as a series of pathological symptoms, probably induced by high levels of virus replication, but the exact pathophysiology is not yet known (Günther and Lenz 2004). Experiments have shown that Lassa virus infections in monkeys are cleared from serum at

latest 14 days and from tissues at latest 21 days after experimental inoculation, but Lassa virus RNA can still be found with RT-PCR in monkey tissues (and particularly the spleen) at least 112 days after infection, although replication-competent virus could at that time not be detected anymore (Fisher-Hoch et al. 2000). Experimental inoculation of mice with LCMV (see 2.2) showed very low titers of replication-competent virus after 80 days, but after 200 days LCMV-specific sequences could still be found as cDNA in the host, although its functional role has yet to be explained (Klenerman et al. 1997). Lassa fever can be treated with Ribavirin (McCormick et al. 1986), but apart from a treatment for Junín, no arenavirus vaccine has as yet been deveoped (Fisher-Hoch and McCormick 2001, Günther and Lenz 2004).

2.5. Mopeia virus

Research on LASV is severely hindered due to its biosafety level 4 (highly dangerous) status. However, there exists a very closely related arenavirus, Mopeia virus, which occurs in East-Africa and shares *Mastomys natalensis* as its reservoir host (Günther et al. in prep., Wulff et al. 1977, Charrel et al. 2008). Experimental inoculations of monkeys have not revealed any pathogenicity, and human disease has never been observed (Günther and Lenz 2004). This makes Mopeia virus (MOPV) a very good alternative for studying arenaviruses in general and LASV in particular. MOPV occurs in East- and Southern Africa (Tanzania, South-Africa, Mozambique, Zimbabwe (Günther et al. in prep., Charrel et al. 2008, Bowen, 1997)), and has not been shown to occur together with LASV (Fichet-Calvet and Rogers 2009). Currently there exists very little specific knowledge about the ecology of MOPV. This study will focus on the ecology of MOPV in *Mastomys natalensis.*

3. Mastomys natalensis

3.1. General

As mentioned above, LASV and MOPV share the same mammal host species: *Mastomys natalensis*, the Natal multimammate mouse (Fig. 1). This species of *Mastomys* (fam. Muridae, o. Rodentia) is known as an important pest for agriculture (Leirs 1994) and a harbinger of diseases like bubonic plague, leishmaniasis and of course Lassa fever (Gratz 1997, Fiedler 1988). It is the most common indigenous rodent in sub-Saharan Africa (excluding South-West Africa) and although it has probably originated in southern savannas, it now often exists commensally in houses and farmlands (Granjon et al. 1997, Leirs 1994). It can be found in

cultivated habitats, human settlements, natural grasslands and bushy habitats (Leirs 1994). When available, they prefer areas with dense undergrowth (e.g. bush, high grass) for cover (Leirs et al. 1996).

Figure 1. ***Mastomys natalensis*** **(picture: Stuart Baird).**

3.2. Population ecology

The multimammate mouse is known for its great variance in yearly population cycles, with irregular outbreaks that can cause extensive crop damage and harvest losses of 50-100% (Mwanjabe et al. 2002). The reproductive cycle is strongly related to rainfall patterns, as shown by Leirs (1994, 1997) for Tanzanian populations. The rainfall peak (around March-May) offsets the main reproduction season, which lasts until the end of the dry season (September). During this reproductive season juveniles grow quickly but do not yet reach sexual maturity and stop growing near the end of the season. Adult animals have by then disappeared. Growth resumes when enough rain has fallen, which usually happens during the first part of the rainy season (October-December). If this period is too dry, growth will be delayed until March. Usually an adult female will produce 5 to 6 litters of 11 pups during the reproductive season. Abundant rainfall in the beginning of the rainy season however can allow for an extra breeding season in December during which an extra litter is produced that exhibits a rapid growth to maturity, allowing the offspring to partake in the following reproductive season. This can result in extreme population outbreaks, such as the 1989 outbreak described by Mwanjabe (1990). As a consequence of this reproductive cycle, population densities vary greatly during the year, with densities of up to 600 individuals per ha at the end of the main reproductive season, to less than 2 animals per ha in June, the end of

the rainy season (Van Hooft et al. 2008). This could have important consequences for density-dependent transmission of pathogens (see below, 4.1).
Dispersal rates during one generation are usually high; 20-100m but sometimes over 400m (Van Hooft et al. 2008). Dispersal does not appear strongly sex-biased (Leirs 1994) although female-biased dispersal has been observed (Van Hooft al. 2008). Daily movements usually do not exceed 20m, although distances of over 100m have been recorded (Leirs 1994). Recent analyses based on genetic differentiation have shown negative density-dependent dispersal and a large effective population size despite the large fluctuations in population density (Van Hooft et al. 2008). This study also showed that *M. natalensis* subpopulations are genetically distinct at scales of at least 300ha (Van Hooft et al. 2008).

4. Virus occurrence and transmission

Identifying the ecological determinants of virus distribution is a crucial step in disease control, necessary for defining potential disease-endemic areas as well as for pinpointing the most effective manner of control. A first obvious factor in virus occurrence is the presence and distribution of its host(s). But although some viruses infect hosts throughout most of the host's geographic range, e.g. Sin Nombre Virus in its primary reservoir host, the deer mouse *Peromyscus maniculatus* (Mills et al. 1997), it is more often the case that viruses can only be found clustered within the host range. When looking on a smaller spatial scale, it is likely that almost every virus-host distribution exhibits a more or less extensive level of incongruence. The occurrence and transmission of viruses in certain habitats or ecotopes depends on biotic factors like host genetics, abundance, behaviour, sex ratio and age, species composition, immune system and local extinction, as well as on abiotic factors such as physical boundaries, temperature, relative humidity, pH, desiccation and UV exposure (e.g. Mills 1999, Sinclair et al. 2008, Kallio et al. 2006). This study will partly focus on the interaction between host factors and virus presence, so a short summary of theories regarding host and transmission dynamics seems useful.

4.1. Transmission dynamics

As this subject is too broad for the scope of this paper, with virus hosts ranging from bacteria (e.g. Brockhurst et al. 2006) to soybeans (e.g. Gildow et al. 2000) to sponges (e.g. Laport et al. 2009), this section will mainly concentrate on the role of rodents as pathogen hosts. In the study and description of pathogen transmission a broad division into two categories is often used, transmission strategies being either *indirect* (needing an intermediate host or

vector) or *direct* (host-to-host). Rodent-borne viral diseases are most often transmitted directly between rodent hosts and between rodents and humans, as exemplified above (2.3) for arenaviruses, so the following theories will be based on direct transmission. In direct transmission, there will in most cases also be a 'short' free-living virus stage - outside the host - as aerosols, on fomites or in excreta. Short in this context means minutes, hours or a few days, but to date there are few studies that have quantified free-living periods of viruses (but see section 3.2 below).

For direct transmission, the rate of production of new infections is proportional to the contact rate (c), the probability of virus transmission in the case of contact (p), the number of susceptible individuals (S), and the proportion of infectious individuals (I) in the population (N): $Rate\ of\ production\ of\ new\ infections \sim c \cdot p \cdot S \cdot \frac{I}{N}$. If this contact rate increases with increasing population density, the transmission is said to be *density-dependent*. The contact rate term then becomes $c \cdot N/A$, where A is area. (Area is usually left out of the equation, because most studies on transmission will use the same study area for the entire duration.) This can for instance be seen for Puumala hantavirus, where prevalence has been shown to increase linearly with bank vole density, which is in turn correlated with habitat factors (Olsson et al. 2005). The contact rate could on the other hand be unaffected by density, as for many sexually transmitted diseases (STD's). This is called *frequency-dependent* transmission, and can for instance be seen for cowpox virus (Begon et al. 1998). The formula above implies the existence of a transmission threshold, determined by c, p, I and S, for which the rate of production of new infections does not increase (Anderson 1982). The basic reproductive rate (R_0) is (for microparasites) defined as the number of new infections that arises from a single infectious host in a population of susceptible hosts (Anderson 1982). The transmission threshold is then given by the condition $R_0=1$. This means that when R_0 is greater than one, the infection will spread, whereas it will disappear when $R_0 < 1$. R_0 will be proportional to the duration (L) of the infectious period of an infected individual, the number of susceptibles in the population, and the contact rate: $R_0 \sim c \cdot p \cdot S \cdot L$. This implies either that when a virus remains infective for long periods the infection can remain in the population even in small populations, or that when the infective period is short a high contact rate or a high number of susceptible individuals is needed. This has important consequences for disease control: when there is information on the factors determining the reproductive rate, disease control can be more efficient. It would for example be possible to determine a critical number of susceptible individuals (S_T) for which $R_0 = 1$, so that management efforts could be aimed toward decreasing the host population below that level, instead of for instance trying to

exterminate all hosts. The more accurately the factors are known, the more accurate and predictive the control measures can become.

Density-dependent and frequency-dependent transmission are however mainly theoretical models, and have proven difficult to observe in nature. This could be due to host variability (e.g. variation in susceptibility), virus qualities (e.g. variability in virulence, local extinction, local adaptation) or the complexity of behavioural responses of the hosts to their environment (environment being defined as any external factor interacting with the host, and the definition of behavioural responses must be interpreted broadly: sexual behaviour, home range use, dispersal, nesting behaviour, aggression, etc.). The outcome of these responses could stay constant in changing conditions (like population density), which would result in a clear transmission pattern like virus prevalence linearly increasing with density and with the existence of a density threshold as can be seen for Puumala hantavirus (Tersago et al. 2008, Olsson et al. 2005), but it could just as well be expected that those responses change with and adapt to changing conditions, in which case the resulting pattern will be more difficult to interpret. Determining the way in which contact rate is affected by a dynamic environment is key in the understanding of the transmission dynamics.

For example, one way in which contact rate can be influenced is species composition. It has been suggested that species richness can influence pathogen transmission through dilution effects (sensu Ostfeld and Keesing 2000) that can be generated by mechanisms including inter-species competition, altering of host dispersal patterns, and lowered abundance of competent hosts. Models and literature reviews suggest that pathogen prevalence is more likely to decrease than increase with higher host diversity (Keesing et al. 2006, Ostfeld and Keesing 2000).

Due to the level of complexity it is not possible here to go into any further detail regarding the way in which biotic factors can influence pathogen occurrence and transmission, but it should now have been demonstrated that a *range* of host factors can be of crucial importance in understanding zoonoses; the ecology of rodent hosts will largely determine the ecology of its parasites. For this reason many studies on the control of rodent-borne diseases will aim to understand rodent life histories. This is also the case for this study, where understanding and applying the population ecology of the multimammate mouse is a central pillar for the construction of hypotheses regarding Mopeia virus distribution.

