IDENTIFYING CAUSES FOR POPULATION DECLINE OF THE BROWN HARE *(LEPUS EUROPAEUS)* IN AGRICULTURAL LANDSCAPES IN DENMARK

PhD Thesis
Trine-Lee Wincentz Jensen

2009

NATIONAL ENVIRONMENTAL RESEARCH INSTITUTE
AARHUS UNIVERSITY

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Data sheet

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Abstract: In recent decades the brown hare (*Lepus europaeus*) in Denmark has undergone a substantial decline, but proximate causes are unknown, and little is known about actual densities. In this thesis, hare populations are investigated with respect to age composition and reproductive parameters in relation to habitat and phenotype, and point transect counts are evaluated in assessing hare densities. Data from culled individuals suggest that contemporary reproductive parameters and juvenile recruitment vary across Denmark, while adult survival seems constant across populations. Juvenile recruitment (indexed as the proportion of hares culled in autumn comprised by individuals < 1 year) is lowest in areas with low densities, but unrelated to the reproductive output of females indexed as the mean number of placental scars, indicating high spatial variation in post-natal survival of offspring. Some variation in the demographic and phenotypic (size, weight) parameters is attributable to habitat composition. In a historical perspective, the proportions of juveniles in game bags dropped significantly between the 1950ies when hare populations where stable and the 1980ies and later, when hare populations according to bag size decreased with 5% annually. Simple matrix population models based on the estimated annual survival for adult females at present and the estimated fecundity for the 1950ies, 1980ies, 1990ies and since 2000, predicted the same population growth rates for each decade as was actually observed in the annual bag records. The model substantiates the supposition that declines in the Danish hare population are caused by reduced juvenile recruitment, and moreover, the model predicts further population decline. Point transect counts are suitable and corrections for detection necessary, when monitoring hare populations, but work is still needed, before the effects of e.g. road avoidance on density estimates are fully clarified. The genetic variation of hares reveals that the population is subdivided and gene flow restricted even between close populations. The genetic differences are caused by a combination of genetic drift, ancient history and translocations. Future research should focus on determining causes of juvenile mortality, and reasons for variation in female fecundity, as well as the genetic consequences of the low densities on the mainland, along with the sustainability of hunting in low-density areas.

Key words: Brown hare, *Lepus europaeus*, recruitment, survival, reproduction, density, genetic variation

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> “Let the earth put forth living
> souls according to their kinds...”
>
> *Genesis 1:24*

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Preface

The present thesis is the results of three years studies on the population ecology of the European brown hare (*Lepus europaeus*) in Denmark. The thesis is proposed to fulfil the requirements for obtaining the PhD degree in biology at the Faculty of Science at the University of Copenhagen, Denmark.

A Danish research project entitled "Wildlife and Landscape" was initiated in 2003 with among many aims, that of gathering knowledge about the factors underlying an observed decline in the brown hare population in Denmark. My thesis was initiated in 2004, as a part of this larger research project, and set out to determine the status of the Danish hare population, with special attention on the population dynamics and on attaining estimates of current population densities.

My thesis contains a synopsis which presents the background for my studies and covers some general aspects of the population dynamics of the brown hare. Also, some central issues related to obtaining density estimates with emphasis on Distance Sampling, as well as some matters of concern in small or subdivided populations are discussed. These subjects are discussed in the context of the results from the six scientific manuscripts presented hereafter.

I owe thanks to several people who in various ways have made it possible for me to complete this study. First and foremost I would like to thank my husband, as without his full support it would not have been possible to complete a PhD with three small children. To my supervisor through the last half of the PhD, Peter Sunde: Thank you for always being an inspiration. I also owe special thanks to my two other, also very competent, supervisors: Henning Noer and Gösta Nachman. Lise-Lotte, thanks for always finding the time for professional as well as moral support. In addition thanks to all the people who were involved with the field work: Tine Sussi, Rikke, Jørn Pagh Berthelsen (I will never forget the lonely stuffed hare in a dark field one late night in Himmerland), Poul Hartmann, Bent Lyng, Maj-Britt Andersen, Camilla, Tommy Asferg and Peter Lange. Michael provided the front page picture, Lise Vølund gave useful comments on the synopsis and Johnny Kahlert gave input to figures. Special thanks to all my co-authors for letting me use our common work in this thesis.

Several master students have gone in and out of the students' office while I conducted my thesis; among these I especially thank Rikke who aged the hares as part of her thesis and Maj-Britt who worked with Point Distance Sampling.

I have enjoyed the working environment at the Department of Wildlife Ecology and Biodiversity at NERI and I have especially taken pleasure in the diversity of people and scientists here, all so dedicated to their specific scientific topics.

Rønde, February 2009
Trine-Lee Wincentz Jensen

1 Synopsis

1.1 Introduction and aims

In Denmark the brown hare (*Lepus europaeus*) is listed on the 1997 Yellow list of species which require special attention and are of national responsibility; a list of species that are decreasing but still numerous enough not to be endangered (Stoltze & Pihl 1998). The game bag of hares has declined through the last decades, suggesting a decline in hare numbers since the 1960ies. The annual bag of hares fluctuated at around 400.000 in the period from the 1940ies to the 1960ies, and hereafter the bag has gradually declined and reached an all time minimum of 57.000 in 2007 (Fig. 1). This equals a decline from a mean national bag of 9.6 km^{-2} to 1.6 hares km^{-2} per year after the turn of the millennium. A similar population trend has been observed in several other European countries (see Fig.1 in Smith *et al*. 2005a).

Until 2006 the Danish game bag was recorded in 14 counties (now 98 municipalities), and this division reveals substantial variation in the bag of hares between regions (Fig. 2a). The regional bag declines along a gradient from the southeast toward the northwest, probably reflecting a similar variation in hare densities (Strandgaard & Asferg 1980). The explanation for this variation is assumed to be differences in climatic conditions and soil productivity along the gradient, with larger bags in areas with good soils and more continental climate (Strandgaard & Asferg 1980). Besides Abildgaard *et al*. (1972)'s renowned capture-recapture experiments on the island of Illumø in the years 1957-1970, no attempts have been made to estimate the densities of hares in Denmark, probably because of the labour-intensive work required to obtain reliable estimates.

Despite the spatial variation in hare densities, the declining population trend has been very consistent across the 14 regions with the exception of one county, an island where the game bag of hares increased for about a decade starting in the mid 1980ies (BOR in Fig. 2b), after the main predator of hares in Denmark, the fox (*Vulpes vulpes*), was extinct due to an outbreak of sarcoptic mange. To optimize management it may be necessary to apply different strategies for high and low density populations.

The fact that hare density varies spatially, even within a limited geographic range such as Denmark, shows that densities are determined by factors acting on a local scale, whereas the simultaneous decline across the Dan-

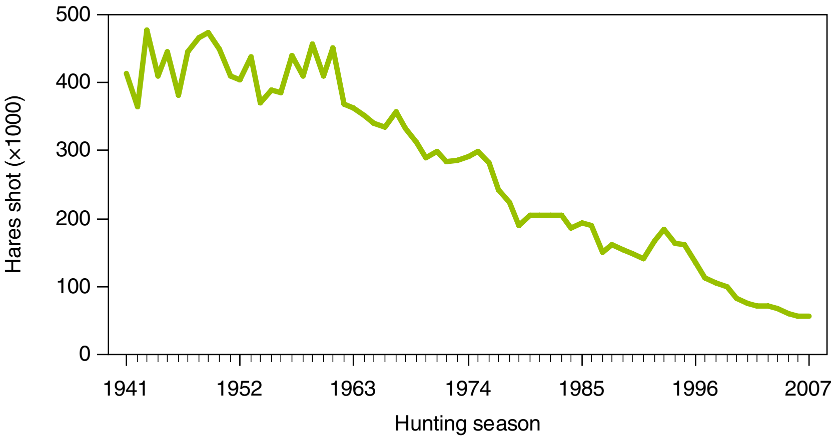


Figure 1. Annual bag of hares in Denmark in the years 1941-2007 (source: The Danish Game Bag Record).

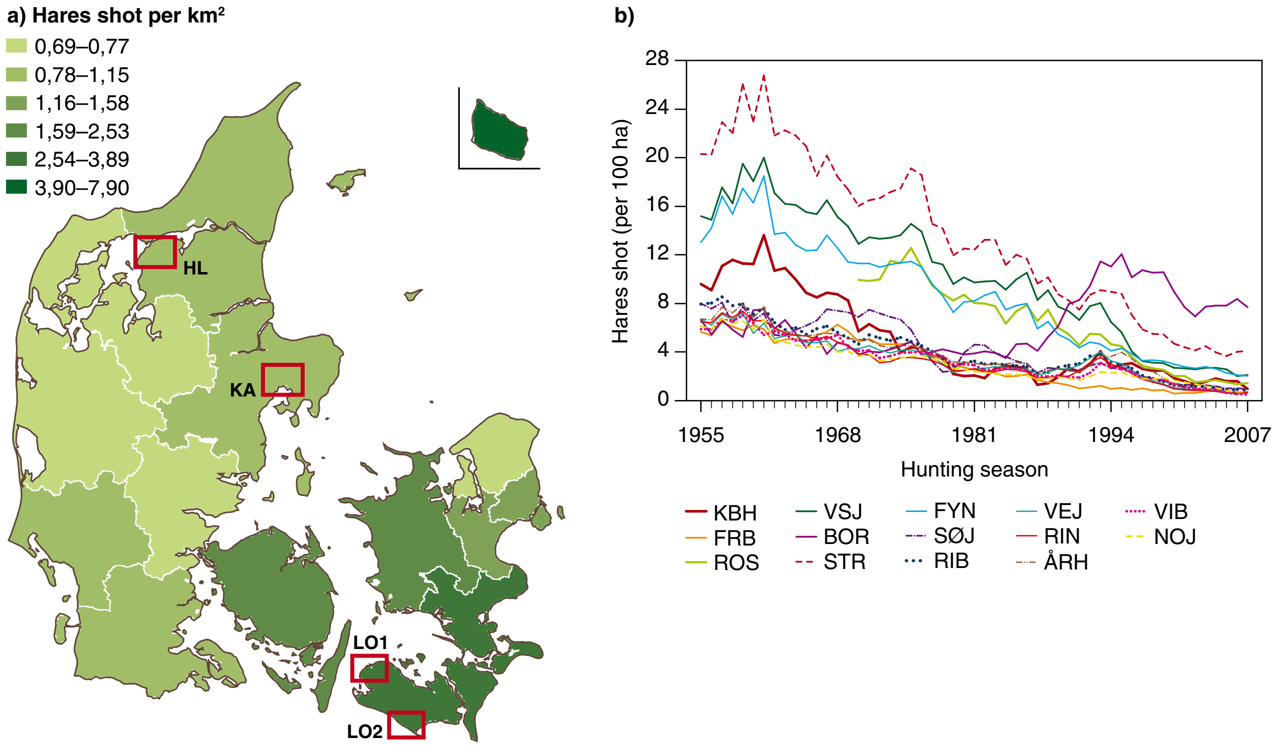


Figure 2. a) The mean number of hares shot per km^2 in 14 counties in Denmark in the years 2003-2007, and red squares indicating four study areas where Point Transect Counts of hares were carried out during the PhD project period. **b)** The bag in the 14 counties in the period 1955-2007.

ish counties as well as across Europe is evidence, that factors of a more universal sort also affect this species. Hence, when studying marginal as well as long term changes in population trends, it is necessary to examine the processes which restrict the performance of individuals on a local scale along with the environmental processes affecting the population as a whole.

To gain an overview of the factors that influence the hare population and in order to identify key areas of the dynamics within this particular species, I set up a conceptual model of the brown hare population dynamics which is visualized in Fig. 3. The individual factors from Figure 3 will be discussed in more detail in the forth coming chapters in the synopsis.

Numerous hypotheses have been put forth and considerable research has been carried out in the pursuit to uncover the causal mechanisms behind the population declines (reviewed in Olesen & Asferg 2006). Research efforts have centred on determining the influences of the key factors in the model above; diseases, climate, predation, hunting, age-specific survival, reproduction, and habitat alterations, in order to estimate their respective effects on hare population sizes. Smith *et al.* (2005a) synthesized results from 77 European hare research papers, and concluded that the ultimate cause for the declining hare populations is habitat alterations in the agricultural landscapes (the preferred habitat of hares) due to intensification of the agricultural practices, whereas effects of climate and predation were enlarged by loss of forage and cover. However the proximate causes, of how the habitat changes affect individual survival and mortality, remain unidentified.

With this knowledge setting the scene, the aims of my thesis were:

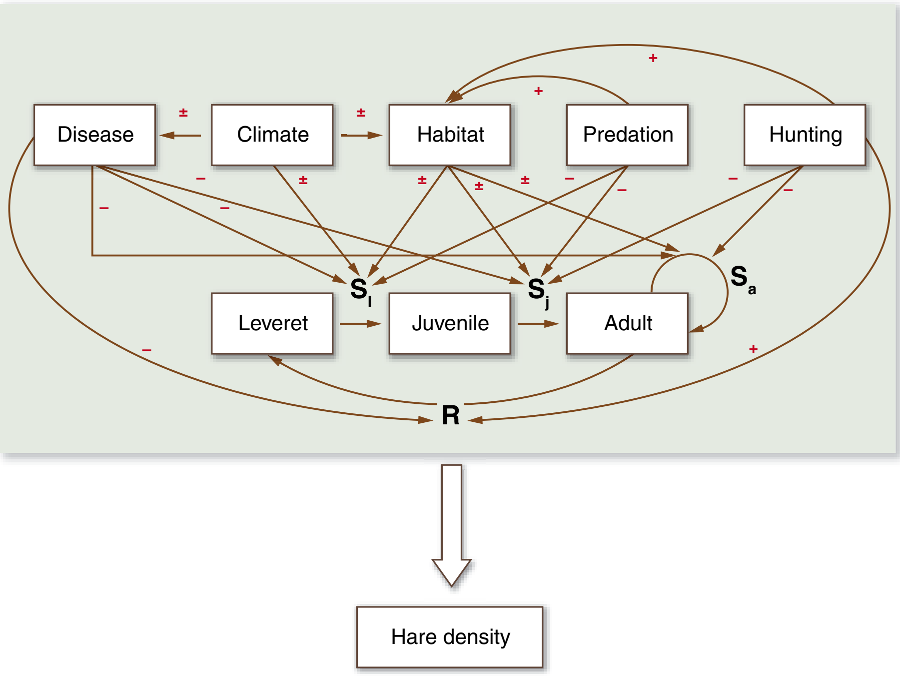


Figure 3. Conceptual model showing the main pathways in the population dynamics of the hare which are discussed in my synopsis. The top row shows the major environmental or external factors affecting all hare populations. The middle row shows the dynamics within each hare population set up as a stage classified life cycle graph (Caswell 1989). S_l indicate survival of leverets, S_j survival of juveniles, S_a survival of adults and R represents the mean reproductive rate of adult females. The environmental factors interact with the dynamics within the population in determining hare density. Arrows indicate direction of influence, signs in parenthesis indicate positive (+) and negative (–) influences. More arrows could be added.

1) To identify the relative importance of survival and reproduction in contributing to the population decline in hares in the Danish agricultural landscapes (Papers I, II).

On the premise that changes in the agricultural habitat have caused the declines in the hare populations, it was investigated to what extent the habitat composition (ultimately: landscape variables, proximately: per capita food supply during growth and reproduction) affected individual's phenotype (size, condition) and demographic traits (age-specific survival, reproductive parameters). This was done by a horizontal comparison of different contemporary populations from different agricultural landscapes (Paper I).

In a vertical investigation of historical data from hunting records dating back 50 years (Paper II) we in particular wished to examine whether juvenile recruitment rates had changed through time in agricultural habitats and to examine, through matrix models, whether a change in recruitment could explain the observed declines in the hare population.

2) To attain reliable density estimates of hares (A) and evaluate Point Transect Counts as a method of obtaining these (B) (Papers III, IV).

A: The overall aim of studying declining populations is obviously to identify ways to reverse the decline, and this calls for information on baseline density to verify with time, that the trend has been reversed. Also, reliable density estimates are essential when optimizing management strategies.

B: As Distance Sampling has mainly been used in audio, aerial and marine surveys, the aim was to critically evaluate, whether the method was applicable for hares. We aspired to assess the effects on density estimates, of possible violations of basic Distance Sampling assumptions when transferring this method to the agricultural habitat.

3) To investigate the genetic population structure of the Danish hares (Papers V, VI).

Genetic variation is requisite for evolutionary adaptation to a changing environment, and besides maintaining population numbers, maintaining genetic variation is a central theme of long-term population management.

We wanted to analyse the amount and distribution of genetic variation within and among populations of hares in Denmark, in order to assess to what degree the population is subdivided, and whether any patterns of genetic differentiation are the result of genetic drift, historical events or recent anthropogenic events (translocations, hare farms, fragmentation).

And finally based on 1-3) the aim was to determine where future research efforts should be concentrated in Denmark.

1.2 The hare in the agricultural habitat

Denmark was colonized by brown hares from Southern refugia less than 10,000 years ago after the last Pleistocene glaciations (Fickel *et al*. 2008, Paper V). In Denmark the hare is traditionally considered a farmland species, but suitable habitat encompasses most open landscape types (Flux & Angermann 1990). Although present in arable habitats throughout Denmark, even within this single habitat type, hare density may vary considerably (3-111 hares per 100 ha, Paper IV). In other European countries (reviewed by Smith *et al*. 2005a), densities at high farming intensity varies around 28±12 hares per 100 ha and at inter-mediate farming intensity around 80±31 hares per 100 ha.

1.2.1 Intensification of agricultural practices

Concomitantly with the decline in hare populations in Europe during the past 50 years, incentives to increase agricultural productivity, has lead to an unprecedented rapid agricultural intensification. This includes a change in planting and cropping regimes toward autumn-sown cereals and silage production (leys) and amplified use of agrochemicals and machinery in the daily management of land (McLaren *et al*. 1997, Robinson & Sutherland 2002). The structural development within the Danish husbandry in this period has gone toward fewer, bigger and more specialized farms, characterized by large field units with less variation in crops (Bach *et al*. 2001). Consequently, landscape uniformity has increased and spatial as well as temporal heterogeneity has been reduced.

Frylestam (1980a) was the first to suggest that these agricultural changes potentially cause hares to experience a food shortage when winter crops mature in large farming areas covered by the same crop. In previous times survival of hares was thought to be limited by food availability in winter. However, autumn-sown cereals and leys are, from hare-perspective, at their “best” in winter, and are avoided during summer (Frylestam 1986, Tapper & Barnes 1986, Reichlin *et al*. 2006). Consequently, the season limited by food appears to be reversed to summer, meaning that a seasonal low in food availability now coincides with the peak reproductive period of the hares. This shortage of food in summer has been hypothesised to be a main contributing factor to the European-wide declines in hare populations (Hackländer 2002b, Reichlin *et al*. 2006).

1.2.2 Agricultural areas as feeding grounds

Hares are selective feeders on green parts of plants, preferring early growth stages of crops and plant parts high in fat (Chapuis 1990, Homolka 1987) and despite their affinity towards agricultural areas, hares positively select weeds/wild species over arable crops (Frylestam 1986, Reichlin *et al.* 2006). Herbicides reduce the standing biomass of weeds, and have caused a decrease in the occurrence of weed species since the 1960ies (Andreasen *et al.* 1996) as well as about a 60% reduction in weed density in the same period (Jensen & Kjellsson 1995).

Increasing field sizes result in reduced field margins and increasing fragmentation of the landscape. Hares prefer edge habitat (Tapper & Barnes 1986, Roedenbeck & Voser 2008), and also, edges toward tree stands have a positive effect on growth (Paper I). This is probably because edges may provide food as the crops mature, while at the same time this habitat provides year-round cover as lack of cover possibly limits juvenile survival in some habitat types (Smith *et al.* 2004, Jennings *et al.* 2006). Also, dense mature cereal stands will physically be difficult for hares to move through. The loss of field margins, copses and hedgerows possibly exacerbates the effects of an encountered food shortage in the summer, which point towards an increased importance of non-cropped habitat in providing corridors as well as feeding grounds in fragmented landscapes.

In Denmark, a shift from a dominance of spring cereals to winter cereals occurred in the 1980ies, a pattern that is recurring across Europe. Schmidt *et al.* (2004) found a negative association between winter cereals and brown hare numbers in Denmark, while it was not possible to confirm any effects of local coverage of winter cereals on age structure on a smaller spatial scale (Paper I).

The areas with grass leys and whole crop have increased dramatically, and grass cutting for silage may occur 3-4 times a year in the same field (Dansk landbrugsrådgivning 2002, J.P. Berthelsen *pers. comm.*). McLaren *et al.* (1997) suggested that silage cutting could increase leveret mortality, but we did not find support of any relationship between the recruitment in autumn and the local coverage of grass leys or whole-crop (Paper I). Only limited data is available on the impact of mechanized harvest and cropping procedures on hare mortality, but this probably mainly occurs in green forage crops for silage, and primarily affects leverets, as silage cutting starts in May and coincides to a great extent with hares' reproductive peak (Kaluzinski & Pielowski 1976, Milanov 1996). Few hares are assumed to be affected by harvest operations in winter cereals, as most young hares are old enough to escape at the time of year these are harvested (Kaluzinski & Pielowski 1976). In heterogeneous landscapes, field operations are spatially and temporally spaced compared to monocultures, which diminish the impact of this kind of mortality.

1.2.3 Dispersal, home range size and structure

Habitat requirements (space, food, shelter) of any species are likely to change over time, and this seasonal change in needs can be fulfilled either by individuals moving between different habitats as they are required, or, by different types of habitats becoming available at the right time (Benton *et al.* 2003). As a sedentary species, the brown hare depends to a large extent on the latter, and although range size may shift seasonally (Reitz & Leonard 1994, Smith *et al*. 2005b), hares do not increase the activity range as the energy content of the forage within their home range decreases (Smith *et al*. 2005b). Tapper & Barnes (1986) showed that hare habitat preference changes in response to crop growth, and that hares select home ranges with access to several fields within a small area. In this manner hares extend their home range to include diversity and home ranges are therefore larger where fields are large. In arable areas, range sizes are consequently larger in more uniform and intensively cultivated areas (Tapper & Barnes 1986, Reitz & Leonard 1994, Kunst *et al*. 2001). Estimated range sizes vary between 37-190 ha, depending on habitat type, estimation method and duration of estimating (Kunst *et al*. 2001, Harris *et al*. 1990).

Though hares are highly philopatric, recent research implies that dispersal may be more common than previously thought (Bray *et al*. 2007). However, most individuals stay close to natal grounds throughout their lives and even those that disperse, often remain within 2 km of the birth site (Tapper & Barnes 1986, Bray *et al*. 2007). Male hares are more likely to disperse, but dispersing females disperse longer than males (Bray *et al*. 2007). Hares show high site fidelity once a home-range is established. It seems likely that the unpredictability of food access in a changing landscape in itself is a quandary, and possibly this philopatry of hares enhances the problem.

The open landscape types in Denmark have decreased by 11% since 1951, the decrease primarily being in the semi-natural grasslands (Levin & Normander 2008). These areas have mainly been transformed to roads and cities. In conclusion; less and less suitable habitat seems available for hares now than 50 years ago.

1.3 Habitat effects on phenotype, survival and reproduction

The importance of identifying the various changes in modern agricultural management lies in quantifying the impact of these habitat changes on the individual hare. The habitat may affect population size by affecting demographic parameters these being affected either directly or mediated by the phenotype (Fig. 4 and Paper I). An individual's phenotype consists of the visible characteristics of the organism such as its morphology (e.g. size, condition, behavior), and is a product of the organism's genetic composition, the influence of environmental factors and possible interactions between the two (Johannsen 1911). Differences in phenotypic traits among populations of the same species are consequently caused by the

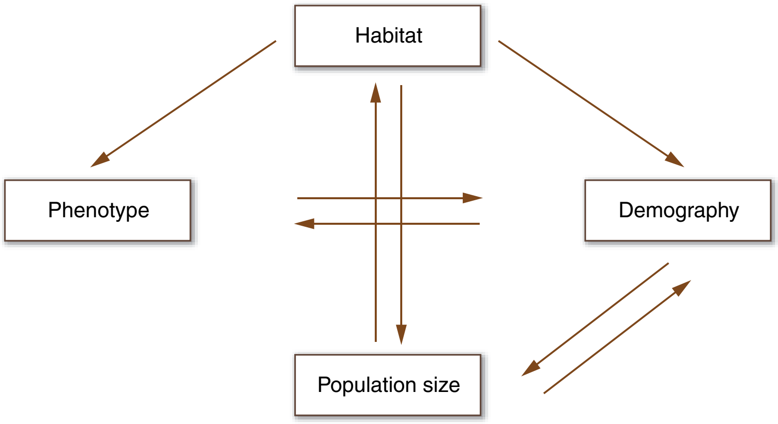


Figure 4. Conceptual model of possible ecological pathways between habitat and population size. Habitat quality affects individuals' phenotype (e.g. skeletal size, body mass) by providing nutrients during growth and to uphold physiological body functions. In turn the phenotype affects demographic parameters such as reproductive success and survival and vice versa. Habitat may also influence on demographic parameters directly, e.g. by improving survival through cover availability. Density dependent effects of population size on demographic parameters and habitat may also occur.

combined effect of variations in local gene pools, and differentiated selection on these genes, invoked from the immediate surrounding habitat (Stearns 1989). The ability of an individual with a particular genotype to change its phenotype in response to changes in the surroundings is entitled phenotypic plasticity (Price *et al*. 2003).

The importance of genetic variation will be discussed in a forthcoming section; the focus here is on how the habitat directly affects size and condition and the eventual effects of these traits on reproduction and survival.

1.3.1 Size-effects on demographic parameters

Habitat provides food and nutrition during growth, and the abundance and quality of food during this stage may directly affect attained adult size (Powel & King 1997, Lindström 1999, Paper I). As a consequence small-scale geographical variation in phenotype is often observed among mammals of the same species (Gortázar *et al*. 2000, Wauters *et al*. 2007, Strickland & Demarais 2008, Paper I).

In some species, larger individuals produce more offspring or offspring of higher quality (Stearns 1992, Begon *et al*. 1996), and size may be linked to sexual maturity, with rapid growth promoting early onset of reproduction and thus increasing fitness (e.g. Albon *et al*. 1987). In mountain hares (*L. timidus*) large size favours early reproduction, and females with large skeletal sizes produce more leverets through the reproductive season (Iason 1990). In some species, large sized individuals gain competitive advantages, which may enhance individual reproduction e.g. when competing for females. Conditions experienced during early development may thus have long-term effects on phenotype and subsequent fitness (the "silver spoon" effect: Grafen 1988, De Kogel 1997, Lindström 1999).

Small individuals will deplete their energy reserves first and these weaker individuals are more susceptible to predation or starvation, which is what leads to selection for large size. However in some instances, large size may be disadvantageous as larger individuals typically have higher absolute energy requirements than small individuals of the same species (Millar & Hickling 1990, Powel & King 1997).

Structural size varies substantially between populations of Danish hares, and attained adult size is related to the local availability of grass leys, an improved fodder crop (Paper I). However, despite significant differences in size, no effects of size on reproduction or survival could be verified in the Danish hares (Paper I).

1.3.2 Effects of condition on demographic parameters

Body condition is an essential habitat-related phenotypic trait, which refers to the energetic state of an individual (Schulte-Hostedde *et al*. 2005). Animals can be heavy because of structural size, but may also be carrying metabolizable tissue, energy reserves. Body condition indices endeavour to determine mass adjusted for structural size. No consensus has been reached as how to measure condition (Garcia-Berthou 2001, Schulte-Hostedde *et al*. 2005) but whichever measurement used, individuals in good condition, i.e. having a high body mass in relation to size, are generally assumed to have higher survival and to reproduce more successfully and thus have higher fitness (Millar & Hickling 1990, Stearns 1992).

Food availability in most temperate habitats varies seasonally, causing similar variation in body condition. At all times the energy available to an individual has to be allocated to uphold differential physiological functions to survive, but at certain times of year also to reproduction. If resources are limited, this may present a compromise allocation of the resources available, a trade-off. Trade-offs are linkages between traits that constrain the simultaneous optimization of two or more traits, and may operate within one individual (intraindividual trade-offs) or between the parent and off-spring (intergenerational trade-offs) (Stearns 1992). Besides the trade-off between present survival and future reproduction, other trade-offs are the allocation of parental effort to current or future breeding attempts or between number and quality of off-spring.

Having a relatively high body mass (i.e. good condition) has shown to be advantageous in terms of high survival and high reproductive success in several mammalian species (e.g. Persson 2005, Wauters & Dhondt 1989, Rodel *et al*. 2005, Wauters *et al*. 2007). Individuals in good condition are more resilient toward food shortages or adverse weather and have relatively more resources available to allocate to reproduction. Also, individuals in good condition are often better competitors, and are superior in escaping predators and less vulnerable to parasites or diseases (Murray 2002, Houston *et al*. 2007). The advantages of carrying fat reserves are compromised by the relative consequences of locomotor inhibition, which may impede predator avoidance.

Reproduction is an expensive occurrence in most animals' lives and the decision to breed or not is related to the body condition of the female, who will adjust her breeding effort in relation to prevailing environmental conditions in order to maximize life time reproductive output (Stearns 1992). A distinction is made between two general tactics; Income breeding in which offspring provisioning takes place by using energy gained concurrently; and capital breeding, where provisioning offspring occurs by using energy stores accumulated at an earlier time (Drent & Daan 1980, Houston *et al*. 2006). Income and capital breeding are at the two extremes of a continuum of strategies and optimal tactic depends on the predictability of resources (Jönsson 1997). Although hares to some extent build up reserves during the winter which are depleted during reproduction (Flux 1967), hares obtain the large majority of the energy invested in their off-spring during the reproductive season, and are classified as income breeders.

