Dispersal in *Mastomys natalensis* mice: use of fine-scale genetic analyses for pest management

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Mastomys natalensis is the major pest rodent in sub-Saharan Africa. In this study, population genetic techniques were used to gain new insights into its dispersal behaviour, a critical parameter in pest management. Using 11 microsatellites, 272 individuals from a 300 ha area in Tanzania were genotyped. Genetic diversity was high, with no isolation by distance and little differentiation between field plots far apart, indicating a large effective population size and high dispersal rates in agreement with ecological observations. On the other hand, genetic differentiation between nearby field plots, isolation by distance within a single field plot and kin clustering were also observed. This apparent contradiction may be explained by yearly founder effects of a small number of breeding individuals per square area, which is consistent with the presence of linkage disequilibrium. An alternative, not mutually exclusive explanation is that there are both dispersing and sedentary animals in the population. The low-density field plots were characterized by low relatedness and small genetic distances to other field plots, indicating a high turnover rate and negative density-dependent dispersal. In one field plot female-biased dispersal was observed, which may be related to inbreeding avoidance or female competition for resources. Most juveniles appeared to be local recruits, but they did not seem to stay in their native area for more than two months. Finally, possible implications for pest management are discussed.

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Population genetic methods offer information on the degree of genetic diversity in individuals, social groups, family groups, and populations, as well as genetic similarities and differences. Factors contributing to such diversity include population size and density, population fragmentation, dispersal (including reproductive success of migrants), and social and mating structure. Therefore, population genetic methods have become a valuable augmentation to field experiments. Capture–mark–recapture experiments (CMR) can be time consuming, difficult to perform, and are often restricted to small areas. Dispersal behaviour across larger areas is difficult to study as recapture probability decreases with geographic distance. Although the population genetic approach has been used successfully for decades to characterize wild populations, it has only recently become sufficiently sophisticated to be applied to pest management (Rollins et al. 2006).

The African multimammate mouse, *Mastomys natalensis*, is the major rodent pest in sub-Saharan Africa. It regularly causes severe agricultural damage, with 50–100% harvest losses in some outbreak years (Mwanjabe et al. 2002). *M. natalensis* occurs in natural grasslands, bushy areas, cultivated areas and human habitations. It exhibits yearly population cycles and irregular outbreaks, which are strongly related to rainfall (Leirs et al. 1994). Within one year, population densities may vary by a factor of 20 (Leirs 1995; Mwanjabe et al. 2002).

Earlier studies in Morogoro, Tanzania (Leirs et al. 1993, 1994; Leirs 1995; Julliard et al. 1999), have resulted in a thorough basic knowledge of the local population ecology and the development of population dynamic models (Leirs et al. 1997a; Stenseth et al. 2001). These studies have contributed greatly to the improvement of pest management strategies. In Morogoro, the main breeding season lasts from April–May until September, and females produce on average five to six litters of 11–12 young (Leirs et al. 1993). There is multiple mating by both males and females. Multiple paternity occurs frequently and there is a large variation in male reproductive success (Kennis et al. 2008). In the breeding season, relatively many males die and the adult female:male sex ratio becomes 2.5–9 (Leirs 1995). Generally the lowest population densities (both

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sexes) are observed in June, sometimes less than two individuals per ha. There is generally little overlap between consecutive generations. In years with abundant rainfall, however, two generations can occur within one year, resulting in outbreaks. (This was not the case for the year sampled in this study).

Dispersal rates and distances over the course of one generation are high. Between September and December, the monthly immigration rate for a 1 ha area is about 24% (Leirs 1995). As a result, the fraction of locally-born (1 ha) breeding individuals is only 0.4–12% (Leirs et al. 1993). About 1% (85/7650) of the animals captured in various field plots (7.9 ha in total) from a 125 ha area in Morogoro consisted of known dispersers with no clear sex bias (unpubl. data). Fifteen percent of these crossed a distance of more than 400 m (62% of the plot pairs separated by this distance). Females are sedentary during the middle of the breeding season, but show home range displacement at the start and at the end of the season (Leirs 1995). Sexually active males migrate slightly farther than non-breeding males (Leirs 1995).

In this study, fine-scale genetic analyses were conducted on a spatial scale smaller than individual *M. natalensis* dispersal, using samples from a CMR study conducted in Morogoro between March 1998 and March 2002. The primary goal was to clarify to what extent population genetics can improve insight into the dispersal behaviour of *M. natalensis*. A detailed understanding of dispersal is vital for the improvement of pest management strategies (Rollins et al. 2006), especially when eradicated animals are quickly replaced by immigrants, as is the case with *M. natalensis* (Leirs et al. 1997b). Three research questions were addressed:

Are there density effects on dispersal? Ecological research shows that there are field plots with relatively low local population density, possibly due to poor habitat quality. Animals appear to prefer field plots with denser vegetation rather than open areas (Leirs 1995; Leirs et al. 1996). In field plots with relatively low local population densities, high turnover rates were anticipated (Leirs 1995).

Is dispersal sex-biased? Dispersal in mammals is generally male-biased (Prugnolle and de Meeus 2002; Handley and Perrin 2007). Ecological research indicates male-biased dispersal of *M. natalensis* during the breeding season (Leirs 1995). The extent of sex-biased dispersal over the course of one generation remains unknown. Although recapture data do not indicate a clear bias, genetic techniques may be more accurate.