4.2. Survival outside the host

Although viruses do not replicate outside animals or plants, there is almost always a free-

living transmission stage outside the host. The longer the free-living stage of the virus life cycle, the more important the effect of environmental non-host factors will be. A relatively small number of studies have so far focused on the presence and survival of viruses outside the host. Sinclair et al. (2008) have recently reviewed the abiotic factors influencing the persistence of category A agents (infectious diseases prioritized by the Centers for Disease Control as highly dangerous potential bioterrorism agents (Rotz et al. 2002)) in the environment. They found that viral aerosols decay rapidly once exposed to air, mainly due to freezing, dehydration and UV exposure. Vaccinia virus for instance was shown to exhibit an initial die-off within a few minutes after aerosolization, although its survival is mainly determined by relative humidity and temperature, with 9h needed for a decrease of initial titers by 99% (Harper 1961; Harper 1963). Biological half-lives of aerosolized Lassa virus at both 24 and 32°C ranges from 10 to 54min, which is sufficient for considerable dispersion distances (Stephenson et al. 1984).

It has been shown that certain fomites can for some pathogens have a positive influence on survival, depending on the surface characteristics. Survival of *Yersinia pestis* for instance is more than 10 times higher on paper particles than on glass particles (Rose et al. 2003). It can be speculated that viruses that are transmitted via fomites, like arenaviruses, can be affected by the particle structure in similar ways.

Some viruses are known to survive inside microbial spores (e.g. Lubicz et al. 2007) that can survive for long periods decades to centuries) in the environment. Although water-borne transmission is important for many disease agents (Blawat et al 1976, Essbauer et al 2007, Kallio et al. 2006), it has not been investigated for arenaviruses. Kallio et al. (2006) tested the survival of hantaviruses in cell culture, and found that Puumala virus and Tula virus remained infectious up to 11 days at room temperatures, and up to 18 days at 4°C, thereby showing that the virus is adapted to a transmission stage outside the reservoir host, yet still intracellular. Unfortunately very little information exists about the survival of arenaviruses outside the host, but it could be expected that adaptations will exist that allow prolonged survival outside the host to increase changes of transmission. As mentioned above (2.4) Lassa virus is mainly transmitted through rodent excreta, but it is not known how long the virus remains infective once shed. The next paragraph will elaborate on abiotic factors that can influence virus survival indirectly through effects on their habitats.

4.3. Habitat

As mentioned above (4) it is usually the case that viruses are distributed over only a small part

of the host's distribution, indicating a complex interaction of abiotic and biotic factors. This phenomenon has been studied for Lassa virus. Fichet-Calvet and Rogers (2009) showed that LASV requires a particular combination of reasonably high rainfall, with a specific variability and seasonality, although the host (*Mastomys natalensis*) has a much wider distribution and can tolerate a much larger range of rainfall patterns (Makundi et al. 2005, Monadjem 1999). One reason for this could be the fact that humidity affects the duration of LASV aerosol survival in the environment, with better survival times at relative humidities around 30% (Stephenson et al. 1984). The correlation with rainfall and humidity can be expected to also be linked to certain habitats, although the former study did not find a significant effect of habitat on LASV prevalence. This could however be due to the way in which habitat was defined, as they used enhanced thematic mapper satellite images to create a vegetation index, a method that does not easily allow for defining structural properties like undergrowth density that have already proven important for rodent distribution and abundance (Leirs 1994).
Puumala hantavirus does show a significant association between habitat and occurrence, mainly through the effects of habitat and landscape structure on host density (Heyman et al. 2009, Tersago et al. 2009, Langlois et al. 2001) but possibly also due to abiotic effects like temperature (Kallio et al. 2006). A review by Jackson and Jackson (2008) highlighted the importance of particle surfaces, temperature and flooding regime in determining virus abundance in wetlands, indicating that small-scale habitat differences can be important in regulating virus survival and transmission.

5. Research questions

In order to understand and eventually control Lassa virus, Mopeia virus provides an excellent and safe study model due to its close phylogenetic relationship with LASV and the fact that they both have the multimammate mouse as their reservoir host species. As explained in this introduction, one of the main factors that needs to be elucidated is the ecology of the virus. This study will focus on one of the basic ecological questions: what determines the distribution of Mopeia virus?
We investigated the occurrence of MOPV in contrasting ecotopes, aiming to provide clues about the central factors - transmission dynamics, abiotic factors and host population ecology - that determine MOPV distribution.

II. Material and methods

1. Study sites

All rodent trapping was performed around Morogoro (Tanzania, 6.84°S 37.65°E, Fig. 2), between the 13th of October and the 3rd of December 2008, which is the period between the end of the dry season and the first part of the rainy season. Around this time *Mastomys natalensis* densities are usually high, depending on previous rainfall and population densities (Leirs 1994, Leirs et al. 1997). Laboratory work was performed locally at the Pest Management Center of the Sokoine University of Agriculture (SUA). Trapping sites were chosen based on several conditions: a) one of three contrasting ecotopes where *Mastomys natalensis* is known or expected to occur; b) distance from human settlement of at least 20m; c) location within a radius of ± 20km from the laboratory where all measurements were performed; d) surface of the ecotope at least 120 x 120m, the size of the trapping grids plus 20m to avoid edge effects. Ideally the ecotopes should be larger, but available locations were unfortunately limited.

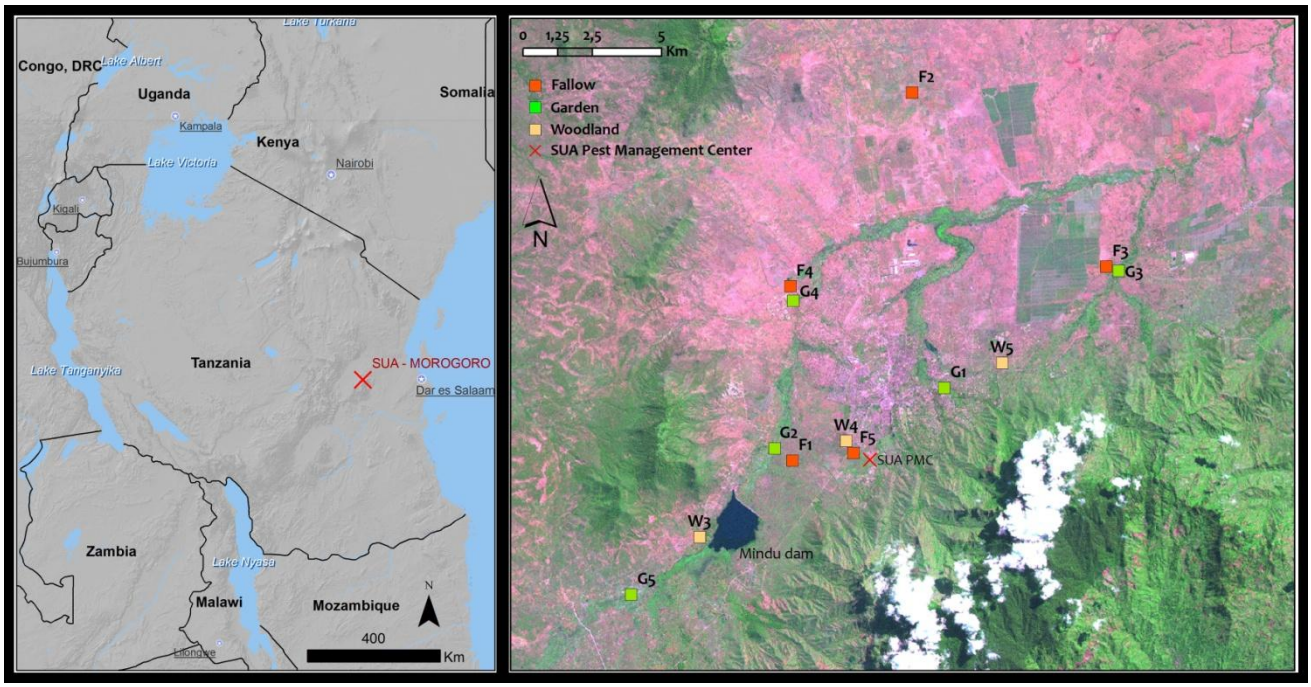

Figure 2: Left: map showing the location of the study area; Right: map showing the sampling sites. W3-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites.

Three different ecotopes were sampled (Fig. 3): woodland with bushy undergrowth, vegetable garden (humid, year-round farming of mixed crops like spinach, banana, coconut, chili, potato, etc.) and fallow field (a few months to about 1 year after last maize growth). These sites differ greatly in several factors: (-) humidity: gardens are irrigated year-round and often near a river; (-) soil: woodland generally has more sandy soil which retains less water than the clay and loam soils in the gardens and fallow fields. This has consequences for soil humidity as well as for burrowing possibilities, as it is impossible to dig an underground burrow in dry sandy soil; (-) plant composition and structure: woodland generally has bushy undergrowth and high (2-10m) young acacia trees, while gardens have patches with dense undergrowth (growing vegetables) mixed with open, recently plowed patches. Fallow fields provide little cover, as they usually have young herbs and old dead maize plants.

For each ecotope 5 sites at separate locations were sampled (Fig. 2). In only three out of seven candidate woodland sites sufficient (if any) *M. natalensis* individuals could be captured to use for analysis. Unfortunately other sites were not available, and there were no other possible ecotopes in the area besides sisal (*Agave sisalana*; a plant used for the production of fiber, common around Morogoro) plantations, of which one location was sampled but the trapping result was too poor.

Figure 3. Examples of the different ecotopes. From left to right: woodland, garden, fallow land.