Being stationary as well as an income breeder, brown hares rely heavily on habitat quality during the breeding period (Reitz & Leonard 1994, Jönsson 1997). Females in good habitats are likely to produce more litters, larger litters or larger off-spring at birth (Vaughan & Keith 1981, Broussard *et al*. 2005, Persson 2005). In European rabbits (*Oryctolagus cuniculus*) and snowshoe hares (*L. americanus*), onset of breeding is delayed after harsh winters, and females in poor condition reproduce later than those in good condition (Vaughan & Keith 1981, Rodel *et al*. 2005). While the winters in Denmark are rarely harsh enough to cause food shortage *per se*, this does however, demonstrate the importance of food availability on reproduction, since a female in good condition will be able to produce litters with shorter intervals, and hereby increase the total number of litters produced during the breeding season. Frylestam (1980b) found that lighter hares produced fewer litters, and similarly, Jennings *et al*. (2006) found poor body condition and lower reproductive output in hares in pastural areas compared to arable areas. Danish female hares with a high body mass relative to size (i.e. good condition) in autumn were more likely to have bred in the previous breeding season (Paper I), and as for structural size, the mean condition was positively affected by the coverage of grass-leys and the amount of edge habitat towards tree-stands in the sampling area (Paper I). Condition (in conjunction with time of birth) may also determine whether a female hare will be able to reproduce in the year of birth in which case individual fitness is highly increased.

The most energetically demanding component of maternal care in mammals is lactation, and in hares, physiological limits of energy transfer is reached at normal sized litters (Martin 1984, Hackländer *et al*. 2002b). Female hares on high fat diets eat less than females on low-fat diets, but still assimilate more energy and produce more and better milk, hereby transferring more energy to large litters (Hackländer *et al*. 2002b). In addition, females on high fat diets may wean their leverets 7-8 days sooner, and may thus in theory produce litters at shorter intervals. Reversely, insufficient food supply may impair lactation, resulting in fewer weaned leverets, prolonged weaning or lower weight of weaned leverets (Hackländer *et al*. 2002b). Although hares may practice superfoetation, due to the impact of reproduction on the females' physiological condition, i.e. the depletion of the females' body reserves, the size of the first litter may have a negative effect on the consecutive litters (Flux 1970, Iason 1990, Stearns 1992).

Little research is available on the effect of female condition on the body mass of off-spring at birth for hares, except one study of caged mountain hares, where birth weights were reduced when feeding was suboptimal (Pehrson 1984), and hares of equal weights but fed different quality diets produced equal-sized leverets under experimental conditions (Hackländer *et al*. 2002b). Giving birth to precocial off-spring (i.e. born at an advanced stage, with eyes open and capable of temperature regulation), large size at birth is an advantage due to a smaller surface:volume ratio, and large leverets may be better at obtaining nutrients and at surviving extreme weather conditions, hereby bettering their chance of survival to reproductive maturity (Hackländer 2002a).

1.3.3 Other habitat features affecting demography

The spatial distribution of hares is determined by a multitude of factors, and features besides food availability may determine habitat quality. The habitats' structural composition in terms of year-round access to shelter from weather and predators as well as exposure risk to parasites or diseases and accessibility to mates are equally vital. The agricultural intensification processes have increased landscape homogeneity and Frylestam (1980a) found that low landscape diversity leads to lower body weights and higher mortality rates. In some habitats, hares may be limited by access to cover (Smith *et al*. 2005a, 2005b, Jennings *et al*. 2006).

No effects of landscape heterogeneity on hare size, weight or reproductive parameters were identified in our investigation, perhaps because the index was operating at the wrong scale (Paper I). In contrast, woodland, hedges and edge habitat between forest and agricultural areas are features of the habitat that has emerged as important factors in several studies, possibly due to the dual function of this habitat type providing year-round shelter as well as food during summer when crops mature (Tapper & Barnes 1986, Roedenbeck & Voser 2008, Paper I).

As habitat quality affects individual quality, it is consequently of great importance to identify links between landscape composition, i.e. the food availability, the resulting effect on individuals' body condition and the derived consequences on reproductive parameters and survival rates.

1.4 Hare population dynamics

Fluctuations in population size are caused by changes in the demographic processes of birth, death, immigration, and emigration (Begon *et al*. 1996). Due to the geographical composition of Denmark; being surrounded by water barriers, the Danish hare population can be considered as a more or less closed population, with only a minor exchange with German hares (Paper V). Assuming that immigration counteracts emigration, a declining population is the result of the mortality rates exceeding the reproductive rates in the population. This is either caused by an increased mortality, a decreased reproductive success or both. In order to assess the relative influence of survival and reproduction on the current declines in Denmark, estimates of the two were obtained. Also, by analysing data on age ratios (juveniles vs. adults) from hunting journals dating back 50 years, we investigated the effects of changes in the juvenile recruitment on population growth rate using population matrix models and elasticity analysis (Leslie 1945, 1948, Caswell 1989, Papers I and II).

To account for the differences in hare bags between NW and SE-areas in Denmark (Fig. 2a) which are likely to reflect differences in density (see also Paper IV), the sampled hare populations were indexed along a NW-SE going gradient (Paper II). The cartographic gradient reflected the variation in the game bag well (Fig. 5a), and was useful in revealing some spatial patterns in the dynamics of the Danish hares.

1.4.1 Reproduction

Placental scar counts provide reliable estimates of reproductive activity of female hares (Bray *et al*. 2003). In Denmark hares usually produce 3-4 litters in the period January to October. Litter sizes vary (1-6) with small litters early and late in the season and larger litters in the peak of the breeding season. Around 73% of Danish leverets are born in 2nd and 3rd litters which peak in April-May and July-August respectively (Hansen 1992).

The mean number of implantation sites in reproductively active females was 10.4, but 20% of adult females did not reproduce in the latest breeding season (i.e. had no scars) (Fig. 6). The overall mean adult productivity was therefore 8.3 leverets, but with variation between the sampled populations. Productivity did not vary between adult age-classes as documented in other countries (Frylestam 1980b, Bensinger *et al*. 2000, Hackländer *et al*. 2001, Paper I). The mean productivity corresponds well with what can be deduced from other studies (e.g. Table 4 in Olesen & Asferg 2006).

Infertility in adult females was related to senescence in Germany (Bensinger *et al*. 2000) and possibly Austria (Hackländer *et al*. 2001), but differences in age structures were not the cause of variation in productivity between Danish populations, as adult age structures were similar between populations (Paper I) however, sample sizes from some areas were small. In Austria, more females from low-density areas were infertile while, al-

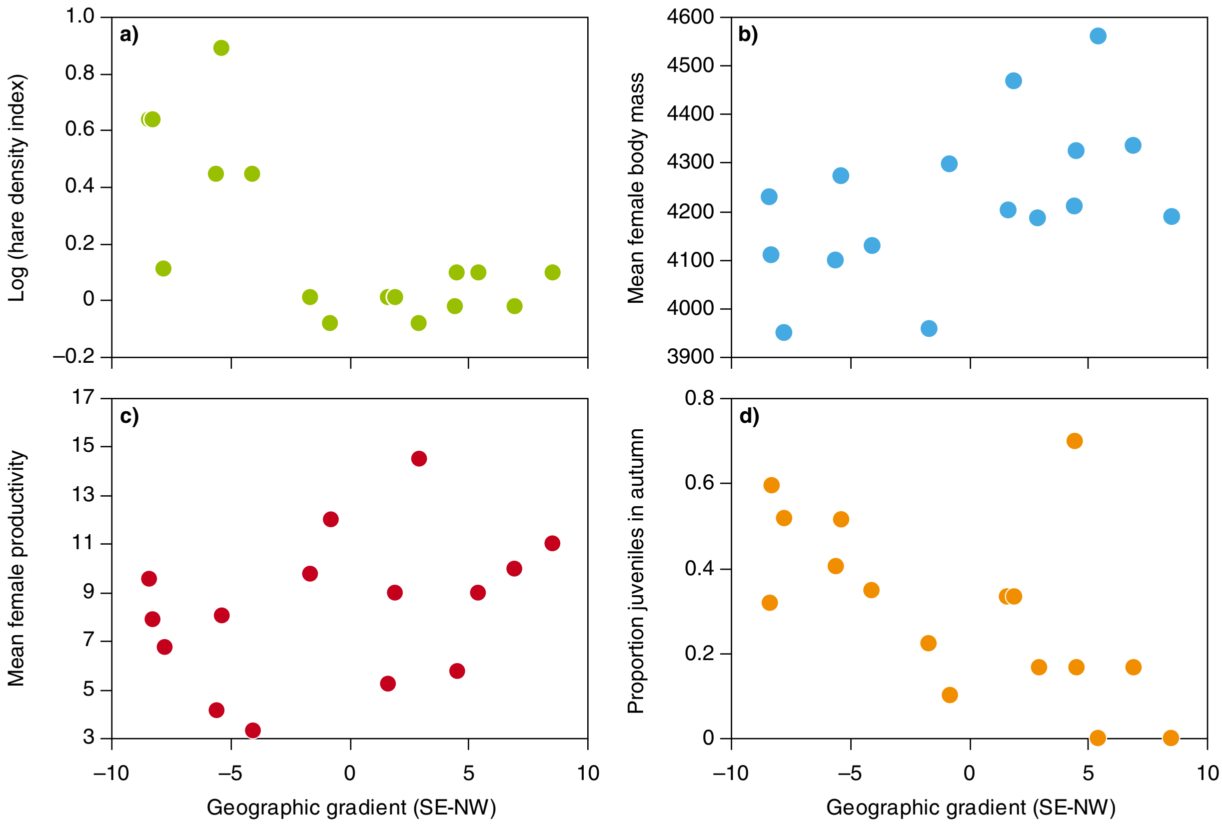


Figure 5. Relationship between the sampling position along a cartographic gradient from NW to SE and **a)** Hare density index (log to the number of bagged hares per 100 ha) (R^2=0.49, P=0.003), **b)** Mean female body mass (R^2=0.30, P=0.04), **c)** Mean female productivity (mean number of placental scars including infertile females) (R^2=0.13, P=0.19) and **d)** The proportion of juveniles in autumn hare bags (R^2=0.33, P=0.02). Regressions were not weighted according to sample size.

though not significantly higher, nothing indicates that productivity is lower towards the low-density NW in Denmark (Fig. 5a,c). Females in good condition were more likely to breed, which is not surprising, given the previous discussion on condition. Somewhat higher mean body masses of females were found towards the NW (Fig. 5b) and this was not due to the hares being structurally larger here as spatial variation in size was unrelated to the cartographic gradient. The differences in female productivity between the investigated populations were to a large degree, a result of differences in the proportion of breeding females. Pathological changes were shown to affect female breeding activity in German hares, but the role of this remains to be investigated in Denmark (Bensinger *et al*. 2000). Infertility of adult females has been documented for 30 years, and is unlikely to be the major cause of the population decline (Hewson & Taylor 1975) and spatial variation in hare productivity is also documented within other countries (e.g. Frylestam 1980b, Marboutin *et al*. 2003).

Of 100 examined uteri from juvenile hares sampled in the years 2003-5, only one contained placental scars, which is similar to German hares (Bensinger *et al*. 2000). Higher reproductive activity of juveniles are found in areas with longer breeding seasons south of Denmark, and most likely, the few juveniles that breed within the birth-year originate from the 1st litters of the year (Marboutin *et al*. 2003)

Obvious from these figures is the high reproductive potential of this species, and the growth potential should allow hare populations to recover fast from extrinsic perturbations, given optimal conditions. Despite this high reproductive potential, among the lagomorphs, the hare is considered a K-specialist (MacArthur & Wilson 1967, Swihart 1984).

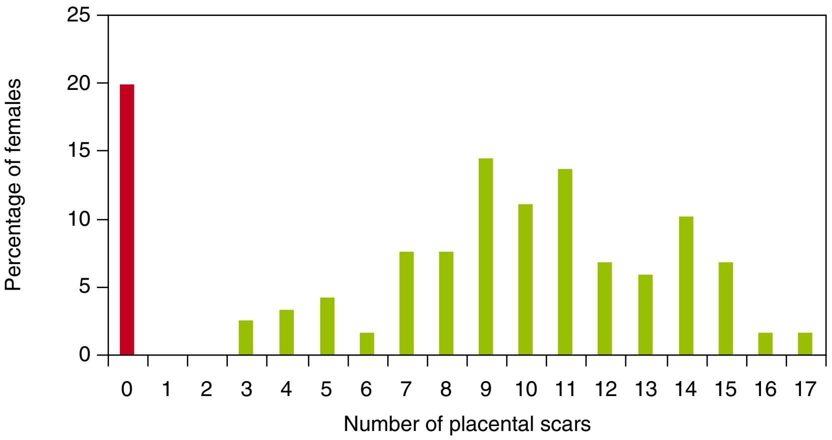


Figure 6. Distribution of scars in 172 adult female hares examined in the present project period. Zero scars indicate that a female did not reproduce in the previous breeding season.

1.4.2 Leveret survival

The high reproductive potential of the hare is counteracted by low leveret survival; only a fraction of the leverets produced during a reproductive season survives to the opening of the hunting season (Papers I and II). Already back in 1976, Pielowski and Raczynski remarks that the high mortality of leverets before the first autumn is worth considering as a tool to improve population growth and that lowering this, could potentially greatly affect on population growth rate.

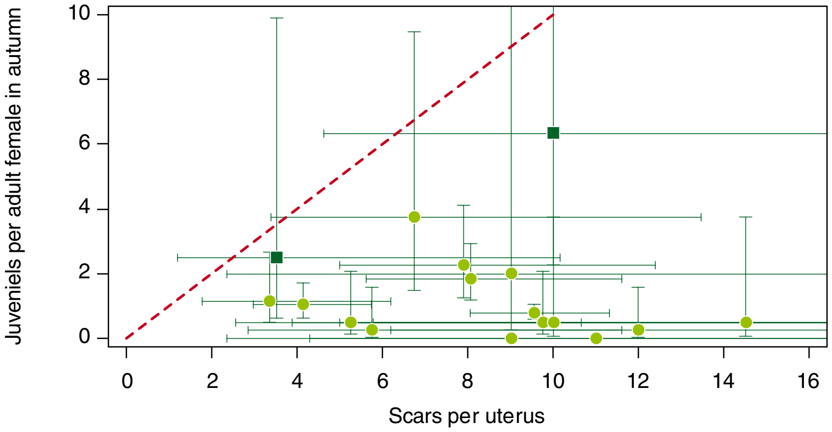
In autumn it is not possible to visually discriminate young of the year from adults at a distance. Juveniles may reliably be distinguished from adults by the degree of epiphyseal suture closure, at best evaluated from cleaned bones, or by the weight of the dry eye lens, the latter method being the most accurate (Stroh 1931, Walhovd 1966, Suchentrunk *et al*. 1991).

A rough index of the birth to autumn survival can be obtained by comparing the per capita productivity of females (i.e. including those that do not reproduce) with the number of juveniles per adult female in the autumn game bag. For 549 hares sampled in Denmark between 2003-5, this provided an index of leveret survival to autumn of 14%, but with spatial variation as also apparent in other countries (Pepin 1989, Paper I). Recruitment rates were lowest in areas with low densities, despite the fact that productivity there, tended to be higher, and females were heavier (Fig 5c). Besides the low recruitment in areas of low density, a central result was, that the proportion of juveniles in hunting bags, the realized fecundity, seemed unrelated to the mean number of leverets produced in an area (Fig. 7), pointing toward different juvenile survival probabilities between areas.

The recruitment of juveniles was significantly affected by soil quality, which however also varies significantly along the cartographic gradient (Paper I). The mechanism of soil quality affecting juvenile recruitment remains to be studied. Similar indices of leveret survival to autumn have yielded values ranging between 9-32%, but with the present results ranging among the lowest (Frylestam 1980b, Wasilewski 1991, Hansen 1992, Marboutin *et al*. 2003).

Leveret survival is related to birth weight, with higher survival of larger leverets. Birth weight is inversely related to litter size, indicating a trade-off for mothers as how to allocate resources. For the same reason smaller

Figure 7. The number of juveniles per adult female brown hares in autumn bags (±90% CI; $r_{13}=0.11$, $P=0.71$, excluding islands), plotted against mean number of placental scars in female hares (including females without scars) from 17 Danish areas (adjusted from Paper I). Squares indicate two island populations. The dashed line shows the expected number of juveniles, if all scars had produced a surviving leveret.



litters are born in the beginning of the breeding season, where weather impacts are expected to be more severe.

Despite the observed low recruitment rates in several studies (references above, Pepin 1989, Marboutin & Peroux 1995), frustratingly few studies document causes for juvenile mortality, probably attributable to the extensive field work required. Nonetheless, identifying causes for juvenile mortality appears central in understanding the population declines.

1.4.3 Adult survival

Age in years of adults can be determined by counting periosteal growth lines in the lower mandible, and the between year survival probability of adult hares can then be approximated through the age distribution of the aged adult hares ((Frylestam & von Schantz 1977, Caughley 1977). It is generally accepted that survival of adults is constant between years (Caughley 1977, Broekhuizen 1979). We estimated mean age of adults to 1.9 years for males and 2.4 for females which is similar to results from the literature (Abildgaard *et al*. 1972, Marboutin & Peroux 2003). A reasonably high mean yearly survival rate of 0.57 for females, and somewhat lower survival of 0.40 for males was found for the combined sample, with no variation between subpopulations. As different methods of calculations have been applied, comparisons between studies are difficult, but to provide perspective on the obtained figures, the survival rates in a non-harvested population without mammalian predators were 0.50-0.61 for adult males and 0.44-0.56 for adult females (Marboutin & Hansen 1998). Adult females reached up to 8 and males up to 5 years of age (Nielsen 2006), and as adult survival in addition appeared similar between populations, it is likely to play a minor role in population declines (Paper I).

1.4.4 Matrix models and models in ecology

Once parameters are estimated, the relative contribution of age-specific survival and reproduction to the current population development may be estimated by projection matrix models. Matrix models have become a standard tool used to evaluate different management options in structured populations, as they allow evaluation of interactions between age- (or stage-) dependent survival rates and fecundity. In addition, sensitivity and elasticity analysis may identify which life history stages are the most critical for the populations' viability (Caswell 1989, McLeod & Saunders

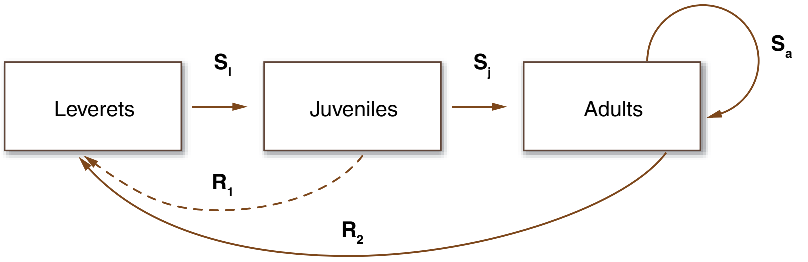


Figure 8. A life cycle graph of the stages a hare passes through in a life time. Within the population, leverets survive to become juveniles (i.e. independent of mothers) with the probability S_l, juveniles in turn survive to become adults (i.e. enter the breeding population of the consecutive time projection) with the probability S_j. Some juveniles reproduce in their year of birth, (R_1). Adults survive another year with the probability S_a and reproduce at the rate R_2.

2001, Bieber & Ruf 2005). Life stages consist of categories of individuals within the population that resemble each other with regards to reproduction and survival (Caswell 1989, McNamara & Houston 1996). We applied such analysis to the Danish hare, based on a condensed version of the life stages in Fig 8. (detail from Fig. 3) adjusted to the data at hand.

Each box in Fig. 8 represents a stage, and each box is supplied with arrows that symbolize the contribution one stage will give to the next, either in terms of age specific survival probabilities ("S"s) or age specific reproductive rates ("R"s). The breeding population in the consecutive year is made up of surviving adults (Sa), and recruitment of juveniles from the preceding year ($S_l \times S_j$).

In population projections incorporating the constant survival rate estimated in Paper I, assuming that contribution of juvenile reproduction was insignificant and entering the realized fecundity of adult females (estimated as the ratio of juvenile females to adult females in autumn bag as matrices only consider females), we estimated the growth rate in the hare population after the turn of the millennium to 0.94 (Paper II). A growth rate of one indicates a stable population while growth rates above or below one indicates increase or decrease, respectively. In other words, based on the present estimates of survival of adult females as well as the actual observed recruitment of juveniles by autumn, the population will proceed declining.

By repeating the above population projection with data on realized fecundity from hunting records dating back to the 1950ies, the growth rates resulting from the model reflected the historic development in the population very well. The model even predicted population increase in the 1990ies that corresponds with registered increases in the hare bags in the same period (probably a result of an outbreak of sarcoptic mange in the Danish fox populations). The fact that we were able to predict the observed development in bags in four decades, simply by varying the realized recruitment, strengthens the supposition that lacking recruitment plays a significant role in causing the declines.

Population growth rate shows variable sensitivity to recruitment, depending on population age structure (Marboutin & Peroux 1995). It is therefore likely, that management strategies must be tailored to fit different regions, depending on local hare dynamics. In regions with low recruitment the growth rate is most sensitive to survival of the adult breeding stock, whereas maintenance is less important in areas with high recruitment rates. This pattern was confirmed in the Danish hares (Paper II). Hence, in areas where recruitment is low, populations are more sensitive to mortality posed on adults, e.g. induced by hunting. However, infertility as well as mortality eliminates adult females from the breeding stock, and in some areas, large proportions of adult females were reproductively inactive. The impact of infertile adult females will be greater in populations with high mortality and high birth rates (Barlow *et al*. 1997). Therefore both aspects; increasing adult survival and identifying causes for adult infertility, should receive attention in the future.

Models are valuable tools in pointing in the direction where future research should be directed, but all models are simplified versions of what happens in the wild. While the advantage of simple models is that they are easy to interpret, the fact that they only incorporate few factors entail that they may not include all or even the most significant factors, in which case model predictions are inaccurate, at best. Complex models conversely provide more realistic results, as numerous factors may be incorporated. However predictions from complex models may be equally intricate to interpret and the choice of model complexity depends on the aim. The use of complex models in ecology is increasing, and to some extent replaces large scale experiments, which are often cost–inefficient.

A multitude of factors interact in shaping the present dynamics in the hare population and the relative influence of these factors are likely to interact in confounding ways. Complex models such as agent-based models (e.g. ALMaSS: Topping *et al*. 2003, submitted) allow for evaluating the impact on populations, of changing one or multiple factors separately or simultaneously. Models of this kind allow in silico experimentation with factors and systems that would be difficult or impossible in the real world and if used iteratively in conjunction with data collection and model reformulation provide a potentially important way to improve systems understanding. However, as models frequently need detailed biological data on the species in focus, field work is often well spent at identifying population mechanisms which will aid in parameterizing models or verifying model predictions.

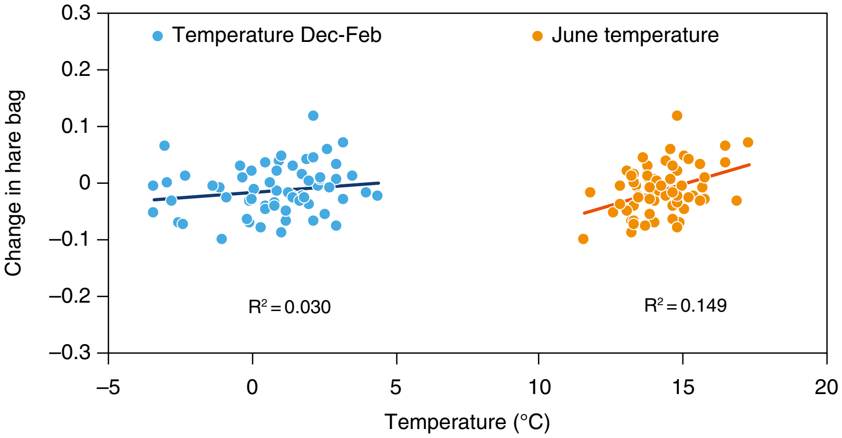
1.5 External factors

None of the external factors from Fig. 3 were investigated *per se* in the present thesis, and they are only discussed here in brief in relation to their potential effects on reproduction and survival with special reference to Denmark.

1.5.1 Climate and weather effects on hare dynamics

Climate and weather may affect individuals and populations directly through physiologic (metabolic) or reproductive processes, or indirectly by affecting the ecosystem (food, competition, predators) (Stenseth *et al*. 2002). When investigating effects of weather or climate on ecological systems, ecologists can select local weather measures like precipitation and temperature or large scale indices (e.g. the North Atlantic Oscillation, NAO, Hurrell 1995). It is widely accepted that climate affects the population dynamics of various species, and several studies have documented effects of e.g. NAO on life histories and population dynamics in mammals (moose, white-tailed deer, wolves: Post & Stenseth 1998, Canadian lynx: Stenseth *et al*. 1999, Soay sheep: Coulson *et al*. 2001).

Figure 9. The effect of the mean temperature in winter (December through February, $R^2 = 0.030$, P=0.17)) and in June ($R^2 = 0.149$, P = 0.002) on the change in hare bags between two consecutive years in the period 1941-2007. The change in bag between years is calculated as $Log10(N)_t$-$Log(N)_{t-1}$.



The effects of single weather components on mammals are likewise undisputed, and in the case of the hare at least previously, hare population growth or, ”good hare years” were expected after mild winters, and when temperature was high and precipitation low during the peak breeding period (e.g. Andersen 1957, Nyenhuis 1995). While mild winters lead to increases in food availability, the changes in crop rotations during the recent decades have resulted in hares seldom experiencing food shortages during winter in Denmark. The ground is rarely ever covered by snow for prolonged periods, and temperature in winter has no significant effect on the hare bag in the forthcoming hunting season (Fig. 9). Mild winters and springs may, though, facilitate transmission of disease (Rattenborg 1994).

Low spring temperatures can delay reproduction while high temperatures in autumn may prolong the breeding season (Frylestam 1979, 1980b, Vaughan & Keith 1981). Especially leverets are vulnerable to adverse weather as well as to the bacterial and parasitic infections which may be promoted by certain meteorological circumstances (Goszczynski & Wasilewski 1992, Hackländer *et al.* 2002a, infections: see later paragraph). Weather conditions during the peak breeding period are thus more likely to affect the population size in autumn (Fig. 9). Consequently, extreme weather conditions cause short term variations in density.

As climate affects all trophic levels in the ecosystem, analysing separate weather components may not reveal effects of interactions between weather components imposed on ecological processes or populations. In some cases, large scale indices are more useful in providing a holistic account of the weather composition, much similar to the use of Principal Components Regressions (Hastie *et al.* 2001, cited in Stenseth *et al.* 2003, Hallett *et al.* 2004, Stenseth & Mysterud 2005). Intangible influences of weather may be identified when entering NAO indices in models, but while they may reduce the residual variation from models, they at times prove difficult to interpret (Paper II).

Climate is predicted to change at an increasing rate in the future, especially in the northern hemisphere, and population dynamics is expected to change with it. A future year-round increase in temperatures between 0.6-4.6 °C along with a reduction of summer precipitation and increasing risks of drought in summer is predicted for Denmark (DMI 2007). The latter could pose additional risks for the hare in some years. Increasing spring temperatures may affect the onset of breeding, which has been seen to advance over very short time spans (Reale *et al.* 2003).

1.5.2 Hunting the doomed surplus or a doomed population

The hare was the most frequent mammalian game in The Danish Game Bag Record until 1999 when roe deer entered the top position, and at the same time hare bags for the first time in the history of the records amounted to less than 100.000 hares.

Initial game management theory was founded on the philosophy that game (or prey) was limited by habitat constraints and each year a "doomed surplus" was produced which was not expected to survive the seasonal bottleneck. This doomed surplus could thus be harvested before the bottleneck arose, supposedly without consequences for the population size (Errington 1945). According to this, it could be argued that even in populations experiencing decline, hunting would not affect the population size, in so far only animals otherwise expected to succumb to natural mortality before the next breeding season were removed.

Another principle addresses compensation for the harvested animals through density dependent responses in the population. Once the population is reduced below the carrying capacity, it may experience decreased mortality rates due to relieved food competition (compensatory survival), or, the increased food availability may result in an increased per capita reproduction (compensatory natality) (Boyce *et al.* 1999). Such responses are well-documented for a variety of species and simple harvest models predicts that populations must be reduced below the carrying capacity to generate density-dependent increases in recruitment, and consequently produce a surplus that may be harvested (Caughley 1977). The central issue regarding sustainability of hunting is, thus, when this mortality factor is additive to natural mortality agents (i.e. reduces the breeding stock), or when the populations are no longer capable of compensating for the losses.

Given the observed long-term declines in the Danish hare population, the sustainability of hunting has been disputed. In Denmark the open season for hares spans from October 1st to December 15th, and was reduced by 16 days in 2004. No doubt, the low density estimates which have been obtained from some of the study areas during this project (Table 1) will add to the discussion regarding the sustainability of hunting in these areas. Especially since recruitment rates also appear lowest in the regions with lowest densities (Fig 5d).

The densities in the two areas in Jutland (Himmerland and Kalø) are below 10% of those on the island of Lolland, and bag records from Jutland indicate that much higher proportions of the populations here are shot each year.

Table 1. Estimated densities in spring in (2005-6 [95% CI]) (Paper IV) in the four study areas, numbers of hares shot in the respective counties (mean of 2005-6), and percentage of the spring density hares, that are shot during the hunting season in four Danish study areas.

	Estimated spring density (100 ha)	Hares shot per 100 ha	%
HL	3.14 [1.8-4.4]	0,77	24% (41–17)
KA	5.96 [3.2-8.7]	0,78	13% (24–9)
LO1	65.23 [28.6-101.9]	3,83	6% (13–4)
LO2	111.17 [49.7-172.7]	3,83	3% (8–2)

These figures demonstrate that the influence of hunting is very likely to vary spatially and that the proportions of the populations removed by hunting differ between high and low density areas. As the reproductive output and the juvenile recruitment vary spatially (Paper I and Fig. 5d), numbers should not be compared between regions. At least in healthy populations, population densities are expected to increase during the course of the breeding season, which would decrease the estimated proportions shot.