What are the dispersal rates of juveniles on a ha-scale and how do these compare with those of adults? Little is known about the dispersal of juveniles because they are rarely trapped during the first months of life. Considering the high adult dispersal rates, it was expected that most juveniles would be dispersers as well.

MATERIAL AND METHODS

Study area and tissue collection

Tissue biopts, consisting of toe clippings, were obtained at the start of a CMR study executed between March 1998 and March 2002 at the campus of the Sokoine University of Agriculture in Morogoro (06°51′S, 37°38′E), Tanzania. The area is a mosaic of maize fields, fallow grassland and small patches of dense bushes, but no trees. There are a few footpaths and vehicle tracks crossing the area, but no roads. There are no landscape elements that could be considered barriers for the studied species. Rodent pest management is only rarely practiced in the area and then only by a few individual farmers in their own fields. Given that the area is a mixture of fields and fallow land and that at most only on a few fields rodenticides may have been applied the year before, we are confident that population sizes in the area are not affected.

Eight field plots were selected in a 300 ha area. Six plots measured 70 × 70 m (49 traps per field plot), one field plot (MOSA) measured 100 × 300 m (300 traps), and one field plot (MONO) was 100 × 100 m (100 traps). All field plots were cultivated with maize except MOSA, which contained a mosaic of 33% maize and 67% fallow land. The location of the field plots is shown in Fig. 1. The geographic distances between field plots varied between 60 and 2015 m.

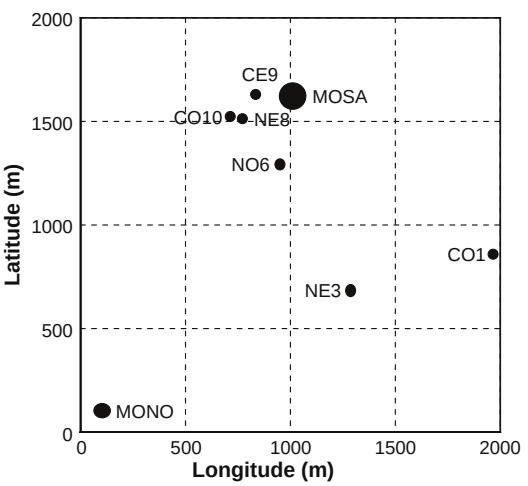

Fig. 1. Map with field plot locations in Morogoro, Tanzania.

Live traps (Sherman LFA Live traps, 7.5 × 9.0 × 23.0 cm) were placed in grids with traps every 10 m. Traps were baited with a mixture of peanut butter and chicken feed in late afternoon and checked in early morning. Rodents were live-trapped for three consecutive nights every fourth week. Captured rodents were marked at first capture by toe clipping. Toes were stored in 100% ethanol at room temperature. These field methods were accepted under the animal ethics permits to the Danish Pest Infestation Laboratory. A total of 8800 individuals were captured.

Sampling strategy

Genotyping was performed on 272 tissue biopts (Table 1), mostly on adults from March 1998. Individuals were chosen randomly per field plot from all available captures in that month (197 genotyped individuals). Forty-seven percent of the genotyped individuals were females. Most tissue biopts were selected from the first month of the CMR study to provide baseline data for future genetic studies, not yet affected by the ecological experiments that later were applied in the study area. Furthermore, March is at the end of the reproductive year, just before the start of a new breeding season. Data from this time period, therefore, demonstrate whether there is still population structure after one generation of homogenizing gene flow. Individuals from the same month were used because preliminary analyses indicated that some genetic parameters might vary on a monthly basis (data not shown).

Sixty-eight additional adults from May–July 1998 (weight ≥25 g during breeding season) and 20 juveniles from July–August 1998 (weight <21 g during breeding season) were selected from the from the MOSA field plot. In *M. natalensis* there is a linear relation between age and body weight in the first weeks after birth. Thus, during the breeding season, animals below 21 g are less than 45 days old and are certain to be juveniles (Leirs et al. 1993; Leirs 1995). These additional animals were genotyped for parentage analysis and estimation of juvenile dispersal. MOSA was selected because of its relatively large size, enabling isolation by distance analyses and sampling of a large number of individuals. The 68 adults and 20 juveniles selected for genotyping constituted 75% of all available samples from those months. No PCR was performed on the remaining 25% because of low DNA quantity (no visible DNA on agarose gel) and quality.

Table 1. *List of genotyped individuals (n = 272) of Mastomys natalensis. Individuals are adults captured in March 1998 unless otherwise indicated.*

Field plot	Sample size[a]
C01	12
NE3	13
NO6	29 (1)[b]
NE8	27 (1)[b]
CE9	28
CO10	31
MONO	16
MOSA	41 (11)[b]
MOSA adults, May–July	68
MOSA juveniles, July–Aug	20

[a]number of genotyped individuals.
[b]number of individuals also captured in MOSA between May and July, including two immigrants captured earlier in NO6 and NE8.

