



University of Zürich
Zoological Museum

Fitness consequences of natal dispersal in the snow vole (*Chionomys nivalis*)

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View of study site at Jochalp/Chur in summer 2007

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1 Abstract

*Fitness consequences of natal dispersal in the snow vole (*Chionomys nivalis*)*

Dispersal has a major impact on population genetics and structure. According to classical models, depending on the mating system, dispersal is often more prevalent in one sex than in the other. Several hypotheses such as avoiding inbreeding and escaping kin competition at the local patch have been suggested to explain dispersal patterns. The present study investigated the reproductive consequences and possible proximate causes of natal dispersal in a single snow vole (*Chionomys nivalis*) population in the wild. Mark-recapture data and the use of molecular markers for identity and parentage analysis revealed a promiscuous mating system in the snow vole due to their reproductive enhancement with multiple matings. As commonly found in mammals, having a breeding system with female-defense polygyny or promiscuous, I observed a male-biased dispersal pattern. No difference in annual reproductive output between dispersed (i.e. immigrant) and resident males was found within the studied area. However there was a trend in annual reproductive success increasing with distance traveled from the natal site in males even though statistically insignificant. This implies that within-population dispersal could be under selection in males. Furthermore, I proposed that female snow voles might be adapted locally, since females breeding closer to their mothers' site, had more offspring. Such local adaptation may be sustained by cooperation in the use of local resources within females aided by kin selection. I argue that the spatial scale under investigation can be of importance when considering the adaptiveness of dispersal.

2 Introduction

2.1 Dispersal and its evolution

The movement of an individual from its natal environment or previous breeding patch to its current breeding patch is a widely used definition for natal- and breeding dispersal, respectively (Cheptko-Sade & Halpin, 1987; Clobert *et al.*, 2001; Bowler & Benton, 2005). Dispersal has important consequences in population demography, genetics and species' distribution, since it directly influences the long-term persistence of populations and genetic differentiation among demes (Clobert *et al.*, 2001).

Major importance is given to dispersal in terms of migration as it brings about gene flow between subpopulations when the dispersed individual breeds in the new population. When migration is low, populations become isolated and genetically different. Both genetic differentiation between populations and co-ancestry (the probability that two mating partners are identical by descent) within populations are enhanced and implied by a genetic structure. Isolated populations run the risk of being inbred and of competing with kin (Clobert *et al.*, 2001). In ecology, dispersal in terms of immigration and emigration can decrease extinction risk through colonisation of an empty habitat or increasing the size of the population. This rescue effect is particularly important in small populations that suffer from genetic consequences of isolation more quickly (*reviewed by* Bowler & Benton, 2005).

To evaluate the evolutionary causes of dispersal, focus is given to the costs and benefits of dispersing for an individual (Cheptko-Sade & Halpin, 1987; Handley & Perrin, 2007). It is widely accepted that dispersal is associated with high ecological costs (Daniels & Walters, 2000, Andreassen & Ims, 2001, Yoder *et al.*, 2004). Therefore, dispersal only becomes a selective advantage to an individual when the overall fitness benefits of movement exceed the costs of movement (Bowler & Benton, 2005).

Factors such as kin structure, inbreeding and habitat quality may cause variation in fitness and thus the evolution of dispersal. At the natal site related individuals will compete for mates (local mate competition) and for resources (local resource competition) and to avoid this competition between close relatives, kin selection may favour dispersal. Competition between relatives reduces indirect fitness and should thus be avoided (Perrin & Mazalov, 2000). Individuals that leave the natal patch free resources for their siblings and thereby increase their indirect fitness (Hamilton & May, 1977). Additionally, inbreeding avoidance can also be a potential selective drive for the evolution of dispersal. Individuals that disperse avoid mating with close relatives at the natal patch and thus reduce the cost of inbreeding depression (Perrin & Mazalov, 1999).

Additionally, factors that determine the intrinsic quality of the habitat such as resources availability can lead to the evolution of dispersal when for example the patch carrying capacity varies over time (Olivieri *et al.* 1995; Bowler & Benton, 2005). In small populations, demographic stochasticity changes in population density and therefore a social and competitive environment can provide enough variability between and within habitats to select for dispersal (Cadet *et al.* 2003). Additionally, local extinction and recolonisation processes can lead to dispersal (*reviewed in* Olivieri *et al.*, 1995).

Furthermore, much insight into the evolution of dispersal was gained by studying its proximate causes. Individuals often disperse as a plastic response to the environment. The strategy is flexible and has the advantage of responding immediately to the variation in the cost and benefits of dispersal (Massot *et al.*, 2002). Such proximate causes depend on food availability, patch quality, isolation and size, interspecific interactions such as with competitors, predators or parasites and social factors such as the presence of opposite sex-kin (*reviewed in* Bowler & Benton, 2005).

2.2 Sex-biased dispersal

Whether dispersal is male or female biased depends largely on the mating strategy of a species. Greenwood (1980, 1983) postulated that the dispersal pattern is determined by

the level of intrasexual competition of resources. In polygynous or promiscuous mating systems which are predominant in most mammals, there is a conflict of interest between the sexes (Greenwood, 1980, 1983). With internal fertilisation, prolonged period of gestation and lactation, the female is constrained and predisposed to care for young. In order to maximise her reproductive success she needs to maximise the rate of processing resources. Males have the opportunity to desert first and their rate of reproductive output is thus limited by the access to females. Males are expected to disperse in such mating system, because at a local patch where individuals are related, local mate competition among males normally exceeds local resource competition among females (Perrin & Mazalov, 2000). A complete bias, where one sex is entirely philopatric while the other sex disperses, is rare in nature. However, male dispersal and female philopatry seems to be an evolutionary stable strategy in mammals (*reviewed in* Handley & Perrin, 2007). Furthermore, the sex-bias in dispersal can be reinforced by inbreeding avoidance where dispersing males reduce the risk that related females will inbreed. Additionally, female mate choice should enhance male-biased dispersal, because females may prefer immigrant males when the inbreeding load exceeds a certain threshold (Lehmann & Perrin 2003; Höner *et al*, 2007).

2.3 Consequences of dispersal: Objectives and hypotheses

Dispersal, as a response to ecological and social conditions on the natal site, and driven by the evolutionary causes hypothesised above, should ultimately result in an improvement of individual fitness, if it were to be a stable strategy. This study focused on the fitness variation within and between sexes affected by dispersal within a single snow vole (*Chionomys nivalis*) population in the wild.

The objectives of the present study were:

- (1) to confirm a male-biased dispersal system in the snow vole by studying its mating system,
- (2) to explore the fitness consequences within and between sexes affected by dispersal,

(3) to examine the effects of possible proximate effects on dispersal.

Since snow voles are likely to exhibit a polygynous or promiscuous mating system, male-biased dispersal is expected to avoid inbreeding and resource competition at the local patch. When male-biased dispersal is adaptive, reproductive benefits to dispersing males are predicted to exceed its costs. Within the population, natal dispersal distance was taken as an explanatory factor. Females have greater parental investment and are therefore expected to benefit in a greater extent from philopatry (Greenwood, 1980). Females closer to their natal site are expected to produce more offspring than dispersed individuals. Social factors such as the presence of opposite sex-kin, body size and age were tested as proximate causes of dispersal.

3 Methods

3.1 Species and study site

A single, semi-isolated snow vole (*Chionomys nivalis*) population was used to study population demography and dispersal patterns. Females have about two litters per year, with a litter size of no more than four young. Juveniles usually do not reproduce in the year of their birth. The population examined in this study is situated in the Swiss Alps at 2000m altitude (Jochalp, Canton Grisons). In the Alps, snow voles prefer rocky environments, such as screes and boulder fields (Janeau & Aulagnier, 1997, Luque-Larena *et al.*, 2002). This habitat preference imposes a spatial structure on snow vole distribution and therefore populations are semi-isolated and ecologically enclosed. Previous results show that snow voles are promiscuous where both sexes increase their reproductive output by several matings (Ravaioli, 2007).

The study site comprises approximately 5ha of loose rock environment. It was divided into five fields, each was divided into 12 squares (30m x 30m). Each area in turn resided nine traps equally set up approximately 10m apart from one another. Per day between 95 and 126 traps were controlled for with first daylight and set up in the late afternoon. Longworth traps (Rogers Manufacturing Co.) served to capture live animals overnight. Hay, pieces of apple and cereal served for isolation, water supplement and food, respectively. Peanut butter was used as bait. In order to mark the location of trapped individuals, the coordinates within the grid were noted. Data was collected from June to September in 2007. These included mark-recapture data for spatial analysis, morphological data such as body length and weight. Trapped individuals were transferred into a plastic bag (30cm x 45cm) and identified by their passive integrated transponder (PIT; ID K162 FDX-B; AEG ID). Ear biopsies were taken using an ear puncher (Hamilton; Thumb Type Punch; 2mm diameter) for genetic data and tissue samples were initially stored in 90% ethanol and then freezed at -20°C. Individuals not recognized by their pit-tag transponder from the previous year were sedated with ether, newly tagged and genetically identified by their ear-tissue sample. Weight was taken using a spring

scale (100g Pesola). Individuals were noted as juveniles with a weight below 34 g (Janeau & Aulagnier, 1997). Further details are provided in Ravaioli, 2007.