It should be emphasized that the estimated hare densities (Paper IV and Table 1) are calculated as numbers of hares per km^{-2} *habitat*, i.e. densities will be somewhat lower when including unsuitable habitat such as housing areas or large forests. The discrepancy between density estimates from the two areas on Lolland, situated 35 kilometres apart, indicates the magnitude of variation in density within counties.

Marboutin *et al.* (2003) modelled hunting sustainability, based on harvest rates and estimates of variability in fecundity and leveret survival in four hare populations in France. By incorporating stochasticity in their model, they concluded that populations below 5 hares km^{-2} could sustain harvest rates below 20%, while harvest rates of ≥35% were never sustainable. In Himmerland this would require a 2 km^2 area for each hare shot and an area of this size would typically include several estates. Others have found brown hare dynamics resilient to hunting pressures of above 40% at spring densities of 23-50 hares km^{-2} (Pielowski 1976, Pepin 1987).

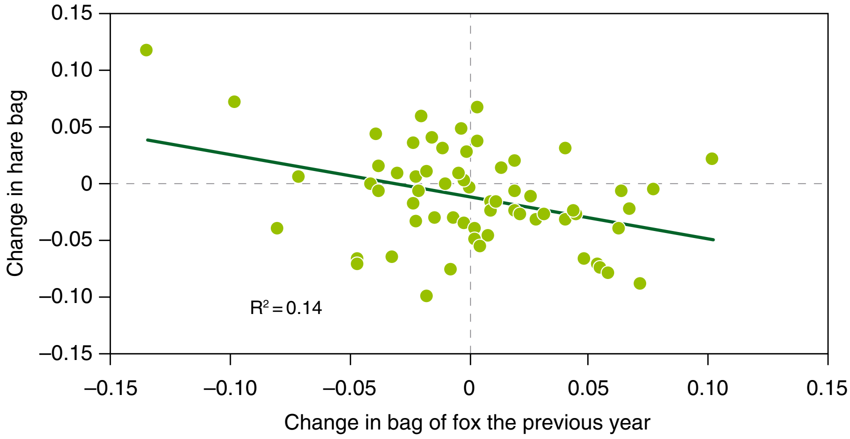
In an agent based model simulating hunting sustainability, Topping & Høye (*submitted*) found disproportionate decreases in hare population size with increasing hunting pressure: An increase in hunting pressure from 0-10% resulted in a 50% decrease in hare density; however the rate of response decreased with increased hunting pressure. Marboutin *et al.* (2003) concluded that overshooting may be an aggravating factor to decline, but was not the primary cause of it and suggested that harvest rates were based on local population dynamic parameters to ensure sustainability. Fickel *et al.* (2005) suggested biannual hunting cycles due to the estimated generation time of 2 years in hares (Marboutin *et al.* 2003, Paper I). A recent simulation of the effects of hunting on hare populations in agricultural areas suggests that density based hunting with a limit density of 15 hares per km^2 could increase the current density in low-density areas with 40% (Topping & Høye *submitted*).

Even if hunting do not cause the decline, and even if protection from hunting (according to future model predictions) proves unable to prevent further declines, hunting a threatened population still raises some moral issues. If hunting is prohibited either fully or partially, we at present have no means of tracking the developments in the population. Prohibitions thus require that other ways of monitoring trends in the population are initiated.

1.5.3 The role of predation

Along with intensification of agriculture, predation is the most mentioned cause for hare declines (e.g. Schmidt *et al.* 2004, Panek *et al.* 2006). In Denmark the hare is preyed upon by the red fox, cats (*Flis catus domesticus*), mustelids (sp.) and avian predators, e.g. buzzard (*Buteo buteo*), the hooded crow (*Corvus c. corone*) and goshawks (*Accipiter gentilis*). The main predator is generally assumed to be the red fox, and predation is believed to mainly affect leverets (Erlinge *et al.* 1984, Goszczynski & Wasilewski 1992).

Figure 10. The change in hare bags between two years as a function of the change in fox bags the previous two years since 1941 ($F_{1,61} = 9.92$, $R^2 = 0.14$, $P = 0.003$). (The change in bag between years is calculated as $Log10(N)_t$-$Log(N)_{t-1}$).



Predation may in the same manner as hunting keep the population below its carrying capacity (Top Down regulation), and much the same theory applies for predation as for hunting. The central issue is, whether the mortality posed on hares is additive or compensatory. High predation in itself is therefore not evidence of predation limiting prey due to the density-dependent compensatory responses (Lindström *et al*. 1986, Reynolds & Tapper 1995, Kauhala *et al*. 1999). Conversely, a small proportion of hares in the fox' diet does not necessarily imply that foxes have no impact on hares (Erlinge *et al*. 1984).

Strong evidence exists of the potential impact of foxes on hare populations, many originating from studies reporting that decreases in fox density (due to disease or control) result in increases in hare densities (e.g. sarcoptic mange: Lindström *et al*. 1994, control: Panek *et al*. 2006) and hare declines when foxes recover (Lindström *et al*. 1994). In Denmark the island of Bornholm is the only place where the brown hare population has increased beyond the year to year variation (as indicated by hunting bags) since 1955, and the increase coincides with the outbreak of mange that eradicated the fox here in the 1980ies (BOR in fig. 2b). Also, the change in the Danish bag of hares between years is negatively correlated with the change in fox bags the previous year (Fig. 10).

Little doubt therefore remains as to the limiting effect foxes may pose on hare populations which may increase many-fold when foxes are removed. The magnitude of the impact depends on factors such as initial density and habitat type.

Essentially, this means that fox predation is an additive mortality factor (Marcström *et al*. 1989, Lindström *et al*. 1994). The question remains then, whether the regulatory effect of predation has changed with time. Such change could occur by increasing predator abundances, which is apparent at least for buzzard (Snow & Perrins 1998) and fox (Mitchell-Jones *et al*. 1999). As apparent from the trends in the game bag, foxes may have increased more or less simultaneous with hare declines in some areas (Fig. 11, left) whereas the relationship between fox and hare bags are somewhat more perplexing in other areas (Fig. 11, right).

Alternatively, increased predator impact could be the result of changes in the configuration of the landscape, for instance reduced cover availability (i.e. increased exposure), or the interactions between prey and predators may be altered by changes in landscape composition (Schneider 2001,

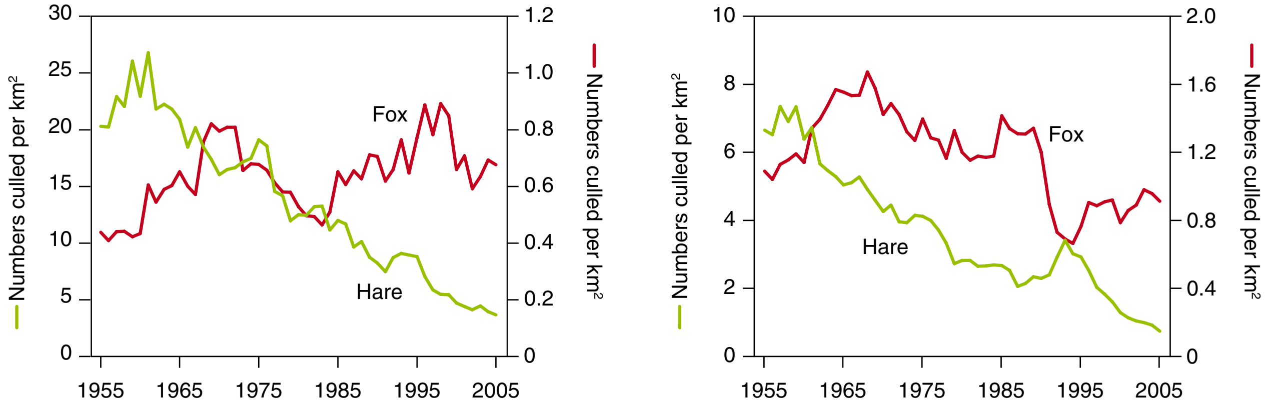


Figure 11. Fox and hare bags in the period 1955-2005 in the county of Storstrøm where the two study areas of Lolland are situated (left), and in the mid-and northern parts of the mainland including the study areas of Himmerland and Kalø (right).

Smith *et al*. 2004, Seymour *et al*. 2004). In the same manner, the importance of predation by fox is likely to vary between landscape types.

Smith *et al*. (2005a) concluded that fox predation was not the main cause of hare population declines, but rather, effects of predation was enlarged by the changes in landscape. From Figs. 10 and 11, it is apparent that while fox predation does explain some of the temporal variation in the hare population size (as indexed by culls), predation can not stand alone. Given the evidence, that juvenile hare survival has decreased, that fox predation may limit hares in some areas and foxes to a large extent prey on juveniles, it is of interest to determine which role the fox – as well as all other post natal mortality factors- plays for the low recruitment of hares.

1.5.4 Diseases and parasites

Brown hares are affected by several diseases and a range of parasites (for a review of the main viral, bacterial and parasitic diseases in the brown hare, see Wibbelt & Fröelich 2005). By definition, parasites and diseases are expected to have injurious effects upon the health and fitness of the host. Studies on several mammalian species show that parasites may reduce body condition, fecundity and/or survival of individuals (e.g. Soay sheep: Gulland *et al.* 1993, Reindeer: Albon *et al.* 2002, mountain hares: Newey & Thirgood 2004). Hereby parasites and diseases potentially affect population dynamics, and might thus be a factor in population decline in the course of their negative effect on individuals' fitness.

When the ultimate effect of a disease is death, the detrimental effect on the population growth rate is obvious. But as parasites rarely kill their host, their effect on the host population is often subtle. When survival and reproduction of infected animals are affected, the prevalence of the parasites in the population determines the importance for population dynamics. Is the prevalence in the populations high (epidemic outbreaks), with many or most individuals suffering from reduced fitness, then dynamics of the entire population may in theory be influenced. Effects on individuals are however difficult to prove in free-ranging populations, and often, the direction of causality is difficult to determine: Do the parasite cause poor body condition, or are individuals in poor condition more prone to get infected. Indirectly, parasites may affect hosts by increasing susceptibility to predation (Ives & Murray 1997, Murray 2002).

Not much research has been conducted on the role of parasites in driving hare dynamics in Europe. Most studies on brown hares present descriptive rather than analytical epidemiology, or has been conducted for the purpose of determining risk of transferring diseases to humans (e.g. Treml *et al*. 2007). Much more literature on this subject is available for mountain hares and snowshoe hares (*L. americanus*) (Murray *et al*. 1997, Newey & Thirgood 2004, Newey *et al*. 2005).

Several of the diseases observed in the brown hare have to be considered in context of environmental conditions like weather, season of year as well as population density and age structure; Some diseases are endorsed by humid weather conditions, some affect juveniles more than adults and others are density dependent. (Lamarque *et al*.1996, Treml *et al*. 2007).

Of 546 Danish hares shot in 2003-2005, 92% were infected by Eimeria and 15% of these infections were severe. The proportion of heavily infected hares varied significantly between eight populations (all with n> 30) (2.8% (Hjelm) - 28.6%(Bornholm)), and was significantly higher in juveniles than adults (25% vs. 9%). In comparison, the infection rate was slightly lower in a sample of 108 hares found dead in the same period: 83% were infected, but here 33% were heavily infected (55% juveniles and 23% adults) (Asferg, Wincentz & Hammer in prep.). These results show, that infection rates in free-ranging hares are quite high, and that autopsies of hares found dead should be interpreted with caution, before generalized to infection rates in the wild population.

Undoubtedly, infectious diseases are a major mortality factor in hares; Haerer *et al*. (2001) suggested that 15% of all hare mortality is caused by infections. However the year to year variation in prevalence of individual infections may vary significantly and this variation may appear without synchrony between nearby populations (Frölich *et al*. 2003). Diseases occur sporadically and parasites are common, but both appear to play a minor role in shaping the dynamics of brown hares; they may lead to additional mortality, but probably do not influence the long-term population trend (Haerer *et al*. 2001, Frölich *et al*. 2003). Rather, the negative impact of disease and parasites on hare populations is seen on a local scale, causing year to year variation in densities. Hence, the negative effects of diseases on hare populations seem to be short term decline.

1.6 Determining density

The hare is a popular game species throughout Europe, and as a consequence, long-term bag records are available for this species. Most evidence for the hare declines are derived from such records as they provide the opportunity to monitor population trends through time. Since hunting records contain information about the amount of hares that died, not how many are alive, using game bags as indicators of trends in the live population is, however, not uncomplicated. Though hunting statistics may reveal relative differences of numbers in time and space, information about their relation to the absolute density of the free-ranging population is not incorporated. In the same manner, we do not know the degree to which a decline in hunting bags reflects a decline in the wild population.

Hunting statistics may only present valid indices of hare abundance in areas where hares are common (Vaughan *et al*. 2003), and bags may be a reflection of shooting effort as much as of trends in the population. Also, in areas of low densities, hunters may carry out voluntary protections which exaggerate the declining trend. There is, though, a general consent that hunting records are good proxies for the amplitude of fluctuations in the wild population as well as for population trends (e.g. Pielowski & Raczynsky 1976, Tapper & Parsons 1984, Hutchings & Harris 1996).

Depending on the size of recording units, density may vary substantially within units, as seen between both pairs of count areas, where density estimates almost varied two-fold (Paper IV). Also, while the mean hare bag in northern counties (e.g. Himmerland) comprises 20% of mean hare bags in southeast (e.g. Lolland), the density in Himmerland is only 3-5% of that in Lolland (Table 1) (however road avoidance, could convert these figures to 8-13% (Paper IV)). Hence, although the bag records indicate large differences in densities between these counties, the relative differences between counties may to a certain extent be disguised by differences e.g. in hunting pressure. To optimize management, firmer estimates are needed.

1.6.1 Why, how and when

Obtaining a reliable estimate of wildlife abundance (population size or density) is a central element in ecological research. Density estimates may provide knowledge about absolute changes in population size over time, and they enable estimations of impacts of e.g. hunting on the population (Marboutin *et al*. 2003, Strauβ*et al*. 2008). Information on density in different landscape types may permit inferences about relationships between density and general habitat characteristics (e.g. Smith *et al*. 2005a), and knowledge about population size allows assessments of whether the threshold of a minimal viable population size has been reached. Finally, monitoring present status in declining populations may provide us with a baseline against which future changes in the density of the populations may be monitored.

Any census technique must be adapted to the species in question, while taking into consideration the availability of resources in terms of manpower and time, as well as the desired level of precision (Sutherland 2006). For the crepuscular hare, timing of surveys depends on the purpose of the investigation. Daytime counts may quantify resting habitats, whereas nocturnal counts (spotlighting) of active hares provides information on feeding habitats. Spring counts may estimate the size of the breeding populations, while autumn counts provides information on population size before winter mortality occurs. In combination with data on breeding parameters, autumn counts may also provide valuable information on the recruitment and mortality rates in the population during the breeding season (Strauβ *et al*. 2008).

Several techniques have been shown to provide reliable hare density estimates (for a review see Langbein *et al*. 1999). Recently, nocturnal spotlight counts appears to have become the most widely used survey method for hares (e.g. Verheyden 1991, Frölich *et al*. 2003, Genghini & Capizzi 2005, Panek *et al*. 2006, Reid *et al*. 2007, Rodenbeck & Voser 2008, Strauβ *et al*. 2008). However different procedures such as point transect (Barnes & Tapper 1985, Verheyden 1991, Péroux *et al*. 1997) or taxations (Roedenbeck & Voser 2008) have been employed.

In terrestrial birds and mammals the awareness towards the “unseen” individuals is increasing (Heydon & Reynolds 2000, Foccardi *et al*. 2002, Diefenbach *et al*. 2003, Reid *et al*. 2007, Newson *et al*. 2008). The number of individuals seen when performing counts (“counts” here referring to enumerations not corrected for the probability of detection) will be a varying proportion of those present, depending on e.g. observer effectiveness, habitat type, weather variables, time periods and the species in question (Buckland *et al*. 2001). Depending on the proportion of undetected individuals, the estimated density will be affected to different degrees; however this problem may be dealt with by Distance Sampling techniques.

1.6.2 Distance Sampling

Generally, Distance Sampling (DS) is applied to point or line transects, and begins with an assumed detectability function, giving the probabilities of detecting animals at different distances form the observer. The strength of DS is that detection of all individuals in not needed, due to this detection function which is fitted to the observed distances of counted individuals, allowing for correction for the missed individuals, and hereby leading to improved density estimates.

The statistical theory and practical application of Distance Sampling is thoroughly described and explained, in a comprehensive manner, by Buckland *et al*. (2001) and the method has successfully been used on many taxa. Initially, the method was applied to duck nests (Anderson & Pospahala 1970) and soon found extensive use in monitoring birds and marine mammals from plane or ship,

In brief, the underlying assumptions for applying DS are (in order of importance):

1) All objects situated at distance 0 to the observer are detected,
2) The probability of detection is a decreasing function of distance to the observer,
3) Individuals are randomly distributed over the observed habitat,
4) Objects are detected at their initial location (none enter or leave the point, none move relative to the observer, and none are counted twice) and
5) Exact radial distances are measured

(Paper III). Besides this, it is supposed that the animals in question distribute themselves randomly in the survey area, and that the sampling points are representative of the survey region (appropriately randomised design).

When applying Distance Sampling techniques in heterogeneous terrestrial habitats, it should in each individual case be critically evaluated, whether the basic assumptions mentioned above are met, and if not, what effects the violations have on the resulting density estimates.

1.6.3 Effect of non-visible subareas

In practise, Point Transect counts (PTC) was applied to active hares at night by visiting predetermined observation points (randomised design), illuminating the surrounding areas within 300 meters with spotlights and measuring distances to observed hares with a range finder. Hares were counted in two different study areas in each of two regions. A quick in-

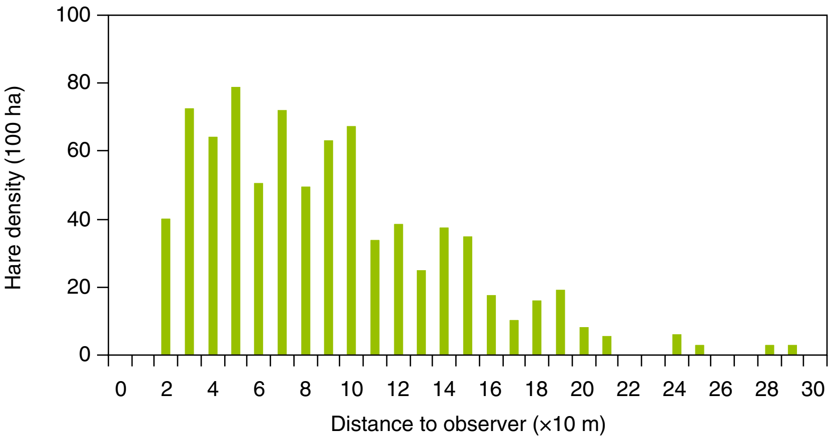


Figure 12. Density of hares in different distance intervals relative to the observer.

vestigation of the measured distances to hares, in relation to the area surveyed at different distances, revealed, that "density" was relatively constant at distances within 100 meters from the observer, while beyond this distance, "density" seemed to decline (Fig. 12): A strong indication that some hares were "missed" at larger distances, and that correction for this was necessary.

Standard DS theory presume that (in our case) visibility in all directions within a given truncation distance is not obstructed by physical objects. In terrestrial habitats this is rarely the case but despite this, DS has commonly been applied to surveys or monitoring programmes of mammals without correcting for the areas that can not be observed. Methods are available to deal with non-visible areas (see e.g. Ruette et al. 2003), but Buckland *et al*. (2001) suggested that modifying standard theory would rarely be necessary, as effects of non-visible areas only in extreme cases would render the estimates unreliable.

In the agricultural landscape in Denmark, hedge-rows, undulating terrains and buildings often obstruct sight, and the terrain in our study areas deviated much from the fully visible circle in the standard case, and on average only 30-50% of the area was visible (Fig.13) (Paper III). We therefore wished to examine the consequences of the non-visible areas on density estimates by comparing estimates obtained when correcting for the unseen areas, with estimates obtained by standard calculation (i.e. assuming full visibility) (paper III).

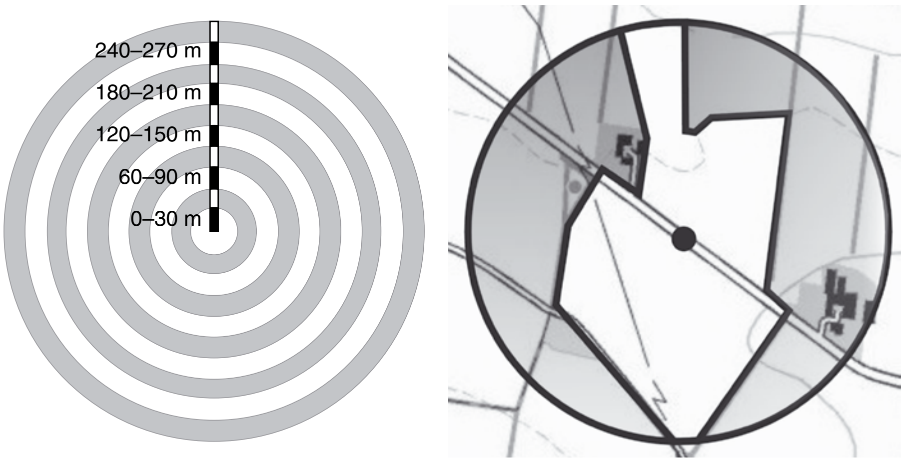
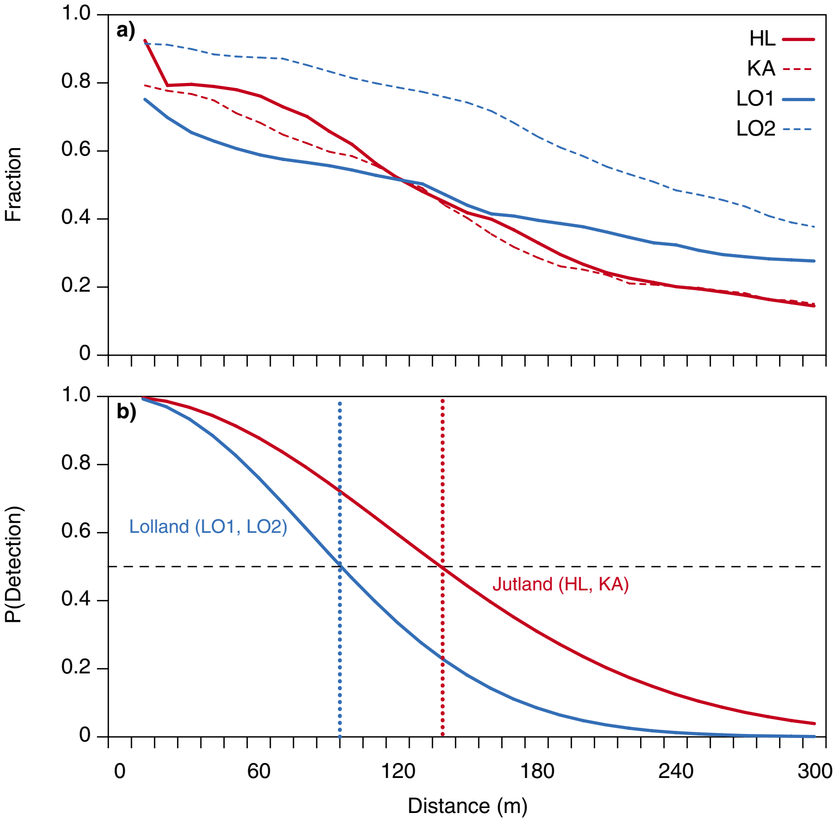


Figure 13. Left: a circle with full visibility in all directions. Right: an observation point from the study area in Himmerland. The observer is placed in the centre, and the shaded areas indicate where vision is obstructed.

Figure 14. a) Mean proportion of the area of a full circle, visible at different distance intervals in four Danish study areas (HL, KA, LO1, LO2, Figure 2 from Paper III). **b)** Detection probability of hares at various distances in two Danish regions. Bars indicate the distances where 50% of the present hares are detected (blue bar: Lolland, red bar: Jutland).



Correcting for non-visible areas increased the estimated proportion of hares seen considerably, but much more so in one region (Himmerland+Kalø), where a lesser fraction of the circle was visible, than in the other region (Lolland, Fig. 14a). Landscapes like the agricultural areas in Denmark, where fields are often separated by hedges and, in our case, around 1/3 of the circle is visible, thus appears to be 'extreme' enough for correction for non-visible areas to be necessary in order to get reliable estimates of detection probability as well as density.

Another, more surprising, result was that when applying the correction for non-visible areas, the detection functions of hares in spring in the two investigated regions became significantly different. Probability of detection was significantly higher in Jutland than on Lolland (Fig. 14b).

The detection function depends on variables like meteorological phenomenon's (light, humidity, temperature (Barnes & Tapper 1985), habitat composition, observer skills and the behaviour of the animals. With regards to the latter, we initially intended to monitor the populations in spring and autumn in order to estimate the population growth rate as in Strauβ *et al.* (2008), but hares clearly displayed different behaviour (less activity) in autumn, when fewer hares were detected than in spring. This was probably related to the season (out of breeding season) as well as nocturnal activity by farmers.

Whatever the cause of the differences in detectability, this result questions pooling of data for estimating one common detection function (which is often done), and in terrestrial habitats it can not be assumed to be constant in space, even for the same species. This has serious implications when using spotlight counts in large scale monitoring programmes if differences in detectability between surveyed areas are not investigated and accounted for.

Despite the above mentioned implications, the estimated densities in the corrected model (i.e. correcting for blind areas) were only moderately higher than those calculated in the standard model. However, though density estimates based on data uncorrected for blind areas may be roughly correct, this is the outcome of committing two oppositely directed errors that tend to cancel out (underestimating the proportion seen and overestimating the counted area) (Paper III).

1.6.4 Effects of road avoidance

The reliability of DS density estimates rely on the choice of detection function and on the assumption, that the counted objects are randomly distributed. The latter assumption will rarely be valid for living creatures in terrestrial habitats, which distribute themselves according to availability of resources and in considering predator evasion or other disturbance effects. A non-random distribution over the surveyed area possibly affects the choice of detection function, which leads to bias in the estimated detection probability and the resulting density estimates, if overlooked.

Although the allocation of observation points in our study areas were randomised, they were all located on roads in order to be readily accessible by car. Reid *et al*. (2007) as well as Roedenbeck & Voser (2008) provided evidence that hares avoid proximity to roads. The confinement of observation points to roads therefore potentially introduces bias in our sampled distances, which in turn influences the detection function. Also, the study areas in the two regions differed with regards to road sizes, and consistent with Reid *et al*. (2007), fewer hares than expected were observed closer than 50 m from paved roads in Himmerland (Fig 15). We therefore wanted to examine whether these differences in road structures caused the differences in detection functions between the two regions, as well as whether and to what extent, road avoidance affected density estimates.

Based on the model correcting for blind areas (Paper III) and the 54 points in Himmerland (Fig. 14a), the region where road avoidance appeared most pronounced, we modelled five scenarios of increasingly strong road avoidance across four detection probabilities (because potential road avoidance will not be equally easy to detect at different detection probabilities).

In the first simulation series, we entered the scale parameter observed in Lolland (i.e. low detection probability) at four levels of road avoidance (see detailed description in Paper IV). For each of 3000 simulated data sets, a new scale parameter was estimated and the expected distribution

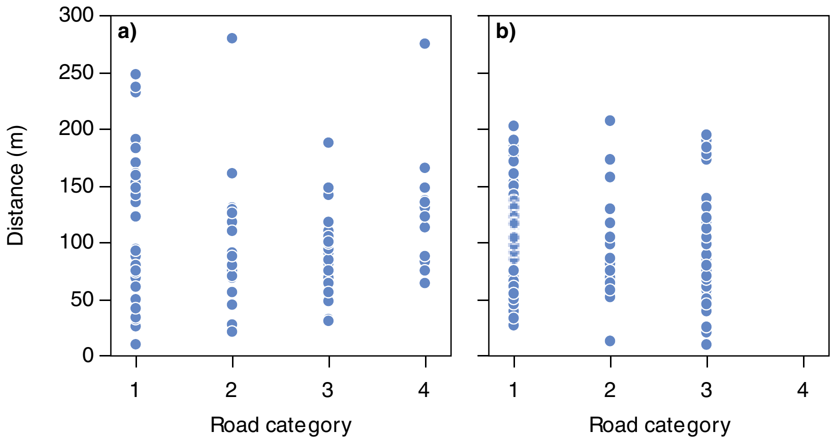
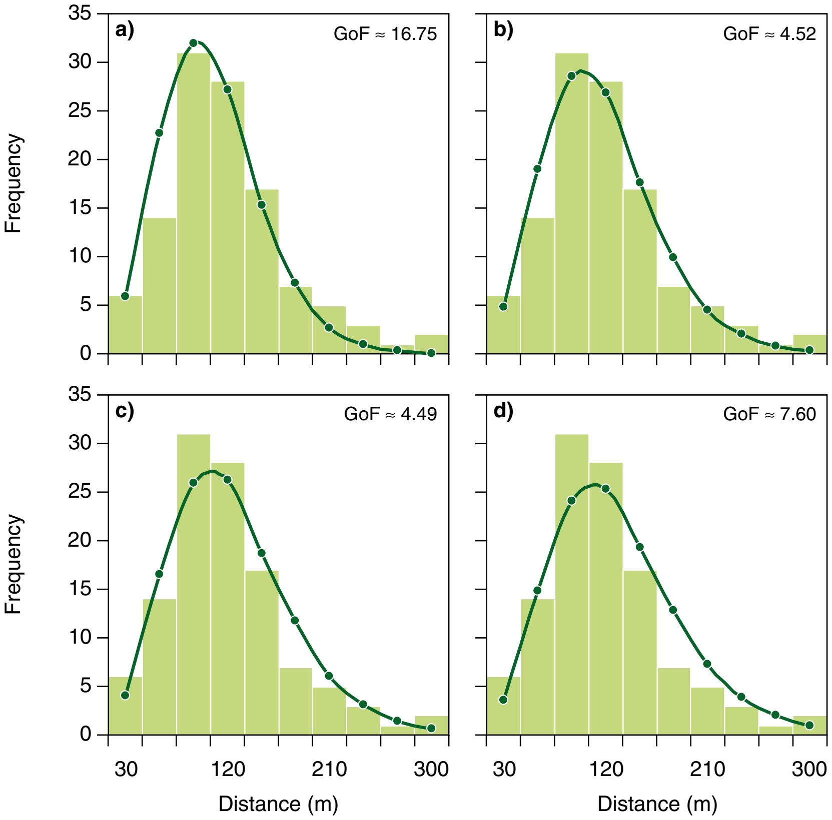


Figure 15. Distances to detected hares measured at different road categories (1: tracks, 2: small, unpaved backcountry roads (2-4 m), 3) small, paved roads (2-4 m), 4) larger paved roads (>4m)). **a)** Pooled data for HL and KA (N = 114) and **b)** Pooled data for LO1 and LO2 (N = 115).
(Source: Figure 2 in Paper IV).