Laboratory analyses

DNA was extracted from one toe per individual using Puregene Kit (Gentra) or Dneasy Tissue Kit (Qiagen), following the manufacturer's instructions. DNA was solved in 100 µl TE. For genotyping, a set of 11 *Mastomys*-specific microsatellites with dinucleotide repeats was used (Galan et al. 2004). The microsatellites were amplified in a single multiplex PCR reaction with the Qiagen Multiplex PCR kit, following the manufacturer's instructions. The number of alleles, allelic range, and heterozygosity per locus are shown in Table 2. One µl of DNA solution was used in an 11 µl reaction volume. Amplification was performed using a Biometra T Gradient PCR machine for 30 cycles with an annealing temperature of 57°C. The forward primers were end-labelled with a fluorescent dye (TET, 6-FAM or HEX). The primer concentration was 0.364 µM for Mh060 and 0.182 µM for the other microsatellites. The PCR products were genotyped on a 6% sequencing gel (Ultrapure Sequagel-6) with

Table 2. *Summary of genetic diversity per microsatellite in Mastomys natalensis using all genotyped individuals (n = 272).*

Locus[a]	Size range (bp)	Allele number	H_o
$Mh146^F$	109–161	18	0.846
$Mh206^F$	163–191	13	0.890
$Mh028^F$	236–266	13	0.670
$Mh174^F$	305–339	16	0.860
$Mh051^H$	106–138	17	0.893
$Mh216^H$	161–183	12	0.879
$Mh141^H$	230–266	19	0.864
$Mh052^T$	85–114	15	0.897
$Mh060^T$	141–229	31	0.897
$Mh030^T$	234–265	15	0.822
$Mh133^T$	284–346	21	0.915

[a]F, H, T: forward primer labelled with either 6-Fam, Hex or Tet.

Genescan 500-Tamra as internal standard and using a 373 automatic DNA sequencer (Applied Biosystems). Data were collected, analyzed, and genotyped using ABI Prism 373 Collection 1.1 and Genescan 1.2.2-1 analysis software (Applied Biosystems).

Only 1.3% of the 2992 (11×272) genotypings were unsuccessful due to insufficient amplification or ambiguous allele sizes. Alleles 246, 247, 248 and alleles 252, 253, 254 (bp) of microsatellite Mh028 were considered as two alleles (246–248 and 252–254). Due to 1-bp differences between these alleles, possibly caused by an indel, it was difficult to determine their exact size. This did not influence linkage disequilibrium; there were similar fractions of locus pairs in linkage disequilibrium after excluding Mh028. Nor were other statistical analyses affected except for a slight lowering of sensitivity in some cases. In essence, this procedure artificially increases the level of homoplasy by ignoring mutations between some related alleles that are close in size. Size homoplasy is common among microsatellites due to the stepwise nature of the mutation process. In general, size homoplasy does not represent a significant problem for population genetic analyses (Estoup et al. 2002).

Local population densities

Closed-model CMR estimates of population size were obtained with the jackknife model for heterogeneous capture probability using the software program Capture (White et al. 1982). Closed-model estimates were used as they generally exhibit negligible sampling correlations with open-model estimates of survival rate, supporting the recommendation to use a robust design in studies of density dependence (Leirs et al. 1997a). The monthly average local population density for each field plot, N_{jkm}, was estimated by averaging the monthly density estimates (number of individuals per ha) during March 1998–February 2001. There was a strong correlation in local population density between years (yearly averages March–February, Spearman rank $r_s = 0.86$–0.95, $p < 0.02$). Therefore, it was assumed that the variation in local population density in the period March 1998–February 2001 was similar to that in the years before sampling, from which no data were available.

Statistical analyses of genetic parameters

The Excel add-in Microsatellite Toolkit (Park 2001) was used for generating input files for the various population genetic software programs. The Spearman rank correlation (r_s) test and various other statistical tests were performed with SPSS 12.0.1. The exact probabilities of r_s were determined according to Zar 1972.

The statistical significance of the genetic differentiation between field plots (without assuming random mating) was estimated with FSTAT 2.9.3.2 (update of version discussed in Goudet 1995). F_{ST} (Weir and Cockerham 1984) and its 95% confidence interval (CI) were estimated with FSTAT using 10 000 randomizations. F_{ST} can be negatively biased when using highly polymorphic microsatellites because the maximum value is always less than the average within population homozygosity (Hedrick 2005). Therefore, a standardized measure (F'_{ST}) was also calculated with Recodedata 0.1 (Meirmans 2006), which recodes the data such that every field plot contains only unique alleles. This measure is unbiased with respect to sample size and number of populations, in contrast to the measure suggested by Hedrick 2005 (Meirmans 2006).

Average expected heterozygosity, H_e (Nei 1987), per field plot and its standard error were estimated with Arlequin 3.0 (Excoffier et al. 2005). Arlequin calculates the variance of H_e per locus (Nei 1987) rather than just the between locus variance like most other software programs. The variance per locus was first calculated. The loci were then combined to obtain the variance of the mean H_e for all loci by dividing the average variance per locus by the number of loci (Shete 2003). The average observed heterozygosity, H_o (Nei 1987), and its standard error were calculated with Microsatellite Toolkit. FSTAT was used to estimate the significance of the linkage disequilibrium between locus pairs using 1100 randomizations. It was also used to estimate allelic richness for all loci (Rs, a measure of the expected number of alleles for a given sample size) and F_{IS} ($1–H_o/H_e$). In the case of F_{IS}, single locus estimates were averaged to obtain a combined estimate for all loci. Its standard error was based on the between-locus variation. Significances of deviations from Hardy-Weinberg equilibrium (HWE) were estimated with FSTAT by randomizing (10 000 randomizations) alleles among individuals within field plots (Weir and Cockerham's $F_{IS} \neq 0$) and among individuals from all field plots (Weir and Cockerham's $F_{IT} \neq 0$).