2. Trapping and sampling

Capture-mark-removal (CMR) was performed at each site for 2 consecutive nights. One hundred live traps (LFA, Sherman Live Trap Co., Tallahassee, FL) were set at 10m intervals in a rectangular grid and labeled rows A-J and columns 1-10. Traps are assumed to cover a trapping area of $10m^2$ around the trap (Leirs 1994). The trapping grids were therefore considered to cover a surface of $100m^2$ or 1ha. Traps were placed every afternoon around

2pm, and removed the following day around 8am. Peanut butter mixed with maize scrap was used as bait. On every first capture day all animals were taken alive to the laboratory. Trap number was noted for every captured animal. Blood was taken from the retro-orbital sinus using 60µL microcapillary tubes, collected on calibrated pre-punched filter papers ("Serobuvard"-cards, LDA 22, Zoopole, Ploufragan, France) and air dried at room temperature. Weight (± 0.5g) was measured and markings were applied using unique toe clipping codes that allow individual identification on the second day. The animals were then released at their exact capture location when resetting the traps for the second night. After the second trapping night all captured animals were taken to the laboratory. Individuals were euthanized using an isoflurane (an inhalational anesthetic) overdose. Blood from non-recaptured individuals was taken. Individuals were carefully brushed from back to front above a yellow plastic water-filled pan using a louse comb to remove ectoparasites. These were stored in 70% alcohol. Ectoparasite load (number of ectoparasites on one individual) was noted to test for correlations between parasite presence (both ectoparasites and MOPV) and body condition. Various measurements were taken: lengths of body, tail, hind foot, and ear, and body weight. Body mass index (BMI; body weight/length^2) was used as a measure of body condition (Tersago et al. 2008). Small pieces of all major organs (heart, lung, kidney, spleen, liver) were collected in 95 % alcohol as well as in RNA*later*™ (Prediger 1999) for RNA preservation for later extraction of viral nucleic acid (not in this study). The digestive tract was collected in plastic zipper bags filled with 70 % alcohol for later macroparasite study (not in this study).

Testes lengths were measured for males, number of embryos was noted for pregnant females. Sexual maturity was noted, where males were considered sexually active when testes were in scrotal position and epididymal gubernacula were externally visible, and females were considered sexually active when a perforated vagina could be observed, when nipples were swollen due to lactation or when they were (visibly) pregnant (Leirs 1994).

Carcasses were preserved in a 10% formalin solution. Eyes were removed and preserved in 10% formalin for later age determination. All samples were taken to the University of Antwerp (Belgium) for storage and analyses.

3. Age determination

After fixation of the specimens, eye lenses were removed from the eyes and oven-dried for 48h at 80°C (Leirs 1994). Dry eye lens weight (± 0.1mg) was used as a measure of age, which could be calculated using the standard curve constructed by Leirs (1994): $a = e^{\frac{(10.46088 + w)}{4.35076}}$

where *a* is age in days and *w* is dry eye lens weight in mg.

4. Serology

Blood samples preserved on Serobuvard papers were used for prevalence estimation based on MOPV NP protein-specific IgG antibodies, using immunofluorescence assays (IFA). Blood samples (one Serobuvard piece contains about 10µL blood) were diluted in a 300µL phosphate buffered saline (PBS) solution, of which 10µL was added to wells containing Vero cells infected with Morogoro virus prepared by the Department of Virology (Bernhard Nocht Institute for Tropical Medicine – Hamburg, Germany). After 1h incubation in a 37°C humid chamber the slides were washed with PBS and 3 x 5min incubated in PBS with gentle agitation. The samples were then incubated with 10µL of 1:100 diluted fluorescein isothiocyanate (FITC)-conjugated mouse secondary antibodies. Anti-mouse IgG cross-reacts with *Mastomys* IgG antibodies. Anti-NP (MOPV and LASV) monoclonal IgG mouse antibodies obtained from the Department of Virology (Bernhard Nocht Institute for Tropical Medicine – Hamburg, Germany) were used as a positive control on the same slides. Samples were considered MOPV-antibody positive when at least about 60% of the cells had cross-reacted (Fig. 4). Ninety samples will be tested twice at different times in order to test the reliability of the technique.

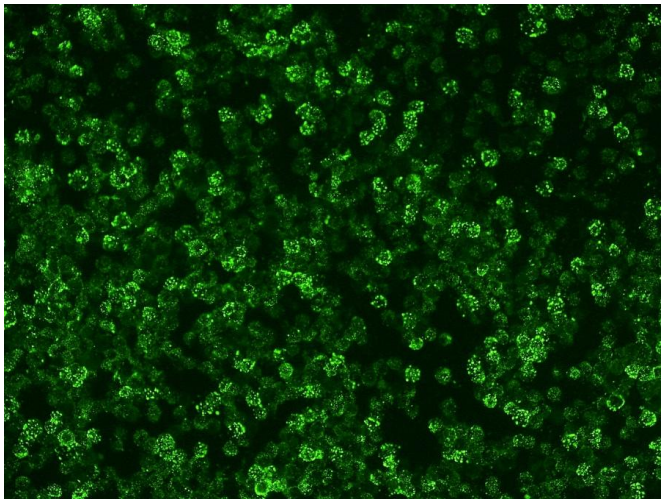

Figure 4. Example of a positive IFA sample. Green fluorescent granules reveal antibody presence.

5. Data analysis

All statistical analyses were modeled using the program "R" (v2.9.0, R Development Core Team 2009), and all analyses, tables and figures include *M. natalensis* only, unless stated otherwise. All Bayesian analyses were performed using 30000 iterations after 5000 burn-in

iterations. Results were considered significant when zero (= no effect) lies outside the 95% confidence interval.

Population density:

Population densities of *M. natalensis* were estimated using the bias-adjusted Lincoln-Petersen estimator, a widely used estimator based on the proportion of marked/unmarked animals that are recaptured on the second trapping night: $\frac{(n_1+1)(n_2+1)}{m_2+1} - 1$, where n_1 is the number of captures during the first trapping night, n_2 is the number of captures during the second night, and m_2 is the number of recaptured animals in the second trapping night (Chapman 1951). Variance and confidence intervals were obtained using bootstrap simulation (10000 iterations) with replacement (R code in Appendix 1). Density differences between habitats were estimated using Bayesian statistics (modeled in WinBUGS, Gilks et al. 1994), which was necessary to account for the strong heteroscedasticity of estimated densities. Density parameters estimated using bootstrap modeling were used as prior distributions, and uniform priors were used for other estimates. Average ecotope densities were estimated using the same model (WinBUGS code in Appendix 2). This method is a good way to take all site variance into account when estimating the average density, as opposed to using a 'normal' weighted average where this variance is not included. Overall density differences between sites were estimated using the non-parametric Kruskal-Wallis rank sum test. For sites the Bayesian method was not used, as this method is time-consuming and the non-parametric Kruskal-Wallis test, which is less sensitive to heteroscedasticity than a parametric ANOVA, gives us the information we need.

Antibody prevalence:

Confidence intervals for site prevalences were constructed using Sterne's Exact method, calculated with the program Quantitative Parasitology (v3.0, Rozsa et al. 2000, Reiczigel 2003).

Individual infection risks were analyzed in WinBUGS using Bayesian statistics. A generalized linear model (GLM) with logit-link function and Bernoulli response distribution was used for analysis of infection risk related to age, density, BMI, ecotope, weight and ectoparasite load. Density estimates and corresponding variances estimated using bootstrapping were used as prior distributions. Other variables were given uniform priors and neutral initial values (WinBUGS code in Appendix 3). A second GLM with logit-link function and Bernoulli response distribution was used for analysis of infection risk related to gender and sexual maturity, with uniform priors for all variables (WinBUGS code in Appendix 4).

Age, weight, ectoparasites and BMI:

Age differences between habitats and correlation between age, density and ectoparasite load were tested using a Bayesian linear regression with a normal response distribution (modeled in WinBUGS; Gilks et al. 1994) using uniform priors for ectoparasite load, weight and age, and estimated priors for densities (WinBUGS code in Appendix 5).

The correlation between age and weight was estimated using linear regression and ANOVA modeling after log transformation. Ectoparasite load was analyzed related to ecotope, BMI and age using a generalized linear model with log-link function and Poisson response distribution. BMI differences between ecotopes were modeled using a linear model and tested using ANOVA. Pairwise comparisons of average ecotope densities were estimated using a Wilcoxon rank sum test without P value adjustment method.

Spatial autocorrelation:

Spatial autocorrelation of prevalence was analyzed in ArcMap (ESRI 2008) using Moran's I index, a frequently used indicator of spatial autocorrelation of data (Moran 1950). The probability for clustered occurrence of prevalence is given by the Z-value: the standard deviation from the mean, which in this case equals random occurrence.

III. Results

1. Population structure

1.1. Trapping results

A total number of 3300 trap nights (number of traps x number of nights) yielded 557 individual animals. Only one trapping night was performed at woodland sites W1 and W2 because trapping results after the first night were too low (3 resp. 2 multimammate mice). These trapping results were therefore not used for density analyses. Three other woodland sites (W6, W7, W8) were excluded from this study because no *Mastomys natalensis* could be found.

Capture results are listed in table 1. Species composition varied across habitats, with *Lemniscomys griselda* (O. Rodentia, F. Muridae) only occurring in woodland, *Crocidura sp.* (O. Soricomorpha, F. Soricidae) captured in gardens only, *Mus nannomys sp.* (O. Rodentia, F. Muridae) in garden and fallow land, and *Gerbilliscus sp.* (O. Rodentia, F. Muridae) occurring in woodland and fallow land. Table 1 lists all captured species.

Table 1. Trapping results for each site. W1-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites.

Species / Site	W1	W2	W3	W4	W5	G1	G2	G3	G4	G5	F1	F2	F3	F4	F5	Total
Acomys sp.			1													1
Crocidura sp.					1	3	1	8	6	1						20
Dasymys sp.							1									1
Lemniscomys griselda	1			2												3
Mastomys natalensis	3	2	4	12	3	39	135	48	19	46	75	23	35	27	39	510
Mus nannomys sp.								1	1	1			3		1	7
Rattus rattus												1				1
Gerbilliscus sp.			1	2							1	5			2	11
Total	4	2	6	16	4	42	137	57	26	48	76	29	38	27	42	557

1.2. Population densities

Estimated multimammate mouse population densities are shown in figure 5 and 6. The range of density estimates was very large, going from an estimated 3.5 *M. natalensis* individuals at woodland site W5 (and no captures at W6, 7 and 8) to a maximum of 582 at garden site G2. Estimated densities differed significantly between woodland and the other two ecotopes (Table 2; Fig. 5). There was no significant overall density difference between the sites (Kruskal-Wallis χ^2_{12}=12, p=0.446).