3.2 DNA extraction, genotyping and parentage analysis

DNA extraction of tissue samples was extracted in a Biosprint™ extraction robot magnetic particle technology using the 96 DNA Blood Kit (Qiagen) following the manufacturers' protocol. Extractions and amplifications were performed in separate rooms. Amplification mixtures were prepared in a UV-sterilised cabinet and pipetting was performed using filtered tips. One control PCR blank was used for each set of amplifications. Thirteen polymorphic microsatellite loci (Wandeler *et al.*, 2007), snow vole specific, were used for identity and parentage analysis. The standard multiplex PCR protocol was used for two Panels with optimized different annealing temperatures and primer concentrations (table 2 in results section, QIAGEN Multiplex PCR Handbook, 2004). A 81 bp fragment of the *Sry*-gene was co-amplified with primers of panel A (table 2 in results section). Male-specific *Sry*-primers (5'-TATGCTGTGGTCTCGTGGTC-3', 5'-TCCCAGTTGCTTGCTTGCTGATCT-3') in order to identify sex was accessed via accession number: Y10322 (Bullejos *et al.*, 1997). Optimized PCR amplifications were performed in a total volume of 6 µl with 3 µl of 2 x Multiplex PCR Master Mix (Qiagen), the primer concentration described in table 2 (results section), and 2 µl template DNA. Amplifications were conducted in a GeneAmp® PCR System 9700 under the following conditions: an initial hotstart denaturation at 94°C for 15 min, followed by 27 cycles of 30 s at 94°C, 90s at 63°C (for Panel A) or 61°C (for Panel B) and 30 s at 72°C. The final elongation step was conducted at 60°C for 30 min. Fragment analysis for PCR products was performed on a 3730 PRISM® DNA Analyser sequencer (Applied Biosystems) using GeneScan-500 LIZ™ size standard and the GENEMAPPER® v.3.7. (ABI) software.

To find matching genotypes, the probability of identity was estimated with the software CERVUS 3.0 (Marshall *et al.*, 1998). The background allele frequencies were defined as the allele frequencies for all genotyped individuals.

Parentage was assigned by analyzing genotypic data using the likely-hood based approach with the program CERVUS 3.0 (Marshall *et al*, 1998, Kalinowski *et al*, 2007). Hereby, the program assigns the two most likely candidate parents on the basis of the natural logarithm with the likelihood difference, termed LOD-score. CERVUS uses the established background frequency of the population to create simulated parents and offspring in order to test the power of loci to distinguish the true parents from other candidate parents in the population (Marshall *et al*, 1998). The input parameters for the simulation of parentage analysis are given in Table 1. Proportion of candidate males sampled in simulations was set to 60% in order to account for male dispersal; however results did not change when less conservative estimates of this parameter (70% and 80%) were used. Parentage analyses were conducted for each year of study separately, where sampled adults with the appropriate sex were attributed to candidate parents and all juveniles within a season served as candidate offspring. For parentage assignment, critical LOD-values were calculated for maternity and paternity separately, with first assigning mothers and then fathers. With a maximum of one allele mismatch allowance, parentage was assigned to those individuals with the highest LOD-score and a delta above 95% (Marshall *et al*, 1998).

2.3 Reproductive success

All individuals born in year 2006 and recaptured as adults in year 2007 were included in the fitness analysis. Adults from 2007, which were not captured as juveniles in 2006 and were only assigned as an offspring in 2006, were also included in the analyses. For all analyses, fitness was measured as annual reproductive success in year 2007. Number of offspring included all assigned offspring. Reproductive success of individuals captured as adults in both years of study were omitted in the analyses to avoid possible age effects (adults reproduced in both years 2006 and 2007). Initially, males were categorised as dispersers (i.e. immigrants) and residents. The following definitions were set: resident males included those males that were

- 1) captured in 2006 and recaptured in 2007,
- 2) captured as adults in 2007 and had parents in 2006.

Season	2006 ^a	2007 ^a
Background Allele Frequency ^b	All individuals	All individuals
No. of simulation Cycles ^c	10 000	50 000
Proportion Loci typed	0.99	0.99
Typing error	0.01	0.01
Confidence Level ^d	95%	95%
No. candidate mothers ^e	44	109
Proportion mothers sampled	0.8	0.8
No. candidate fathers ^e	20	97
Proportion fathers sampled	0.7	0.7

Table 1: Input parameters of CERVUS parentage analysis. ^aSeparate analyses were conducted for each year of study in order to better reflect the number of sampled individuals present. ^b CERVUS uses background allele frequencies to create simulated parents and offspring to better test the power of the loci to distinguish actual parents from other individuals in the population. ^c In year 2007, adults of 2006 and 2007 were taken as potential parents; therefore, the number of simulation cycles were set to 50 000. ^d Some mismatch between parents and their putative offspring is expected due to microsatellite mutation and genotyping error. ^e Candidate parents in 2007 included adults from 2006.

Since the study was carried out only on a single large population and dispersed males were captured, dispersers were defined as the males that immigrated into the population. Immigrant males included all other than resident males, therefore “new” adult males that were captured in 2007. Annual reproductive success was compared between resident and immigrant males. Furthermore, individual annual reproductive success was analysed for each sex.

3.4 Spatial and temporal data

For both sexes, I compared fitness (annual reproductive success) to the natal dispersal distance. The location of capture of an individual was noted in coordinates within a grid

(see Study species and population). Coordinate data was transformed into values with the assumption that voles were captured in the exact middle of a 100m² grid.

The arithmetic mean of all individual trapping locations an individual was captured was referred to as its activity centre. The activity centre of the mother in the previous year was defined as the natal site of an adult individual captured in the following year. Therefore the Euclidean distance from an individuals' activity centre as an adult to its mothers activity centre is taken as the natal dispersed distance of that individual.

Trapping occurred continuously throughout the summer 2006. Date of birth was defined as the date of first capture for individuals born in 2006, since the exact date of birth is unknown.

3.5 Statistical Analysis

All data I analysed using the statistical program R 2.7.0 (R Development Core Team, 2005) and MINITAB 14 (Minitab Inc. 2003). Annual reproductive success was analysed using generalised linear models, where the number of offspring produced by individuals in one year was treated as a continuous variable following a negative binomial distribution (link = log for glm.nb). Explanatory variables were considered to have a significant effect at $P < 0.05$, from ANOVA reports obtained from model comparison by use of χ^2 -tests. However, most models showed a slight overdispersion, with the residual deviance being larger than the degrees of freedom and therefore might influence the credibility of the results.

Models included variables such as distance to natal site, sex, number of mating partner, presence of the mother, time of first capture and adult body length. Final models were selected following a backward deletion, progressively eliminating nonsignificant variables. Nonsignificant factors were presented in the results section separately. For better display and since males and females had differential dispersal behaviour (males dispersed and females were philopatric), I presented data separately for each sex.

However, full model effects are given in the results section. When sample size was too small to deduce distribution of data, non-parametric tests (Mann-Whitney-U) were used.

4 Results

4.1 General Findings

Thirteen microsatellite markers were scored in 334 snow voles (captures of 2006 and 2007). The number of alleles per locus varied from 4 to 16 (Mean $_{(k)}=11.77$, Table 2). None of the 13 loci deviated from Hardy-Weinberg expectations after Bonferroni correction (CERVUS: Marshall *et al*, 1998). Microsatellite markers provided a high resolution for individual identification, because all re-sampled (11), i.e. those that lost their PIT tag, and recaptured (43) individuals in 2007, had a 100% genotypic match.

Multiplex	Locus	Temp(°C)	uM	Flouresc. Label	Fragment size		k	H(obs)	H(exp)	NULL
					min	max				
A	SRY	63	0.075	PET						
	Chni01	63	0.3	6-FAM	110	133	12	0.817	0.814	-0.0028
	Chni03	63	0.3	6-FAM	175	199	13	0.892	0.886	-0.0038
	Chni09	63	0.2	VIC	257	281	11	0.829	0.793	-0.0243
	Chni12	63	0.3	6-FAM	259	281	8	0.74	0.777	0.0248
	Chni13	63	0.2	NED	192	214	9	0.823	0.822	0.0248
	Chni18	63	0.1	NED	100	146	16	0.856	0.846	-0.0063
B	Chni04	61	0.2	6-FAM	103	129	12	0.886	0.86	-0.0070
	Chni05	61	0.2	NED	79	127	15	0.835	0.856	-0.0191
	Chni06	61	0.15	NED	160	196	15	0.871	0.842	0.0103
	Chni08	61	0.3	PET	104	136	13	0.877	0.867	-0.0152
	Chni14	61	0.3	6-FAM	250	276	12	0.826	0.84	0.0067
	Chni15	61	0.15	VIC	167	199	13	0.844	0.857	0.0056
	Chni16	61	0.15	PET	253	267	4	0.722	0.69	-0.0245

Table 2 (4.1): Characteristics of dinucleotide microsatellite loci, based on 334 juvenile and adult snow voles. Table includes description of multiplex panels, marker name, annealing temperature for PCR reaction, concentration of labeled primers, type of flourscent label, fragment size range in base pairs, number of alleles (k), observed ($H_{(obs)}$) and expected ($H_{(exp)}$) proportion heterozygotes and possible frequency of Null alleles. No locus showed significant deviation from Hardy-Weinberg expectations.