Figure 16. Observed (histograms) and expected (curves, means of 3,000 simulated data sets) distributions of distances in the scenarios of weak avoidance of habitat close to roads for a Half-normal detection function with scale parameter: **a)** $\sigma^2 = 6.62$, **b)** $\sigma^2 = 8.62$, **c)** $\sigma^2 = 10.62$ and **d)** $\sigma^2 = 12.62$ (Paper IV).



of observed distances calculated. The average of the 3000 expected distributions was then compared to the observed distribution of distances in the "real" data. Analogue simulation series were performed with increasing detection probabilities, gradually approaching the one observed in the field (Fig. 16).

These results demonstrated that acceptable fits to the actual observations could be produced by more than one model. At one extreme, the observations from HL+KA could be "explained" by a true detection probability corresponding to the one observed at Lolland plus very strong road avoidance. At the other, they might be equally well "explained" by assuming that the different detection probabilities were real while there was no road avoidance. Detection functions with intermediate scale parameters could be inflated to the actually observed level if combined with only weak and moderate avoidance behaviour.

Thus, the differences between regions could be real, or alternatively they could have been caused by road avoidance. It should be noted, however, that if road avoidance was the cause of these differences, strength of reactions must differ between regions.

As expected, based on common sense, road avoidance leads to fewer observations in proximity to roads than expected from a random distribution. This in turn leads to an overestimation of the scale parameter, and reduces estimates of density. However, when the detection probability is high, the influence of road avoidance on the detection function decreases (i.e. the underestimation of density is smaller). Thus, in Himmerland and at Kalø, road avoidance could have deflated the estimated densities to 54% of the actual figures.

An important conclusion from this investigation is, that the non-random spatial distribution of the hares – in this particular case imposed by road avoidance- significantly affects the detection function. Buckland *et al.* (2001) emphasize the importance of fitting the correct function to the observed distances, even suggesting second order functions overlaid on the initial chosen (key) function. Our findings suggest, that fittings of detection functions may in some instances reflect habitat preference rather than pure detection probabilities.

1.6.5 Pros and cons of Point Transect Counts in terrestrial habitats

Some ecologists may assume that the bias when not correcting for undetected individuals is small and a lot of information may in fact be extracted from unadjusted count data. But our results show that at distances of just below 100 m, only 50% of the hares present were detected in one region. Not adjusting count data for detection therefore probability merely provides a minimum estimate of unknown quality.

In spite of the issue concerning road avoidance that cannot be settled on the basis of the collected data, use of simple spotlight counts without distance sampling would have led to even lower figures for densities (Paper IV). Moreover, the amount of visible habitat varied by a factor of two across the four counted areas (Fig. 12 and Paper III). Thus, figures from spotlights counts can not result in reliable density estimates - and particularly so if the area actually counted is not measured carefully. One should also note that the issue of road avoidance, even if presently unsettled, would have affected simple counts as well. Without distance sampling, however, these issues would never have been realised.

The strength of PTC lies not only in the calculations that corrects for the undetected individuals, but the method is non-intrusive, less labour demanding and less costly than e.g. capture-recapture methods, and it is possible to cover quite large study areas. One disadvantage is, that individuals detected between the points are not used; this is especially frustrating when collecting data in regions sparsely populated by hares, and when obtaining sufficient sample size is difficult.

1.7 Security in numbers

The density of a population does in itself not provide much information about population status. Populations may be numerous and declining or small and prospering. But small populations are vulnerable to extinction. This is caused by a negative relationship between population size and temporal fluctuations in population size, as a greater risk of extinction is found in populations of more variable size (Reed & Hobbs 2004, Vucetich *et al.* 2000). A larger temporal variation in small populations is caused by a relatively higher sensitivity to perturbation caused by e.g. stochastic events.

1.7.1 Stochasticity

Stochasticity is variability due to chance or random events. Population persistence is influenced by three forms of stochasticity: demographic, environmental (including catastrophes) and genetic. The importance of all three factors, increase, in their magnitude of effect on population dynamics, as population size decrease (Shaffer 1987)

Demographic stochasticity relates to chance fluctuations in local survival and reproduction rates. Stochastic variation in demographic rates causes small populations to fluctuate randomly in size, and the smaller the population, the greater the risk that the fluctuations lead to extinction. The relative effect of this kind of stochasticity is small compared to other kinds of stochasticity, and only considered relevant for very small populations (Lande 1993), where e.g. the risk of all remaining individuals being of the same sex increases.

Environmental stochasticity includes random or unpredictable changes in weather, resource supply or populations of predators, parasites or competitors: events that may affect individuals simultaneously i.e. are correlated for the population. As described earlier, weather may affect yearly recruitment rates or cause changes in food availability. In large populations, environmental stochasticity may lead to short-term reductions in abundance or density, which will be compensated for when conditions are more favourable. In low density areas however, a single bad year may cause irreversible losses and lead to the extinction of a local population. Also, as soon as a population is debilitated by e.g. extreme weather, other factors such as road mortality may become increasingly import, either as a mortality factor or as a barrier hindering recolonization of vacant habitats (Shaffer 1987). Catastrophes can be considered as the extreme end, of a continuous distribution of environmental perturbations (Reed & Hobbs 2004).

Allee effects are decreases in population growth rate when the population density declines (i.e. inverse density dependence). They are the result of mechanisms such as mate limitation, obligate cooperation or anti-predator strategies based on group living (Allee *et al*. 1949, Courchamp *et al*. 1999). Allee effects in hares could arise through reduced reproduction rates, e.g. caused by an inability to find a mate resulting in unmated females. However, a high intrinsic growth potential allows hare populations to recover fast from extrinsic perturbations, given optimal conditions.

Our study area in Himmerland covers approximately 100 km^2 and is enclosed by two streams and in theory encompasses a closed population. We found a density of 3.14 hares per 100 ha (Paper IV), equalling a total population size of roughly 314 hares. At the current national annual growth rate of 0.94, based on the observed survival and fecundity at present (Paper II), a deterministic model predicts that the population in Himmerland will have disappeared in less than 100 years. However, stochasticity may either augment or decrease the time to extinction. In simulations of endangered population trajectories which included both environmental and demographic stochasticity Marboutin *et al*. (2003) showed that hare populations with densities of 3 hares per 100 ha, would not go extinct if hunting rates were low. A similar result was obtained by McLaren *et al*. (1997), who predicted that closed hare populations were likely to go extinct at densities of 3 hares per 100 ha in areas over 100 km^2, while immigration could buffer populations down to 1 hare per km^{-2}. The sustenance of the hare population in Himmerland thus appears to be on the verge, depending on migration rates.

When evaluating whether a population is large enough to maintain a viable population, with potential for evolutionary adaptation, several issues have to be addressed, some of which are: Initial populations size, the dynamics within the population in question (i.e. intrinsic potential for growth), stochastic processes, fragmentation (which will influence migration rates) and the genetic composition of the population (Soulé 1987). Also, the issue of scale need to be considered. Some will not consider an extinction of the hare population in Himmerland a “real” extinction, as long as patches maintaining the hare in other areas of Denmark persist.

1.7.2 The significance of genetic variation

Genetic variation is requisite for evolutionary adaptation to a changing environment, and maintaining population numbers and genetic variation is a central theme of long-term population management (Lande & Barrowclough 1987).

There are two levels at which to consider genetic variation when examining small populations or in this instance subdivided populations: Level of homozygosity within individuals (i.e. the percentage of loci at which identical alleles are present) and the degree of polymorphism within the populations’ gene pool (i.e. amount of different alleles of the same gene in the population). Loss of this genetic variation in small or isolated populations occurs through genetic stochasticity or genetic drift, and by inbreeding.

Genetic drift is random fluctuations in allele frequencies, and occurs because populations do not exactly reproduce their genetic constitutions through generations; there is a random component of gene-frequency change (Suzuki *et al*. 1989). In small populations the relative importance of genetic drift is higher, and alleles (deleterious, neutral or beneficial) may increase in frequency and become “fixed” due to mere chance, and this decreases the degree of polymorphism.

Inbreeding is breeding between closely related individuals, and may, if practiced repeatedly, lead to an increase in homozygosity, which will expose recessive deleterious alleles to natural selection. Inbred individuals may suffer from reduced fitness through lowered levels of fertility and increased mortality, higher susceptibility to disease and reduced competitive ability, while the overall population fitness is decreased through reduced population growth rate (inbreeding depression) (Lacy 1997). The random fluctuations in small populations’ size may also cause bottleneck effects.

Use of mitochondrial DNA in genetic analyses

Mitochondria are structures (organelles) within cells that contain DNA of their own, so called mitochondrial DNA or mtDNA. Intra-specific differences in certain coding and non-coding areas of the mtDNA are widely used to study dispersal, patterns of colonization, occurrence of bottlenecks, and other related processes in natural populations (Avise 1994, 2000). Due to the rapid mutation rate, mtDNA is useful for studying genetic divergence within species, or between species that are closely related, as the number of differences in the DNA sequence in this case can be easily counted.

Several studies (Fickel *et al*. 2005, Mendez-Harclerode *et al*. 2005, Gunduz *et al*. 2007, Paper V), have focused on the most variable part of the mtDNA, the mitochondrial D-loop region, as here, the substitution rate is higher, than in the remainder of the mtDNA genome (Cann 1984). Different versions of the same region in the D-loop (sequence polymorphisms), referred to as different 'haplotypes', indicates population genetic variability and serves in determining genetic identity and diversity among populations of the same species. MtDNA haplotypes have maternal inheritance (Hutchinson *et al*. 1974), and are usually assumed to be neutral (with respect to natural selection) genetic markers.

Genetic population structure of Danish hares

Genetic structure refers to the amount and distribution of genetic variation within and among populations. Denmark is at the edge of the brown hares' distribution area, and is subdivided into several islands. We therefore expected restricted gene flow across water barriers, as well as a somewhat lower overall genetic diversity compared to central European "core areas", as species on the edge of a distribution range, are more subject to fluctuations in population size, causing genetic drift and bottleneck effects (Brown 1984, Schwartz *et al*. 2003).

We analysed mtDNA from eight Danish hare populations (Paper V) and found low genetic variation, though haplotype diversity and prevalence of unique haplotypes are similar to levels in other European hare populations (Fickel *et al*. 2005) and higher than in Sweden (Thulin *et al*. 1997) and Austria (Hartl *et al*. 1993). The Danish hare population is not panmictic, but consists of several isolated populations, and as expected, hares on islands are locally subjected to loss of genetic diversity due to low population sizes causing genetic drift and isolation. Nevertheless, two hare populations on Zealand Island which are situated less than 50 km apart also showed high genetic differentiation and restricted gene flow between them. Besides the short dispersal distances and the philopatric behavior of hares, and of female hares in particular (Fickel *et al*. 2005), fragmentation of the agricultural habitat seems a probable explanation of limited genetic exchange between such relatively close populations.

Fragmentation may result in a reduced effective population size (defined by Wright (1938) as: "the number of breeding individuals in a population where each individual has the same chance to contribute its own genetic material to the next generation (no selection) and show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration"). The effective population size deviates from the total number of individuals in the population, since not all have the same probability of breeding (some die before reproducing, some produce more offspring than the average individual, some are sterile), and affects the rate of loss of heterozygosity per generation as well as the census population size. When the effective population size decreases, genetic variability is reduced and the differentiation between the remaining subpopulations increases, due to the restricted gene flow between them. In time, the low genetic variability in an isolated population may cause inbreeding depression (Keller & Waller 2002).

Loss of genetic diversity due to subdivision of the total hare population may reduce the adaptive potential of the subpopulations and the ultimate effect may be, that the local gene pool does not suffice to adapt to changes in the landscape. It should be stressed, that genetic diversity does not recover once lost, even if population size increases (bottleneck effects).

To counteract genetic drift, a solution is to increase genetic variability through influx of genes from neighbouring populations, for example by creating corridors in the landscape. Population genetic theory suggests, that one breeding immigrant per generation may be enough to counteract the negative effects of inbreeding; however, this rule of thumb must be evaluated with regards to the social, genetic and ecological characteristics of the migrants, as well as the temporal fluctuations in size of the recipient populations (Wright 1931, Mills & Allendorf 1996, Vucetich & Waite 2000). An influx of genes from neighbouring populations (assumed to be genetically similar) may even enhance population growth rate, as immigrant genes reduce effects of inbreeding in addition to increasing genetic variability (Saccheri & Brakefield 2002) (genetic rescue effect, Keller & Waller 2002). This renders the population less vulnerable to stochasticity in demography as well as in genetics. If the stocked population is locally adapted, introducing new genes may cause the opposite effect, outbreeding depression. If an increase in mtDNA variation is the aim, introduced individuals will have to be females. The sedentary life of especially female hares is obviously a hindrance to natural migration between neighbouring populations.

Anthropogenic effects

The genetic population structure in the Danish hare population is influenced by other anthropogenic effects than fragmentation, as hares have been translocated between Danish locations (Kleist 1995), and escapes of farmed hares appear to have occurred (Papers V and VI). No records of the translocations within Denmark have been kept, neither with regards to the originating or receiving localities. The analysis of evolutionary distances indicates that the translocations are likely to have affected the current genetic population structure, causing the impression of a recent expansion in some of the declining populations. However, probably the present genetic structure reflects both these recent anthropogenic interventions and also historical fragmentation.

Translocation of hares, i.e. creating artificial gene flow between populations (outbreeding enhancement) may hinder local adaptation and even decrease fitness in the receiving population (outbreeding depression) (Dobzhanski 1936).

Introgression of mountain hare mtDNA

Gene flow between introduced brown hares and indigenous mountain hares was first reported in Sweden by Thulin *et al*. (1997), and later on the Iberian Peninsula (Alves et al. 2003). Most recently, indications of the presence of Mountain hare-like mtDNA in several other hare species in Asia and North America suggests, that mountain hare introgression may be general (Alves *et al*. 2008). The finding of mountain hare mtDNA in Danish hares was unexpected, as mountain hare does not occur in Denmark while it is present east of Poland, in northern Russia, northern Scandinavia and isolated populations exist in Scotland, Ireland, England, Switzerland and Italy (Flux & Angerman 1990). Of the examined Danish hares 4% (6 males and 10 females) contained mountain hare mtDNA.

At the time of discovery, two explanations for the occurrence of mountain hare mtDNA in Danish hares seemed probable; either introgressed brown hares crossing the frozen sea between Denmark and Sweden during harsh winters (mountain hares are no longer present in southern Sweden) or, they could originate from introgressed hare-escapees from farms that were initiated with captive reared hares imported from, among other countries, Sweden (Hansen *et al*. 1990). Hare farms were prohibited in 1993, and hares from the farms may have been released into the wild. The recent finding of Alves *et al.* (2008) however could indicate a more ancient origin.

What ever the origin, the significance of the introgressed mtDNA lies in, whether this introgression is neutral or has a deleterial effect on the brown hare population. If the introgression is recent, and given that mountain hares do not live in Denmark and therefore is unadapted to the Danish climate, it could induce a negative effect on the fitness of introgressed individuals, through decreasing vital rates. Thulin *et al*. (2002) suggested that hybrid individuals were likely to have a selective disadvantage in the wild, while the fact that introgressed mountain hare mtDNA is widespread and even appears fixed in some populations, and maybe even species, indicates a selective advantage to the introgressed lineage (Melo-Ferreira *et al*. 2005, Alves *et al*. 2008). The effect of introgressed mtDNA on vital parameters remains to be investigated.

The influence of genes on phenotype and demography

Danish hare populations vary with regards to structural size and reproductive parameters (Paper I), and although these spatial differences were related to differences in habitat, genetics may also be at work. The genetic investigations showed that hare populations are locally subjected to genetic drift; an indication of low exchange of genes between populations. Nonrandom or lack of dispersal may reinforce population differentiation at a small spatial scale (e.g. Garanth *et al*. 2005). The genetic effect on morphological traits remains to be investigated, but mtDNA does not appear to be associated with morphological variation (Hartl *et al*. 1993), which makes this marker unsuitable for investigations of quantitative trait loci (i.e. stretches of DNA that are closely linked to the genes that underlie a trait in question).

As we merely investigated the variation in the D-loop (a neutral marker) and not in any other markers, no statements can be made at present with regards to the influence of (lack of) genetic variation on the observed population declines. If the population remains subdivided, in time, a lower genetic variation will be the result, due to the above described mechanisms. Locally this may cause inbreeding, which eventually will affect population growth negatively.

2 What we learned, and future research

AIM 1: Identifying the relative importance of survival and reproduction in contributing to the population decline in hares in the Danish agricultural landscapes

Variation in hare population dynamics appears to originate in local factors affecting female reproductive parameters as well as leveret survival from birth to adulthood. We found no variation in adult age distribution across populations or at different habitat compositions, and overall adult survival was high, suggesting that this parameter plays a minor role in the hare population declines.

Spatial variation in female reproductive success could be linked to local habitat composition, and within populations, heavy females relative to their size were more likely to be fertile. This implies that good food habitats improve female reproductive success, and grass leys – which are improved fodder crops, appear to positively affect condition. Availability of cover as well as of grass leys positively affected growth, causing spatial variation in skeletal size, but size had no effect on survival or reproductive parameters.

Juvenile recruitment (indexed as the proportion of hares culled in autumn comprised by individuals < 1 year) was lowest in areas with low densities, but unrelated to the reproductive output of females indexed as the mean number of placental scars, indicating high spatial variation in post-natal survival of offspring. Juvenile recruitment was lower in areas with poor soil quality, but the mechanism behind this pattern is not clear.

In a historical perspective, the proportions of juveniles in game bag records dropped significantly between the 1950ies, when hare populations where stable, and the 1980ies onwards, when hare populations according to bag size decreased with 5% annually. Simple matrix population models based on the estimated annual survival for adult females at present and the estimated fecundity (realized juvenile recruitment by autumn) for the 1950ies, 1980ies, 1990ies and since 2000, predicted the same population growth rates for each decade as was actually observed in the annual bag size. This strongly indicates that reduced juvenile recruitment plays a major role in the observed population declines.

The matrix model showed that as recruitment of juveniles decrease, the sensitivity of the population growth rate to adult survival increases. As recruitment is lowest in areas with lowest densities, this raises the issue of protections from hunting in low-density areas. The low population growth rate implies that the hare population does not appear to be approaching a new, lower carrying capacity, wherefore hunting is unlikely to be compensatory. It seems, that reduced recruitment is not confined to the agricultural habitat, and the model predicts further population decline.

The focal point of future research on brown hare population dynamics should be clarifying the mechanisms underlying the low recruitment. The respective roles of prenatal mechanisms (i.e. energy-constraints) as well as post natal mechanisms (energy-constraints as well as other mortality agents) in causing the low recruitment should be identified. Tracking marked leverets daily could aid in identifying mortality agents. Also, reasons for variation in female productivity should be elucidated, especially with regard to the effects of food availability and quality in the reproductive period.

Fecundity and leveret survival are likely to interact with the multivariate effects of agricultural practices and the numerous mortality factors and should therefore be considered collectively, rather than individually. Once the actual mechanisms behind the low fecundity have been quantified, all the above may be incorporated in individual based models.

No studies have been carried out on male reproductive performance in Denmark, and as reduced sperm motility was observed in one-third of male hares in Poland (Seck-Lanzendorph 1997), this field ought to receive attention in the future.

AIM 2: Attain reliable density estimates of hares (A) and evaluating Point transect counts as a method of obtaining these (B)

A) We have obtained estimates of hare densities in four areas in Denmark. The densities were surprisingly low in two of our study areas, and given the observed declines taking place at present, this presents serious issues regarding the sustainability of hunting in some areas, especially since recruitment appears lowest in the regions with low densities. It is suggested, that a model e.g. such as ALMaSS is engaged in assessing the sustainability of hunting at different densities. This will enable estimations of the extent to which complete or partial protections of the hare are necessary, or able, to avoid or postpone local extinctions.

Hare densities vary substantially regionally as well as within counties, and the former counties were too large to be used as management units. From 2006 the counties have been divided into 98 municipalities, which in the future will provide an improved possibility of more detailed analysis of the influences of hunting and predation, as well as of local land management, crop diversity and land cover on hare densities, in for example time series analysis.

B) Point Distance Sampling is suitable and necessary for monitoring hare populations. Even within distances of 150 m, less than 50% of present hares are detected by spotlight counts, thus invalidating use of uncorrected counts for estimating densities. But while spotlight counts are a simple method for obtaining information of hare densities, the interpretation of the data turned out to be highly intricate. For this reason, this part of the thesis entailed much more of the project time than originally intended.

For some habitat types, correcting for unseen subareas is necessary in order to avoid systematic errors. This is well illustrated by the fact, that twice as much area was covered per count point on LO1 than at HL and KA, which invalidates comparisons between counts even before considering detection probabilities. When correcting for blind areas, the estimated density increased. In areas where detection probability is high, the error of not correcting for blind areas affect density estimates less. However this is the cause of two oppositely directed errors- hardly a satisfactory solution.

The simulations of road avoidance behaviour and detection probabilities showed, that if hares avoid roads, this may overestimate the proportions seen by 6-85% and underestimate the density by 16-45%. Hence, care must be taken with regards to the assumption of whether individuals are evenly distributed in the surveyed area.

The detection function is fitted empirically to the observed distances under the sole assumption, that all hares at distance 0 are detected. From the fitted detection function, the proportion of animals that are missed during the survey is estimated, and the choice of detection function may severely impact this estimate. In our work we applied the half-normal detection function, and although this fitted the data satisfactory, the result is of fitting other key-functions to the data should be investigated.

Though there are still some uncertainties with regard to the accuracy of the estimates, at least distance sampling has provided insight into which sources of uncertainties are involved. Despite these, distance sampling provided more realistic density estimates compared to uncorrected counts. For most purposes, counting numbers suffice for estimating density when the scope of an investigation is to obtain magnitudes of densities; insofar counts are properly corrected for unseen individuals.

The consequences of the violations of the basic assumptions are not yet investigated to the full, and more research is needed to identify the effect of e.g. intensity of the spotlight used, time of year or night and weather, on population density estimates. Also the issue of road avoidance remains unsettled. Once the influential factors are determined, a standardized methodological protocol can be developed to transform counts into density estimates.

If, as mentioned above, partial protections of hares are carried out in low density areas, new monitoring programmes of hares must be initiated, in order to keep track of the development in the population, as information will no longer be available from hunting records. PTC has potential as a tool in establishing a nationwide monitoring programme for nocturnal mammals, e.g. by establishing yearly count data from each of the 98 hunting municipalities.

AIM 3: Investigating the genetic population structure of the Danish hares

The hare population in Denmark consists of several separate genetic populations. The restricted gene flow was not only due to the composition of Denmark (islands), and it is also likely, that the observed genetic divergence results from both ancient genetic history (before anthropogenic influence) and genetic drift (possibly caused by fragmentation of the landscape) in combination with translocation of hares between localities in Denmark. The findings of introgressed mountain hare (*L. timidus*) mtDNA into the Danish brown hare were surprising, and the effects of this on population dynamics or the role it may play in population declines are uncertain. Extending a similar investigation to hare samples from the mainland, could shed some light on the origin of the mountain hare mtDNA. This area was not well represented in our study, giving a perhaps counterfeit impression of the introgressed mtDNA being confined to brown hare populations in eastern Denmark. If mtDNA is of ancient origin or the result of hare farm escapees, we expect to find equal amount of introgression in Jutland.

The low exchange of genes even between populations on Zealand, emphasizes the importance of increasing connectivity between scattered populations, e.g. by decreasing landscape fragmentation. This will enhance genetic exchange and strengthen populations by reducing sensitivity towards demographic and genetic stochasticity.

In the future, particularly the population structure of the hares on the mainland should be further investigated. Here, the population densities are low and a larger degree of subdivision of the population can be expected, increasing the risks of local differentiations or extinctions. With a stationary species like the hare, in a fragmented landscape and in areas with densities as low as in Himmerland, recolonization of patches may not occur instantaneously. Such an investigation should ideally be supplemented with microsatellites as genetic markers, as these provide a higher solution of mutation rates due to a higher mutation rate than in mtDNA. They are therefore useful for identifying differences between populations that are not highly differentiated yet and may provide a picture of the ongoing development in the populations. In addition, microsatellites are biparental, i.e. affected by the behaviour of both males and females. With regards to the hare, they could thus provide information of the dispersal and migration rates of males in particular.

Finally, having found inter-populations differences in size, reproduction and parasite loads, it would be a logic next step, to determine to what extent these traits are related to the genetic variation also found.

Photo: Michael Albert Schmidt, VIBI

3 Dansk resumé

I de seneste årtier har den danske bestand af harer (*Lepus europaeus*) undergået en væsentlig nedgang, sandsynligvis relateret til intensiveringer inden for landbruget, men kendskabet til de proximative årsager, samt til faktiske bestandstætheder, er begrænset. I denne afhandling undersøges nutidige hare populationer med hensyn til alderssammensætning og reproduktive parametre set i relation til habitat sammensætning og fænotype. Den genetiske variation undersøges, ligesom "Point transect counts" evalueres med hensyn til anvendelighed til at estimere haretætheder.

Nutidige reproduktive parametre og rekruttering af juvenile varierer, mens overlevelsen af voksne harer er sammenlignelig imellem de danske underbestande. Rekruttering af juvenile er lavest i de områder hvor tæthederne er lavest, men rekruttering i et område viser ingen direkte sammenhæng med antallet af killinger hunner i et område producerer. Dette indikerer varierende overlevelse af årets killinger mellem bestande. En del af den rumlige variation i de undersøgte parametre kan tilskrives lokal habitatsammensætning.

En analyse af andelen af juvenile i jagtoptegnelser fra de sidste 50 år viser, at der med tiden er sket en nedgang i rekrutteringen af juvenile frem til starten af efterårets jagtsæson. Hvad mere er, vækstrater, der afspejler den observerede nedgang i vildtudbyttet gennem de sidste årtier, kan forudsiges via en simpel matrix model, alene ved anvendelse af den observerede rekruttering i jagtoptegnelserne. Modellen underbygger hypotesen om, at de observerede bestandsnedgange er forårsaget af svigtende juvenil rekruttering, og forudsiger yderligere bestandsnedgang.

"Point transect counts" er anvendelige og korrektion for detektionssandsynlighed nødvendig, når man ønsker at estimere haretætheder ved nattællinger. Mere arbejde foreligger dog med at bestemme betydningen af f.eks. harers undvigelse af veje for tæthedsestimaterne.

Den genetiske variation viser, at den danske harebestand er underopdelt og at der kun sker begrænset udveksling af gener mellem selv nærtliggende populationer. De genetiske forskelle mellem underpopulationer tilskrives genetisk drift, harens indvandringshistorie i Danmark, samt de translokeringer der er foregået mellem landsdele.

Fremtidig forskning bør fokusere på at få klarlagt årsager til den manglende rekruttering af juvenile, herunder betydende mortalitetsfaktorer for killinger, samt årsager til, at adulte hunners produktivitet varierer. Endvidere bør de genetiske konsekvenser af de meget lave tætheder der er fundet i Jylland undersøges, ligesom bæredygtigheden af jagt i disse områder bør evalueres.

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Spatial variation in brown hare demography and phenotype in relation to habitat

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Manuscript

Spatial variation in brown hare demography and phenotype in relation to habitat

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Abstract

Brown hare populations in Europe have declined through decades, ultimately as a result of habitat alterations, but the underlying, proximate mechanisms are debated. On the premise that the most decisive demographic parameters for population dynamics are those that vary most across populations and habitats, we investigated demographic (age, reproductive) parameters and phenotypic parameters (size, weight) of 480 brown hares culled in 16 Danish populations. We firstly wished to identify which parameters varied across spatial locations, next, to what extent between-population variation covaried with landscape characteristics. Finally, we analysed the correlations between certain demographic and phenotypic traits of population biological interest within as well as beyond populations. We found no variation in adult age distribution across populations, suggesting equal adult survival across populations and habitat types. Fecundity parameters (prevalence of fertile females, number of placental scars per fertile female, proportion of juveniles in the bag), varied significantly across populations, some of this between-population variation being attributable to habitat traits. Within populations, heavy females relative to their size were more likely to be fertile. The proportion of juveniles in a population cull was not correlated with the population mean number of placental scars per adult female, suggesting the latter being a poor indicator of a population's productivity which instead appears to be sensitive to post-natal mortality. Skeletal size (humerus length) accounted for 30-45% of adult body mass variation and differed between populations, population means being significantly correlated with habitat variables, but not with population means of any demographic parameter. Variation in hare population dynamics originates in local factors affecting female reproductive parameters as well as survival from birth to adulthood: habitat composition affects reproductive parameters, while factors other than nutrition also affect juvenile survival.