The significance of all isolation by distance analyses was estimated with the Mantel G test with the Excel add-in Poptools 2.6.2 (CSIRO, Canberra, Australia). All genetic distances were regressed against the natural logarithm of geographic distance using 10 000 randomizations. Isolation by distance tests were performed not only with $F_{ST}/(1 - F_{ST})$ (Rousset 1997) but also with genetic differences between pairs of individuals as preliminary analyses indicated that F_{ST} may have been influenced by population density, potentially obscuring isolation by distance. These genetic differences

were estimated with the â and ê statistics, by taking their average value between all possible pairs of individuals from two different field plots. We had to average as the coordinates of individual mice in one field plot relative to those in other field plots were unknown. The â and ê statistics were estimated with Genepop 4.0 (update of version discussed in Raymond and Rousset 1995). They can be used to estimate the neighbourhood size, $D\sigma^2$, where D is the population density and σ^2 is the average squared axial parent-offspring distance (Rousset 2000; Watts et al. 2007). In a graph of isolation by distance, using the natural logarithm of geographic distance, $4D\Pi\sigma^2$ is inversely related to the slope of the regression line. The â and ê statistics were not calculated on the within-field plot level as reliable estimates of $D\sigma^2$ are only possible with a sampling of the population in a block of minimal $10\sigma \times 10\sigma$ (Rousset 2000), which would amount to about 2000×2000 m (ecological observations indicate that 50% disperses >200 m during lifetime).

Genetic relatedness between pairs of individuals (R) at a 95% CI (100 bootstraps) was explored with Identix 1.1 (Belkhir et al. 2002) using Lynch and Ritland's R (Lynch and Ritland 1999). The average pairwise Lynch and Ritland's R values within and between groups were estimated with Spagedi 1.1 (Hardy and Vekemans 2002), using global allele frequencies and estimating the standard error by jackknifing over loci.

To analyze density-dependent effects on dispersal various genetic measures were regressed against local population density (N_{jkm}). Density-dependent dispersal may influence genetic diversity (H_e, H_o, and Rs: expected to be positively correlated with immigration rate), relatedness (R: expected to be negatively correlated with immigration rate) and genetic distances to other field plots (F_{ST}: expected to be negatively correlated with immigration rate).

Various tests for sex-biased dispersal between field plots were performed with FSTAT (two-sided tests): mean assignment test, variance assignment test, F_{ST} test, F_{IS} test, relatedness test, H_o test, and H_s test. Goudet et al. (2002) provide details of the principles, methods, and power analyses of the tests. Additionally, we tested for sex-biased dispersal within a single field plot by comparing isolation by distance between individual males and females (Sundstrom et al. 2003) from MOSA using Lynch and Ritland's R. Relatedness has often been used to analyze isolation by distance (Lynch and Ritland 1999) and sex-biased dispersal (Goudet et al. 2002; Prugnolle and de Meeus 2002; Sundstrom et al. 2003). The dispersing sex is expected to show the weakest pattern of isolation by distance. This test has the advantage that individual data are not pooled and that spatial information (coordinates) is taken into account, which may increase sensitivity. The other field plots were too small with too few individuals genotyped for isolation by distance analyses.

For estimating dispersal rates of juveniles it is relevant to known the geographic distance to their putative parents. Parentage analysis was performed with Cervus 3.0 (Marshall et al. 1998; Slate et al. 2000), with the 20 juveniles from MOSA as offspring and all adults (252) as candidate parents. The following parameter values were used in the simulation analysis: number of simulated offspring 100 000, number of candidate parents of either sex: 75 (assuming that most putative parents would be from or nearby MOSA), proportion of candidate parents genotyped: 45%, average proportion of loci per individual not typed 1.3%, average proportion of loci per individual mistyped: 1%, minimum number of typed loci for each individual: 8. The allele frequencies for creating simulated genotypes were obtained by pooling the candidate parents. Candidate parents which were assigned with 95% confidence (exceeding the critical delta score of 0.59 as estimated by the program) were considered as putative parents.

RESULTS

Microsatellite diversity and differentiation between field plots

This section describes results for adults from March 1998 unless otherwise indicated. The 11 microsatellites showed a high genetic diversity (Table 2). The average number of alleles per locus was 17.3 and H_o averaged 0.858 per individual, taking into account all 272 samples. There was no significant deviation from HWE for individual loci (Bonferroni adjusted nominal level $=0.0045$, $F_{IS} > 0$: all loci $p > 0.09$, $F_{IT} > 0$: all loci $p > 0.05$) and the average F_{IS} per field plot was close to zero (Table 3). Out of the 55 locus pairs tested for linkage disequilibrium, 23 (42%) were significant at the 0.05 level. It is unlikely that this was due to close linkage on a chromosome because the microsatellites were randomly chosen with respect to their genomic location.

There was significant differentiation between field plots ($p = 0.0001$); 18 out of 28 pairwise field plot comparisons were significant after Bonferroni corrections ($p \leq 0.0018$), including one pair separated by only 60 m. F_{ST} was small (0.016), though significantly positive (95% CI: 0.011–0.020). F'_{ST} was seven times higher (0.11). Heterozygosity values (H_o and H_e) were high, varying between 0.832 and 0.903 (Table 3).