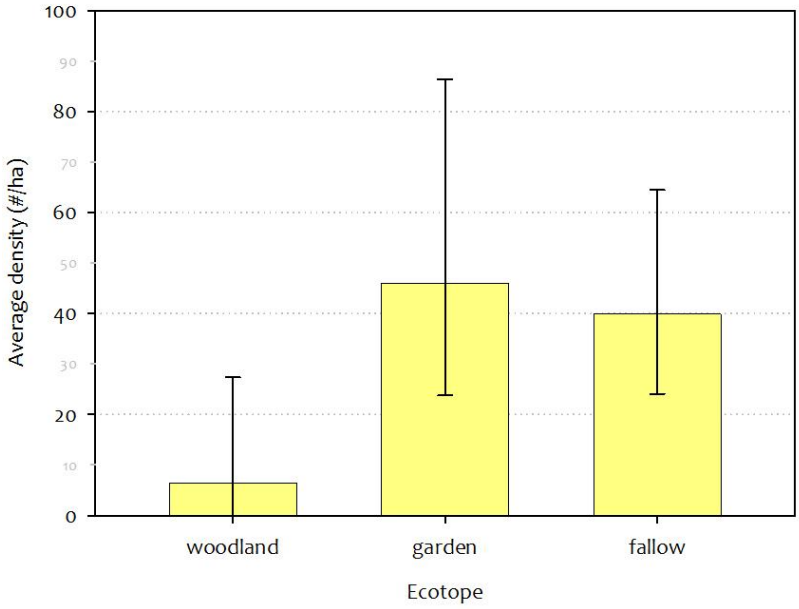

Figure 5. Average population density in each ecotope.

Table 2. Estimated density difference between ecotopes. Results from Bayesian statistics, standard deviation of the estimated difference, and 95% confidence intervals including the median value. Density mean and variance estimates (obtained from bootstrapping) used as prior distributions for estimating ecotope averages.

Sites	Difference (mean)	SD (mean)	2.5% LL	median	97.5% UL
woodland - garden	-39.5	18.1	-85.5	-34.5	-15.7
woodland - fallow	-33.4	13.5	-64.8	-31.8	-8.7
garden - fallow	6.2	16.9	-19.4	3.5	48.0

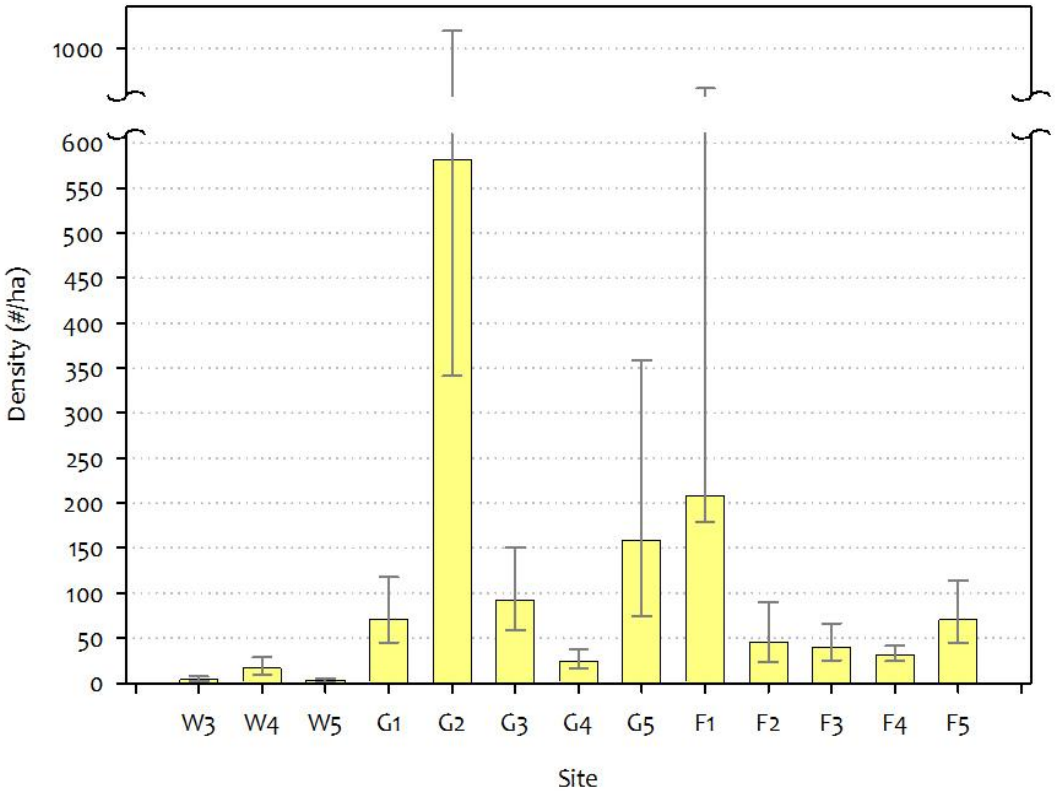

Figure 6. Estimated *M. natalensis* population densities at all sites. W3-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites.

1.3. Age structure

All age estimates were based on lens weight only, as this method has been shown to be more reliable than estimating age based on body weight (Leirs 1994). There is however a significant positive correlation between age and weight (Fig. 7, $F_{(1,237)}$=292.73, R^2=0.55, p<0.0001), which indicates that in our case weight would not have been a completely unreliable age estimate, although the residual fit is rather low. There is a significant age difference between the garden and fallow land ecotopes only (+15.53 days at fallow land, 95%CI: 4.6 to 26.4). Age is negatively correlated with density (effect estimate=-0.057, 95%CI: -0.09 to -0.024). Population age structures seem to be similar in ecotopes (Fig. 8).

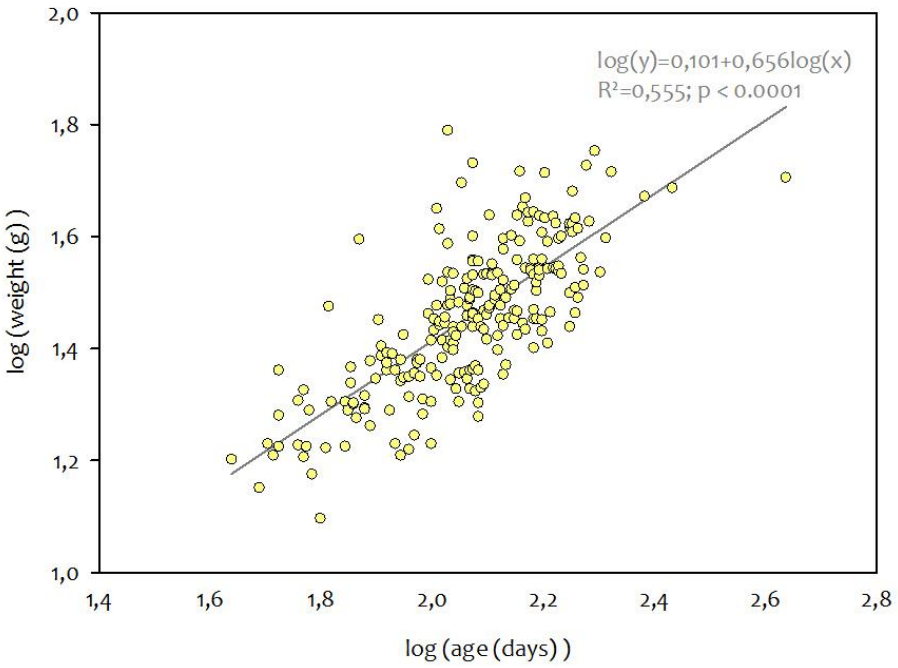

Figure 7. Age vs. weight (after log transform) scatterplot. Fitted linear regression shown with equation, residual fit and p value.

1.4. Ectoparasites and body condition

Ticks, mites and fleas were found on the rodents. Identification on lower taxonomic levels will not be done for this thesis. Total numbers of ectoparasites on one animal ranged from 0 to 48. Ectoparasite load differed significantly between ecotopes (χ^2_2=17.92, p=1.3e^{-4}), with animals in gardens having more ectoparasites than those in fallow land. Ectoparasite load was positively correlated with body mass index (χ^2_1=11.32, p=0.0008), weight (χ^2_1=11.1, p=0.9e^{-3}) and age (χ^2_1=17.97, p<0.0001).

BMI's did not differ significantly between habitats ($F_{(2, 226)}$=2.44, p=0.089).