Introduction

To understand why a species vary in density across landscapes or over time, the underlying population dynamic processes, and the factors constraining individual performance must be identified. Investigating how demographic and phenotypic parameters of sampled individuals are inter-related and vary across and within study sites can reveal which parameters vary in general, and identify possible causal links between them and indicators of environmental variation. Phenotypic traits related to the early developmental period, e.g. body size which reflects nutritional condition during growth, might have a direct effect on reproductive performance or survival later in life (Albon, Clutton-Brock & Guiness 1987; Iason 1990, Powel & King 1997, Lindström 1999).

Populations of European brown hares (*Lepus europaeus*) have declined dramatically over the past 50 years. The ultimate causes appear to be habitat changes caused by intensification of farmland management practices (Smith, Vaughan & Harris 2005 and references therein) but the proximate causes remain unidentified. The present need is to identify how demographic parameters are affected by the on-going habitat changes and restrict the population's growth potential.

Among the hypotheses proposed to explain the declines is a change in seasonal availability of forage, in favour of winter accessibility but at the expense of availability during the reproductive period (Frylestam 1980a, Schmidt, Asferg & Forchhammer 2004, Reichlin, Klansek & Hackländer 2006). Several authors have focused on predation by the red fox (*Vulpes vulpes*) (Erlinge *et al*. 1984, Reynolds & Tapper 1995, Panek, Kamieniarz & Bresinski 2006), while others have examined the prevalence of disease, hunting and female reproductive performance to evaluate their relative effects on population declines (Bensinger *et al*. 2000, Hackländer 2001, Marboutin *et al*. 2003, Wibbelt & Frölich 2005).

If population dynamics are driven by factors influencing reproductive parameters, we expect these to vary across populations. Also, we expect that between-population differences possibly correlate with landscape features representing their varying influence. If nutritional constraints on reproducing females and weaned leverets are an important mechanism regulating the reproductive output by autumn, we expect between-population variation in the production of young per female, provided that nutritional constraints vary between populations. In that case, should the number of juveniles per adult female (corrected for summer mortality of adult females) in the autumn bag of hunts conducted at the same time in different localities correlate positively with the mean number of newborn per female the preceding summer, unless other factors, not strictly related to resource availability (e.g. predation) also plays important roles for post-natal survival.

If nutritional constraints on juvenile development vary across populations we should expect mean body (skeletal) sizes to be affected (e.g. Powel & King 1997). Likewise, female size and reproductive output might vary, within as well as between populations, possibly as a function of overall landscape features such as soil fertility or crop composition. We test these predictions, which are not mutually exclusive, on 480 individual hares from 16 Danish hare populations.

We analyze the variation within and across populations with respect to size, weight, age structure and reproductive performance. In addition to (1) identifying the general level of variation within these traits, we aim to (2) investigate the amount of variation in hare body weight (the conventional measure of size) that is attributable to variation in skeletal size. Having separated effects of structural size and condition (size-adjusted weight) statistically, we (3) investigate whether these phenotypic measures are related to variation in demographic traits. Knowing the landscape characteristics of the sampling locations, we (4) explore whether the means of these traits covary with landscape features such as crop composition.

Materials and methods

Carcass collection

During autumn hunts (October-December) of 2003-2005, hares were collected at 67 sampling sites within 16 Danish populations, separated by water barriers or minimum 40 km distance (Fig. 1, appendix 1). Based on the degree of epiphyseal closure evaluated from cleaned bones (Walhovd 1966), the hares were classified as adults or juveniles (born prior to or in the year of sampling respectively). Juveniles over 7 months, which is less than 15% of leverets, will not be identified by this method (Hansen 1992). Adult age (years) of hares from 2003 (83 males, 110 females) was determined from periosteal growth lines in the lower mandible (Frylestam & Schantz 1977). Three hares with closed sutures were determined to be juveniles and were reassigned to the juvenile age-class.

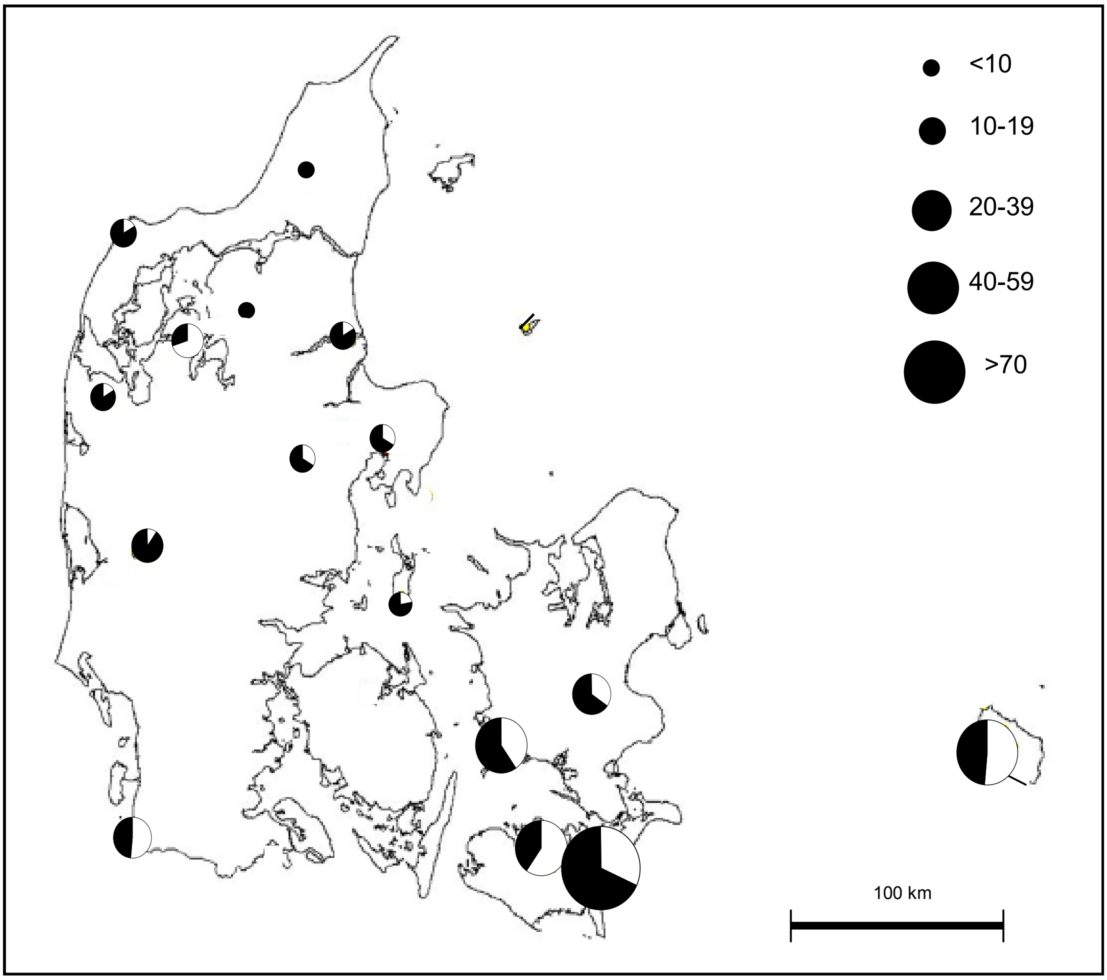

Fig. 1. Map of Denmark showing the 16 populations from which 480 brown hares were sampled. Circle sizes indicate sample size. The white area within each circle signifies the proportion of juveniles.

The date of death ± 1 day was known for 219 adult hares and to the nearest month for 12 hares (date set to the 15th in the month of death). Sampling location was known with a precision of ± 100 m for 107 adult hares, within 500-1500 m (estate) for 155 adult and within 1500-3000 m for 33 adults. Skeletal size of adults were measured as the zygomatic width of the skull (ZW) (n=216) and the humerus length (HL) (n=211) with a digital calliper to the nearest 0.1 mm (repeated measurements on HL and ZW from 18 and 27 hares showed close to 100% agreement: R^2=0.99).

Reproductive output in the latest breeding season of 158 adult female hares was determined by counting the total number of placental scars following Bray *et al.* (2003). Yearly variation in demographic parameters was not considered due to small sample sizes. As adults originate from different cohorts, time-effects on size were considered insignificant.

Landscape composition at the sampling sites

Based on knowledge of natal dispersal distance and adult home range size (Tapper & Barnes 1986, Reitz & Leonard 1994, Smith *et al*. 2004, Bray *et al*. 2007) we measured the landscape composition within a 2-km radius of the estimated sampling site centre, which should be sufficient to ensure the majority of the sampled hares having spent all their life within this circle. Under all circumstances, in the flat, Danish rural landscapes, areas bordering the 2-km circles were of similar composition. We indexed landscape heterogeneity, agricultural coverage and shelter availability in terms of edge habitats which also may provide access to weeds; these being important to hares in agricultural landscapes (Smith *et al*. 2004, Reichlin *et al*. 2006, Jennings *et al*. 2006). Landscape data was extracted from GIS, ArcMap (ESRI 2006) using a raster grid land cover map (resolution 25 x 25 metres, Groom & Stjernholm 2001) while a polygon layer of soil structure was used to index soil productivity (Table 1a).

a) Landscape variables	**Description**
Uncut grass	Proportion of land covered with uncut grass or non-pastural grass areas
Arable	Proportion of land covered with arable land
Edge arable	Summed length of edges (ha^{-1}) between arable land and contrasting land cover categories
Edge pasture	Summed length of edges (ha^{-1}) between pasture and contrasting land cover categories
Edge forest-arable	Length of edge habitat between tree-stands (forest or copses) and arable land
Heterogeneity	Landscape heterogeneity indexed by calculating the number of raster-squares ha^{-1} that borders a differing landscape category
Soil productivity[a]	The potential plant productivity indexed by the percentage of good quality soil
b) Crop variables	
Root crop	Sugar beets or potatoes
Semi-natural grass	Area covered with permanent short, in general native, grass species. Unfertilized, unplowed, grazed or ungrazed areas
AOR	Area out of rotation
Corn	Primarily for stock food
Spring cereals	Spring sowed cereals (rye, oat, wheat)
Winter cereals	Autumn sown cereals (rye, barley, wheat)
Grass ley	Mixtures of improved grass (e.g. Lolium, Festuca, Poa sp.) with e.g. Trifolium sp. or Vicia sp. cut several times a year for silage or occasionally grazed. In 1-3 yr rotation
Wholecrop	Vegetation as in 'Grass ley', but intensively managed and heavily fertilized, for silage

Table 1. Landscape variables a) and crop variables b) used as predictor variables in models on HL_{mean}, mean body mass, mean size-adjusted mass (condition), reproductive parameters and age structure in 16 Danish brown hare populations. [a]Source: http://www.djfgeodata.dk/website/DJFGeodata/viewer.htm.

For the averagely 69% of the 2 km-radius areas that were cultivated, information of agricultural cover (Danish Plant Directory, Ministry of Food, Agriculture and Fisheries) was extracted from a polygon layer of field block data from 2005 in GIS. Prior to the statistical analysis, the initially 19 cover types were lumped into eight categories (Table 1b). The remaining area constituted minor agricultural cover (e.g. horticulture) or non-agricultural areas (roads, buildings, forest). Some landscape variables and all the crop variables (area units) were log transformed to ensure linearity.

The different habitat variables were loaded as individual variables rather than as principal component axes as the derived variables from a principal component analysis had low explanatory power and low component loadings.

Indices of regional fox and hare densities were obtained from The Danish Game Bag Record (mean number shot hunting region^{-1} ha^{-1} in the years 2003-2005, http://vildtudbytte.dmu.dk/dmu5c.asp).

Statistical analysis

All statistical analyses were conducted with SAS 9.1 software (SAS Institute). Significance was set at $P<0.05$ (two-tailed tests throughout).

Skeletal size

To the analyses of whether HL and ZW varied across populations and as function of factors operating within a given population (sex, age, sampling site) we used the individual hare as the statistical unit in general linear mixed models (GLMM, Mixed procedure), entering population and sampling site nested within population as random effects (explicitly tested by using the 'Method=Type3' statement). As the allometric relationship between the two skeletal measures differed between populations and HL explained most of the variation in body weights in the subsequent analyses, we chose to leave out detailed analysis of ZW.

The analyses of whether the population mean body size (HL_{mean}) correlated with landscape and crop variables (Table 1) was conducted with a general linear model (GLM, GLM procedure in SAS), weighing individual observations ($n=16$ HL_{mean} obtained as least square means from the previous analysis) by the square root of the number of hares (as males and females had similar skeletal sizes, HL_{mean} values were estimated from male as well as female data) contributing with information to the mean size estimate because the SE of the individual population means are defined as $SD/\sqrt{n}$.

Firstly, all predictor variables (Table 1) were each correlated with HL_{mean}. Those variables that came out as significant after this initial round were then selected as potential predictors of HL_{mean} in a model with multiple predictors. The choice of the model best describing the variation in HL_{mean} was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004).

Body mass and size adjusted body mass ('condition')

Body mass variation was analysed separately for males and females as females are heavier than males and the two sexes might differ in their energetic ecology.
Following the same analytical principles as in the analyses of structural size, we analysed whether body size and size-adjusted body mass (i.e. a measure of condition: García-Berthou 2001), varied across populations and as a function of factors operating within a given population (age, sampling site), using the individual hare as statistical unit in a GLMM, entering population and sampling site nested within population as random effects. Following the same analytical procedure as in the analysis of structural size, the 16 population means of total weight and size adjusted weights of females were then correlated with landscape and crop variables.

Placental scars

As one fifth of all adult females had no placental scars, the remaining usually having many, the probability of being fertile (having scars) and the number of placental scars if being fertile were analysed separately, as different factors might influence the probability of being fertile and leveret production if being fertile.
Using generalised linear mixed models (GLIMM, Glimmix procedure, using restricted maximum likelihood as estimation method), the probability of being fertile was modelled as a binomial outcome with a logit link, while the total number of scars in fertile females were analyzed using the negative binomial distribution (log link) enabling a proper handling of the right-skewed distribution of placental scars per female (checked with residual diagnosis analyses). The statistical significance of variation between populations and sites within populations was explicitly tested by means of log-likelihood tests, treating populations and sites as systematic factors.
The proportions of fertile females aggregated for each of the 16 populations were correlated with landscape and crop variables (Table 1) using logistic regression (Proc logistic procedure), adjusting for within-population variation by scaling the deviance for

overdispersion. As the response variable consisted of the number of fertile females out of the total number of adult females, populations were automatically weighted according to their relative contribution to the total data set. To achieve comparable AICc-values of different models, we applied same scaling factor of the deviance (to be as conservative as possible, we used the scaling factor of the base model containing only an intercept).
The populations' mean numbers of scars (least-square means predictions) were correlated with landscape and crop variables (Table 1) in a GLM following the same principles as the previous GLM-analyses.

Age structure

The proportion of juveniles in the autumn bag in the different populations was modelled with GLIM, using the binomial distribution and a logit link in exactly the same way as described for the analysis of proportion of fertile females. However, as additional covariates that might influence a population's proportion of juveniles, we also tested for the potential effects of road density (≥6 metres wide) (data extracted from a line polygon theme of roads in ArcGIS) and regional fox and hare abundance. on age structure on the proportion of juveniles was examined by correlations with the density of roads ≥6 metres wide (m/ha) within a two km radius of sampling sites and with indices of regional fox and hare densities.

We modelled the effects of sex, sampling site and population on adult mean age (a proxy for annual survival after the first year of age) with a GLIM with a log-link and negative binomial distribution. In a subsequent analysis, predicted population mean ages (n=16 least square means, which followed a normal distribution) were then correlated with population means of landscape and crop variables (table 1) in a GLM following previously described GLM procedures. In addition to land use variables, predicted population mean ages were also correlated with HL_{mean}, mean size-adjusted body mass, road density and indices of regional fox and hare densities.

Results

Skeletal size

Skeletal sizes (HL) of 208 adults sampled at 67 sampling sites in 16 populations varied significantly between populations ($F_{15, 47.915}$=2.12, P=0.025, R^2=0.12, grand mean 102.61 mm; range of means: 99.44-105.18), but not between sampling sites within populations ($F_{43,149}$=0.76, P=0.85), and was independent of sex and age (additive effects to population differences: $F_{1,148}$=1.78, P=0.18 and $F_{1,101}$=0.52, P=0.47, respectively). A population's mean skeletal size (HL_{mean}) was best described as a combined, increasing function of the amounts of grass leys and edge habitat between forest-arable (Fig. 2a,b, Appendix 2).

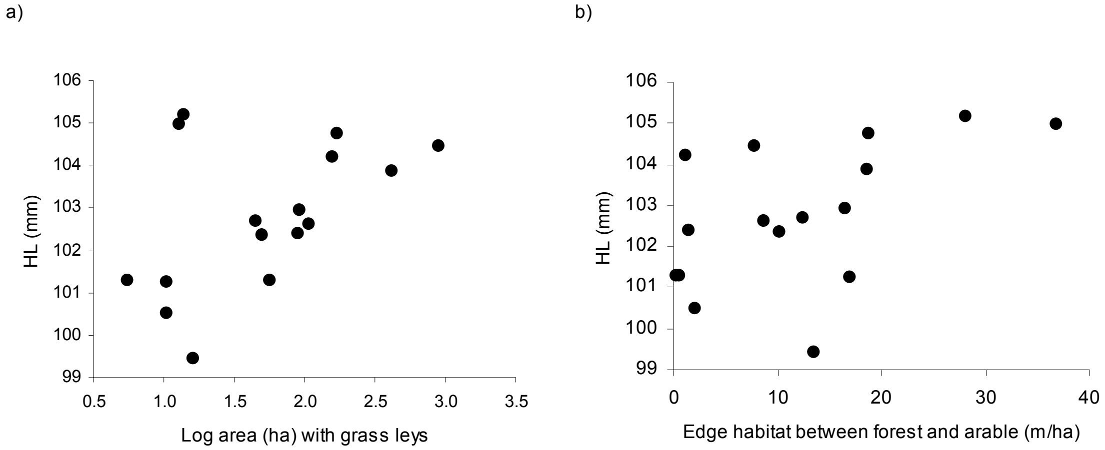

Figure 2. Population mean humerus length (HL) as a function of a) the coverage of grass leys (χ^2_1=4.74, P=0.030) and b) edge habitat between forest and arable land (χ^2_1=5.34, P=0.031). Regressions were weighted by √n (n=number of individuals within each population).

Body mass and condition (size adjusted body mass)

On a national basis, adults females averagely weighed 4200g (95% CI accounting for population differences: ±53 g) and males 3877 (±54) g ($F_{1,293}$=68.7, P<0.0001, R^2=0.19).

Female raw body mass was not conditional to sampling date ($F_{1,85}$=2.23, P=0.14), nor did it vary significantly across populations ($F_{13,38.33}$=0.63, P=0.82) or sampling sites within populations ($F_{23,85}$=1.41, P=0.13) but was strongly correlated with HL ($F_{1,116}$=49.1, P<0.0001, R^2=0.30; additive effect of ZW after accounting for effect of HL: $F_{1,79}$=0.54, P=0.47). After having adjusted for size, female body masses (i.e. condition) varied significantly between sampling sites within populations ($F_{22,52}$=1.76, P=0.048, R^2=0.27) but not between populations ($F_{12,29.03}$=0.82, P=0.63, R^2=0.11).

Population means of female condition correlated positively with the amount of area with grass leys (Fig. 3a, Appendix 3a).

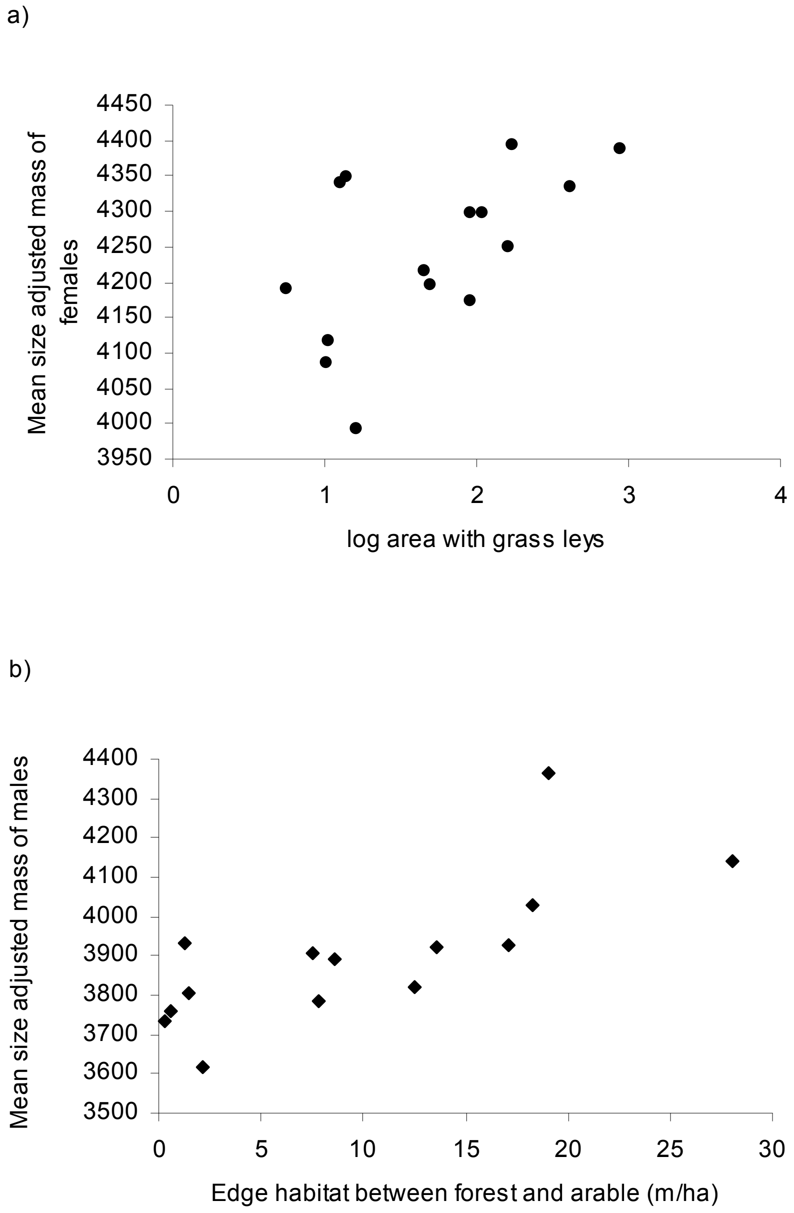

Figure 3. a) Mean size adjusted female body mass (indexed condition) plotted against mean coverage of grass leys ($F_{1,14}$=6.4, P=0.025). b) Mean size adjusted male body mass (indexed condition) plotted against edge habitat between forest and arable land.
($F_{1,13}$=5.75, P=0.032). Regression weight: √n (n=number of individuals within each population).

Male raw body mass did not vary significantly with date ($F_{1,62}$=0.02, P=0.89), between populations ($F_{12,25.38}$=1.50, P=0.19) nor between sampling sites within populations ($F_{32,62}$=0.85, P=0.69), but correlated positively with HL ($F_{1,88}$=26.60, P<0.0001, R^2 =0.23) as well as ZW ($F_{1,92}$=33.0, P<0.0001, R^2=0.26) these two skeletal measures explaining 45% of the total body mass variation in males ($F_{2,49}$=19.7, P<0.0001). Male condition (body mass adjusted for HL and ZW) did not vary between populations ($F_{12,21.21}$=1.01, P = 0.48) or between sampling sites within populations ($F_{18,21}$=1.86, P=0.09), but population means of male condition correlated positively with the amount of edge between forest and arable (Fig 3b, Appendix 3b).

Reproduction

Overall 79% of Danish female hares >1 year contained placental scars (95%CI: 64-89% if accounting for inter-population variation). The prevalence of breeding females varied significantly across populations (χ^2_{15}=41.1, P=0.0003, R^2=0.23) but not between sampling sites within populations (χ^2_{33}=32.0, P=0.52). In a multiple regression model, incorporating spatial variation between populations (random effect) the prevalence of breeding females was negatively affect by skeletal size and positively by (size adjusted) body mass (Fig. 4, $F_{1,97}$=6.70, P=0.011 and $F_{1,97}$=7.41, P=0.008, respectively). Upon incorporation of size and weight effects, the between-population variation in prevalence of breeding females remained highly significant (χ^2_{14}=29.47, P =0.009). The proportion of breeding females in a population correlated negatively with coverage of semi-natural grass (χ^2_1=5.29, P=0.021, Appendix 4).

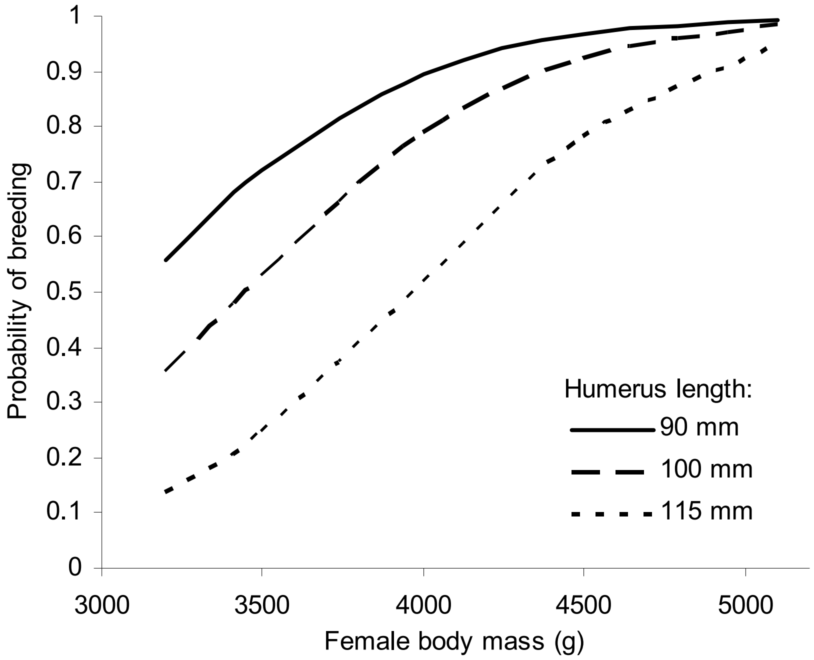

Figure 4. Probability of female hares breeding as a function of body weight and size (humerus length). Predictions from model accounting for variation between populations.

On national level, fertile females averagely carried 10.4 (95% CI: 9.8-11.1) placental scars, but means varied between populations (χ^2_{14}=24.91, P=0.035) as well as between sampling sites within populations (χ^2_{22}=40.97, P=0.008). Accounting for spatial effects, placental scar numbers were independent of HL ($F_{1,49}$=2.84, P=0.10), age ($F_{1,42}$=0.34, P=0.56) and weight ($F_{1,70}$=1.02, P=0.32).

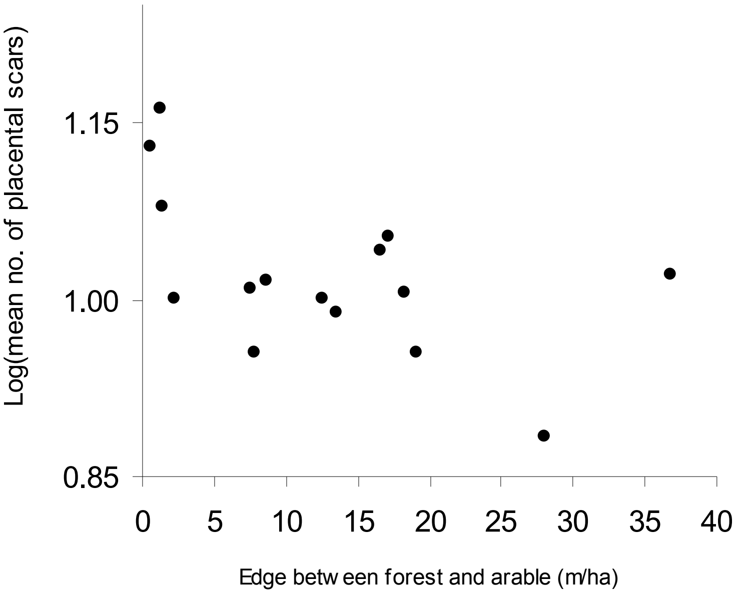

Figure 5. The mean number of placental scars in reproducing female hares as a function of the amount of edge habitat between forest and arable areas (χ^2_1=4.44, P=0.035, regression weight: √n (n=number of individuals within each population).

Differences in the mean number of scars in breeding females among populations were best described by a negative correlation with the edge habitat between forest-arable (Fig. 5), but also correlated positively with the coverage of semi-natural grass (χ^2_1=4.25, P=0.039, appendix 5).

Age structure

Of the total sample 185 (39%) were juveniles. This corresponded to an estimated national mean proportion of 0.38 (95%CI: 0.31-0.45) juveniles when accounting for a considerable variation across populations (χ^2_{15}=41.71, P=0.0002, R^2=0.09). The proportion of juveniles did not vary between sampling sites within populations (χ^2_{57}=7.22, P=0.82).

The population proportion of juveniles did not correlate significantly with the population's mean female size (χ^2_1=0.02, P=0.88), mean female condition (χ^2_1=0.02, P=0.63) or the mean number of placental scars (including infertile females, χ^2_1=1.21, P=0.27). The ratio of juveniles per adult females did not increase linearly with increasing mean number of placental scars (including non-breeding females, Fig. 6).