Table 3. *Summary of genetic diversity at the field plot level for Mastomys natalensis individuals captured in March 1998.*

Field plot[a]	N_{jkm}	$H_e \pm SE$	$H_o \pm SE$	$F_{IS} \pm SE$	Rs[b]
MOSA	50.4	0.861 ± 0.007	0.842 ± 0.017	0.021 ± 0.026	9.26
CO1	30.4	0.878 ± 0.011	0.833 ± 0.032	0.052 ± 0.044	9.23
NE3	59.6	0.884 ± 0.012	0.849 ± 0.031	0.037 ± 0.051	9.58
NO6	73.3	0.871 ± 0.007	0.903 ± 0.017	−0.038 ± 0.016	9.14
NE8	77.0	0.845 ± 0.008	0.871 ± 0.020	−0.032 ± 0.016	7.77
CE9	101.7	0.846 ± 0.009	0.890 ± 0.018	−0.053 ± 0.019	8.40
CO10	88.1	0.838 ± 0.009	0.832 ± 0.020	0.008 ± 0.028	8.07
MONO	51.3	0.863 ± 0.012	0.860 ± 0.027	−0.002 ± 0.029	9.02
Mean ± SE[c]		0.861 ± 0.006	0.860 ± 0.009	−0.001 ± 0.013	8.81 ± 0.23

[a]sample sizes are mentioned in Table 1.
[b]per 11 individuals (smaller than minimum sample size due to missing genotypes).
[c]standard error based on between field plot variation.

No isolation by distance was observed at the between-field plot level ($F_{ST}/(1-F_{ST})$, â, ê: $p > 0.09$). The genetic distances between the three field plots farthest apart were very small (MOSA, MONO and CO1: average geographic distance = 1669 m, average $F_{ST} = 0.0019$, average $F'_{ST} = 0.014$). On the other hand, highly significant isolation by distance was observed with R between individuals within the MOSA field plot ($p = 0.0001$, Fig. 2). The average R decreased from 0.023 at distances ≤ 10 m to –0.015 at distances ≥ 250 m.

Density-dependent dispersal

This section describes results for adults from March 1998. The data were examined for correlations between N_{jkm} and R, pairwise F_{ST} and genetic diversity (H_e, H_o and Rs). A significant positive correlation was observed between N_{jkm} and R in each field plot ($p < 0.005$, Fig. 3). In the four high-density field plots R was significantly greater than zero ($p < 0.0014$), indicating kin clustering. A significant positive correlation was also found between N_{jkm} and pairwise F_{ST} (Spearman rank test, N_{jkm} vs average pairwise F_{ST} with each of the other field plots, $p < 0.005$; Mantel test, average N_{jkm} per field plot pair vs pairwise F_{ST}, $p = 0.0010$, Fig. 4). This could not be explained by an accidental positive correlation between geographic distance and N_{jkm}; that correlation was in fact negative (Pearson $r = -0.66$). Thus, pairwise F_{ST} was related to local population density rather than to geographic distance. No significant correlations were observed with genetic diversity (H_e, H_o and Rs, $p > 0.05$).

Sex-biased dispersal

Tests done with FSTAT did not indicate sex-biased dispersal at the field plot level among the adults from March 1998 ($p > 0.26$, two-sided tests). On the other

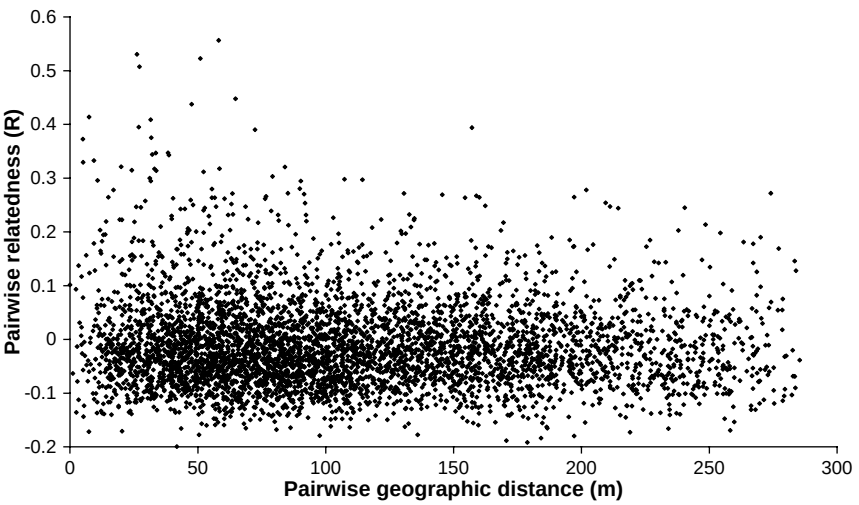

Fig. 2. Correlation between pairwise geographic distance (m) and pairwise relatedness (R) among individual mice from MOSA ($p = 0.0001$).