In total, 152 individuals (128 juveniles, 24 adults), including immigrants and residents, were captured within the season of 2007 in a total of 21 trapping nights. Trapping

probability per day was 20 - 30%. At least thirteen individuals have escaped from traps during the night, got away during handling or due to holes in plastic bags. Forty-three individuals (31 females, 12 males), captured in 2006, were recaptured in 2007. In total, two males and eight females that have been captured as adults in year 2006 were recaptured in year 2007. Five (two females, three males) juveniles were reported dead in the field. Migration into the study population was estimated as 13% (One female and eight male immigrants of 67 adult snow voles in 2007) of the voles within one year with males' immigration rate of 30% (eight of 26). As predicted, 97 % (40 of 41) of females were philopatric, i.e. recaptured, within the study population. The adult population in the year/season 2007 consists of all new adults (immigrants: one female, eight males) captured during that season, together with all recaptured individuals in 2006 (31 females, 12 males) and all individuals found in 2006 being parents of juveniles in 2007 (nine females, six males). Animal abundance remained similar between years 2006 and 2007 (Fig. 1). In both years, significantly more adult females than adult males were captured ($\chi^2=5.55$, $P<0.01$, Fig. 1a). Among the juvenile population, there was equal sex ratio in both years ($\chi^2=0.376$, $P=0.721$, Fig. 1b). However, numbers might be an underestimate because about 36% of unmarked juveniles in 2006 have been found as parents in 2007.

Corresponding to the capture probability, paternity assignment in 2006 was 64% (93 offspring of 146 had fathers); where as maternity assignment included 85% (124 of 146). Paternity assignment in 2007 was considerably higher with 84% (108 offspring of 128 had fathers), and 91% (117 offspring out of 128 had mothers) of offspring had assigned mothers.

Reproductive success was not taken of all adults. Snow voles captured as adults (eight females, two males) in both years of study, 2006 and 2007, were omitted in the fitness analysis to avoid possible effects of age. Annual reproductive success did not differ between males and females (Mann-Whitney-U: $w=923.5$, $P=0.59$, Median_{males}=2.5, Median_{females}=2.0, Fig. 2). Yet, males have an intelligibly larger variance than females (Levene's test for equal variances: Females: $\sigma^2=4.74$, Males: $\sigma^2=10.32$, $P<0.05$, Fig. 2).

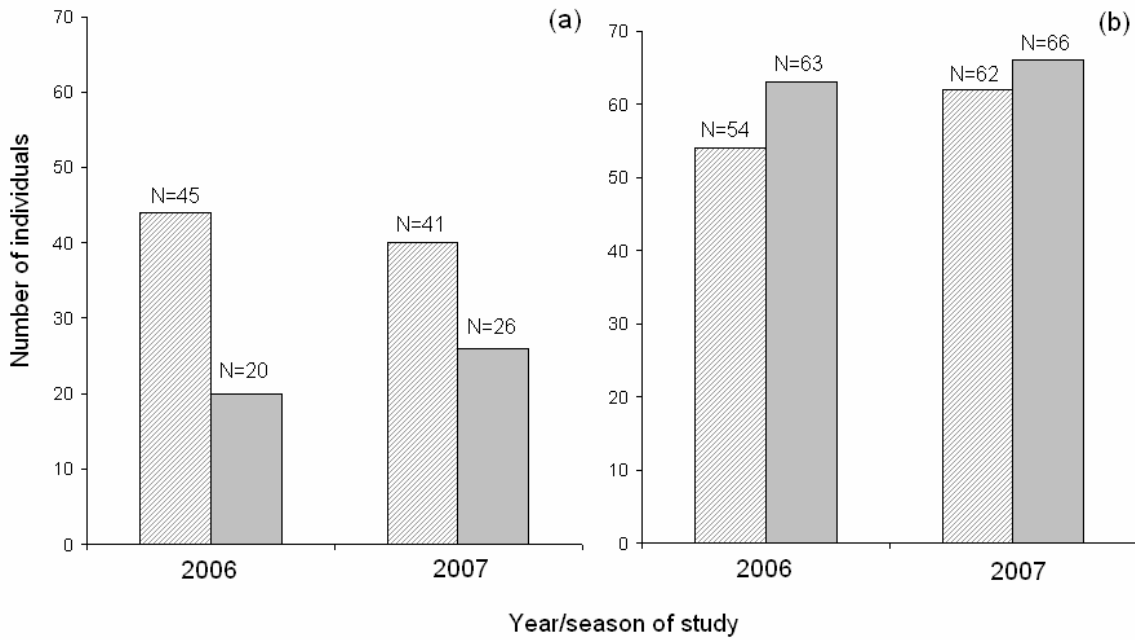


Figure 1 (4.1): Number of female (striped) and male (grey) snow voles in 2006 and 2007 (a) adult and (b) juvenile populations.

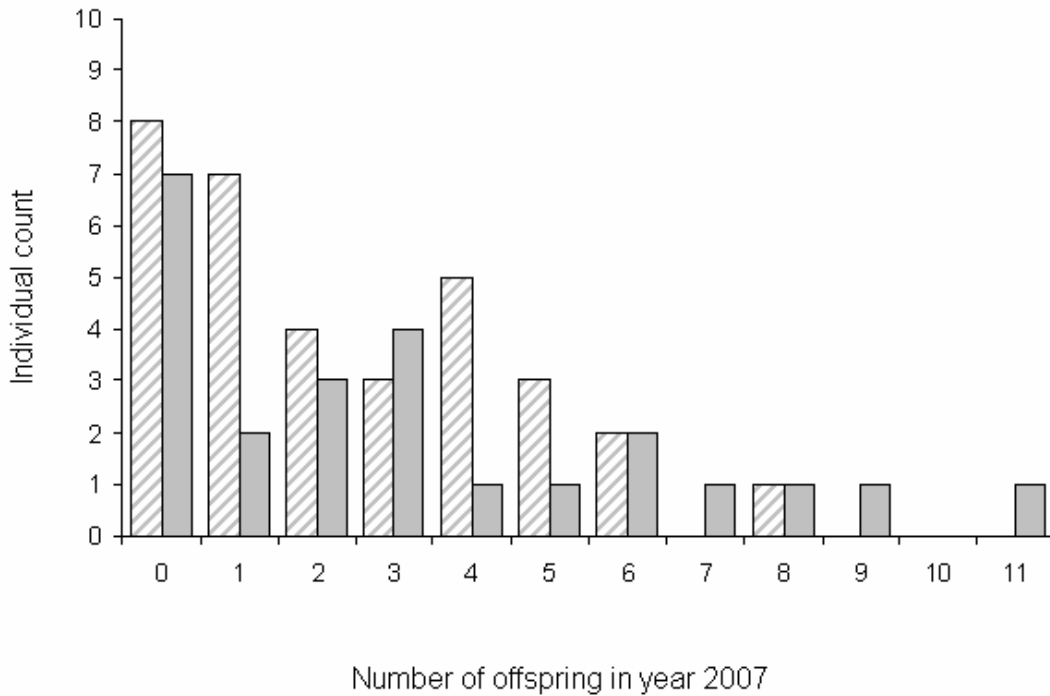


Figure 2 (4.1): Frequency of annual reproductive success (2007) in male (grey bars, N= 24) and female (striped bars, N=33) snow voles.

4.2 Reproductive success of male dispersers and residents

No difference was found comparing reproductive success of male immigrants and residents (Mann-Whitney-U: $w=102.0$, $P=0.926$; Median_{immigrant}=2.5, Median_{philopatric}=2.5, Fig. 3). However, given that immigration rate was low and the sample size of male dispersers was small ($N_{immigrant}=8$, $N_{philopatric}=16$), natal dispersal distance within the study population was used to explain fitness variation.

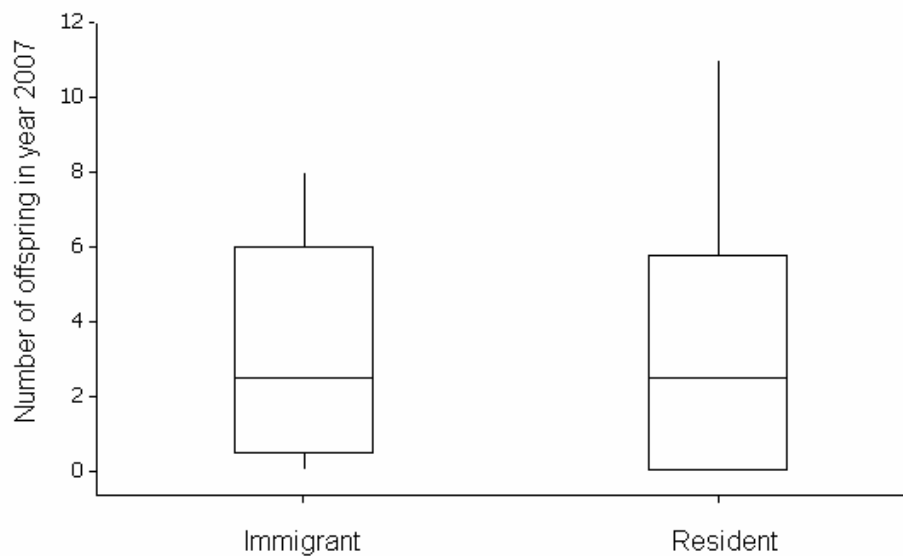


Figure 3 (4.2): Annual number of offspring for immigrant ($N=8$) and philopatric ($N=16$) male snow voles.