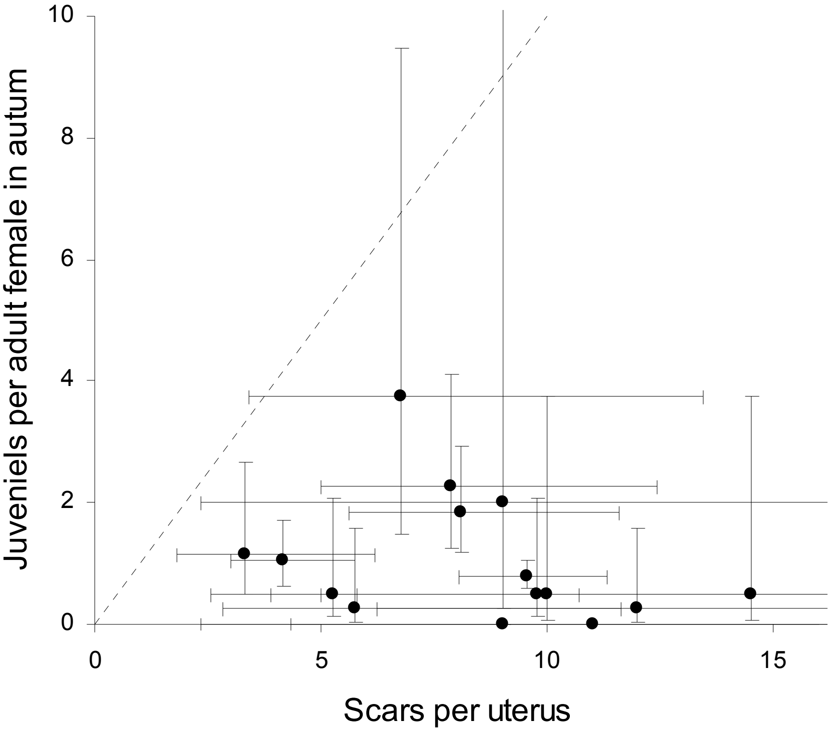

Figure 6. The number of juveniles per adult female brown hares in autumn, plotted against mean number of placental scars in female hares (including females without scars) from 15 Danish areas (+/-90% CI; χ^2_1=2.24, P=0.13 in a logistic regression modelling juveniles/(juveniles+adult females). The dashed line shows the expected number of juveniles, if all scars had produced a surviving leveret.

Of environmental variables, the proportion of juveniles increased with increasing soil productivity (P=0.040, Fig. 7, appendix 6), but was unconditional to road, fox or hare density (all P>0.12).

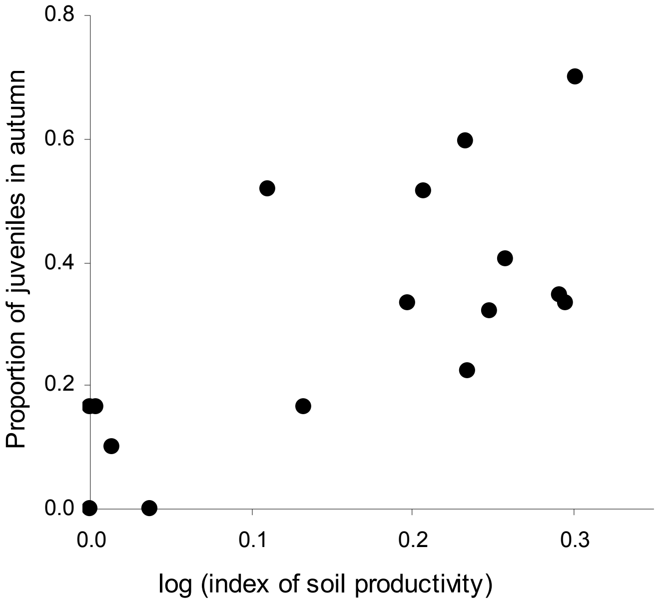

Figure 7. The proportion of juvenile hares in samples from 16 Danish populations plotted against mean index of soil productivity in a radius of 2km.

Females were averagely older than males (2.38 ±0.27 vs. 1.90 ±0.21 year [95% CIs], t_{191}=4.12, P<0.0001), corresponding to an annual, adult survival rate of 0.57 for females and 0.40 for males. Mean adult age did not vary between populations (χ^2_{14}=8.02, P=0.89) or between sampling units within populations (χ^2_{35}=34.93, P=0.47). Population mean ages (least-square-means adjusted for sex-effects) did not correlate with HL_{mean}, mean condition, density of roads, index of fox or hare density or any habitat variables (all P>0.16).

Discussion

We discovered considerable variation in phenotypic and reproductive parameters as well as in juvenile proportions in autumn, while adult age varied little between populations (Table 2).

	Individual hares as observation units		**Population means as observation units correlated with habitat variables**
Response variable	**Within-Population variation**	**Between-population effects**	
Skeletal size (HL)	NS	*	(+) Grass ley + (+) edge arable/forest***
Body Mass	Sex **** HL **** ZW **** (males only)	NS	(no analysis)
Female Condition	*	NS	(+) Grass ley*
Male Condition	NS	NS	(+) edge arable/forest*
Prevalence of fertile females	(-) HL* (+) Body mass**	***	(-) Semi-natural grass*
Scars per breeding female	**	*	(-) edge arable/forest* (+) Semi-natural grass*
Proportion of juveniles	NS	***	(+) Soil productivity-index*
Mean adult age	Sex ****	NS	None

Table 2. Summary of the main results from this study. 'Within-population variation' lists significances of factors correlated with the response variable in question. 'Between-population effects' gives significances of differences in response values between the 16 populations. In the last column are listed habitat variables that correlated significantly with population mean values of the different response variables. Brackets indicate direction of effects, stars indicate level of significance (*: P<0.05, **: <0.01, ***: <0.001, ****: < 0.0001).

While the variation in structural size between populations was related to habitat composition, it was unrelated to demographic parameters. This suggests that the factors driving hare dynamics are related to the reproductive parameters and the survival of juveniles.

Skeletal size

Our result indicates that habitat composition, i.e. nutrition but also possibly shelter, restricts juvenile development during the growth period in some areas. The positive effect of grass leys on growth may be a favourable response to legumes; as such effects are well-documented in livestock (e.g. Pace *et al*. 2006). Roedenbeck & Voser (2008) found that edge habitat, especially in conjunction to forest, is the most important habitat factor for hares, and lack of shelter possibly limit juvenile survival in some habitats (Smith *et al*. 2004, Jennings *et al*. 2006). While we were able to explain observed size-differences between hare populations by differences in habitat composition, these size-differences had no effect on any of the investigated demographic parameters, and are unlikely to play any role in regulating dynamics.

Body mass and condition

The mean body mass of hares did not vary between populations despite significant size-differences; this was probably caused by significant variation in condition within the populations. The mean condition was positively affected by grass leys, which is not surprising, considering that these are refined for the purpose of stock feed. Hence, matters determining condition act on a small spatial scale, most likely reflecting local food availability.

Hare body mass is to a large extent (R^2=30-45%) a function of skeletal size. Discriminating size from condition is relevant when interpreting results from former studies; e.g. Frylestam (1980b) and Marboutin *et al*. (2003) found increased output of young by heavy females, but it is unclear whether this was caused by size or condition. Similar to our results, repeated means of free-ranging brown hares from Illumø showed that 66-75% of body mass variation from several recaptures related to the individual, indicating that individual mass is highly related to size regardless of seasonal weight variation (T. Wincentz, unpublished data).

Reproduction

The proportion of breeders and the mean number of scars in breeding females varied between populations, the latter also within populations. Where tree stands were abundant breeding females had fewer scars, while breeding females in semi-natural grassland had many placental scars. On the other hand, many females did not breed at all in semi-natural grasslands. Brown hares are income breeders (i.e. depend on a steady supply of high-quality food throughout the reproductive period), and impaired reproduction may indicate poor nutrition (Hackländer 2002a). Given the apparent positive effect of edges toward tree stands on growth, poor leveret production is hardly due to food shortage in these habitats. Rather, it is possible that females compensate for early litter loss in poor habitats, i.e. semi-natural grasslands, by producing additional litters. Semi-natural grasslands being suboptimal habitat could also explain the fact that more females were unproductive here. Lower condition and fewer lactating females in winter were found in pastural areas by Jennings *et al*. (2006). We found no relationship between crops containing legumes and female reproduction, while Stott & Wight (2004) found evidence of phytoestrogenic infertility presumably caused by grazing on legumes. The reproductive parameters estimated in this study (prevalence of breeding females, mean number of placental scars), are lower than found by Marboutin *et al.* (2003) but comparable to results from other studies (e.g. Frylestam 1980c, Bensinger *et al*. 2000, Hackländer *et al*. 2001), although the latter authors document age-effects on reproduction.

Age structure

When the reproductive output varies between populations we expect that the populations with the highest offspring production also have higher proportions of juveniles in autumn. Despite a considerable variation in the proportion of juveniles between populations, this variation was unrelated to the mean offspring production per female in the populations. This implies that aspects unrelated to reproduction per se, i.e. differences in juvenile survival or adult summer survival affect local recruitment rate. However, adult age varied insignificantly between populations, signifying that afflicted mortality on adults is similar between areas. Relatively constant adult survival is generally found in hares (e.g. Marboutin & Peroux 1995). The sensitivity of a population's growth rate to adult survival will vary with juvenile recruitment, being more sensitive toward this in areas with low recruitment (Marboutin & Peroux 1995). Leveret survival depends on climate (Andersen 1957, Häcklander, Arnold & Ruf 2002b, Smith *et al*. 2005) disease (e.g. Wibbelt & Frölich 2005) and predation (e.g. Erlinge *et al*. 1984). McLaren, Hutchings & Harris (1997) suggested that silage cutting could reduce leveret mortality, but we found no relationship between the juvenile proportion and grass leys or whole-crop. In our study the proportion of juveniles was unrelated to the fox density but increased with the soil productivity index, indicating higher juvenile survival in areas with good soil, possibly affecting food quality.

Conclusion

Our study shows that factors affecting adult hare survival are relatively unimportant in shaping population dynamic dissimilarities between areas; rather, reasons for variation in hare dynamics are found in the reproductive period. This includes factors that affect the ability of adult females to produce leverets and providing for them until independence, as well as the odds of weaned leverets surviving until adulthood. Our results suggest that the nutritional situation imposed on individuals during growth through habitat composition may directly affect adult body size and body condition, which in turn affect females' chances of being able to reproduce.

In order to impede the population decline in the hare, the present need is to identify causes of juvenile mortality, and to find ways to optimize nutritional conditions for hares during the reproductive season. Also, as populations with low recruitment are sensitive to maintenance, our findings stress the importance of minimizing adult mortality in areas where recruitment is low.

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Appendix 1. Numbers (N) of adult and juvenile hares sampled of each sex, mean adult body mass, mean zygomatic width of the skull (ZW), mean humerus length (HL), mean age of adults (determined from periosteal growth lines), number of adult uteri examined, the proportion of breeding females, and the mean number of placental scars in breeding female hares, in 16 Danish populations.

Population	Sex	Adult N	Juvenile N	Mean Body mass	Mean ZW	Mean HL	Mean adult age	Uteri examined	Proportion breeders	Mean no of scars
1	Male	15	16	3919	46.2	103.2	1.7			
1	Female	20	21	4271	47.0	104.3	1.3	17	0.76	8.1
2	Male	12	9	3864	47.3	101.5	1.9			
2	Female	22	14	4104	46.8	103.2	2.9	22	0.45	4.2
3	Male	5	0	3878	46.4	101.9	2.0			
3	Female	4	1	4296	46.9	102.9	2.7	4	1.00	12.0
4	Male	3	0	3938	46.0	99.7	2.0			
4	Female	4	2	3967	45.5	99.2	2.0	4	1.00	9.8
5	Male	3	0	3809	46.5	103.4	2.3			
5	Female	2	0	4192	45.3	102.4	1.0	2	1.00	11.0
6	Male	3	1	4139	48.4	106.7	3.0			
6	Female	2	0	4188	47.0	102.9	2.5	2	1.00	14.5
7	Male	0	1							
7	Female	4	1	4204	47.2	105.0	2.8	4	0.50	5.3
8	Male	8	3	3762	46.6	101.7	2.3			
8	Female	7	5	4134	46.1	104.1	2.0	7	0.43	3.3
9	Male	52	31	3948	47.1	102.9	1.9			
9	Female	76	29	4231	47.1	102.0	2.7	72	0.94	9.5
10	Male	3	0	3627	45.1	104.5	1.0			
10	Female	1	2	4462	46.7	105.6	3.0	1	1.00	9.0
11	Male	10	9	3578	45.2	100.8	1.7			
11	Female	4	6	3961	44.4	103.2	1.0	4	0.50	6.8
12	Male	6	9	3968	46.4	101.4	1.2			
12	Female	11	16	4115	46.6	101.1	3.4	11	0.82	7.9
13	Male	1	1	4146	44.5	107.1	1.0			
13	Female	4	0	4322	46.0	104.7	1.8	4	0.75	5.8
14	Male	2	4	3592	45.0	101.3	2.0			
14	Female	1	3	4213			3.0	1	1.00	*
15	Male	3	1	3689	45.5	100.5	1.3			
15	Female	2	0	4334	46.8	100.6	3.5	2	1.00	10.0
16	Male	4	0	3901	45.5	104.1				
16	Female	1	0	4550	47.5	105.1		1	1.00	9.0

Appendix 2. Habitat effects on mean humerus length (HL_{mean}) of brown hares in 16 Danish areas. All landscape parameters and agricultural categories were each correlated with HL_{mean}. Those variables that came out as significant after this initial round were then selected as potential predictors of HL_{mean} in a model with multiple predictors. The choice of the model best describing the variation in HL_{mean} was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004). The models were weighted by √(n). P is the significance of the model improvement calculated in a log likelihood test compared to the null-model.

Landscape parameters	**k**	**-2LL**	**AICc**	**D_AICc**	**wi**	**P**
Edge forest/arable	3	54.62	62.62	000	40%	0.03
Uncut grass	3	56.14	64.14	1.52	19%	0.08
Null-model	2	59.23	64.15	1.53	19%	
Edge arable	3	58.67	66.67	4.05	5%	
Heterogeneity	3	58.75	66.75	4.13	5%	
Soil productivity	3	59.04	67.04	4.42	4%	
Edge pasture	3	59.18	67.18	4.56	4%	
Arable	3	59.20	67.20	4.58	4%	

Agriculture categories	**k**	**-2LL**	**AICc**	**D_AICc**	**wi**	**P**
Grass ley	3	54.49	62.49	0.00	42%	0.03
Null-model	2	59.23	64.15	1.66	18%	
Whole-crop	3	57.58	65.58	3.09	9%	
Corn	3	58.13	66.13	3.64	7%	
Root crop	3	58.67	66.67	4.18	5%	
Winter cereal	3	58.72	66.72	4.23	5%	
AOR	3	58.88	66.88	4.39	5%	
Semi-natural grass	3	58.98	66.98	4.49	4%	
Spring cereal	3	59.04	67.04	4.55	4%	

Final model	**k**	**-2LL**	**AICc**	**D_AICc**	**wi**	**P**
Edge forest/arable + Grass ley	4	45.63	57.27	0.00	85%	0.001
Grass ley	3	54.49	62.49	5.22	6%	0.03
Edge forest/arable	3	54.62	62.62	5.35	6%	0.03
Null-model	2	59.23	64.15	6.89	3%	

Appendix 3. Habitat effects on population mean condition (size-adjusted body mass) of adult female (a) and adult male (b) brown hares from 16 Danish areas. All landscape parameters and agricultural categories were each correlated with size-adjusted mass. Those variables that came out as significant after this initial round were then selected as potential predictors of size-adjusted mass in a model with multiple predictors. The choice of the model best describing the variation in size-adjusted mass was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004). The models were weighted by √(n). P is the significance of the model improvement calculated in a log likelihood test compared to the null-model. The correlation with semi-natural grass was caused by a single population.

a) Landscape parameters	k	-2LL	AICc	D_AICc	wi	P
Null-model	2	183.29	188.21	0.00	31%	
Uncut grass	3	181.43	189.43	1.22	17%	
Edge forest/arable	3	181.79	189.79	1.58	14%	
Edge arable	3	182.70	190.7	2.49	9%	
Edge pasture	3	182.78	190.78	2.57	8%	
Heterogeneity	3	182.87	190.87	2.66	8%	
Arable	3	183.21	191.21	3.00	7%	
Soil productivity	3	183.25	191.25	3.04	7%	

Agriculture categories	k	-2LL	AICc	D_AICc	wi	P
Grass ley	3	177.91	185.91	0.00	46%	0.02
Semi-natural grass	3	180.50	188.50	2.59	13%	0.09
Null-model	2	183.29	188.21	2.30	15%	
Whole-crop	3	181.86	189.86	3.95	6%	
Root crop	3	182.16	190.16	4.25	6%	
Corn	3	182.35	190.35	4.44	5%	
Winter cereal	3	183.17	191.17	5.26	3%	
Spring cereal	3	183.29	191.29	5.38	3%	
AOR	3	183.29	191.29	5.38	3%	

Final model	k	-2LL	AIC	D_AIC	wi	P
Grass ley	3	177.91	185.91	0.00	76%	0.02
Null-model	2	183.29	188.21	2.30	24%	

b) Landscape parameters	k	-2LL	AICc	D_AICc	wi	P
Edge forest/arable	3	187.55	195.55	0	51%	0.02
Null-model	2	193.04	197.96	2.41	15%	
Edge arable	3	191.12	199.12	3.57	9%	
Arable	3	191.34	199.34	3.79	8%	
Uncutt grass	3	191.60	199.60	4.05	7%	
Soil productivity	3	192.35	200.35	4.80	5%	
Edge pasture	3	192.92	200.92	5.37	3%	
Heterogeneity	3	193.04	201.04	5.49	3%	

Agriculture categories	k	-2LL	AICc	D_AICc	wi	P
Null-model	2	193.04	197.96	0	20%	
Winter cereal	3	190.37	198.37	0.41	16%	
Spring cereal	3	190.37	198.37	0.41	16%	
AOR	3	190.66	198.66	0.70	14%	
Root crop	3	190.96	198.96	1.00	12%	
Corn	3	192.03	200.03	2.07	7%	
Whole-crop	3	192.17	200.17	2.21	6%	
Semi-natural grass	3	192.74	200.74	2.78	5%	
Grass ley	3	192.89	200.89	2.93	5%	

Final model	k	-2LL	AIC	D_AIC	wi	P
Edge forest/arable	3	187.55	185.91	0	77%	0.02
Null-model	2	193.04	188.21	2.30	23%	

Appendix 4. Habitat effects on fertility (presence or absence of placental scars) of adult female brown hares from 16 Danish areas. All landscape parameters and agricultural categories were each correlated with fertility. Those variables that came out as significant after this initial round were then selected as potential predictors of fertility in a model with multiple predictors. The choice of the model best describing the variation in fertility was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004). To achieve comparable AICc-values of different models, we applied same scaling factor of the deviance (to be as conservative as possible, we used the scaling factor of the null-model containing only an intercept).
P is the significance of the model improvement calculated in a log likelihood test compared to the null- model.

Landscape parameters	k	-2LL	AICc	D_AIC	wi
Null- model	1	57.09	59.39	0.00	27%
Soil productivity	2	55.73	60.66	1.28	14%
Edge pasture	2	55.96	60.88	1.50	13%
Edge forest/arable	2	56.14	61.06	1.68	12%
Uncut grass	2	56.49	61.41	2.03	10%
Edge arable	2	56.87	61.79	2.41	8%
Heterogeneity	2	57.02	61.94	2.56	8%
Arable	2	57.07	61.99	2.61	7%

Agriculture categories	k	-2LL	AICc	D_AIC	wi	P
Semi-natural grass	2	51.40	56.32	0.00	51%	0.02
Corn	2	54.22	59.14	2.82	12%	0.09
Null- model	1	57.09	59.38	3.05	11%	
Root crop	2	54.97	59.89	3.57	9%	
Spring cereal	2	56.49	61.41	5.09	4%	
AOR	2	56.84	61.76	5.44	3%	
Whole-crop	2	56.98	61.90	5.58	3%	
Grass ley	2	57.02	61.94	5.62	3%	
Winter cereal	2	57.05	61.97	5.65	3%	

Final model	k	-2LL	AICc	D_AIC	wi	P
Semi-natural grass	2	51.40	56.32	0.00	82%	0.02
Null- model	1	57.09	59.38	3.05	18%	

Appendix 5. Habitat effects on the mean number of scars in reproducing adult female brown hares from 16 Danish areas. All landscape parameters and agricultural categories were each correlated with the mean number of scars. Those variables that came out as significant were selected as potential predictors of mean number of scars in a model with multiple predictors. The choice of the model best describing the variation in mean number of scars was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004). The models were weighted by √(n). P is the significance of the model improvement calculated in a log likelihood test compared to the null-model.

Landscape parameters	k	-2LL	AICc	D_AIC	wi	P
Edge forest/arable	3	-46.01	-38.01	0.00	33%	0.04
Arable	3	-45.02	-37.02	0.99	20%	0.06
Null-model	2	-41.57	-36.65	1.36	17%	
Soil productivity	3	-43.10	-35.1	2.91	8%	
Heterogeneity	3	-42.76	-34.76	3.25	6%	
Edge arable	3	-42.69	-34.69	3.32	6%	
Uncut grass	3	-42.64	-34.64	3.37	6%	
Edge pasture	3	-41.59	-33.59	4.42	4%	

Agriculture categories	k	-2LL	AICc	D_AIC	wi	P
Semi-natural grass	3	-45.83	-37.83	0.00	34%	0.04
Null-model	2	-41.57	-36.65	1.18	19%	
Root crop	3	-43.74	-35.74	2.09	12%	
Spring cereal	3	-42.74	-34.74	3.09	7%	
Winter cereal	3	-42.41	-34.41	3.42	6%	
AOR	3	-42.39	-34.39	3.44	6%	
Whole-crop	3	-42.19	-34.19	3.64	6%	
Grass ley	3	-42.00	-34.00	3.83	5%	
Corn	3	-41.57	-33.57	4.26	4%	

Final model	k	-2LL	AICc	D_AIC	wi	P
Edge forest/arable	3	-46.01	-38.01	0.00	36%	0.04
Semi-natural grass	3	-45.83	-37.83	0.18	33%	0.04
Null-model	2	-41.57	-36.65	1.36	18%	
Edge forest/arable + Semi-natural grass	4	-47.77	-36.13	1.88	14%	

Appendix 6. Habitat effects on proportion of juveniles (j/n) in brown hares sampled at 16 Danish areas. All landscape parameters and agricultural categories were each correlated with the proportion of juveniles. Those variables that came out significant were then selected as potential predictors of proportion of juveniles in a model with multiple predictors. The choice of the model best describing the variation in proportion of juveniles was based on Akaike's information criterion corrected for small sample size (AICc) (Burnham & Anderson 2004). To achieve comparable AICc-values of different models, we applied same scaling factor of the deviance (to be as conservative as possible, we used the scaling factor of the null-model containing only an intercept). P is the significance of the model improvement calculated in a log-likelihood test compared to the null-model.

Landscape parameters	k	-2LL	AICc	D_AIC	wi	P
Soil productivity	2	212.00	216.92	0	39%	0.04
Uncutt grass	2	213.65	218.52	1.59	18%	
Null-model	1	216.23	218.57	1.65	17%	
Edge forest arable	2	215.63	220.55	3.63	6%	
Arable	2	216.18	221.10	4.18	5%	
Heterogeneity	2	216.19	221.11	4.19	5%	
Edge arable	2	216.22	221.14	4.22	5%	
Edge pasture	2	216.23	221.15	4.23	5%	

Agriculture categories	k	-2LL	AICc	D_AIC	wi
Null-model	1	216.23	218.52	0.00	29%
Semi-natural grass	2	215.35	220.27	1.76	12%
Corn	2	215.86	220.78	2.27	9%
Whole-crop	2	215.91	220.83	2.32	9%
Spring cereal	2	215.97	220.89	2.38	9%
Root crop	2	216.00	220.92	2.41	9%
AOR	2	216.00	220.92	2.41	9%
Grass ley	2	216.11	221.03	2.52	8%
Winter cereal	2	216.23	221.15	2.64	8%

Landscape parameters	k	-2LL	AICc	D_AIC	wi	P
Soil productivity	2	212.00	216.92	0.00	69%	0.04
no parameters	1	216.23	218.52	1.59	31%	

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Evidence of lowered recruitment rate in brown hare hunting bags through 50 years: effects on growth rate

Wincentz, T. & Sunde, P.

Manuscript

Evidence of lowered recruitment rate from brown hare hunting bags through 50 years: effects on growth rate

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Evidence of lowered recruitment rate from brown hare hunting bags through 50 years: effects on growth rate

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ABSTRACT

1. The game bags of brown hares in Denmark indicate long-term population declines. Changes in the agricultural habitat have been hypothesized to cause high leveret mortality (i.e. reduced recruitment) in brown hare populations. Bag records may provide information on changes in population parameters through time if information on e.g. sex, age and body mass of bagged individuals is recorded.

2. We investigate sex and age ratios (juvenile/adult) as well as body mass in Danish bag records from four decades and three habitat types, with the aim of identifying changes in these parameters through time, and to determine to what extent changes are confined to the agricultural habitat.

3. Juvenile recruitment declined during the past 50 years, varied spatially and declines were not confined to the agricultural habitat. Mass of juveniles and adult female hares was unchanged through time, indicative of unaltered body condition. Sex ratio of adults showed declines in male proportions.

4. Simple matrix population models based on the estimated annual survival for adult females at present and the realized fecundity (i.e. proportions of juvenile females to adult females in bag records) for the 1950s, 1980s, 1990s and since 2000, predicted the same population growth rates for each decade as was actually observed in the annual bag records.

5. Elasticity analysis showed that during the past 50 years, reduced recruitment rates have caused increasing sensitivity of the population growth rate to adult survival. Maintenance is essential where recruitment is low, and impact of adult mortality agents, including hunting, should be assessed and reduced in such areas. The model substantiates the supposition that declines in the Danish hare population are caused by reduced juvenile recruitment, and moreover, the model predicts further population decline.

Key words: body mass, Denmark, fecundity, *Lepus europaeus*, matrix model, sex ratio

INTRODUCTION

Agricultural intensification in Europe through the second half of the 20th century has caused a decline in several taxa related to farmland habitat, including plants, birds, insects and mammals (Robinson & Sutherland, 2002 and references therein). Population declines can be explained by a general reduction of suitable habitat in species which are associated with the agricultural habitat. Alternatively, the declining populations are reacting to changes in their surroundings which cause them to be unable to uphold self-reproducing populations. If this is the case we should observe changes within the dynamics of these populations (i.e. changes in reproductive parameters or vital rates) through time.

Hunting statistics provide data on population trends, but may also provide valuable information on changes in population dynamics through time if information on e.g. sex, age and body mass of bagged individuals is recorded.

Bag records indicate that brown hare (*Lepus europaeus*) populations in Europe have been declining since the 1960s (Tapper & Parsons, 1984; Smith, Vaughan & Harris, 2005). Today's intensively managed agricultural landscape has been suggested to cause a nutritional bottleneck for hares in summer (Frylestam, 1980; Tapper & Barnes, 1986; Hackländer, Arnold & Ruf, 2002a). The hare is an income breeder, and as poor food availability in summer coincides with the peak breeding period for hares, and an insufficient diet, may impair female reproductive performance (Hackländer, Tataruch & Ruf, 2002b). Examinations of placental scars do not indicate a impaired leveret production (Bensinger *et al*., 2000; Hackländer *et al*., 2001), although reproductive parameters appear to be related to local habitat composition (Wincentz *et al*., *unpublished*). Instead, several investigations point towards high leveret mortality being the cause of the decline (Hackländer *et al*., 2001, 2002a; Jennings *et al*., 2006; Marboutin *et al*., 2003; Wincentz *et al*., *unpublished*).

Three, not mutually exclusive, hypotheses could explain an impaired juvenile recruitment in the hare; First, poor food availability in agricultural areas during lactation or the earliest months of independency could cause low juvenile survival (Frylestam, 1980; Hackländer *et al*., 2002b), second; increased predation on juvenile hares primarily by foxes (*Vulpes vulpes*) due to a general increase in fox numbers, but also as a result of changed habitat configurations of the agricultural landscape (Mitchell-Jones *et al*., 1999; Panek, Kamieniarz & Bresinski, 2006; Schneider, 2001). Thirdly, climatic changes in the last half century may have affected hare dynamics as weather parameters such as precipitation influence hares (Andersen, 1957; Nyenhuis, 1995).

In this paper we investigate hunting records from four decades which include information on age (juvenile vs. adult) and sex composition, body mass of individuals and habitat type at the sampling locations. Our aims were to 1) evaluate whether the realized fecundity, indexed as ratio of juveniles to adults, has declined since the 1950s, 2) to determine whether any changes in juvenile adult ratios through time differs between biotope types and 3) to explore the development in brown hare sex ratios and body mass through time in different habitat types in relation to the effects these may have on recruitment rates. We relate all data to fox culls and climate indices.

We predict that the proportion of juveniles in autumn game bags have experienced a decline since the 1950s. If the agricultural intensification causes a reduced recruitment of hares, we expect a stronger decline in juvenile to adult ratio through time in agricultural areas than in other biotope types. If the present constitution of agricultural areas result in a suboptimal habitat for hares during lactation and growth compared to past times, we predict a reduction of hare body mass with time in agricultural areas, but not in other biotope types. As the survival rate of adult female hares is larger than for adult males (Wincentz *et al.*, *unpublished*) we expect a slightly skewed sex ratio in favour of females.

Finally, we analyze the consequences of changes in the proportion of juveniles on the hare populations' growth rate in a stage classified matrix model (Leslie, 1945, 1948; Caswell, 1989), using fixed contemporary values of survival rates, with the aim of determining a critical value for the recruitment of juvenile hares.