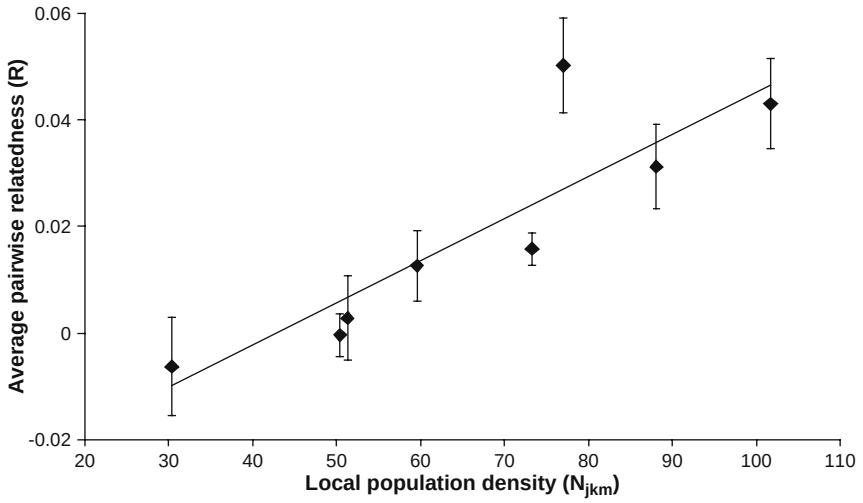

Fig. 3. Correlation between local population density (N_{jkm}) and average pairwise relatedness per field plot. Error bars correspond to ± 1 SE ($p < 0.005$).

hand female-biased dispersal was observed among the individuals from MOSA captured in March, May, June and July 1998. The R values showed significant isolation by distance among males ($p = 0.012$, $n = 51$, Fig. 5) but not among females ($p = 0.14$, $n = 47$).

Juvenile dispersal

This section describes results from MOSA for juveniles and for adults captured in May–July 1998 unless otherwise specified. For the juveniles, H_e, H_o and Rs were 0.860 (± 0.008), 0.841 (± 0.025) and 8.66, respectively. There was no significant deviation from HWE ($F_{IS} = 0.026 \pm 0.036$, $p > 0.2$). Significant isolation by distance was observed with R ($p < 0.0001$, Fig. 6), which decreased by 0.18 across a distance of 250 m. This measure was stronger than among the adults (see section Microsatellite diversity and differentiation between field plots) because a relatively large number of closely related animals were found at short distances.

Seven putative parents (two mothers, five fathers, no couples) were identified for 45% of the juveniles (three daughters, six sons). No putative parents with a genotype mismatch were observed. This percentage is a minimum estimate because some adults may have died, not been trapped, or already emigrated before sampling. Also, not all trapped adults could be genotyped. No putative parents were observed in other field plots or in MOSA in March 1998. Although the presence of linkage disequilibrium

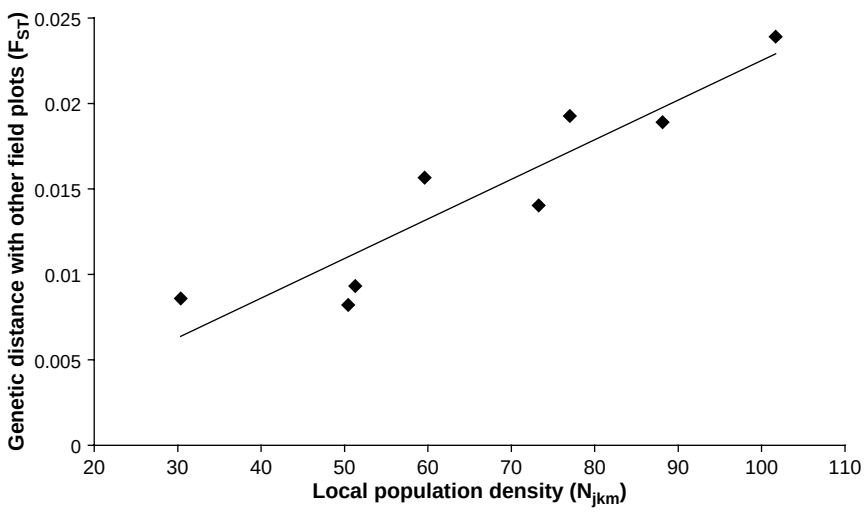

Fig. 4. Correlation between local population density (N_{jkm}) and genetic distance to other field plots. Genetic distance was calculated by averaging the pairwise genetic distances (F_{ST}) with all other field plots ($p < 0.005$).

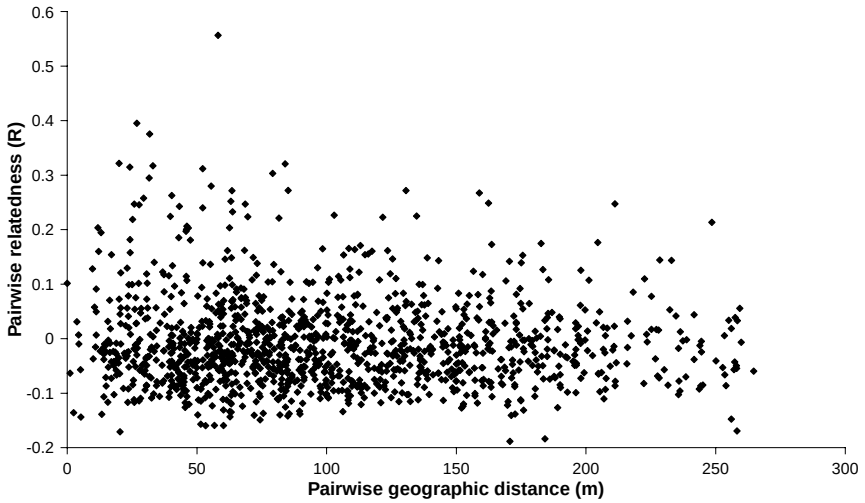

Fig. 5. Correlation between pairwise geographic distance (m) and pairwise relatedness (R) among male individuals from MOSA ($p = 0.012$).