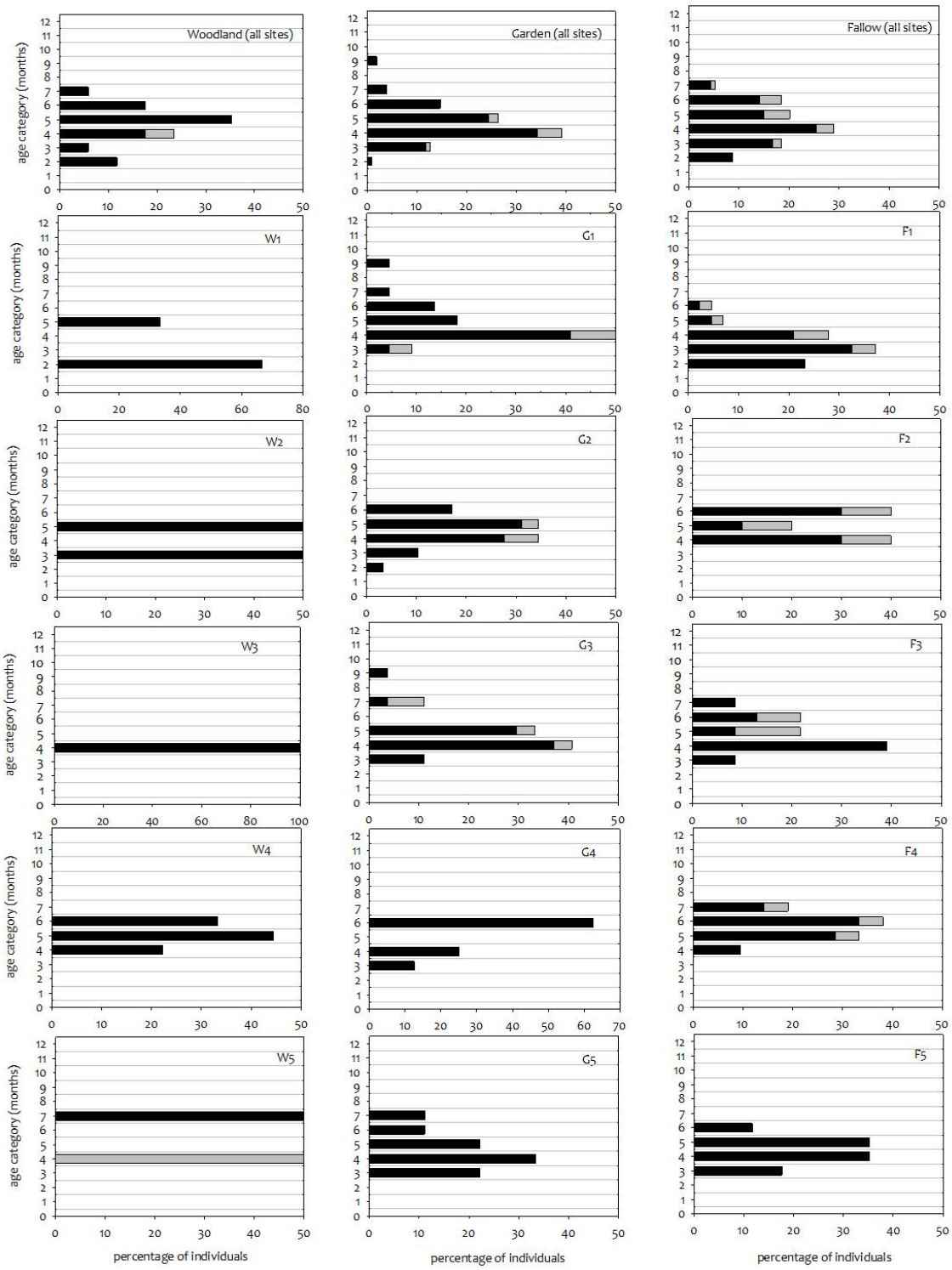

Figure. 8. Population age structure at different ecotopes. Y-axis shows age categories, each category indicating estimated number of months since birth (1 = 0-30 days, 2 = 31-60 days, etc.). X-axis shows the percentage of individuals in each category. The grey portion of the bars shows the percentage of antibody-positive individuals. The upper row shows all sites combined for each ecotope, while the lower five rows show population age structure for each site. W1-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites. Note that the graphs of G4, W1 and W3 have a different X-axis range.

2. Antibody prevalence

2.1. IFA results

Mopeia virus anti-NP antibodies were present in 58 out of 416 sampled *M. natalensis* individuals (Table 3). When including all species and all analyzed samples (including W1, W2, and sisal), 61 out of 516 individual tested positive for antibodies. Other species with MOPV antibodies are *L. griselda* in woodland site W1 and *Mus nannomys sp.* in garden G3. Also, we found one out of two *M. natalensis* individuals captured in the sisal field to be antibody positive. The immunofluorescence assay technique proved quite reliable, with 2 out of 90 re-tested samples showing a different result. Antibody prevalence was not significantly different between ecotopes (Table 4). Site and ecotope prevalence are shown in figures 9, 10 and 11.

Table 3. Mopeia virus anti-NP antibody prevalence in all sites.

Site	Prevalence in %	Nr. positive/ Nr. tested
W3	0	0/4
W4	0	0/12
W5	33	1/3
G1	8	3/39
G2	14	19/132
G3	13	6/45
G4	11	2/19
G5	5	2/41
F1	13	9/68
F2	23	5/22
F3	18	6/33
F4	17	4/24
F5	3	1/37

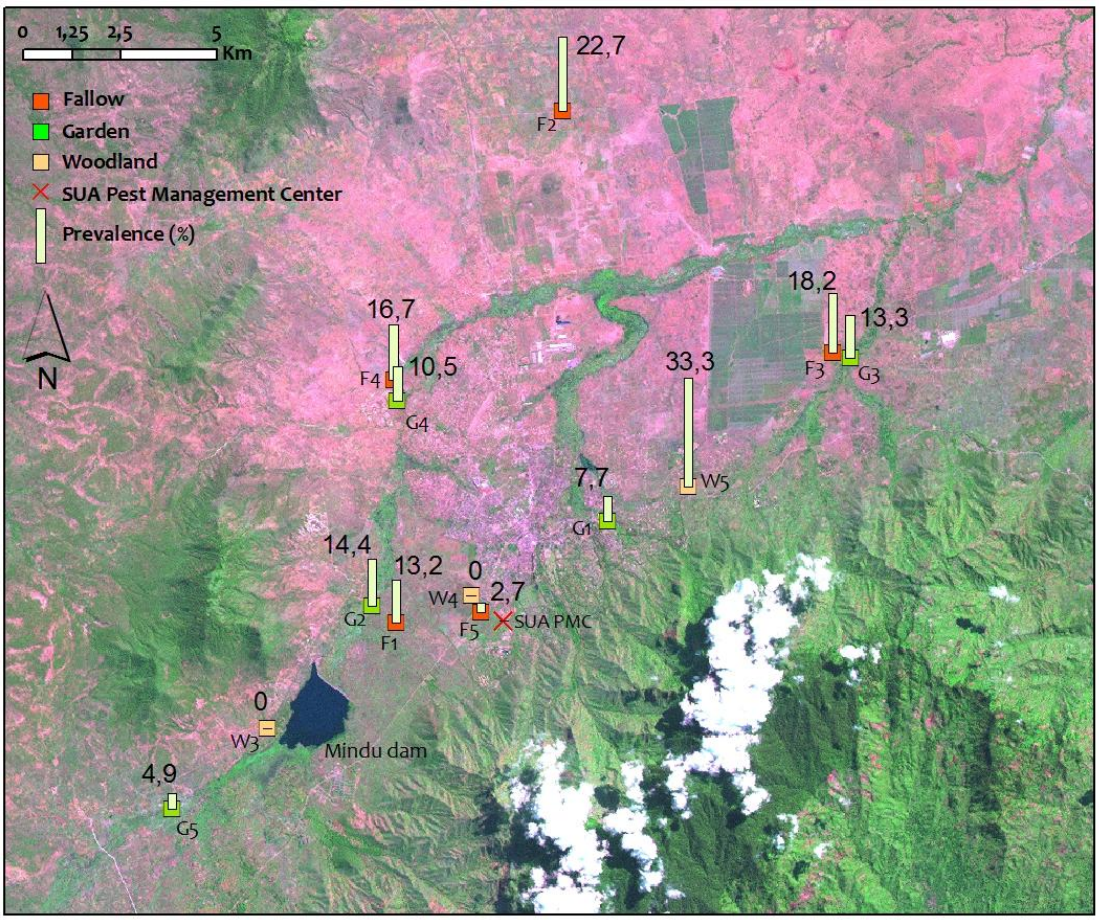

Figure 9. Map depicting location and IgG prevalence at each site. W3-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites.

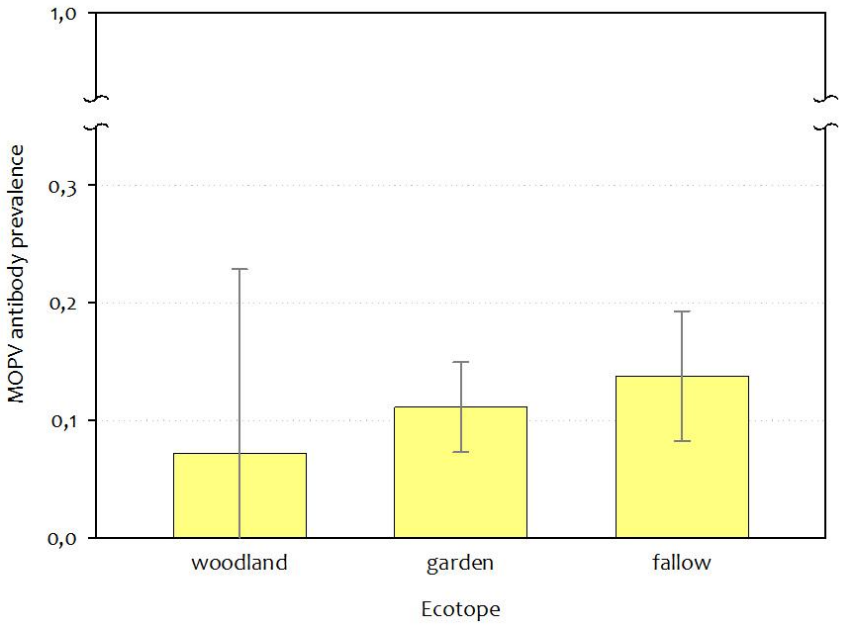

Figure 10. Average IgG prevalence for each ecotope.

Table 4. Difference in IgG prevalence between ecotopes. Results from Bayesian stats: estimated intercept difference of the regression between prevalence and independent variables (age, bmi, ectoparasite load, interaction between ectoparasite load and habitat, density, weight), standard deviation of the estimated difference, and 95% confidence intervals including the median value.

	Intercept		2.5%		97.5%
Sites	difference (mean)	SD (mean)	LL	median	UL
woodland - garden	1.83	1.48	-0.66	1.72	5.03
woodland - fallow	2.9	1.71	-0.1	2.84	6.59
garden - fallow	-1.06	0.54	-2.12	-1.07	0.02

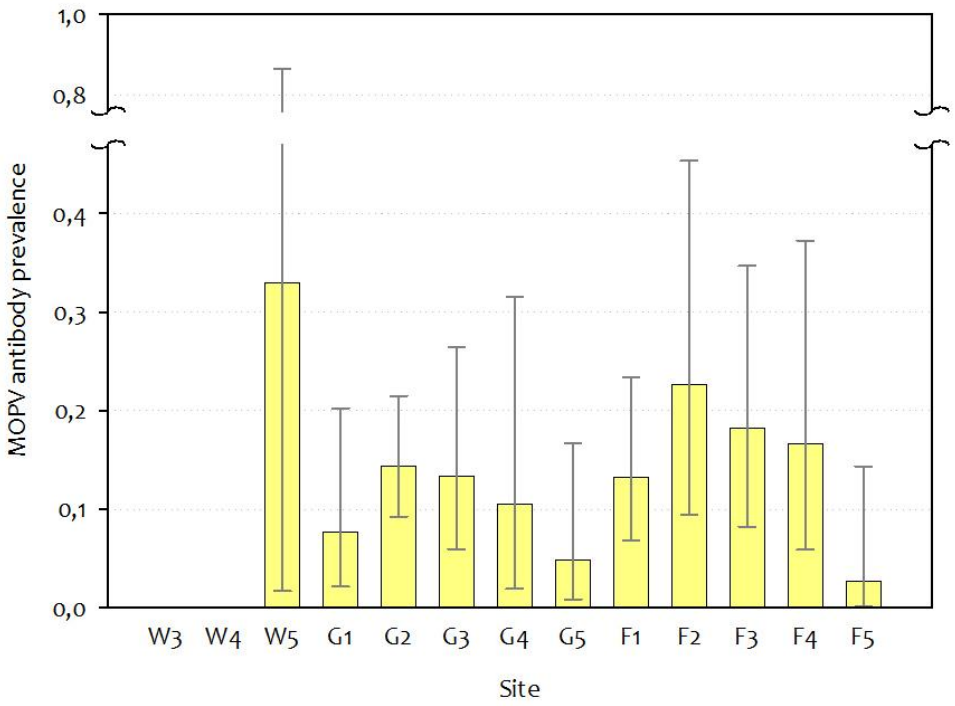

Figure 11. Graph showing IgG prevalence at each site. W3-5 = woodland sites; G1-5 = garden sites; F1-5 = fallow land sites.