MATERIAL AND METHODS

Data

We used records from 265 customary autumn hunts all over Denmark during 1951-2006 (Table 1), containing information on sampling date and location, individual body mass (hares 1951-1993 were weighted in the field, after 2000 during autopsy following freezing), sex and age (1950s evaluated from Stroh sign (Stroh, 1931), 1980-90 evaluated from Stroh sign and visual examination of growth lines (Walhovd, 1966, Frylestam & Schantz, 1977), after 2000 from visual examination of the cleaned bones).

Decade	samples	mean n	range n	median n	Adults	Juveniles	Total hares	Mean sampling date	J/(A+J)
1950	157	59	1-418	22	4243	5015	9263	313	0.54
1980	18	56	4-216	34	530	476	1008	305	0.47
1990	49	60	1-192	49	1592	1352	2944	301	0.46
2000	41	13	1-74	7	304	201	514	295	0.40
Total	265	52	1-418	17	6669	7044	13729	308	0.51

Table 1. Overview of the number of brown hare samples, mean sample size (n), range, range and median of samples, and the distribution of adults and juveniles in 265 hunting journals from Denmark from four decades, which were analysed in the present paper. Mean sampling date is Julian Day.

According to the knowledge of the shooting locations each sample was assigned to one of three habitat types (agriculture, shore-meadows, forests), to one of two categories of land type: small islands (all hares on the island assumed to belong to the same population), or large islands/mainland as these were not significantly different. Sampling date was coded as "Julian Day", "Year" and "Decade" (1950, 1980, 1990 and 2000). For each sample, the proportion of juveniles, the sex ratio of juveniles and adults respectively and the mean body mass of adult males, adult females, juvenile males and juvenile females were calculated.

We used the winter state of the North Atlantic Oscillation (NAO: Hurrell, 1995) to index winter severity, and average winter NAO from the year and the two years before sampling, to index winter severity during an adult hares' life. The NAO for the months June-August indexed climatic conditions during the juveniles' growth period as well as effects of summer climate on adult body mass (condition). The summed precipitation in March-August inclusive was calculated (DMI, 2007), as precipitation has been shown to affect juvenile survival (Andersen, 1957; Nyenhuis, 1995).

Indices of national fox densities were obtained from the National Game Bag Record (http://vildtudbytte.dmu.dk/dmu5c.asp). Islands known to be without foxes were assigned a 0-value.

To be able to account for an increase in the bag of hares from the NW toward the SE in Denmark, probably reflecting similar declines in hare densities (Strandgaard & Asferg, 1980), we created an index of position along a NW-SE going gradient by placing a Cartesian coordinate system across the country in the direction SE-NW. The index yielded negative values for populations located in the SE and increasingly positive values for populations toward the NW.

Some of the investigated material has previously been examined by Hansen (1992) and Wincentz *et al*. (*unpublished*).

Statistical methods

Analyses were performed using the Statistical Analysis System (SAS) Enterprise Guide 4. Sample means were used as the statistical unit, and significance was set at $P<0.05$ (two-tailed tests throughout).

The proportion of juveniles and the sex ratios of adults and juveniles respectively were examined in generalized linear mixed models (GLIMMIX, Binomial distribution, log link). We corrected for overdispersion (random_residual_statement).

The mean body mass of adult males, adult females, juvenile males and juvenile females were examined in General linear mixed models (The MIXED Procedure). We weighted each sample mean (n=265) by the square root of sample size, because the SE of the individual population means are defined as $SD/\sqrt{n}$. Predictions of response variables were made for October 1st, which is the opening of the hunting season in Denmark.

The protocol of analysis (Table 2) was to; keep sampling year nested within decade as a random variable in the model at all times, and then:

A) Adjust for significant effects of land type (omitting small islands from the analysis if these deviated from the remaining samples), sampling date, gradient and decade. Main effects and the interaction terms were kept in the model if significant, while insignificant terms were removed manually.

B) Expansion of the final A model to include habitat. As we were not interested in differences between habitat types per se, but rather in differences interacting with habitat, we only included habitat interaction terms. We removed insignificant terms manually. To test the robustness of the B-model we hereafter entered:

C) the index of fox density, including all interaction terms, again removing insignificant terms manually and, finally,

D) entered the climate indices to the final B model along with interaction terms and only including significant terms. NAO-average was not entered in the models on juvenile sex ratio and body mass.

PROTOCOL	EXPLANATORY VARIABLES	DESCRIPTION	TYPE OF VARIABLE	VARIABLE LEVELS
Random	**Year(decade)**	Sampling year nested within sampling decade	-	Decades: 1950, 1980, 1990, 2000. Years: 1951-1956, 1958,1984-1986, 1988-1993,2003-2005
A	**LAND TYPE**	Small islands: all hares assumed to belong to one population. Main land/large island	categorical	Main land/ large island vs. small island
	SAMPLING DATE	Julian day	continuous	274-365
	GRADIENT	The position of a sampling location on a gradient going from North-West to the South-East, with negative values indicating locations more towards the SE.	categorical	Mean: 0.04, Median:0.72 (-6.88-10.02).
	DECADE	Decade in which a hare was culled	categorical	1950, 1980, 1990, 2000
B	**HABITAT***	Biotope type in which a hare was culled	categorical	agriculture, shore-meadows, man-made forests
C	**FOX TOTAL**	Total number of hares shot in Denmark in a given year	continuous	Mean: 37446 Median:41075 (0–53504)
D	**NAO-summer**	NAO in June-August	continuous	Mean: -0.09, Median:-0.17 (-2.46–2.10)
	NAO-winter	NAO (Dec.-March) starting from the year previous to the sampling year	continuous	Mean: 0.37, Median:0.18 (-2.52–5.08)
	NAO-average	Average (Dec.-March) NAO from the year a hare was sampled, and two years back	continuous	Mean: 0.63, Median:0.32 (-1.3 –3.36)
	PRECIPITATION	Summed amount of precipitation in the months March-August	continuous	Mean: 329, Median:335 (244–410)

Table 2. Description of the explanatory variables entered in the models on the dependent variables (proportion of juveniles, sex ratios, and mean body mass), order in which the variables were entered (A-D). Variable levels designate the used categories for categorical variables, and mean and median (range) for continuous variables. *We only examined habitat interaction terms.

The response variables were examined for correlation (Pearsons r).

Projection matrix model

The sensitivity of the populations' growth rate to changes in the realized female fecundity (estimated as the proportion of juvenile females to adult females in bags) in early November (mean sampling date) was examined using a stage classified matrix model with a time projection of 1 year (Figure 1a; Caswell, 1989). Due to the structure of our data we applied a two-stage model, only including juveniles and adults.

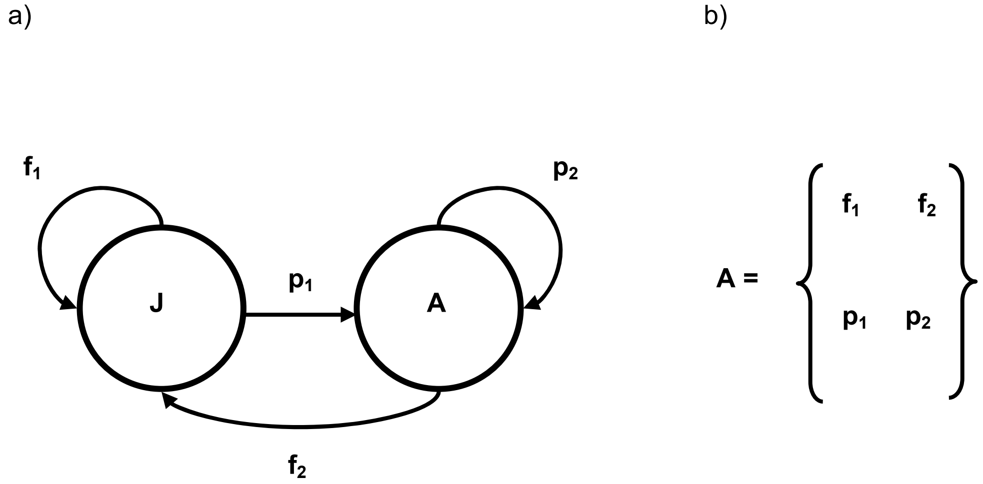


Figure 1. a) Stage classified (J=Juveniles, A=Adults) life cycle graph for hares on November 1st b) The matrix corresponding to the life cycle in a).

The asymptotic rate of population growth is given by the dominant eigenvalue (λ) of the matrix **A**.

Sensitivity analysis estimates the potential impact of a change in the vital parameters on λ, whereas elasticity analysis estimates the effect of a proportional change in the vital rates on population growth rate λ. Elasticities have the desirable property that they sum to 1.0, and thus represents the proportional contributions of each element in the matrix to λ.

In Denmark, only one in 100 juvenile females reproduce in their birth year (Wincentz, *unpublished data*) hence their contribution to annual growth is negligible and juvenile fecundity (f_1 in figure 1) was set to zero. The survival of juveniles to adults (p_1 in figure 1) does not differ from that of adults (Wincentz, *unpublished data*; Broekhuizen, 1979). The constant yearly survival rate for adult female hares (p_2 in figure 1) is 0.57 1 (Wincentz *et al.*, *unpublished*).

We entered the mean of the observed proportions of juvenile females to adult females in the bag records from each decade in the matrix (i.e. varied f_2 in figure 1b) and calculated the resulting growth rate (λ) and elasticity. The resulting growth rates were then compared to the observed development in hare bags in each sampling period. The projection matrix model was restricted to include data from arable habitat on mainland and large islands (i.e. omitting small islands).

RESULTS

Proportion of juveniles

A) The juvenile ratio was significantly higher on small islands than on the main land/large islands ($F_{1,255.5}$=49.7, P<0.0001); hence we excluded small islands from the analysis. The juvenile ratio decreased significantly with sampling date (Table 3). The proportion of juveniles in samples on October 1st was significantly larger in 1950, compared to the three recent decades which did not differ significantly from each other (Table 3, Figure 2).
The juveniles comprised significantly smaller proportions of the samples from the NW (Table 3). The decrease in the proportion of juveniles with sampling date interacted with the gradient, with the juvenile proportion being higher in the SE on October 1st, but then decreasing at a higher rate than in the NW (Table 3).

	Type I			Type III		
Effect on prop juv	**DF**	**F**	**P**	**DF**	**F**	**P**
JulDay	1,77.8	3.5	0.064	1,183.4	0.01	0.91
Decade	3,18.7	4.7	0.013	3,180.5	4.1	0.0077
Gradient	1,140.2	8.4	0.0044	1,185	5.3	0.023
JulDay*Gradient	1,184.8	5.9	0.016	1,184.5	5.0	0.027
JulDay*Decade	3,175.2	2.7	0.045	3,182.2	3.7	0.012
Habitat*Decade	6,177.9	6.1	<.0001	6,176.8	6.0	<.0001

Table 3. The effect of sampling date, sampling decade, geographic gradient (NW-SE) and habitat type on the proportion of juveniles in samples of brown hares collected through four decades in Denmark.

B) We found a significant habitat*decade interaction as the proportion of juveniles decreased significantly with time in arable areas as well as in shore meadows (Table 3, Figure 3).

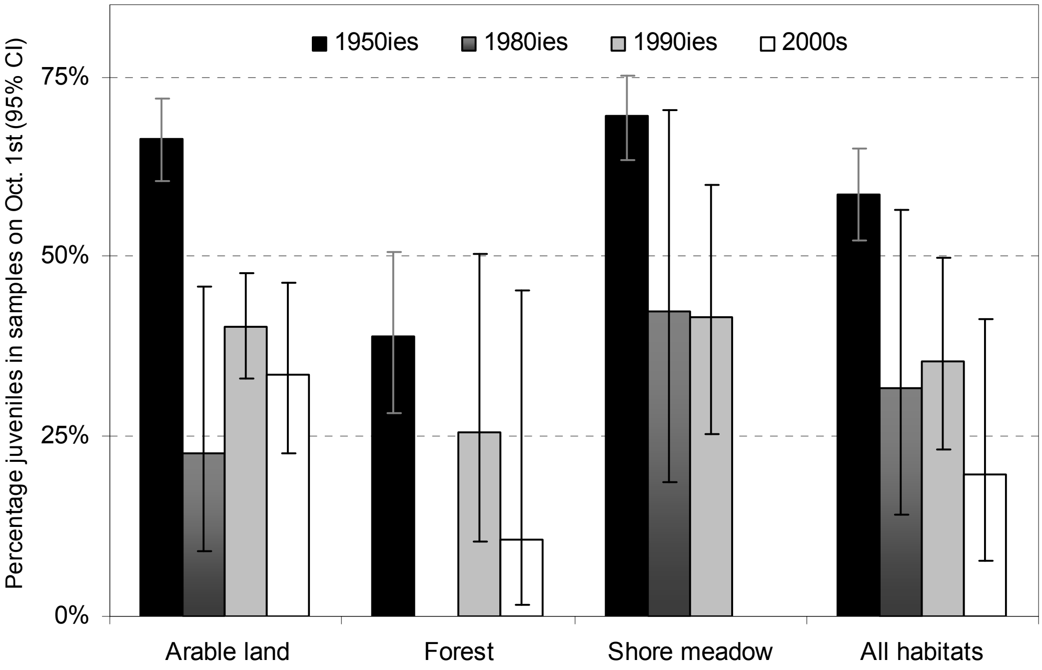


Figure 2. The percentage of juvenile hares in samples on October 1^{st} in four decades and in three habitat types (+/- 95%CI) (Least-square-means adjusted for effects of sampling date, decade, gradient and interaction terms from model).

C+D) The results were robust even when entering effects of fox or yearly variation in climate indices, while these factors themselves were not significant (all P>0.10).

Juvenile sex ratios

A) The proportion of juvenile males to juvenile females decreased with increasing sampling date ($F_{1,213.8}$=7.9, P=0.0056), but did not vary significantly between land types ($F_{1,209}$=0.1, P=0.71) or with gradient ($F_{1,214.5}$=0.1, P=0.72). When testing the effect of decade on the juvenile sex ratio, we were forced to also enter year as a systematic variable in order to make the model converge. There was no significant effect of decade ($F_{1,198}$=0.4, P=0.53) while the sex ratio of juveniles varied between years ($F_{16,198}$=1.7, P=0.048). The effect of year on the sex ratio of juveniles remained significant when entering the habitat*decade interaction ($F_{8,179}$=0.5, P=0.89), fox (total) ($F_{1,198}$=0.9, P=0.35) or the fox*habitat ($F_{3,184}$=0.5, P=0.71). D) When analyzing the effects of climate indices, year was removed from the base model and instead entered as a random variable (leaving only sampling date in the base model). NAO-summer had a marginally positive effect on male ratio ($F_{1,18.5}$=4.6, P=0.046), while the remaining models did not converge.

Juvenile body mass

A) + B) Juvenile body mass was lower on small islands for both males ($F_{1,163}$=16.7, P<0.0001) and females ($F_{1,172}$=22.3, P<0.0001), hence island hares were omitted from this analysis. The mean body mass of all juveniles increased significantly with sampling date (males: $F_{1,128}$=11.9, P=0.0008; females: $F_{1,116}$=14.6, P=0.0002).

After correcting for the effect of sampling date, the body mass of juvenile females did not vary with gradient ($F_{1,134}$=0.4, P=0.54), between decades ($F_{3,127}$=1.7, P=0.16) or habitat*decade types ($F_{8,39.3}$=1.1, P=0.31). The index of fox(total) density had a significantly negative effect on the body mass of juvenile females ($F_{1,74.9}$=8.9, P=0.0039), while none of the climate indices affected juvenile female mass (all P>0.10).

After correcting for the effect of sampling date, juvenile males from the NW were significantly lighter than juvenile males in SE ($F_{1,128}$=11.9, P=0.0008, Figure 5). We found a significant habitat*decade interaction affecting the body mass of juvenile males which was caused by a significant decrease in body mass in forests with time ($F_{8,109}$=2.1, P=0.044). The effect of the habitat*decade interaction on mass of juvenile males was robust to fox(total) which in itself had no effect on mass ($F_{1,108}$=0.04, P=0.85). The habitat*decade interaction was not robust to the entered climate indices (all P>0.18) while the effect of gradient remained significant.

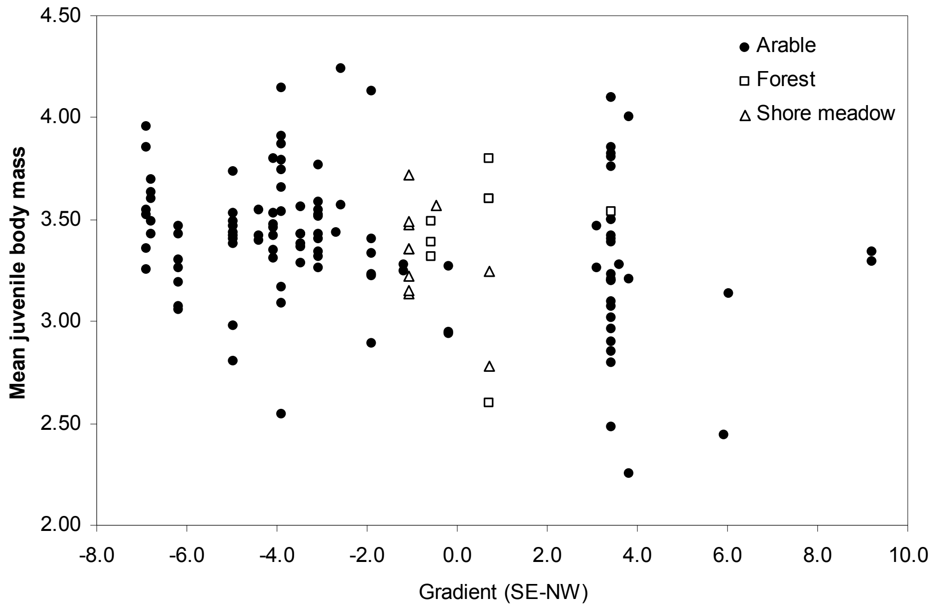


Figure 5. Mean body mass of juvenile male hares from 130 sampling sites, as a function of the sampling sites' position along a NW-SE going cartographic gradient (Least-square-means correcting for effects of sampling date).

Adult sex ratio

A) There were no effects of sampling date ($F_{1,192.6}$=1.1, P=0.29), land type ($F_{1,233.1}$=1.1, P=0.29) or gradient ($F_{1,235}$=0.1, P=0.76) on the sex ratio of adults. The sex ratio all samples combined varied between decades. Fewer males were found in samples from the 1950s compared to 1980 and 1990, and with more males in 2000 compared to 1980 ($F_{3,233}$=3.5, P=0.016). The interaction between decade and habitat was not significant ($F_{7,215}$=1.8, P=0.083), however the proportion of males in shore-meadows increased significantly from 1950 to 1990 and 2000, while the proportion of males had decreased significantly in arable land in 2000 compared to the three previous decades (Figure 3).

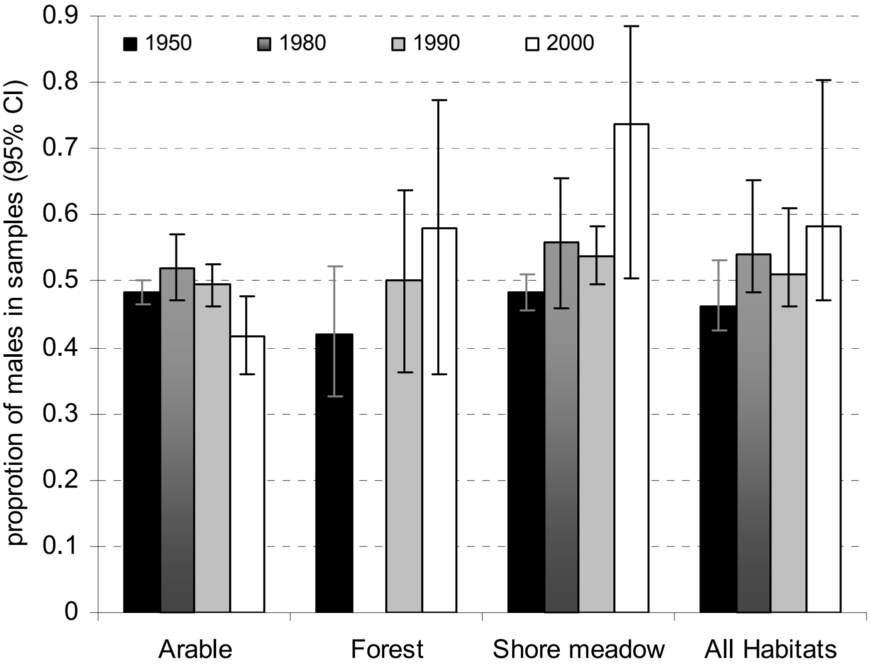


Figure 3. Proportion of males in hare samples on October 1st from three habitat types in four different decades (ls-means)

C+D) The effect of decade persisted after including index of fox density and some climate variables (NAO-summer, precipitation) while these factors were not themselves significant. However, when entering the climate variables NAO-winter and NAO-average, the effect of decade was no longer significant (neither was the added terms).

Adult body mass

A) Hares from small islands were excluded from the models on mean adult body mass as they were significantly lighter than the remaining hares (Males: $F_{1,185}$=53.6, P<0.0001; Females: $F_{1,188}$=27.5, P<0.0001). The body mass of adult females decreased significantly with sampling date while this decrease was not significant for males (females: $F_{1,78.6}$=5.2, P=0.025; males $F_{1,152}$=0.5, P=0.50). The adult body mass was not affected by gradient (Females: $F_{1,134}$=2.6, P=0.11; Males $F_{1,152}$=0.1, P=0.80), while body mass differed significantly between decades for both sexes (females: $F_{3,20.2}$=3.5, P=0.033; males: $F_{3,5.99}$=4.9, P=0.048).

B) Habitat interacted with decade in predicting body masses of both males and females (males: $F_{5,131}$=2.9, P=0.017; females: $F_{5,134}$=6.9, P<0.0001), as in arable land, adult male body mass was significantly lower in 1980 and 2000 compared to 1950, while female body mass in arable land was significantly lower in 1980 than in the remaining decades (Figure 4). There were no significant differences in body weights between decades within shore-meadows or man-made forests.

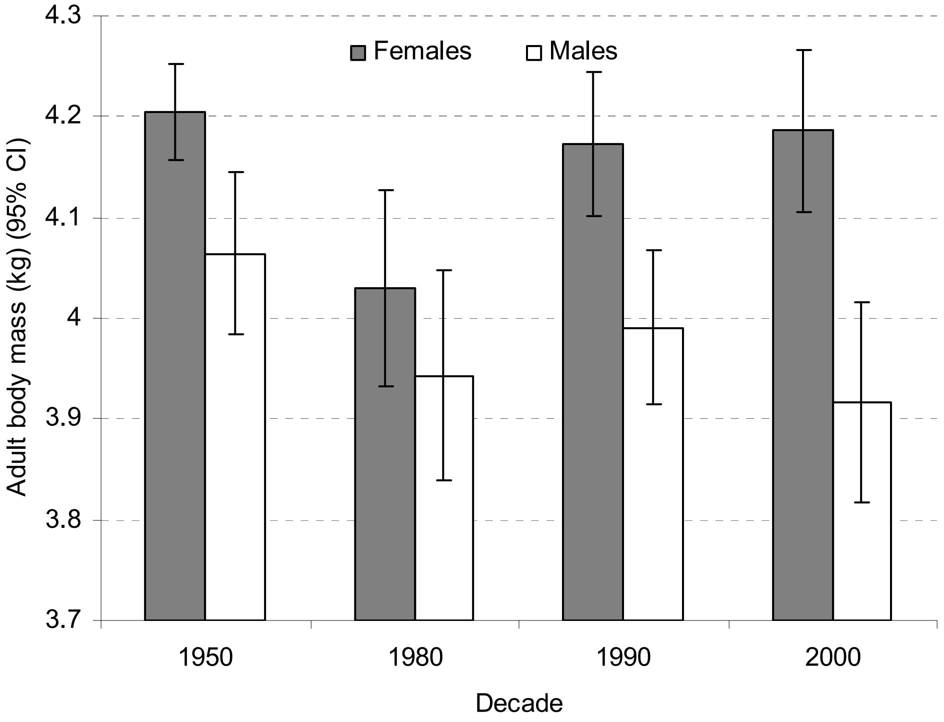


Figure 4. Mean adult body mass of female (ls-means adjusted for sampling date) and male hares in arable land through four decades.

The effect of decade*habitat was not weakened by entering fox or climate variables for either sex. However NAO-average had a negative effect on male body mass ($F_{1,133}$=4.9, P=0.029).

The inter-relationships between response variables are shown in Table 4.

	J/N	Sex ratio Adults	Sex ratio juveniles	Adult male mass	Adult female mass	Juvenile male mass
Sex ratio adults	-0.04	-	-	-	-	-
Sex ratio juveniles	**0.15** *	0.04	-	-	-	-
Adult male mass	-0.02	**-0.22** **	-0.14	-	-	-
Adult female mass	-0.05	**-0.19** **	0.12	**0.22** **	-	-
Juvenile male mass	-0.04	-0.13	**-0.20** **	**0.35** ***	0.06	-
Juvenile female mass	0.001	**-0.19** *	-0.14	**0.34** ***	**0.20** *	**0.40** ***

Table 4. Interrelationships (Pearson's r, n=121-238) between the response variables analyzed in the present study.

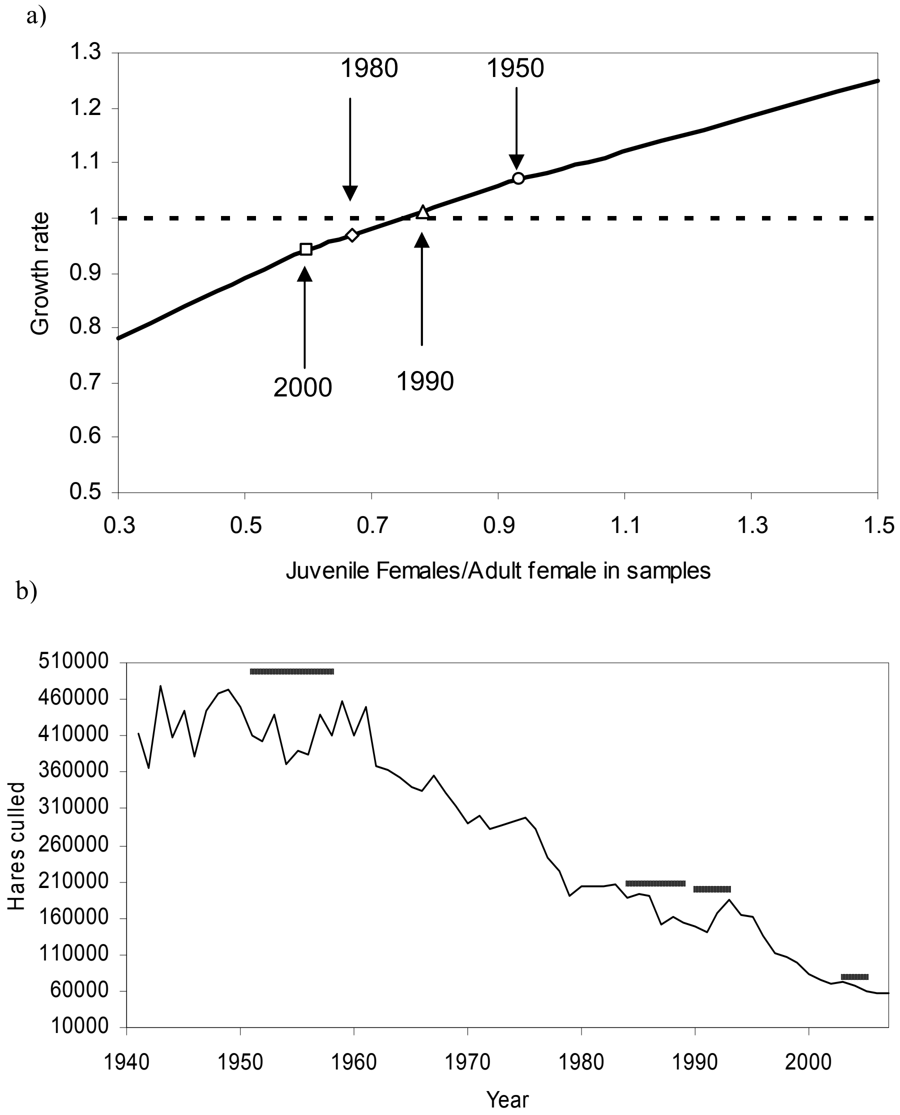
Projection matrix model

The mean ratio of juvenile females to adult females in arable samples ranged between 0.93 in the 1950s to 0.60 in the 2000s, and with a constant survival rate of 0.57, the populations were stable (λ =1) with a realized fecundity of 0.76 (Table 6, Figure 7a).

Decade	Median sampling day	Adult Females	Juvenile Females	Ratio juv/ad F	95% CI	λ
1950	321	1164	1088	0.93	0.86-1.02	1.07
1980	304	136	91	0.67	0.51-0.87	0.97
1990	280	353	276	0.78	0.67-0.92	1.01
2000	292	147	88	0.60	0.46-0.78	0.94

Table 5. Median sampling day (Julian day), total number of adult and juvenile females, ratio of juvenile to adult females (realized fecundity) in samples from arable habitat and the resulting growth rate (λ) from matrix projections at a constant adult and juvenile survival rate of 0.57.

The present game bag is approximately 14% of that in 1960 (57.000/400.000), equalling a constant annual λ of 0.96.



Figur 6. a): The development in the Danish hare bag (culls) from 1941 to 2006. The horizontal lines indicate sampling periods. B): Sensitivity of the population growth rate to the fecundity of adult females; the realized fecundity in the four sampling periods are indicated. The dotted line marks the baseline of $\lambda=1$.