violates one of the assumptions of the Cervus program, the results can be regarded as reliable considering that there were no mismatches, and that all putative parents came from MOSA but none from March 1998. The average geographic distance between putative parent-offspring pairs was 19.8 m, with a maximum of 40.3 m ($n = 15$). In contrast, the average pairwise geographic distance among all genotyped adult-juvenile pairs ($n = 1960$) was 114 m; only 13% were less than 40.3 m.

DISCUSSION

Population size and density

The field plots showed a high genetic diversity as indicated by an average H_e of 0.861 and an average number of alleles per locus of 17.5. This genetic diversity is considerably higher than that of commensal *M. natalensis* populations in Senegal ($H_e = 0.678$) (Brouat et al. 2007), indicating a relatively large effective population size (N_e) at this study site. H_e values greater than 0.82 are not extraordinary for rodents and have also been observed in *Apodemus flavicollis*, *Clethrionomys glareolus* (Gockel et al. 1997), *Dicrostonyx groenlandicus* (Ehrich et al. 2001a), *Peromyscus leucopus* (Mossman and Waser 2001), *Mus musculus* (Yu and Peng 2002), and *Arvicola terrestris* (Berthier et al. 2005). The high genetic diversity in *M. natalensis* indicates a large N_e despite yearly periods of strong genetic drift caused by low local population density, skewed sex ratio and large variation in male reproductive success. It is likely that high dispersal rates counterbalance the effects of genetic drift (Berthier et al. 2006). The lack of

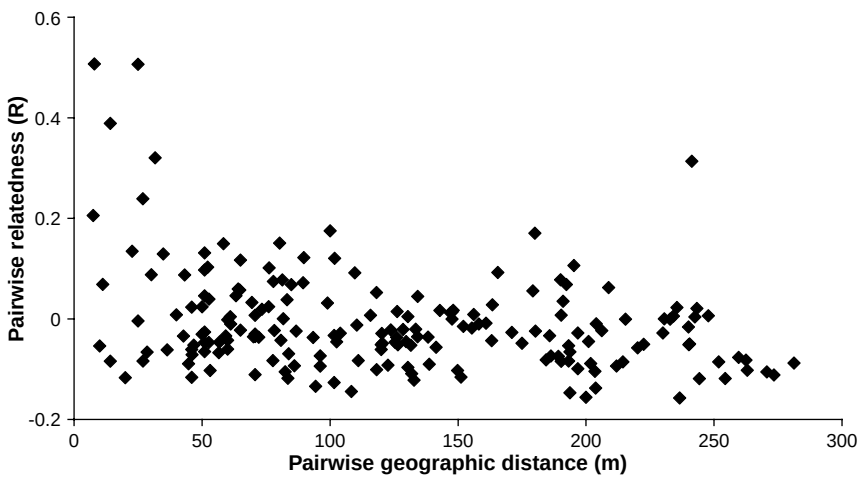

Fig. 6. Correlation between pairwise geographic distance (m) and pairwise relatedness (R) among individual juveniles from MOSA ($p < 0.0001$).

isolation by distance and the small genetic distances between the three most distant field plots ($F'_{ST} = 0.014$ at 1669 m) indicate that moderate genetic differentiation ($F'_{ST} > 0.05$, (Hedrick 2005; Hartl and Clark 2007)) in *M. natalensis* populations occurs at a scale larger than 300 ha.

There was genetic differentiation between field plots at geographic distances as small as 60 m, isolation by distance among individuals within the MOSA field plot and significant kin clustering in the high-density field plots. These observations are surprising considering the estimates of extensive dispersal provided by ecological observations, supported in this study by the high genetic diversity and absence of isolation by distance between field plots. The most likely explanation for this population genetic structure is yearly founder effects by a small number of breeding individuals. The plots used in this study have yearly periods of low local population density and a skewed sex ratio, implying a small number of breeding individuals per ha. The founder effect causes isolation by distance and kin clustering at a small geographic scale, while long distance dispersal results in its absence at larger geographic scales. Such microgeographic variations are also known as "chaotic genetic patchiness" (Planes and Lenfant 2002). Strong genetic drift due to the founder effect and kin clustering probably explain the presence of linkage disequilibrium (Hartl and Clark 1989; Planes and Lenfant 2002) and the positive correlation between F_{ST} and local population density. An alternative, not mutually exclusive explanation is that there are both dispersing and sedentary animals in the population; animals that breed at a young age show a greater tendency to breed close to their site of birth (Leirs 1995).