4.3 Reproductive success and dispersal within the study area

Within the area (~5ha) studied, natal dispersal distances ranged from 3.5 meters to 210 meters (Median=33.85, Mean=47.34, $N=40$) showing a skewed distribution (Fig. 4). Dispersal frequency was largely determined by sex, with males dispersing further than females (Median_{males}=67.2, Mean_{males}=88.2, Median_{females}=19.91, Mean_{females}=27.62; Mann-Whitney-U: $w=433$, $P<0.001$, Fig. 4).

There were no significant effects on reproductive success in the full model, including fitness explained by sex and distance. However, visually female offspring number decreases with dispersal distance (insignificant: $\chi^2=1.35$, $P=0.245$, $df=1$, Fig. 4a). Even though distance did not have a statistically significant effect on reproductive success in males ($\chi^2=2.55$, $P=0.279$, $df=1$), there was a positive trend (Fig. 5b).

4.4 Proximate effects on fitness and dispersal

The full model, explaining the annual reproductive success between sexes, included proximate factors such as the number of mating partners, presence of the mother, time of first capture and body length. Statistics followed backward elimination and I presented the minimum adequate model. There was a positive relationship between the number of offspring produced with the number of mating partners ($\chi^2=45.31$, $P<0.001$, $df=1$, Fig. 6a, 6b). There was no significant influence of sex in the model, meaning that both sexes increase their reproductive success in the same manner ($\chi^2=0.114$, $P=0.734$, $df=1$).

The presence and absence of a mother in the area did not have a significant effect on reproductive output in females or males (Mann-Whitney-U; Females: $w=468$, $P=0.262$, Males: $w=263$, $P=0.968$; Fig. 7a,b). Furthermore, the presence of a mother did not influence the dispersal distance of the offspring either (Females: $F=0.24$, $P=0.622$, $df=1$; Males: $F=0.004$, $P=0.949$, $df=1$). Those females that were found to live longer than one year showed no difference in offspring number between two successive years ($\chi^2=0.03$, $P=0.861$, $N=8$).

In male and female snow voles, the time individuals were born the, i.e. date of first capture had no effect on the number of offspring individuals had as first year adults ($\chi^2=1.587$, $P=0.207$, $df=1$; Fig. 8a,b).

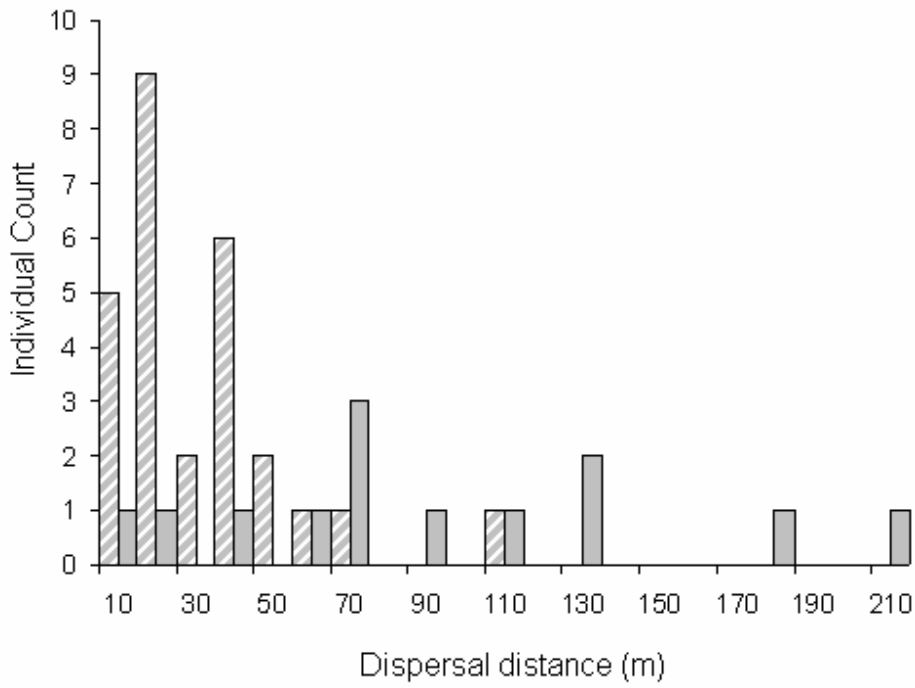


Figure 4 (4.3): Natal dispersal distances in meters in female (striped bars; N= 27) and male (grey bars; N= 13) snow voles.

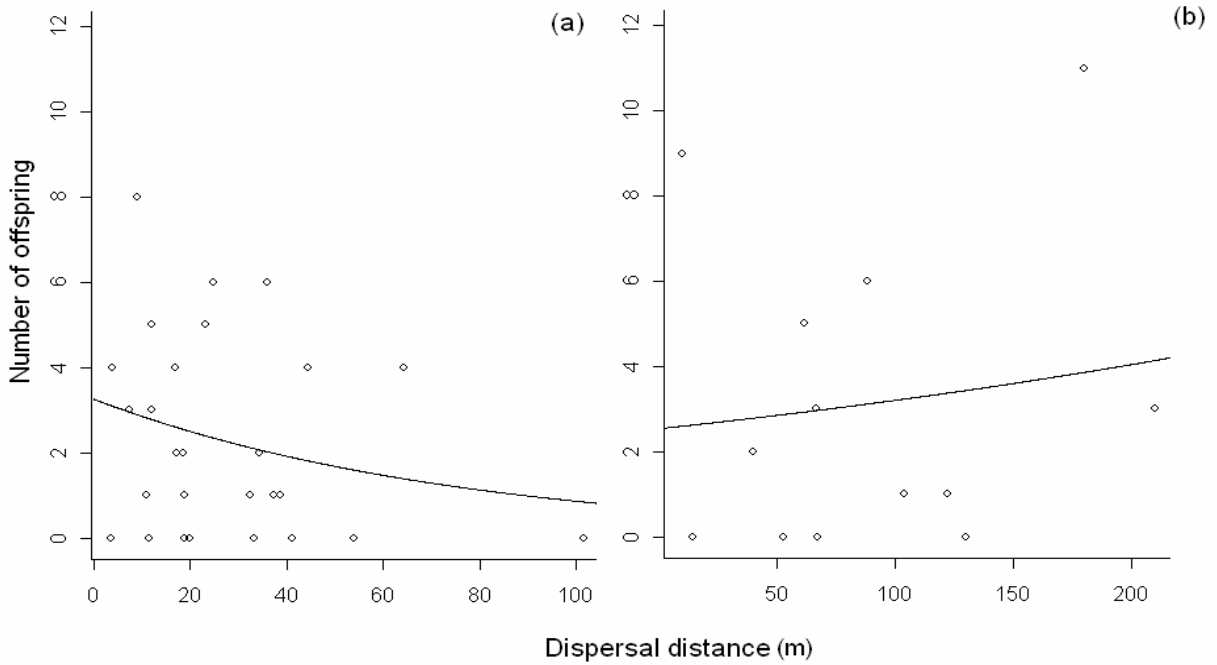


Figure 5 (4.3): Annual reproductive success of female (a) and male (b) snow voles in relation to natal dispersal distances in meters.