2.2. Spatial distribution

The spatial autocorrelation test indicated a spatially non-clustered prevalence distribution (Moran I index=0.16, z-score=1.25, p=0.21). Statistical analyses did therefore not have to take spatial autocorrelation into account.

2.3. Density dependence

No evidence for a significant correlation between density and antibody prevalence could be detected (effect estimate=0.0012, 95%CI: -0.4 to 0.003). As can be seen in figure 12, no sensibly discernible pattern can be seen.

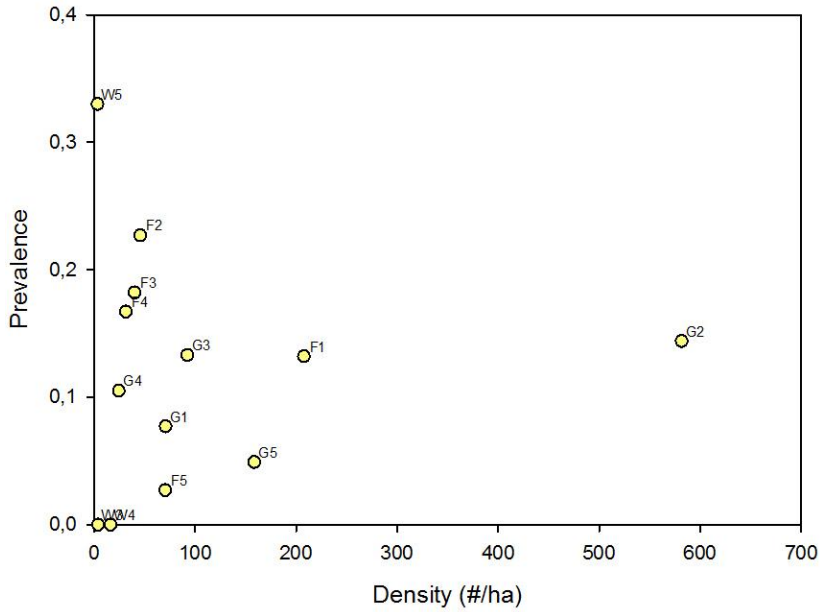

Figure 12. Antibody prevalence vs density scatterplot, site labels shown for corresponding data points.

2.4. Correlation with age, weight, sexual maturity, BMI and ectoparasite load

There was no significant correlation between age and antibody presence (Fig. 13, effect estimate=0.006, 95%CI: -0.004 to 0.017), which means that all measured age classes have a similar probability of having MOPV antibodies. The youngest individual to have antibodies has an estimated age of 66 days, while the youngest found individual was estimated to be 43 days old.

There was no significant correlation between antibody presence and weight (Fig. 13; effect estimate= -0.014, 95%CI: -0.027 to 0.053).

Antibody presence was not significantly different between males and females (effect estimate= -0.135, 95%CI: -0.828 to 0.568), nor between sexually inactive and sexually active animals (effect estimate= -1.456, 95%CI: -3.421 to 0.568). There was no significant interaction between sex and sexual maturity (effect estimate=1.063, 95%CI: -0.377 to 2.417). BMI did not have a significant effect on antibody presence (Fig. 13; effect estimate=0.3, 95%CI: -1.19 to 1.74).

There is no significant correlation between antibody presence and ectoparasite load (Fig. 13; effect estimate=0.1, 95%CI: -0.15 to 0.325), and there was no significant interaction between

habitat and ectoparasite load (effect estimate=-0.07, 95%CI: -0.17 to 0.03).

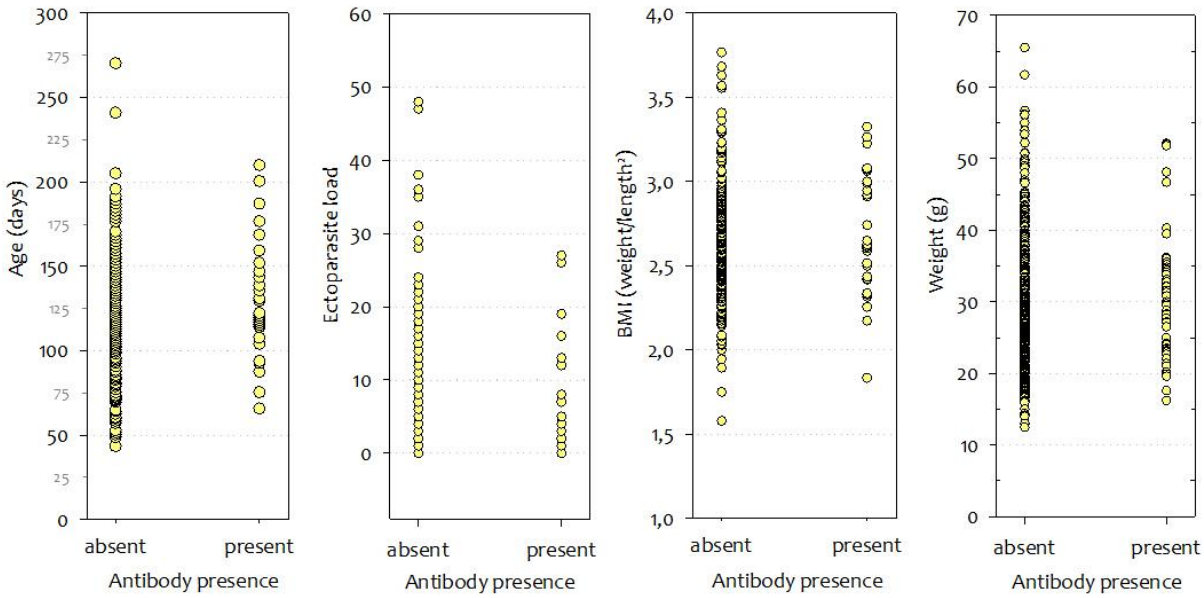

Figure 13. Age, ectoparasite load, BMI and weight of all individuals (for which this data was available) plotted against IgG presence.

IV. Discussion

1. Transmission

Determining whether or not transmission dynamics are dependent on host density is crucial for understanding virus ecology. For many directly transmitted (no intermediate host) infectious diseases a density dependent mode of transmission is assumed or at least expected, while transmission of sexually transmitted parasites is known as the prime example of frequency dependent transmission (Begon et al. 2002). One of the purposes of this study is to get an idea of which mechanism best describes MOPV transmission. A thorough understanding of transmission dynamics requires long-term data of at least one complete host life cycle (but preferentially data of several generations) because this makes it possible to see changes in transmission patterns and because it allows discerning transmission dynamics from temporal variance. As our study only provides a snapshot in the course of these dynamics, MOPV transmission dynamics have to be derived indirectly. In this context, there are several factors we will now discuss: the correlation between density and prevalence, clues for sexual transmission, and correlation between age and prevalence. We will then move on to the roles of abiotic factors and dispersal behaviour.

1.1. Density dependence

Our results show that the probability of having anti-MOPV antibodies is not significantly correlated with *M. natalensis* densities at the time of capture (Fig. 12). The fact that antibody-positive animals were found at sites with very low estimated densities (1 out of 3 captured individuals in woodland site W1, and 1 out of 2 in the sisal field) seems to imply that the probability of carrying MOPV is, at this study scale and at this moment in time, independent of density. Correlations between density and transmission however must be made with care, as this snapshot-study is not able to incorporate transmission dynamics during earlier periods where densities might have been different. The fact that *M. natalensis* populations are known to exhibit large density fluctuations related to rainfall and previous densities (Leirs 1997) adds to the large uncertainty about making inferences based on momentary data only. Therefore the density-related patterns this study provides must be interpreted with care and as educated suggestions at best. The main importance of these results then lies in providing background

information for future studies.

In the light of density dependence our data could be interpreted as MOPV having a low S_T (the threshold number of susceptible individuals below which the basic reproductive rate R_0 is lower than 1, meaning that the infection would die out. See also introduction section 4.1), which would explain the survival of MOPV in populations with low densities. An interesting observation is the presence of antibodies in one *Lemniscomys griselda* (in W1) and one *Mus nannomys* sp. (in G3). They are both, like *M. natalensis*, members of the Old World mice (Murinae) subfamily (Steppan et al. 2005). A serosurvey by Günther et al. (in prep.) around Morogoro also reported arenavirus antibodies in non-*Mastomys* Murinae (*Arvicanthis* sp., *Lemniscomys* sp. *Mus* sp.) and even Soricidae (*Crocidura* sp.). Proportions of antibody-positive animals were always much lower than those found in multimammate mice. The role of these other species in the MOPV life cycle is unknown, but if they turn out to actually be suitable hosts that participate in MOPV replication and transmission instead of being dead-end hosts that are unable to infect other hosts, they should be considered part of the host population used to describe and estimate transmission dynamics. The greater abundance of multimammate mice compared to that of other possible MOPV host species (see also Günther et al. in prep.) and the higher antibody prevalence in multimammate mice however validate the fact that *M. natalensis* is considered the main reservoir host, and that conclusions about transmission dynamics should be drawn based on *M. natalensis* population ecology.

2. Host characteristics

Host behaviour will affect transmission dynamics. It can be expected that certain behaviours important for virus transmission will change in proportion to density (e.g. maybe aggression between animals increases at higher densities. If then MOPV is transmitted through aggressive encounters, via biting for instance, infection rates will increase with density) while other behavioural traits will be independent of density (e.g. maybe sexual contact rate does not increase at higher densities). For these reasons a more likely scenario would be a mix of both density dependent and density independent transmission, the resultant of which will determine how transmission will change with density and other factors. It is important to note that this relationship may not be a linear one, as it is possible that certain behaviours, like aggression or sexual encounters, will not increase until a certain density threshold is reached,

after which transmission by means of this behavioural trait becomes density dependent.
Our data is not able to give us this information directly, but again some of this information can be deduced indirectly.

2.1. Sexual maturity

The probability of having antibodies is not significantly different between males and females, nor between sexually active and sexually immature mice, which suggests that if sexual transmission does occur, it is probably not of major importance in determining virus transmission patterns. This agrees with the findings of Fichet-Calvet et al. (2008), who investigated but did not find a correlation between fecundity and LASV prevalence.