Interestingly, a combination of high H_e and significant linkage disequilibrium has also been observed in other rodents with large regular fluctuations in population densities. These include *Peromyscus leucopus* (Kesner and Linzey 1997; Mossman and Waser 2001), *Dicrostonyx groenlandicus* (Ehrich et al. 2001a), *Lemmus trimucronatus* (Ehrich et al. 2001b) and *Myopus schisticolor* (Vuorinen and Eskelinen 2005). In general, these studies did not provide explanations for the combination of high H_e and linkage disequilibrium. Vuorinen and Eskelinen (2005) did raise the possibility of selection. But results presented here suggest it may be due to a small number of breeding individuals per ha and high dispersal rates.

Density-dependent and sex-biased dispersal

The positive correlations between N_{jkm} and both R and F_{ST} indicate a high turnover rate in low-density field plots leading to low R and F_{ST} values. The high turnover rate implies negative density-dependent dispersal. This pattern has been observed in many other fluctuating small rodents (Ims and Andreassen 2005; Berthier et al. 2006). Possible explanations for negative density-dependent dispersal are low habitat quality, inbreeding avoidance and scarcity of mates (Ims and Andreassen 2005). The absence of significant positive F_{IS} values despite kin clustering, with the high-density field actually showing negative F_{IS} values, may indicate inbreeding avoidance (Mateo 2003).

Female-biased dispersal in MOSA was not anticipated because this phenomenon is rare among mammals and has only been observed in a few other rodent species (Davis-born and Wolff 2000; Handley and Perrin 2007). Inbreeding avoidance has been suggested for most cases. An alternative explanation for *M. natalensis* is female competition for resources i.e. relative avoidance of a field plot as a territory to breed or to raise offspring (Sweitzer and Berger 1998; Handley and Perrin 2007) because of low habitat quality. It is not known to what extent the direction of sex-biased dispersal is dependent on local habitat characteristics. For example, MOSA is the only field plot that includes fallow land. Male-biased dispersal in other field plots or other times of the year (ecological research indicates male-biased dispersal during the breeding season, (Leirs 1995)) can therefore not be ruled out.

Juvenile dispersal

Considering the high adult dispersal rate, it was initially expected that most juveniles would be immigrants. However, most juveniles in this study were likely to be local recruits. Several lines of evidence support this conclusion. First, a high fraction of juveniles had local putative parents ($\geq$ 45%). Second, geographic distances among putative parent-offspring pairs were much smaller than among random adult-juvenile pairs. Third, there was a relatively strong decrease in pairwise juvenile relatedness with geographic distance. The high percentage of local recruits may be due to juveniles accompanying their mothers or having had less time to disperse over large distances. Capture data suggest that the young accompany their mothers until they are weaned, which is around 23 days in captivity (Leirs 1995). Most juveniles do not seem to stay in their native area for more than two months, as putative parents were observed only among animals captured between May and July but not those captured in March. However, the possibility that this is (also) caused by parental dispersal cannot be ruled out.

Implications for pest management

This study provides knowledge of the geographic scale at which *M. natalensis* populations are genetically distinct, which appears to be at least 300 ha. The large effective population size and high dispersal rates make pest management on a ha-scale difficult because eradicated animals will be quickly replaced by immigrants. This has also been observed in ecological studies (Leirs et al. 1997b). The dispersal rate may even increase after eradication due to its negative density dependency. Considering the low number of breeding individuals per square area, effective pest management may be possible when population numbers are low. Such a strategy has been proposed earlier (Stenseth et al. 2001), but must be done at a scale of hundreds of hectares to prevent rapid recolonization.

Short-acting measures (e.g. prophylactic rodenticide application to protect a short but vulnerable crop stage) may be efficient over smaller areas that correspond to the rodents' dispersal ranges. Isolation by distance among adults was observed at distances shorter than 300 m, indicating that the average individual dispersal distance over the course of one generation is of the same order of magnitude. Because most juveniles do not seem to stay in their native area for more than two months, it is futile to control local rodents two months or longer before the vulnerable period. The rodents will have moved, and damage will be done by new immigrants. According to a bio-economic model in which recruitment was only due to local reproduction (Skonhoft et al. 2006), optimal control strategies for *M. natalensis* would involve rodenticide application at several times during the year. The direction of sex-biased dispersal may also need to be considered when selecting a pest management strategy. For example, female-biased dispersal may increase the rate of recolonization due to the effect of reproduction on the rate of net recruitment.

For the moment, there are no other alternative rodent management strategies known for control of this species, other than the application of rodenticides. Attracting raptors with owl nest boxes or raptor perches did not result in lower rodent population sizes (Vibe-petersen et al. 2006). Trapping is not feasible over large field areas and it is not possible to attract the rodents over longer distances to a central trapping area. Given the opportunist and generalist characteristics of *M. natalensis*, it is unlikely that a landscape can be organized such that rodent dispersal would be made difficult. On the other hand, the use of rodenticides could be minimized and made more effective by application at specific times (Stenseth et al. 2001) or by coordinating rodenticide application over larger areas, as also suggested by the present study.

The results presented here demonstrate the importance of including dispersal in simulation models for pest management, even when it is assumed to operate on areas much larger than a single field. Furthermore, a better understanding of the determinants of habitat quality and their association with local population density and dispersal behaviour is needed. Although this study provided relevant new insights into the dispersal behaviour of *M. natalensis* in Tanzania, additional population genetic studies are needed to better quantify dispersal. Dispersal may be analyzed at different spatiotemporal scales by inclusion of genetically distinct populations (scales >300 ha), different generations, and different life-history stages.

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