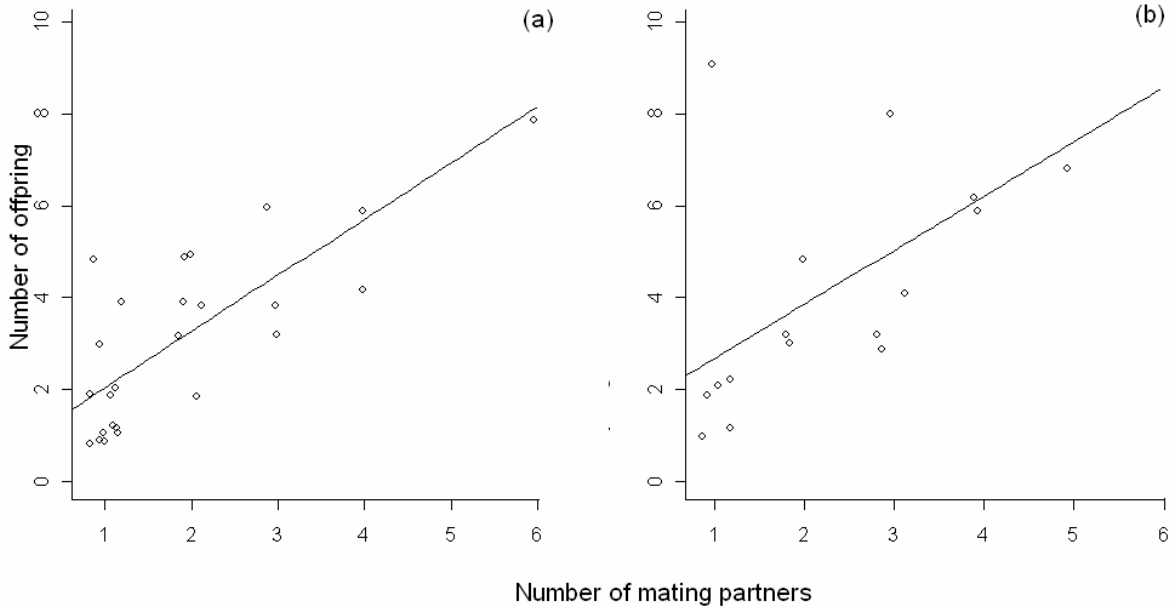


Figure 6 (4.4): The number of offspring produced in a year correlates with the number of mating partners in both (a) female and (b) male snow voles. The line was fitted using the identity function on a negative binomial distribution in generalized linear model statistics.

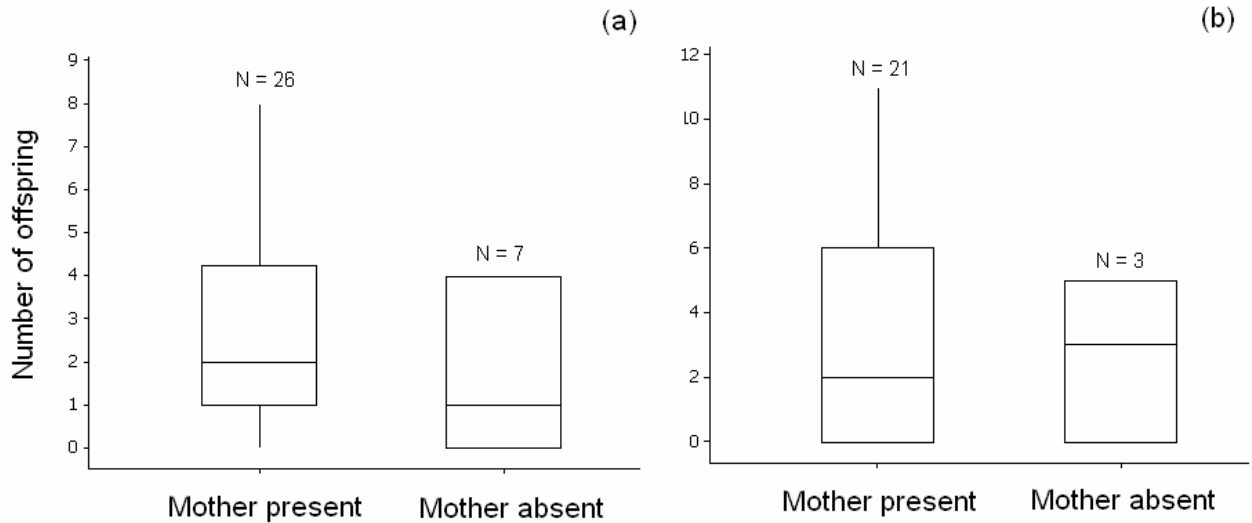


Figure 7 (4.4): Number of offspring of a) female and b) male snow voles tested against the presence and absence of their mother in the season.

There was no correlation with body weight, varying from 19grams to 26gram, of captured individuals and their date of capture. Data on body length was not available for juveniles captured in 2006. Therefore controlling for body size or using a body size index in the analysis to account for variation in capture probability of newborns was not possible.

Adult males with an average of 117.23 mm were larger in body length than females with an average of 114.21 mm ($t=-3.19$, $P=0.003$, $df=38$). There is a positive relationship in the number of offspring and adult body length in both male and female snow voles ($\chi^2=7.56$, $P=0.023$, $df=2$; Fig. 9). There is no effect of body size on dispersal distance ($F=0.261$, $P=0.506$, $df=1$).

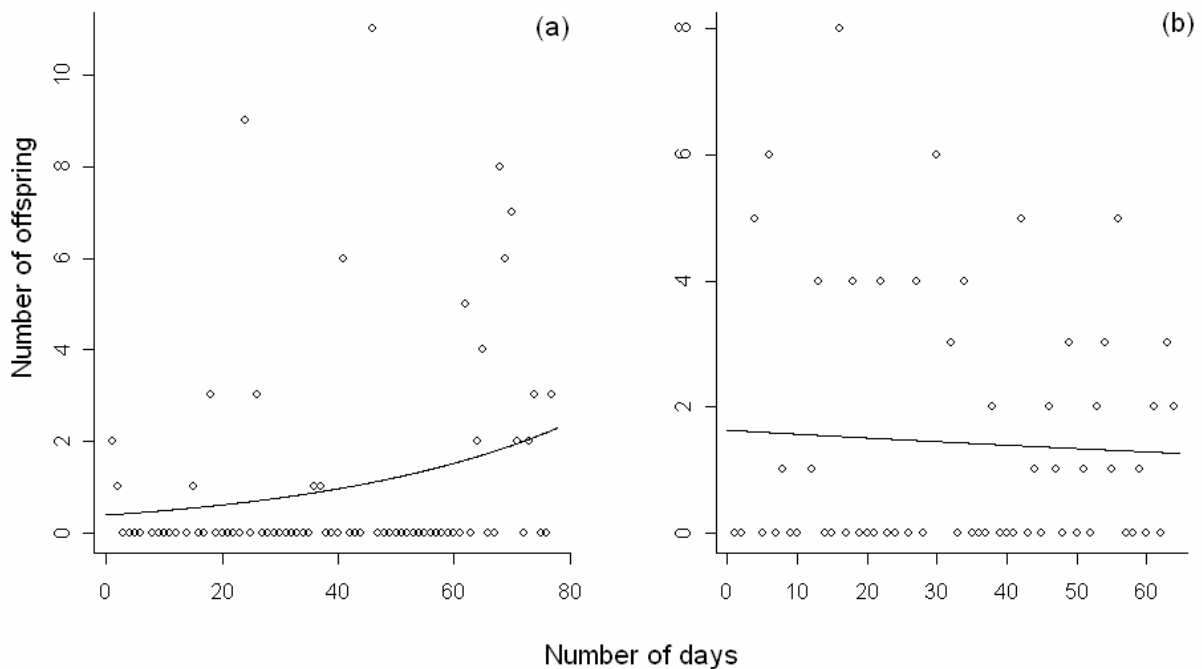


Figure 8 (4.4): The time of first capture had no significant effect on the offspring produced as adults.

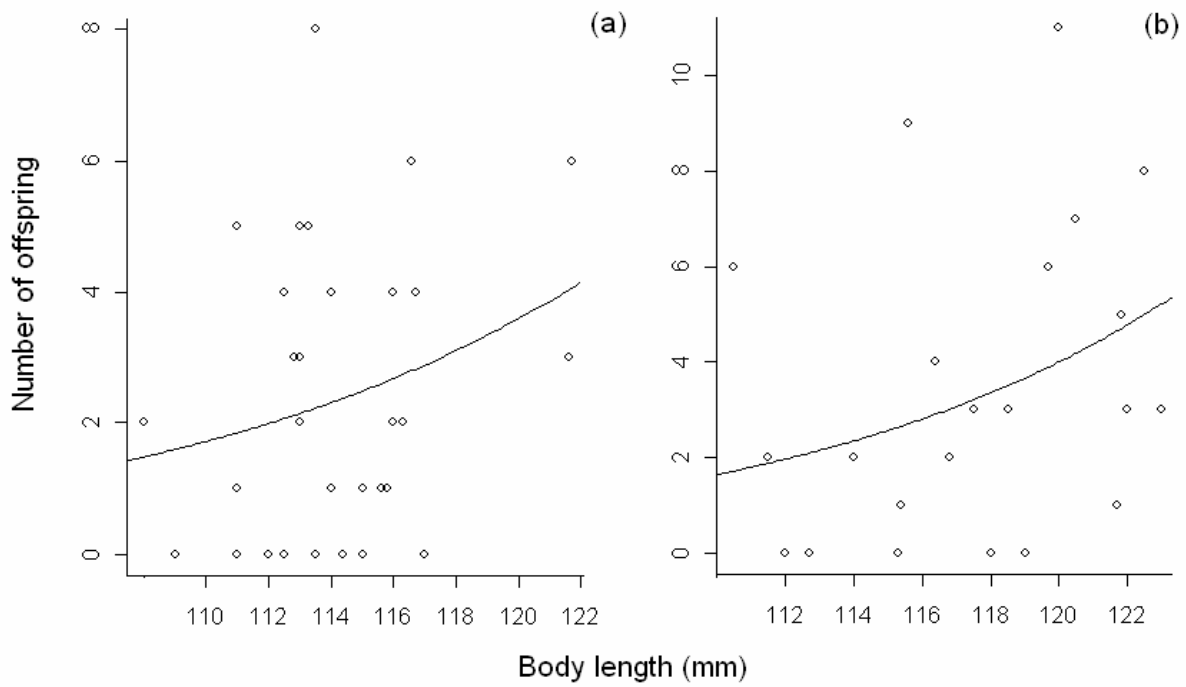


Figure 9 (4.4): The annual reproductive success in female (a) and male (b) snow voles is influenced by adult body length.