2.2. Age

None of the antibody positive individuals was younger than 66 days, while the youngest captured individual was 43 days old. Considering the fact that it generally takes about 8-16 days for IgG antibodies to develop in sufficient amounts to be detectable (Hauge et al. 2007, Izui et al. 1981, Schaible et al. 1991), and the fact that, for LASV, antibodies remain detectable for at least 3 months (Walker et al. 1975), this means that individuals younger than 50 days (66-16) are not likely to have been infected with MOPV, and that therefore vertical transmission (mother-to-offspring) is an unlikely mode of transmission. Walker et al. (1975) found that LASV can be transmitted vertically, and that infected neonatal animals develop a persistent tolerant infection, which can also be observed in other arenaviruses. Our findings however do not support these observations, as it would then be expected that anti-MOPV antibodies can be detected in all age classes, which is not the case in our study. This suggests that vertical transmission either does not occur for MOPV or that if it does, maternal antibodies are sufficient to clear the infection without the development of a persistent infection. An absence of vertical transmission would leave horizontal transmission as the only other possibility. The fact then that we did not detect antibodies in the youngest individuals might be due to the lower probability of young animals to have encountered the virus during their shorter lifetime.
Walker et al. (1975) found that LASV can persist in multimammate mice (mainly in liver and spleen, but also in blood) for at least 74 days even in the presence of high titers of antibodies. This persistent infection provides a relatively long period during which LASV transmission can occur. A similar mechanism may be present for MOPV, but our study is unable to clarify this as we only examined antibodies and not actual virus presence. Detection of virus material by PCR methods is underway, but this falls beyond the scope of this thesis.

Although there was no significant correlation between prevalence and age or weight (Fig. 13), both effect estimates gave a positive slope (+ 0.97 for weight and +0.01 for age) with the 2.5% confidence limit near zero. This indicates that if there is any correlation between prevalence and age/weight, it would be a positive one, meaning that chances of having anti-MOPV antibodies increase when animals get older. As mentioned earlier, it is expected that chances of encountering MOPV and developing antibodies are cumulative over time. The positive age effect estimate is therefore the expected outcome, only it is not significant which is possibly due to insufficient statistical power (only the portion of the individuals that were sacrificed could be used to estimate age from eye lens weight).

2.3. Body condition

There was no observable significant correlation between body condition (BMI) and antibody prevalence. A minimal negative effect of a parasite on the fitness (through survival/body condition or through reproduction) of its main reservoir host to which it has adapted and fine-tuned (in an evolutionary sense) its survival is an observation that would be expected from a co-evolutionary perspective. Unless survival and transmission of a parasite depends on the reduced condition or death of the host - for instance in the case of hairworms (Nematomorpha) that at one stage of their life-cycle need an aquatic environment to survive into adulthood and for this purpose induce their insect host to commit suicide by jumping into water (Thomas et al. 2002) - parasites generally co-evolve with their hosts in such a way that the parasite is still able to survive inside the host, but minimizes its harmful effects in order to maximize time for transmission and reproduction. When diseases do cause detrimental body conditions and high mortality rates (e.g. Lassa virus, Ebola virus), it is usually because the parasite has infected the 'wrong' host and caused unexpected harmful effects because it has not adapted to that host. Lassa virus for instance does not seem to strongly affect *M. natalensis* to which it has adapted (Walker et al. 1975, Demby et al. 2001), but does cause pathological effect in other mammals. Experimental infection of guinea pigs (*Cavia* sp.) for instance can cause respiratory insufficiency and high mortality rates (Walker et al. 1975). Because of the refined adaptation between LASV and *M. natalensis* a long asymptomatic virus presence (Walker et al. 1975) is possible, increasing chances for LASV to infect other mice. It is likely that this is also the case for MOPV, seeing as we did not observe any sign of diminished body conditions in antibody-positive animals.

We found a significant correlation between the number of ectoparasites and BMI, body

weight and age. This can be explained by the fact that as individuals live longer, chances of having encountered ectoparasites are higher. This is then combined with the increase of body size with age, providing a larger surface for ectoparasites to occupy. The relationship between BMI and ectoparasite load is a positive one, although one might expect ectoparasites to have a negative effect on body condition, and/or vice versa, that animals with a decreased body condition are less able to remove ectoparasites. If this is true in our case, then this effect is not observable with the dataset we have, as the effect of increased weight is probably greater than that of the relationship between ectoparasites and the weight/length^2 (BMI) ratio. Individuals in gardens had significantly more ectoparasites than the ones in other ecotopes. Conditions in gardens (such as higher and more constant humidity, and dense undergrowth) may be more favourable for both ectoparasite existence and transmission of ectoparasites.

3. Mopeia virus distribution

3.1. Spatial distribution

The spatial distribution of prevalence is rather random, with MOPV occurring in rodents at most sites and at least once in each ecotope (Fig. 9). This random distribution is also supported by the spatial autocorrelation test which indicates no significant clustering of prevalence.

3.2. Habitat dependence

No significant difference in MOPV prevalence could be found between ecotopes (Fig. 10). This suggests two hypotheses: (**1**) Abiotic environmental factors are, compared to host occurrence, of relatively little importance in determining MOPV distribution; or (**2**) Certain abiotic factors do determine the occurrence of MOPV, but they are similar in the three different ecotopes. The next paragraph will consider these ideas in more detail.

3.2.1. Importance of abiotic factors

So far there has been very little research on the abiotic factors that determine arenavirus occurrence and survival outside the host (Sinclair et al. 2008, Stephenson et al. 1984). This study suggests that the distribution of MOPV is random in the three ecotopes, which could mean that the free-living stage of MOPV is very short and/or that the influence of abiotic factors like humidity, temperature and UV, at least within the ranges observed here, is minimal for the population dynamics of MOPV. Because there is a free-living stage of the virus, there must however be at least some influence of these factors, however small it is.

There is no indication that humidity is an important factor, as antibody-positive individuals have been found in both dry (woodland, fallow field) and humid (garden) areas, and prevalence is not significantly different in the three ecotopes. This observation does not correspond with recent findings in a study that analyzed spatial patterns of LASV in West-Africa, where rainfall regime and quantity was shown to be a reasonable predictor of LASV occurrence through direct effects on LASV, as *M. natalensis* tolerates a much wider rainfall regime than that suggested to be important for LASV occurrence (Fichet-Calvet and Rogers 2009). An other study showed increased LASV prevalence in *M. natalensis* during the rainy season in Guinea, West-Africa (Fichet-Calvet et al. 2008). This may possibly be due to an increase in humidity, but another possible reason is the fact that multimammate mice in West-Africa aggregate commensally in houses during the rainy season (Günther and Lenz 2004), which would increase contact rates and in that way transmission rate. Our study can not reveal temporal patterns, so it is still possible that a link between humidity and MOPV infection rates does exist: if transmission of MOPV is linked to rainfall like it seems to be the case for LASV (Fichet-Calvet and Rogers 2009, Fichet-Calvet et al. 2008), then transmission rates could be correlated with periods of higher rainfall, during which all ecotopes would have a more similar humidity. This would result in a sudden increase in antibody-presence some time after this suitable period and a stable prevalence afterwards when there is less or no MOPV transmission. The pattern we would then expect to see is at least one (depending on the number and timing of transmission periods) age group in which antibodies can be detected (the individuals that were alive during the transmission period). This age group would of course be different depending on the sampling period. To better explain this theory here is an example of the least complex seasonal transmission scenario: if transmission would only be possible in February due to certain conditions, all individuals alive during that period can get infected. If one would then study antibody prevalence 60 days later, chances of observing antibodies in individuals older than 60 days (those alive during the transmission period) would be significant, while no antibodies would be present in younger individuals. Of course, real transmission is very likely to be more complex, and year-round transmission may be possible, but the existence of seasonal transmission (as may also be the case for LASV (Fichet-Calvet et al. 2008)) is not a far-fetched scenario.

If transmission rates would remain similar during the year, a positive correlation between antibody-presence and age would be expected because of the cumulative chance of being infected. The age pattern we observed (Fig. 8) does not match the cumulative model, but does match the rainfall/age group model, suggesting that MOPV transmission may be seasonal. In

order to have a more conclusive answer to this question a long-term follow-up of antibody presence in different habitats is necessary, related to local rainfall regime.
An indirect way in which transmission can be seasonal is through changes in host population ecology. The large density fluctuations that can occur in multimammate mouse populations (Leirs 1994, 1997) may cause seasonal transmission if the susceptible population threshold (S_T) is exceeded during periods of high density, while transmission is decreased or impossible outside those periods. This effect would then depend on the ecotope, as gardens and fallow fields seem to maintain fairly high population densities and local transmission may be less influenced by density changes, while a temporary density increase may have large effects in low density woodlands.

As mentioned in the introduction (4.2), the biological half-lives of aerosolized Lassa virus at both 24 and 32°C ranges from 10 to 54min (Stephenson et al. 1984), and this decay is probably due to UV damage (Sinclair et al. 2008). Since the virus is transmitted through aerosolized excreta and secreta (Günther and Lenz 2004), this period of say half an hour would probably be sufficient to infect susceptible hosts if they are not too far from the infected host when the virus is shed (e.g. inside the burrow), and it would also be enough in the case of transmission through aggressive contact or sexual transmission. If abiotic factors during the free-living virus stage are important, then they would influence the rate at which the virus can be transmitted in different habitats: higher temperatures due to less cover from sunlight could mean faster evaporation and more time spent in aerosolized form, whereas UV exposure would be higher in less covered habitats. A high relative humidity would give a slower evaporation rate, so more time in non-aerosolized form.
The way in which these abiotic factors work is however largely hypothetical, and our observations are inconclusive regarding their role. The main importance of the proposed ideas however lies in the line of reasoning, and in order to properly quantify these mechanisms and their relative importance for MOPV/LASV transmission and distribution, carefully set-up controlled experiments guided by field observations such as ours are necessary.

3.2.2. Uniform abiotic determinants across habitats

A second hypothesis that can be deduced from the similar prevalence in the three ecotopes is that the abiotic conditions important for determining MOPV distribution are similar in the three ecotopes, even though the ecotopes had been chosen on the basis of having contrasting abiotic conditions. This must then mean that MOPV can tolerate a reasonably large range of

abiotic conditions, as several factors differ between the habitats: all gardens were a lot more humid than both woodland and fallow land because of irrigation and/or vicinity to a river; cover from direct solar radiation (UV and direct heating) differs greatly between the ecotopes, with most fallow lands having little or no cover, while gardens and woodlands offer a significant amount of cover; daily temperature fluctuations will be greater in fallow lands than in gardens, as outgoing slow-wave radiation from the earth will be more efficient at uncovered locations; soil types of the different ecotopes are very different, ranging from very loose dry sand soils in some woodlands to humid loam and clay in gardens and fallow lands. This could have an effect on burrowing possibilities, plant composition and daily temperature fluctuations (humid soils will change temperature more slowly due to the high specific heat capacity of water).