5 Discussion

5.1 General findings

Mammals usually show male-biased dispersal due to promiscuity or polygyny, males being limited by mating opportunities and females bearing the costs of internal fertilisation (Greenwood, 1980, 1983, Sutherland *et al.*, 2000). I observed that snow voles are polygynous, because both sexes increased their reproductive success with multiple matings. As predicted within such mating system, natal dispersal was male-biased. This is well documented in rodents (Boonstra *et al.*, 1987; Gundersen & Andreassen, 1998; Randall *et al.*, 2005). Also, male juveniles traveled on average further distances from their natal area than females. A further indication to a male-biased dispersal system served the adult population sex-bias. Sex-ratio was balanced at birth, and as adults in the population, more females than males were captured. Within the studied population, philopatry occurred in more than 95% of females and observed genetic structure using mitochondrial DNA (Ravaoli, 2006) further confirms male-biased dispersal in snow voles.

The immigration rate of males into the area with about 30% might be an overestimate in this study, since not all males might have been captured; relying on the finding that capture probability in 2006 was less than 65%. As heterozygosity levels were as expected, this low immigration rate suggests that the study population is ecologically isolated. In fact, unsuitable habitats often prevent migration over long distances, since costs in terms of energy expenditure and exposure to predation can increase as the animal moves greater distances (Roland *et al.*, 2000, Bowler & Benton, 2005). This is supported by Haddad (1999), who suggests that it is only when the surrounding environment is more hospitable that individuals should exhibit a greater emigration rate (Haddad, 1999). In this study, the investigated area was predominantly characterized by rocks and boulder, the preferred habitat of snow voles in alpine regions (Janeau & Aulagnier, 1997, Luque-Larena *et al.*, 2002), and the edge of the area changed to grass fields and meadows providing less protection from predators. Therefore low immigration rate could have been restricted by an environment more resistant to movement. Other factors that influence

immigration - not investigated in this study - include isolation and habitat cues as discussed by Bowler and Benton (2005).

In total, eight females and two males were captured as adult over the two seasons studied, meaning that snow voles can live longer than one year and that some reproduced in both years as adults. This is concordant with the description of population dynamics of the snow vole by Janeau & Aulagnier (1997). Although the age at which individuals reproduce can affect individual fitness (Stearns, 1992), there was no difference in reproductive success between the seasons in adult female snow voles. Also, the time of the year an individual was born did not influence reproductive output in the following season. However, the sample size was probably too small in order fully interpret the result. Furthermore, the date of birth was taken as the date of first capture. Capture probability of newborns is likely to vary highly between individuals and unfortunately body size data (body length and weight) of juveniles was not sufficient to correct for growth rate.

In root voles (*Microtus oeconomus*), it was found that larger males associated more often with females than smaller males, due to factors such as territory defence (Gunderson *et al.*, 2002). Although male home ranges probably encompass several female home ranges in snow voles (Ravaioli, 2006), there was a no significant relationship between body length and dispersal distance. Studies have shown that dispersing individuals are smaller than residents, indicating an influence of social dominance locally, because larger individuals are more competitive locally (in birds: Pasinelli & Walters, 2002; in mammals: Bowler & Benton, 2005). This is contrary to other studies that demonstrated that dispersers are larger, because they are capable to immigrate into a new competitive patch (Gunderson *et al.*, 2002). However, it may well be that individual characteristics as proximate causes of dispersal differ between species, or at least taxa, given the difference in dispersal behaviour in birds and mammals.

Offspring number of male and female snow voles differed in variance. This can imply sexual selection. A greater variance in offspring number in males could be the result of

reduced investment in gametes and parental care by males, increasing their potential rate of reproduction (Clutton-Brock, 2007). Usually, high variance in male breeding success causes females to be choosy. This in turn selects for secondary sexual characteristics (*reviewed by* Clutton-Brock, 2007). Body length was larger in males than in females, indicating that male snow voles exhibit slight secondary sexual characteristics. Furthermore, I found that there was a positive correlation between body length and reproductive success in males, which is supportive for sexual selection in snow voles (Clutton-Brock, 2007). Sexual selection in voles can be studied more thoroughly by considering factors such as choice of mating partners, multiple paternity per litter, intensity of intra and intersexual competition.

5.2 *Is dispersal adaptive?*

Two major factors have been proposed to cause dispersal behaviour in animals. Resource competition among females and mate competition among males should be avoided at the local patch, where individuals are related, to escape indirect fitness costs. Similarly, animals should disperse to avoid inbreeding at the natal site. Males are more prone to disperse in a system where they are not limited by resources and where females can benefit from philopatry. For dispersal to be adaptive, fitness consequences of dispersed individuals must exceed that of their philopatric counterparts. Studies on birds (Bensch *et al.*, 1998, Townsend *et al.*, 2003, Hansson *et al.*, 2004) and mammals (Gunderson & Andreassen, 1998) support the adaptiveness of dispersal in the dispersing sex.

Due to the opposing trend in reproductive success over distance in male and female snow voles I suggest that dispersal distance might be under selection, at least under the spatial scale considered. It is recognised that the spatial scale of a study can determine the description of dispersal movements (Bowler & Benton, 2005). This can be important when investigating the causes of dispersal, since optimal travel distances to avoid inbreeding and escape resource competition are likely to differ from the optimal travel distances required to colonise a new patch. Costs and benefits are predicted to vary over different scales (Bowler & Benton, 2005). Many studies rely on distance criteria to categorise dispersed and residential individuals, relying on data such as definite breeding

places or territories (Johannesen & Andreassen, 1998; Lacey, 2004, Martin *et al.*, 2008). In the present study, I used dispersal distance within the study area as a continuous variable and I observed that with distance, offspring number can change between the sexes. Since offspring only disperse as adults, this might imply that dispersal distance is be under selection.

Generally, it is hypothesised that individuals at the local patch compete for resources. Female snow voles seem to be adapted locally, as all females closer to their natal site had a higher offspring number. It is assumed that females with greater parental investment benefit from philopatry due to familiarity with the natal area and due to reduced energy expenditure and likelihood of predation (Daniels & Walters, 2000, Yoder *et al.*, 2004). Other studies found that females can also benefit from space sharing against threat from males (Root voles (*Microtus oeconomus*): Andreassen & Gundersen, 2006), food acquisition, communal care of young and defence against predators (Desert voles (*Rhombomys opimus*): Randall *et al.*, 2005). Therefore, local resource competition can be prevented when there are benefits of local resource cooperation, and female philopatry and strong mother-offspring associations can be selected for (Perrin & Goudet, 2001; Devilliard *et al.*, 2004). Microtine rodents often behaviourally tolerate female offspring and share space and food resources with kin (Ims & Andreassen, 1999; Solomon, 2003). In a study on root voles (*Microtus oeconomus*), it was suggested that frequent movement of daughters can disrupt a potential mother-daughter association, since short-distance dispersal of daughters was triggered by the presence of the mother (Gundersen & Andreassen, 1998). The study used an experimental setup and therefore small patch size used in unnatural environmental conditions might have changed the natural behaviour of voles. In my study, the maternal residency did not cause natal dispersal in female offspring. Similarly, in another study on root voles, the presence of the mother at the natal site did not cause dispersal of female offspring either (Le Galliard *et al.*, 2007), suggesting mother-daughter cooperation at the natal site.

Suppression of female sexual maturity is common in voles (Kawata, 1987). Yet, Le Galliard and co-workers found that root vole offspring remaining in contact with their

mothers were only weakly reproductively suppressed (Le Galliard *et al.*, 2007). In fact, in the laboratory, strong reproductive suppression between relatives in prairie and meadow voles is absent (Wolff *et al.*, 2001). This is concordant with the present findings, in that maternal residency did not have an effect on reproductive success on female offspring. Therefore, as reproductive success itself was not diminished by the presence of the mother at the natal site, local resource cooperation among female snow voles is possible.

Furthermore, a spatial autocorrelation analysis showed a significant fine-scale genetic structure among females seen from mitochondrial data (Ravaioli, 2006). This implies the presence of matrilineal female clusters. Le Galliard and co-workers argue that kin clusters among philopatric females are the basic determinant of the social structure in the root vole (Le Galliard *et al.*, 2006). Since reproductive output of daughters was not lowered and natal dispersal was not caused by maternal residency, evidence of matrilineal female clusters in snow voles suggest that mother-offspring associations are further selected for by kin cooperation (Solomon, 2003). However, it would be important to demonstrate that snow voles can recognise kin, yet likely in social species (Perrin & Lehman, 2001; Handley & Perrin, 2007), in order to imply that female philopatry is aided by kin selection.

Since local resource competition among females in snow voles is probably prevented by local resource cooperation (possibly via kin selection) and environmental benefits of philopatry, local mate competition among related males could induce male-biased dispersal in snow voles, to avoid indirect fitness costs (Perrin & Mazalov, 2000; Handley & Perrin, 2007). Dispersal within the study site might be selected for in male snow voles, as within the area, the males that moved greater distances from their natal site showed a trend of higher reproductive success. However, immigrant and resident males did not differ in reproductive output. This implies that short-distance dispersal, i.e. the movement away from the natal site within the population, might be adaptive for males. In fact, short-distance dispersal is probably sufficient to avoid kin competition, whereas long distance dispersal might function to colonize new habitats or escape crowding (Handley & Perrin,

2007). However, there was no direct indication that males disperse in order to escape mate competition among kin.

The male-biased dispersal itself, found in this study is in accordance with the inbreeding avoidance hypothesis. A further indication for the inbreeding avoidance hypothesis would be the influence of maternal presence on male-biased dispersal (Wolff 1992, Gundersen & Andreassen, 1998; Bowler & Benton, 2005). However, in this study, the presence of the mother did not have an influence on male dispersal, being contrary to the predictions. A similar result was found by Gundersen and Andreassen on root voles (Gundersen & Andreassen, 1998). In their study, root vole populations were manipulated experimentally in order to disentangle adaptive responses of dispersal. Dispersal was male-biased and philopatric male root voles were often reproductively inactive, which supports the inbreeding avoidance hypothesis. However, dispersal was not triggered by opposite sex-kin (Gundersen & Andreassen, 1998). Additionally, reproductive success was not different between individuals that were born earlier or later in the season, which indicates that there is no strong pressure to disperse prior to sexual maturity. This is expected when dispersal is selected to avoid inbreeding (Bowler & Benton, 2002). The results on the time of birth in snow voles and their reproductive success further do not support inbreeding avoidance by males. Even though, as mentioned above, the measurements on time of birth (date of first capture) had its caveats, male-biased dispersal in snow voles is unlikely to be solely to avoid inbreeding.

5.3 Conclusions

Short-distance dispersal within the studied snow vole population might be under selection due to the trend I observed. There was no difference in reproductive success between immigrants and residents, so proposed selective pressures that cause short-distance dispersal within the studies area, might differ from those that cause long-distance dispersal or inter-patch movement. In fact, dispersal costs such as likelihood of predation in an unsuitable habitat like meadow in snow voles are predicted to increase with inter-patch movement (Bowler & Benton, 2005). Inter-patch movement costs can also explain the observed low immigration rate into the snow vole population.

Female philopatry in snow voles could be caused by resource cooperation, aided by kin selection. However, in order to provide a solid clue on kin selection, it needs to be demonstrated that snow voles can recognize kin. It is difficult to distinguish whether male-biased dispersal evolved via means of avoiding local mate competition or inbreeding (Perrin & Goudet, 2001). Juvenile dispersal and philopatry is not influenced by the presence of the mother and therefore synergetic effects of kin competition and inbreeding avoidance to select for male biased dispersal in mammals proposed by Perrin and Mazalov (2000) is not supported. However, a simple analysis of presence/absence of close kin in natal sites may not be enough to test the inbreeding avoidance hypothesis by male-biased dispersal, because philopatric individuals might also mate with genetically similar individuals nongenealogically related (Ortego *et al.*, 2008). Lambin and colleagues conclude in their review of empirical evidence that there is hardly a single factor that accounts for dispersal patterns, since factors vary with individual life history and its interaction with the environment (Lambin *et al.*, 2001), given that there can be many more influences on dispersal such as density, food availability, sociality, female mate choice and habitat cues (Bowler & Benton, 2005, Höner *et al.*, 2007). Perrin and Goudet (2001) point out to potential causes of dispersal as interacting forces rather than alternatives (Perrin & Goudet, 2001).

5.4 Limitations and future implications

Generally, the inferences about the adaptiveness of dispersal in the present study need to be taken with caution, due to the small sample size and statistically insignificant results. Additional proximate factors, such as developmental stage, habitat cues using geographical information systems (GIS) and interspecific interactions, such as predators and parasites can be collected. Rigorous sampling over additional seasons should disclose a more confident picture on the fitness consequences of dispersal in male and female snow voles.

Moreover, the fitness costs of dispersal were not evaluated in this study, which are important determinants in the evolution of dispersal. Fitness is mainly composed of and usually measured as adult survival during the breeding season and their reproductive

success. Mortality however could not be investigated in this study, which might have masked some effects on the adaptiveness of dispersal in snow voles. For example, a study on Northern Bobwhites (*Colinus virginianus*) showed that dispersers benefit from higher survival rates, but dispersal distance has little influence on the reproductive output of the bird (Townsend *et al.*, 2003). Nonetheless, in my study, measured annual reproductive success is probably biased towards an underestimation. Therefore a difference detected would be unlikely due to mortality, since this would require an overestimation of mortality. Different costs of dispersal include unfamiliarity with the area, energy expenditure and likelihood of predation (Daniels & Walters, 2000, Yoder *et al.*, 2004). However these factors can be difficult to measure in a wild population and would require experimental setup and direct observation. Costs and benefits of dispersal are likely to depend on the scale of movement (Bowler & Benton, 2005, present study), and thus, should be differentiated accordingly.

6 References

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7 Appendix

*An investigation of individual identification of snow voles (*Chionomys nivalis*) via faecal samples*

Abstract. I proposed to use DNA from faecal samples to identify individuals that have escaped during field work on my study on fitness consequences of dispersal in the snow vole (*Chionomys nivalis*). Faecal samples were stored in ASL buffer solution and as dried samples using silica gel beads. Low DNA was extracted using a standard protocol for stool samples and subsequently PCR co-amplified with snow vole specific microsatellite markers. PCR amplification rate was low and genotypes of dried and ASL buffer preserved faecal samples did not match genotypes obtained from tissue samples. Reasons are discussed.

7.1 Aims of the investigation

Individual identification via non-invasive sampling is a useful tool in wild animal populations and widely applied in conservation genetics and behavioural ecology, since it does not require handling of animals (Woods *et al.*, 1999; Taberlet & Luikart, 1999). Non-invasive material used as source of DNA are usually shed hairs, shed feathers or faeces (Taberlet *et al.*, 1997).

In my study on dispersal behaviour in snow voles (*Chionomys nivalis*), individuals often escaped traps during the night or got away during handling or due to holes in plastic bags. Knowing which individuals escaped, can increase sample size for mark-recapture data and parentage analysis. A non-invasive sampling approach using faecal samples might be used to identify individuals that have escaped during capture and therefore optimize sampling effort. Here I present an initial investigation on genotype error rate and PCR (polymerase chain reaction) amplification rate, using a simple approach to compare known genotypes from tissue samples with known genotypes obtained with the non-invasive approach. Furthermore, two preservation methods were tested to compare

extraction results via PCR products and genotypic match. The objectives of this investigation were

- 1) to verify that genotypes obtained from faecal samples match the genotype obtained from a tissue sample and thus to deduce genotype error rate,
- 2) to compare two collection methods to assess performance of sample preservation.

7.2 Methodology

Faecal sample collection and preservation. Snow voles were captured during the night using Longworth traps (Rogers Manufacturing Co.) (further details on trapping are described in Methods section, page 7). Three to four pellets of stool (1 – 2mg) were collected from the inside of life traps using a clean spatula of thirteen un-captured animals, i.e. voles that have escaped, and of seven individuals with known genotype (from ear biopsies) as comparative samples. During field work, collected faecal samples were a) transferred in 2ml tubes prepared with 0.8 ml Buffer ASL and b) dried in 2ml tubes containing 10g of silica gel beads (SIGMA S7625) (Wasser *et al.*, 1997). Samples were stored at room temperature for both methods investigated.

DNA extraction. DNA of faecal samples was extracted using the QIAamp[®] DNA Stool Mini Kit (Qiagen) following the manufacturer's protocol for isolation of DNA from stool for pathogen detection, page 15. I added 0.6 ml of Buffer ASL to samples stored in 0.8 ml Buffer ASL. Dried faecal samples were homogenized in 1.4 ml Buffer ASL. For both, dried samples and samples stored in Buffer ASL, I used the same extraction procedure as described by the manufacturer, except that I used half the amount of InhibitEX tablets for each sample and half the Proteinase K solution than recommended, as the amount of stool used in protocol was about 1 – 2mg only.