Although it is still impossible to assess the exact way in which abiotic factors influence MOPV distribution, the apparent non-preferential occurrence of MOPV does show that even if the abiotic environment is somehow important for MOPV, this influence is less important on a regional (10-20km^2) and local (< 10km^2) scale.

3.3. Dispersal

A recent study by Van Hooft et al. (2008) investigated dispersal patterns of multimammate mice in a 300ha area in Morogoro using fine-scale genetics (11 microsatellites). They found a high genetic diversity, indicating a large effective population size and high dispersal rates, and some degree of local isolation by distance and kin clustering. This pattern is in agreement with behavioural observations that have seen high levels of dispersal of both males and females (Christensen 1996, Leirs et al. 1996, Van Hooft et al. 2008) and promiscuous mating behaviour (polyandry as well as polygyny, see Kennis et al. 2008). These behavioural similarities between males and females can also account for our observation that antibody-presence is not significantly different between the sexes.

A large effective population and high dispersal rates fit the MOPV distribution patterns we observed. The fact that antibody-positive individuals can be found in areas with very low local densities (W5 and sisal field; Table 3, Fig. 9), combined with the observation that prevalence is not significantly clustered, can be explained by the dispersal behaviour. In this case there are two possible scenario's: (**1**) a very small number of residents occupy the woodland ecotopes, giving low densities and thus possibly unsuitable conditions for density-dependent transmission of MOPV. High dispersal rates of woodland populations into neighbouring

more suitable ecotopes and/or vice versa can then increase transmission rates, resulting in the presence of MOPV in low-density ecotopes; (**2**) the woodland ecotope is unsuitable for permanent *M. natalensis* occupation, but passing individuals from neighbouring regions can temporarily be present (passing through, exploring, or foraging) and caught. Assuming that individual variation in dispersal behaviour exists (Van Hooft et al. 2008), there would be some individuals that are more dispersal-prone and therefore more likely to get infected with MOPV because they have increased chances of encountering infected animals or excreta. This might then also increase chances of trapping one of these "adventurous" animals.

A consequence of both of the proposed scenarios is that a large effective population should be considered when describing MOPV transmission dynamics. This has consequences for studying MOPV ecology, as the spatial scale of the study will need to be taken into account when trying to understand the correct nature of the transmission dynamics. This also implies that virus-control measures will need to take large spatial scales into account because of the combination of high dispersal rates, a low susceptible population threshold (S_T), and long virus presence in the hosts, which can result in a rapid spread and long-term survival of MOPV. Possibly the artificial year-round stability of gardens results in different population dynamics than at sites that are not constantly irrigated and maintained by humans, and in that way support a permanent MOPV infection. These ecotopes could then serve as MOPV infection sources, transmitting MOPV to surrounding sites (woodland, fallow, crops) whenever conditions are suitable for supporting larger rodent populations.

V. Conclusion

In our study, we found that multimammate mice carrying MOPV antibodies occurred in three very different habitats, with no significant prevalence differences between those ecotopes. Thus, environmental factors are not able to explain MOPV occurrence, at least at the scale of our study. There was no significant relation between antibody prevalence and host density either, but since the study took place at a single moment in time and concerned the presence of antibodies (demonstrating earlier infection) rather than ongoing infections, we are careful not to over interpret the results. A plausible explanation, linked to the high dispersal rates of *M. natalensis,* is that seropositive animals move from "source" areas of Mopeia virus infection (where densities are high enough to maintain virus transmission) into "sink" habitats where densities would otherwise possibly be too low or abiotic conditions unsuitable for virus transmission.

Our conclusions show that future studies must take into account the large spatial scale on which transmission seems to operate, and highlight the importance of long-term longitudinal observations.

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VIII. Appendices

Appendix 1: R code - Site density estimation (bootstrap)

```
site<-"F5"
n1<-25
n2<-21
m2<-7
nres<-10000  # iterations
density<-(((n1+1)*(n2+1))/(m2+1))-1 #lincoln petersen estimate
captures<-matrix(c(rep(1,n1),rep(0,(density-n1)),rep(0,(n1-m2)),rep(1,n2),rep(0,(density-(n1-m2)-n2))),nc=2)
captures
recaptures<-matrix(data=NA,nrow=length(captures[,1]),ncol=1,dimnames=NULL)
for (j in 1:length(captures[,1])){
recaptures[j]<-ifelse((captures[j,1]+captures[j,2])==2,1,0)}
testdensity<-(((sum(captures[,1])+1)*(sum(captures[,2])+1))/(sum(recaptures[,1])+1))-1
resamples<-matrix(data=NA,nrow=nres,ncol=1,dimnames=NULL)
new<-matrix(data=NA,nrow=length(captures[,1]),ncol=length(captures[1,]),dimnames=NULL)
recaps<-matrix(data=NA,nrow=length(captures[,1]),ncol=1,dimnames=NULL)
system.time(  # returns simulation processing time
for (i in 1:nres){
for (j in 1:length(captures[,1])){
for (k in 1:length(captures[1,])){
index_j<-ceiling((runif(1,min = 0,max = 1)*length(captures[,1])))
index_k<-ceiling((runif(1,min = 0,max = 1)*length(captures[1,])))
new[j,k]<-captures[index_j,index_k]
recaps[j]<-ifelse((new[j,1]+new[j,2])==2,1,0)
}}
resamples[i]<-(((sum(new[,1])+1)*(sum(new[,2])+1))/(sum(recaps[,1])+1))-1
} # end simulation
) # system time calculation stop
x<-order(resamples)
y<-resamples[x]
results<-
matrix(c("site","2.5%","bootstrap_mean","lincolnpetersen_est","97.5%","var","stdev","number_of_iterations",site,
y[nres*0.025],mean(y),testdensity,y[nres*0.975],var(y),sd(y),nres),nc=2)
results
```

Appendix 2: WinBUGS code - Average ectotope density estimation

```
 model{
for (j in 1:3){
a[j]~dnorm(0.0,0.000001)
}
for (i in 1:13){
tau.dens[i]<-1/vardens[i]    #precision = 1/var
mean[i]~dnorm(a[habitat[i]],taumean)
meandens[i]~dnorm(mean[i],tau.dens[i])
site[i]~dnorm(0.0,0.01)  # not used, only estimated to be able to use the data matrix
}
diffwg<-a[1]-a[2]    #density difference between woodland (1) and garden (2)
diffwf<-a[1]-a[3]    # density difference between woodland (1) and fallow (3)
diffgf<-a[2]-a[3]    # density difference between garden (2) and fallow (3)
overallmean<-mean(a[])    # overall density average
taumean~dgamma(0.001,0.001)    #uninformative prior for mean precision
}
```

Appendix 3: WinBUGS code - GLM 1

```
model{
for (i in 1:476)
{
prev[i]~dbern(mu[i])
logit(mu[i])<-
A+Bhabitat[habitat[i]]+Bweight*weight[i]+Bdensity*density[i]+Bage*age[i]+Bbmi*bmi[i]+Bectopar*ectopar[i]+
Bectoparhabitat*ectopar[i]*habitat[i]
density[i]~dnorm(meandens[i],taudens[i])I(0,)
taudens[i]<-1/vardens[i]
age[i]~dnorm(meanage,tauage)I(0,)
ectopar[i]~dnorm(meanectopar,tauectopar)I(0,)
bmi[i]~dnorm(meanbmi,taubmi)I(0,)
}
habitat2_3<-(A+Bhabitat[2])-(A+Bhabitat[3])     #verschil tussen habitat 2 en 3
A~dnorm(0,0.000001);
Bhabitat[1]<-0;
Bhabitat[2]~dnorm(0.0,0.0001);
Bhabitat[3]~dnorm(0.0,0.0001);
Bweight~dnorm(0,0.000001);
Bdensity~dnorm(0,0.00001)
```

```
Bbmi~dnorm(0.0,0.000001)
Bage~dnorm(0.0,0.000001)
Bectopar~dnorm(0.0,0.00001)
Bectoparhabitat~dnorm(0.0,0.00001)
meanage~dnorm(100,0.000001)
tauage~dgamma(0.001,0.001)
meanectopar~dnorm(5,0.00001)
tauectopar~dgamma(0.001,0.001)
meanbmi~dnorm(2,0.00001)
taubmi~dgamma(0.001,0.001)
}
```

Appendix 4: WinBUGS code - GLM 2

```
model{
for (i in 1:476)
{
prev[i]~dbern(mu[i])
logit(mu[i])<-A+Bhabitat[habitat[i]]+Bsex*sex[i]+Bsexact*sexact[i]+Bsexsexact*sex[i]*sexact[i]
}
habitat2_3<-(A+Bhabitat[2])-(A+Bhabitat[3])    #verschil tussen habitat 2 en 3
A~dnorm(0,0.000001);
Bhabitat[1]<-0;
Bhabitat[2]~dnorm(0.0,0.0001);
Bhabitat[3]~dnorm(0.0,0.0001);
Bsex~dnorm(0.0,0.000001)
Bsexact~dnorm(0.0,0.001)
Bsexsexact~dnorm(0.0,0.00001)
}
```

Appendix 5: WinBUGS code - LM 1

```
model{
for (i in 1:506)
{
age[i]~dnorm(mu[i],tauage)
mu[i]<-A+Bhabitat[habitat[i]]+Bdensity*density[i]+Bectopar*ectopar[i]
density[i]~dnorm(meandens[i],taudens[i])I(0,)
taudens[i]<-1/vardens[i]
ectopar[i]~dnorm(meanectopar,tauectopar)
}
```

```
vhabitat<-(A+Bhabitat[2])-(A+Bhabitat[3])    #verschil tussen habitat 2 en 3
tauage~dgamma(0.001,0.001)
meanectopar~dnorm(5,0.00001)
tauectopar~dgamma(0.001,0.001)
A~dnorm(0.0,0.00001);
Bhabitat[1]<-0;
Bhabitat[2]~dnorm(0.0,0.000001);
Bhabitat[3]~dnorm(0.0,0.000001);
Bdensity~dnorm(0.0,0.00001)
Bectopar~dnorm(0.0,0.00001)
}
```