DNA amplification and genotyping. Extractions and amplifications were performed in separate rooms. Amplification mixtures were prepared in a UV-sterilised cabinet and pipetting was performed using filtered tips. Negative control PCR blanks, to monitor contamination, and seven DNA faecal reference samples of individuals with known

genotype (from previously extracted tissue samples) were used for each set of amplifications. The faecal reference samples of individuals with known genotype were multiplexed in same plate as faecal samples of unknown animals (thirteen). A multi-tube approach, (Taberlet *et al.*, 1996) recommended for low DNA samples, was used by repeating the 20 samples twice per plate per panel. In this approach, each amplification was repeated independently two times, and the genotype was scored by analyzing the set of trials. Thirteen snow vole specific microsatellite loci (Wandeler et al, 2007) and a *Sry*-primer to identify sex were selected and separated into panel A and B. Multiplex PCR reactions (panel A, panel B) and PCR amplification followed the same procedure as for tissue DNA (as described on page 8). Reactions were repeated twice with 35 cycles and twice with 38 cycles for each sample stored in Buffer ASL. In total there were four runs for samples stored in buffer ASL, one trial for dried samples. Reference genotypes of tissue samples were known from previous analysis. PCR products were detected and analysed on an ABI 3730 PRISM[®] DNA Analyser sequencer (Applied Biosystems).

7.3 Results and Discussions

In total, for all faecal samples, including samples of unknown individuals (N=13) and samples of individuals with known genotypes (N=7), average PCR amplification across thirteen microsatellite loci was successful for only 47% of samples. PCR product (loci) detection included all alleles detected, i.e. also those that did not correspond to the genotype detected via tissue samples. The loci amplification success rate did not differ between samples that were dried (65% success) and samples stored in buffer (35 cycles: 58% success, 38 cycles: 56% success) (Fig. 10). Number of cycles in amplification did not make a difference to loci detection in samples stored in Buffer ASL (Fig. 10).

Male-specific *Sry*-marker were amplified correctly for 79% for samples that were preserved in ASL buffer, and 71% for samples that were dried (statistically indifferent, $F=0.12$, $P=0.731$, $df=1$). Of those genotypes that could be scored successfully across all microsatellite loci, for samples preserved in ASL buffer, on average there was 22% allelic match per individual (N=7) with the genotype obtained from the tissue DNA sample.

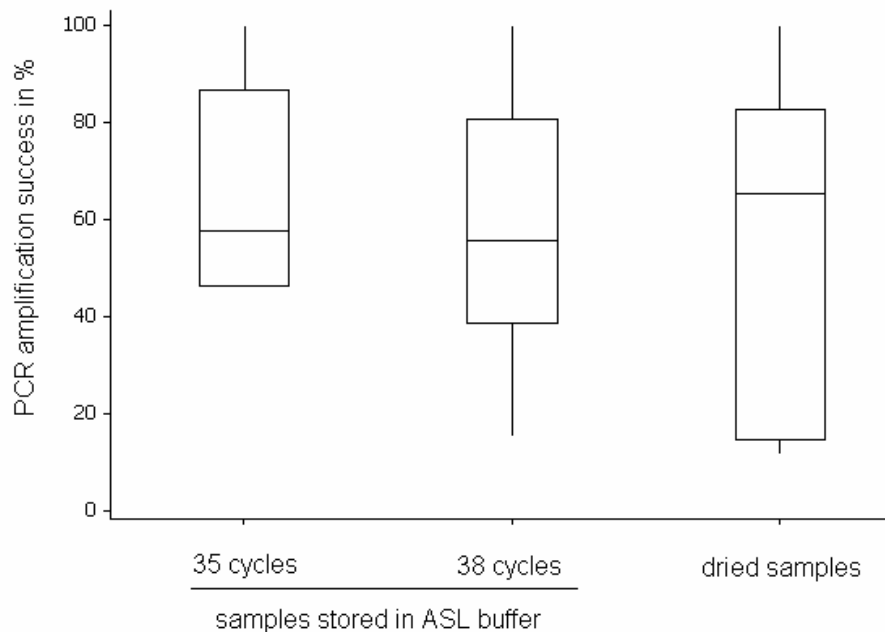


Figure 10 (7.3): Comparison of amplification success rate of two different storage methods: samples stored in ASL buffer at room temperature and samples dried using silica gel beads. Samples stored in ASL buffer were amplified using 35 and 38 cycles in the PCR reaction.

Five percent of scored genotypes of dried sample DNA matched genotypes obtained from tissue sample. The average genotype error rate per microsatellite loci was estimated as 0.81 for samples preserved in ASL buffer and 0.93 for samples that were dried. The average homozygote-heterozygote ratio – a homozygote detected for a heterozygote – was 0.38 for each heterozygote loci detected.

Individual identification of snow voles using faecal samples was not possible. Lampa and co-workers pointed out that for successful microsatellite analysis of faecal samples, there are four successive steps that are crucial: 1) sample collection, 2) the sample preservation method, 3) DNA extraction and 4) DNA amplification using PCR (Lampa *et al.*, 2008). The overall low PCR amplification success for faecal samples found in this investigation can be due to one of those factors. Amplification success was equally low for sample preserved in buffer or in silica gel beads. Failure of detection might therefore be due to the storage methods used in this investigation. However, DNA was extracted from low

amounts of collected scat (1 – 2mg). Although, the QIAamp[®] DNA Stool Mini Kit (Qiagen) protocol is usually a reliable extraction method for faecal samples (Lampa *et al.*, 2008), the protocol normally deals with 180 – 220mg of stool. Due to low collected amounts of faeces per individual, DNA was extracted only once. No DNA quantification was done after extraction, because of technical difficulty. DNA of the species is usually co-purified with large amount of bacterial DNA (Taberlet & Luikart, 1999). Therefore, failure in PCR amplification could be due to low amount of extracted DNA, which can also be highly degraded. Additionally, faecal sample amplification might have failed because of herbivore inhibitors present in faeces (Taberlet & Puikart, 1999).

Since only a small amount of DNA extracts can be used as a template, the number of PCR cycles can be increased in order to obtain a detectable product (Taberlet & Luikart, 1999). Here, I did not observe a difference in PCR product detection using a higher number of PCR cycles, meaning that more amplification cycles did not improve results. However, it might be that 38 cycles are still not sufficient to obtain enough target DNA to be detected and analysed.

Genotypes of successfully amplified loci showed mostly only a weak signal and further did not correspond to the genotype scored from tissue samples. Match for correct genotypes were extremely erroneous and did not differ between dried samples and samples stored in ASL buffer. It is realized that when DNA is extracted using non-invasive sampling techniques, PCR often allows detection of only one allele of a heterozygous individual (Taberlet *et al.*, 1996). This is referred to as allelic dropout, which produces false homozygotes. However, in this study, I estimated the allelic dropout rate for heterozygote loci as 0.38. Most often, within the independent genotyping experiments (multi-tube approach), using the same sample storage and DNA extraction method, PCR amplified different alleles or maximal one correct allele from the reference genotype (obtained from tissue sample). Genotyping error could be explained purely by sampling stochasticity: samples were not mixed thoroughly during pipetting, so that only one allele is amplified. Some control samples were not free of PCR product, which strongly suggests contamination. However, in most successfully amplified samples, two

or one allele was detected, which contradicts contamination. Having “wrong” alleles amplified is an indication that samples were exchanged and falsely tracked during pipetting. DNA of faecal samples was extracted using single tubes instead of a plate, because there were so few samples. This can enhance pipetting mistakes and therefore contamination between samples. In fact, when I did an identity analysis for each scored genotype, similar genotypes (allowing nine mismatches and three fuzzy matches) did not correspond to the individual they should represent. Unfortunately DNA extraction could not be repeated due to little material. I suggest that genotype error is mainly due to exchanged samples during DNA extraction and PCR preparation. Additional error can be explained by contamination and allelic drop-out.

Faecal sample collection combined with tissue sampling of snow voles in the field can be advantageous, because eluded individuals could be identified non-invasively. Knowing individuals that were able to escape could greatly improve sample size for mark-recapture data and parentage analysis. As faecal sampling in the snow vole has a major drawback due to the low amount and quality of the DNA obtained, an appropriate pilot study on non-invasive genetic sampling seems to be crucial in order to estimate technical errors.

Taberlet and Luikart propose guidelines of a more time-intensive pilot study on non-invasive genetic sampling and subsequent individual identification, so that the potential of non-invasive sampling prior to an extensive analysis can be realised. The proposed steps include an estimation of the probability of identity using preliminary data, simulations including the probability of identity and choosing a technical error rate, and lastly optimize PCR experiments (Taberlet & Luikart (1999). Furthermore Zhang and co-workers recently published an applicable protocol for DNA isolation from faecal samples. They tested a new protocol against the QIAamp[®] DNA Stool Mini Kit in herbivore species and conclude that they proposed protocol is not only cheaper, but also better to extract DNA from herbivorous species than the commercial kit (Zhang *et al.*, 2006). Piggot and colleagues propose a two-step PCR method in order to increase quality and quantity of the desired DNA template (Piggot *et al.*, 2004).

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Finally, I really hope that molecular tools (including non-invasive sampling techniques) will be in use more often in conservation biology and management in the future, because of their incredible powerful potential to unravel animal life histories, their behaviours and evolution.

For now, I will leave the lab and my next step in the impressive world of biology is to explore sampling techniques in ecology. I would like to perform baseline research with motivated students to discover the diversity of life in an area of the Kalahari. Together with passionate friends and my family, I am establishing a volunteer organisation (<http://brinknamibia.googlepages.com>) with the aim to protect an area of wilderness in Africa (Kuzikus) by the use of sustainable farm and wildlife management.