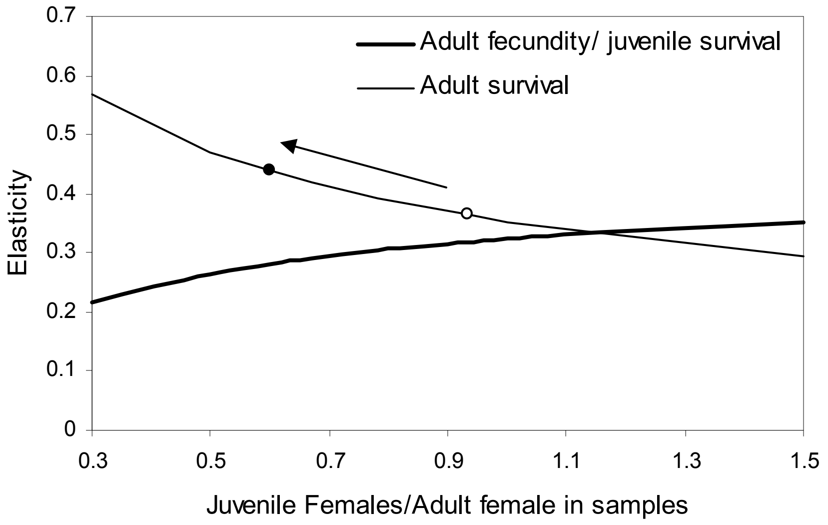


Figure 7. Elasticity of λ to the changes in the realized adult fecundity, juvenile survival of adult survival. Circles denote the observed ratio of juvenile to adult females in samples (Open circle 1950s, closed circle 2000s. Arrow indicates the direction of development through time.

The elasticity of λ to the survival of adult females increases as the proportion of juveniles declines (Figure 7).

DISCUSSION

In the 1950s Stroh sign was used to age the hares. Stroh sign discriminates juveniles from adults through October, with 84% juveniles being correctly identified through November, but in few juveniles the Stroh sign disappears at 4 months of age, thereby underestimating the proportion of juveniles (Suchentrunk, Willing & Hartl, 1991). The juvenile ratio may therefore have been even higher in the samples from the 1950s. But as 73% of leverets are born from early May to late July (Hansen, 1992), the majority of leverets will be discernable in samples from October onwards. More accurate ageing in the three recent decades were done by examining visually, the symfyses (Walhovd, 1966).

Changes through time

Juveniles

The proportion of juvenile hares in autumn game bags has declined since the 1950s.
The decline was not confined to the agricultural habitat as expected in the instance that population declined is connected to agricultural intensification. The reduced recruitment was already apparent by the 1980s, and has not significantly advanced since. As also documented

by Wincentz *et al*. (*unpublished*) recruitment was lower in north-western areas, while it did not appear to be affected by precipitation or fox abundance.

If food availability is compromised during summer, or if the survival of the different litters during the reproductive season had changed, we would expect body masses of juveniles to change through time, but no consistent change in juvenile body mass between decades were found. However, male juveniles were significantly lighter in the NW, where recruitment is low. This possibly appoints the poor recruitment in north-western areas to poor body condition, although the same pattern was not apparent for female juveniles.

The juvenile sex ratio varied between years and was possibly affected by climate in summer, but it did not vary systematically between decades. Juvenile males comprised a larger proportion in samples early in the season, as documented in other studies. That female and male juveniles should be differentially affected be external factors seems unlikely, and an analysis for the sexes combined will be carried out in the future.

Adults

As female hares live longer than males Wincentz *et al*. (*unpublished*), an increased adult survival would create a pronounced skew in favour of females in the sex ratio. This was in fact observed in agricultural areas. A general increased survival could explain the relatively high yearly survival rate of females found by Wincentz *et al*. (*unpublished*), and could be caused by relieved hunting pressure, e.g. due to public awareness of the declining populations. However, the matrix model showed that the current survival rates of females in combination with the realized recruitment yielded realistic growth rates.

An alternative explanation of the changing sex ratio could be reduced male survival perhaps caused by increased mortality during dispersal in arable areas. Males disperse more often than females (Bray *et al*., 2007), and genetic studies from Denmark show, that exchange between populations is limited possibly as a result of fragmentation (Andersen *et al*., *accepted*). Interestingly the development in sex ratios seemed to go in opposite directions between agricultural and other habitat types.

If the agricultural habitat is suboptimal feeding habitat for hares at present compared to the past, a decrease in either size or condition could result (Wincentz *et al*, *unpublished*). While body mass of male hares significantly decreased in the studied period, this was not apparent for females, who with regard to recruitment are the most interesting.

Without having a proper explanation the decreased male body mass should possibly be seen in connection with the decrease in the overall male proportions in samples.

Projection matrix model

Our aim was to investigate whether reduced recruitment could explain the observed declines in the Danish hare populations. In the history of keeping, the hare bags have shown marked yearly variation, but were stable until the 1960s when declines started. In the sampling periods in the 1980s, and after year 2000, the game bag records indicated population declines, while population increases were indicated in the early 1990s probably due to an outbreak of sarcoptic mange among the red foxes. These population trends in game bags in the four decades were all reflected in the estimated growth rates based on the observed realized fecundity of adult females, and with present adult survival rates. This is a very strong indication that the reduction in juvenile recruitment by the onset of the hunting season is a major driver in the population decrease.

Population growth rate shows variable sensitivity to recruitment, depending on population age structure (Marboutin & Peroux, 1995; Marboutin *et al.*, 2003). Elasticity analysis showed that during the past 50 years, the reduced recruitment rates have caused the sensitivity of the population growth rate to adult survival to increase. In populations with low recruitment the growth rate is most sensitive to maintenance of the adult breeding stock, whereas recruitment rate is less important in populations with high maintenance (Marboutin & Peroux, 1995). As the recruitment varied spatially in Denmark, it is likely, that management strategies must be tailored to fit local dynamics, with emphasis on protecting the breeding stock in areas with low recruitment. This implies determining the impact of hunting in these areas.

CONCLUSION

The proportions of juveniles in game bags dropped significantly between the 1950s when hare populations where stable and the 1980s and later when hare populations according to bag size decreased with approximately 5% annually, and not only in agricultural areas. Simple matrix population models based on the estimated annual survival for adult females at present and the estimated fecundity for 1950s, 1980s, 1990s and since 2000, predicted the same population growth rates for each decade as was actually observed in the bag records. The model substantiates the hypothesis that declines in the Danish hare population are caused by reduced juvenile recruitment. The present parameters of survival and recruitment predict further declines.

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III


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Point transect counts of hares: Distance sampling in partially visible habitats

Noer, H. & Wincentz, T.

Manuscript

Point transect counts of hares: Distance sampling in partially visible habitats

By

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Summary

1. Use of counts for estimates of animal numbers or densities tacitly assumes that all individuals are seen. By proposing a method for estimating the proportion actually detected, the theory of distance sampling offers a way to correct for unseen individuals.
2. Standard theory assumes that all habitat within some truncation distance w can be observed. For point transect counts of hares in four farmland areas, restricted observability enforced selection of points where only 30-50% of the area within 300 m could be seen. We assessed the implications by extending the theory for half-normal detection functions into a model correcting for blind areas.
3. This correction increased estimated proportions seen by 50-250% and density estimates by 10-70%. All variances of estimates increased, though coefficients of variation were basically unchanged. Depending on the visible proportion of the area, 30-100% increases of sample sizes would be needed to achieve a precision equal to a situation with 100% observability.
4. After correction, estimated scale parameters differed significantly, and strong support was found for pooling data sets into two pairs. Estimated proportions seen were c. 18% in two areas and 40% in the others. Behavioural differences such as road avoidance and/or reactions to predators may be the explanation of this difference.
5. *Synthesis and applications*. Spotlight counts are a simple method for obtaining information of e.g. hare densities, but the interpretation of the data is highly complex. For some habitat types, a method correcting for unseen subareas will undoubtedly be necessary in order to achieve valid estimates. Further, in heterogeneous environments the assumption of individuals distributing randomly over the observable area may be violated. If so, care must be taken when fitting detection functions. Finally, differences in detection probabilities raise serious issues with respect to the point counts used in many monitoring programmes.

Key-words: Distance sampling, density, brown hare, *Lepus europaeus*

Introduction

Visual counts are presumably the simplest and oldest method to get information on animal numbers or densities, but a major intrinsic problem is that not all individuals may be seen. The theory of distance sampling (Buckland *et al.* 2001) proposes a method to deal with this problem from measurements of distances to detected individuals. Assuming that the unseen proportion will increase with distance x from the observer, a socalled detection function g(x) can be fitted to the data, and further assuming that the objects counted are evenly distributed over the observed area, the overall proportion seen - P_a - can be estimated by means of the detection function. Once an estimate of P_a is available, estimation of total numbers present from numbers counted is straightforward.

Distance sampling offers an ingenious method to assess the proportion detected, and not surprisingly its use is becoming more widespread. However, while it has become a standard tool for e.g. ship transect counts of marine birds and mammals, there are still fields where it is not widely implemented. As a result, the practical experience needed for evaluating model performance is lacking.

Though still being common, the brown hare *Lepus europaeus* has decreased in numbers throughout Europe over the past 50 years (Vaughan *et al.* 2003 and references therein). Evidence for these decreases is mainly based on hunting statistics, while actual density estimates are available from a few older capture-recapture studies (Abildgaard, Andersen & Barndorff-Nielsen 1972, Marboutin & Péroux 1995) and a number of studies based on spotlight counts (Verheyden 1991, Péroux *et al.* 1997, Langbein *et al.* 1999). Distance sampling methods have been used for diurnal counts of brown hares by Hutchings & Harris (1996), and for spotlight counts by Peroux *et al.* (1997) and – in a study of Irish hares (*L. timidus hibernicus*) - by Reid *et al.* (2007).

In order to assess the feasibility of estimating densities of hares from spotlight counts, one of us (TLW) carried out a study in 2005 and 2006. However, a number of problems in applying standard distance sampling to this habitat type arose already during planning. Use of the more efficient line transect protocols (Buckland *et al.* 2001, Ruette, Stahl & Albaret 2003) was prevented by the terrain, and counts had to be based on point transects (Buckland *et al.* 2001). Moreover, while standard theory assumes that all habitat around a point is fully visible, the structure of these habitats interfered strongly with observability. The standard remedial action - to leave out complete angular sections ψ from full circles (Buckland *et al.* 2001, Reid *et al.* 2007) – was not applicable. Unless very small truncation distances were chosen, too much area that actually could be seen would have been excluded.

Use of standard methodology in such cases was discussed by Buckland *et al.* (2001). By ignoring unobservable subareas, the resulting 'detection' function g(x) will be composite – say g(x)e(x), where e(x) reflects the decreasing proportion of the total area that can be seen. Thus, the detection function will reflect not only the fall-off in detectability with distance, but also the decreasing proportion of individuals that are potentially detectable.

Although noting that in some cases this effect might be sufficiently extreme to render such modelling unreliable, the authors stressed that the circumstances under which such modifications were likely to prove worthwhile would hardly be common (Buckland *et al.* 2001). In the absence of real data, these statements were necessarily very general. Therefore, we could not be certain whether the habitats in this study were 'sufficiently extreme'. To evaluate this, standard methodology had to be extended to include a correction for blind areas.

The purpose of this paper is to present a model extending standard distance sampling estimates for point transect counts and half-normal detection probabilities to correct for unseen parts of the habitat, and to compare the results with estimates resulting from the standard method in order to assess the extent to which application of standard methodology will affect estimated densities.

Material and methods

COUNT AREAS

Four typical farmland areas in Denmark were censused: a 100 km^2 area in Himmerland (HL, center 56°52′ N, 9°20′ E), a 50 km^2 area around the estate of Kalø (KA, 56°20′ N, 10°30′ E), and two areas of c. 20 and 10 km^2 on the island of Lolland (LO1, 54°55′ N, 11°05′ E, and LO2, 54°42′ N, 11°20′ E). Distance between the two former was c. 70 km and between the two latter c. 30 km.

Habitats consisted mainly of arable land, with typical NW-European crops. All areas are heavily infrastructured, and the density of roads ensures that nearly all habitat in question is less than 500 m from the nearest one. 'Roads' ranged from larger ones with fast and dense traffic to unpaved backcountry roads.

Point transect counts were the only realistic option (Wincentz & Noer *unpublished*). Inspection of maps and localities revealed that buildings, hedgerows, undulating terrains and in some cases also crops would prevent hares from being seen in large fractions of the areas around points. Thus, points had to be placed where the view was not too restricted, providing a fair coverage of count areas. In the final planning, 54 points in HL, 27 at KA, and subsequently 12 and 8 at LO1 and LO2 were selected (Tab. 1).

COUNTS AND MEASUREMENTS OF DISTANCE

Counts were conducted from cars with roof-mounted spotlights during spring and autumn periods in 2005 and 2006. Most measurements were made late March-April, but measurements from August-September were included since no differences between spring and autumn could be detected. Counts were conducted on nights with suitable weather conditions, from 30 min to 4 hours after sunset, when foraging activity in fields is assumed to be highest (cf. Verheyden 1991). Overall, 9 counts were conducted in HL and KA, and 4 at LO1 and LO2 (Tab. 1). Further details of counting protocols and techniques are given by Wincentz & Noer (*unpublished*).

Table 1.

Count area	Counts	Points	N_p	N
VH	9	54	486	57
KA	9	27	243	57
LO1	4	12	48	63
LO2	4	8	32	52
Total	26	101	809	229

Table 1. Survey of the material collected for each count area, with respectively number of counts, number of count points, total number of points counted (N_p) during surveys, and total number of distances measured (N).

Excepting a few individuals very close to the observer(s), all individuals were detected from reflection of light from eyes. Detected individuals usually froze in a crouching posture when illuminated. A few individuals moved slowly, and for these the distance to the point where they were first detected was used. None were seen running. At HL and KA, all observations were of single individuals. At LO1 and LO2, some observations were made of hares either with leverets or too close to rule out gregariousness. For the purpose of this paper, these have all been treated as a single observation (= the individual first detected), and the sample size accordingly was reduced from 136 to 115. All distances were measured in m by binocular laser range finders (Leica GEOVID 7x42 BDA).

VISIBLE SUBAREAS AND FRACTIONS

Visible areas were mapped on a GIS-platform (Arcview), followed by verification of groundtruth. Visible proportions were measured in 10 m segments, and since no observations were made of hares more than 300 m from the observer(s) observations were truncated at that distance. The potential area covered from a point would thus be $\pi 300^2 = 282{,}743$ m^2.

This mapping confirmed that the configuration of observable areas was complicated (Fig. 1). It further confirmed that limiting counted areas to angular sections ψ, for which all area to the truncation distance could be seen,would have led to exclusion of major parts of the observable habitat (cf. Fig. 1).

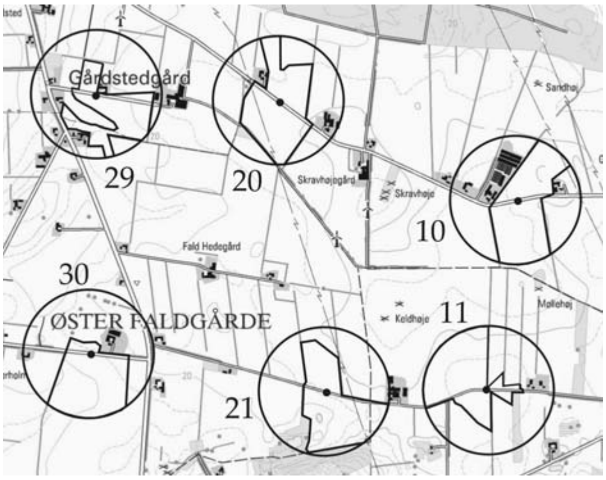

Fig. 1. Six of the 54 count points in HL, truncated at 300 m.Visible subareas are indicated by heavy lines.

ESTIMATION OF DETECTION PROBABILITIES

Preliminary fitting clearly indicated that the half-normal detection function (Buckland *et al.* 2001) was a suitable choice of key-function. For a half-normal detection function g(x) with scale parameter σ^2, the probability density function of observed distances from point transects is $f(r) = 2 \pi r\, g(r) / \nu$. For data truncated at distance *w*, the correction factor will be $\nu = 2 \pi \sigma^2 (1 - \exp\{- w^2/2\sigma^2\})$. To simplify notation, we write g(x) instead of $\exp\{- x^2 / (2\sigma^2) \}$ in the following.

For data grouped into intervals and truncated at distance *w*, the probability that the distance r of a detected hare falls in the interval [a ; b [is

Eqn 1 $$P\{a \le r < b\} = (g(a) - g(b)) / (1 - g(w))$$

If intervals of equal width are chosen and scaled to a width of one measuring unit (i.e. *w* is an integer), and N measurements indexed by i (= 1,2,…,N), the i'th distance – say h(i) - will belong to the interval [h(i)-1; h(i) [, (h(i) = 1, 2, …, *w*). Disregarding multinomial coefficients, the Likelihood-function of N observations will be

Eqn 2 $$L(\sigma^2) = \prod_{i=1}^{N} (g(h(i)-1) - g(h(i))) / (1 - g(w))$$

For count points with blind areas, however, only a fraction R_h of the total area $\pi (h^2 - (h-1)^2)$ of the interval [h-1;h [is visible. This fraction, moreover, will differ between points (cf. Fig. 1). Though conceptually simple, this extension of the standard model renders indexing tedious. If N_p points are indexed by j (j = 1, 2, 3, …, N_p), the probability that the i'th observed distance, made at point j(i), falls in the interval [h(i)-1 ; h(i)[will be

Eqn 3 $$P\{ h(i)-1 \le r < h(i) \} = R_{j(i),h(i)} (g(h(i)-1) - g(h(i))) / \nu_{j(i)} ,$$

Where for any point j

Eqn 4 $$\nu_j = \sum_{h=1}^{w} R_{j,h} (g(h-1) - g(h)) .$$

Once more disregarding multinomial coefficients, the Likelihood-function for N measurements can be written as

$$\text{Eqn 5} \qquad L(\sigma^2) \qquad = \qquad \prod_{i=1}^{N} R_{j(i),h(i)} \, (g(h(i-1)) - g(h(i))) \, / \, \nu_{j(i)} \qquad ,$$

Maximum-likehood estimators of σ^2 from Eqn 5 are derived in Appendix S1 (see the supplementary material). Solutions were found numerically by Newton-Raphson iteration, and variances were estimated by $\mathrm{var}(\sigma^2) = -[d^2 (\log L(\sigma^2)) / d\sigma^2]^{-1}$ (e.g. Seber 1973).

We used Monte-Carlo simulations to check whether these modifications changed the basic statistical properties of estimators. Estimates were still roughly unbiased, and the overall characteristics of their distributions did not change, though skewness became more pronounced. However, variances of estimates increased somewhat after correction for blind areas, i.e. for the same number of measured distances. To reduce variance to the level of the standard situation, sample sizes would have to be increased 30-100%, depending on the percentage of the total areal that could be covered. Examples of these simulated results are given in Appendix S2 (see supplementary material).

ESTIMATION OF DENSITIES

The ultimate purpose of distance sampling is to estimate density from numbers counted, by providing an estimate of the proportion of individuals that is detected, P_a . Given that, when N_{obs} individuals were counted, estimation of the number present (whether detected or not) follows directly, $N = N_{obs} / P_a$. When a detection function g(r) has been chosen, $P_a = \int_0^w g(r)u(r)dr$, where u(r) is the distribution of individuals over distance (Buckland *et al.* 2001).

Assuming that individuals distribute randomly, the overall distribution u(r) is simply determined by the visible area. Therefore, calculations can be averaged over points (see Buckland *et al.* 2001). We write $R_h = \sum_j R_{j,h} / N_p$ for the average proportion of habitat visible in the interval [h-1; h [. In this case, $\nu = \Sigma R_h(g(h-1) - g(h))$.

Because for point transects any distance interval [h-1 ; h[actually represents an annulus, the probability of detecting an individual present at an arbitrary point within this annulus is affected by a larger proportion of the area being close to the outer rim (distance = h) than to the inner (distance = h-1). Thus, the expected probability P_h of detection in the interval [h-1;h[is

$$\text{Eqn 6} \qquad P_h = [\pi(h^2 - (h-1)^2)]^{-1} \int_{h-1}^{h} 2\pi\, r \exp\{-r^2/(2\sigma^2)\}\, dr$$

$$= 2\,\sigma^2 (g(h-1) - g(h)) / (h^2 - (h-1)^2)$$

For the uncorrected model, the distribution u(r) of individuals present at a count point (within distance *w*) is $u(r) = 2\pi r$. The probability that an observed distance is in the interval [h-1 ; h[is $P\{ h-1 \le r < h\} = \pi (h^2 - (h-1)^2) / \pi w^2$. For a half-normal detection function, therefore,

$$\text{Eqn 7} \qquad P_a = \sum_{h=1}^{w} [\, 2\,\sigma^2 (g(h-1) - g(h)) / (h^2 - (h-1)^2)]\, (\pi (h^2 - (h-1)^2) / \pi w^2)$$

$$= 2\sigma^2 (1 - g(w)) / w^2 \quad .$$

If the count point contains blind areas, the distribution of observable individuals changes to

$$\text{Eqn 8} \qquad u(h) = R_h\, \pi (h^2 - (h-1)^2) \Big/ \sum_{h=1}^{w} R_h\, \pi (h^2 - (h-1)^2) \quad ,$$

and the estimated overall probability of detecting an individual is:

$$\text{Eqn 9} \qquad P_a = \sum_{h=1}^{w} 2\,\sigma^2 (g(h-1) - g(h))\, u(h) \,/\, (h^2 - (h-1)^2)$$

COMPARISON BETWEEN MODELS

Densities are usually expressed as estimated density at distance 0 (Buckland *et al.* 2001). For the comparisons between the uncorrected and corrected models in this paper, however, we prefer the intuitively more clear estimated proportion seen, P_a. P_a, obviously, depends on truncation distance. Except when otherwise stated, values for P_a given below are for a truncation distance of $w = 300$ m.

Since density D is estimated as $D = N_{obs}/(AP_a)$, where A is the total area counted, the variance of D is $var(D) = D^2 \{ var(N_{obs})/N_{obs}^2 + var(P_a)/(P_a)^2 \}$ (Buckland *et al.* 2001).

Because 2-9 counts were made in each area, we used the formula given for replicate points by Buckland *et al.* (2001) for estimating var(N). For the proportion seen, P_a is a function of σ^2 (Eqn 9), and an approximate expression for the variance of P_a can therefore be found by $var(P_a) \approx (dP_a/d\sigma^2)^2 var(\sigma^2)$, (Seber 1973).

For the uncorrected model,

Eqn 10 $$dP_a / d\sigma^2 = 2(1-g(w))/w^2 - 2 w^2 g(w) / \sigma^2 w^2 \quad .$$

For the corrrected model,

Eqn 11 $$dP_a/d\sigma^2 = \sum_{h}^{w} (2(g(h-1) - g(h)) + 2((h-1)2g(h-1)/2\sigma^2 - h^2g(h)/2\sigma^2)) u(h)/(h^2 - (h-1)^2) \quad .$$

We compared density estimates derived from respectively standard and corrected models by means of Monte-Carlo simulations. One advantage of this was that assumptions were met, i.e. individuals were randomly distributed over areas and the detection function was half-normal. For each count area we used the estimated value of σ^2 from field data, and simulated 3,000 data sets of N observations made at the actual count points with partially visible habitat. For each data set the resulting estimates of P_a and D were found, with and without correction for blind areas.

Results

COUNT POINT GEOMETRY

The configuration of visible areas was highly variable between count points, and unrestricted view to 300 m was only found for very small angular sections ψ, covering less than 20% of the total area (Fig. 1). Truncation would of course increase this percentage – but not substantially unless at truncation distances of c. 100 m (cf. Fig. 1).

Average visible proportions of 10 m segments decreased from 75-90% at the center to 15-40% at 300 m (Fig. 2). HL, KA, and LO2 were rather similar in this respect, while LO1 differed by having a somewhat higher proportion of visible areas (Fig. 2). Of the 28.27 ha potentially visible for a point truncated at 300 m, 9.79 (31%) were visible in HL, 8.46 (30%) at KA, 16.40 (59%) at LO1, and 10.74 (38%) at LO2. These percentages compare to those reported by Verheyden (1991) for Bocage-landscapes in NW-France.

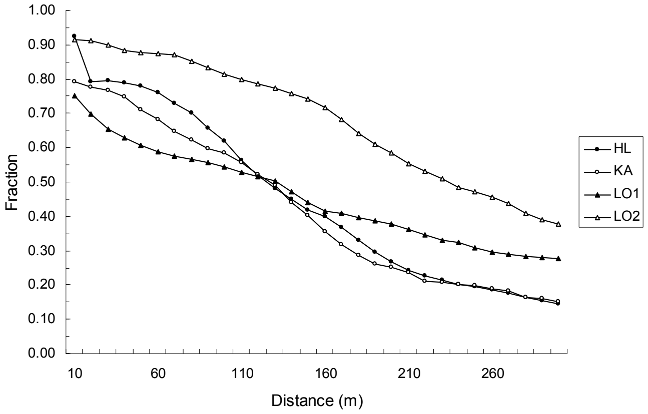

Fig. 2. Mean observable fractions of 10 m segments as a function of distance to observer, for the four counted areas.

Compared to standard point transect geometry, the visible parts of the counted habitats are considerably closer to the observer in this kind of landscape (Fig. 3). Since these models assume that individuals distribute randomly over habitat, this figure also models the expected distributions of individuals over distances to the observers. Thus, in all cases the observable individuals were closer to the observer than assumed by a standard model (Fig. 3). While for the latter, 25% of the area is within 150 m from observers, this percentage was in fact 46% for HL and KA, and 35% for LO1 and LO2. Notably, though the two latter differed in proportions visible, they had quite similar distributions of proportions visible over distance from observers.

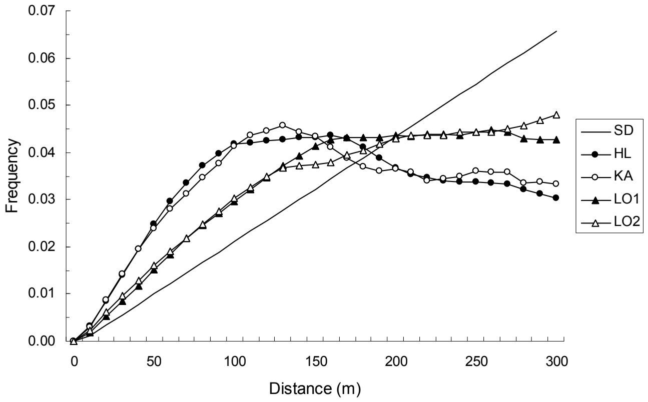

Fig. 3. The distribution of visible habitat 0-300 m from an observer, for the standard point transect model (SD) and the actual terrains counted. The distribution for the standard model would be unchanged if angular sections ψ were excluded. Under the assumption that objects are randomly distributed, the graphs would be identical to the distribution u(r) of individuals over distances from the observer.

FIT OF MODELS

Choice of interval width influences Goodness-of-Fit (see discussion in Buckland *et al.* 2001). For models corrected for blind areas, however, the chosen resolution of 10 m for the spatial mapping limited possibilities for adjustments.

We checked the sensitivity by repeating the analyses for data grouped into intervals of 20, 30 and 50 m. In all cases, the corrected and uncorrected models showed surprisingly identical fits to the data, predicting nearly identical numbers of observations for all distances. Since results were so similar, we only discuss Goodness-of-Fit tests for the corrected model.

We chose grouping of measurements into 30 m intervals as the best choice. Following this choice, the visible proportions – shown in Fig. 2 for 10 m intervals - were recalculated into 30 m intervals. For 30 m intervals, Goodness-of-fit tests accepted the half-normal detection function for all areas (HL: $\chi^2 = 12.13$, df = 6, $0.050 < P < 0.100$), KA: $\chi^2 = 1.45$, df = 6, $0.950 < P < 0.975$, LO1: $\chi^2 = 3.19$, df = 6, $0.80 < P < 0.90$, and LO2: $\chi^2 = 1.31$, df = 5, $0.90 < P < 0.95$).

Parameter estimates may also be sensitive to inclusion of outlying observations (observations made at unusually large distances). For this reason, robust estimation is generally recommended to be based on truncated data sets, with 5-10% of the largest measurements being excluded from analyses (Buckland *et al.* 2001). We checked this by truncating gradually down to 200 m. The only effect was that the Kalø estimate actually increased with truncation, because of a slightly excessive number of observations in the interval 150-180 m. In this case, therefore, the only effect of truncation was to destabilise estimation for one area, and we decided to maintain the originally chosen truncation distance of 300 m.

DETECTION PROBABILITIES

The uncorrected model yielded nearly identical estimates of σ^2 for LO1 and LO2, and somewhat higher values for HL and KA (Tab. 2). An overall Likehood-ratio test for identical scale parameters accepted the null hypothesis ($\chi^2 = 4.38$, df = 3, $0.200 < P < 0.300$), though if count areas had been pooled in two groups - (VH+KA) vs. (LO1+LO2) - as an intermediate step this would have been just significant (Tab. 2, $\chi^2 = 3.85$, df = 1, $0.050 \approx P$).

Correction for blind areas, as expected, increased all estimates. But while estimates for LO1 and LO2 only increased slightly, there was a much more marked increase for HL and KA (Tab. 2). For the corrected model, the hypothesis of identical detection functions was highly significantly rejected ($\chi^2 = 16.18$, df = 3, $0.001 < P < 0.005$), while pooling of data from (HL+KA) versus (LO1+LO2) increased the significance markedly ($\chi^2 = 15.57$, df = 1, $P < 0.001$). Clearly, therefore, correction for blind areas indicated that pooling of data sets should be done with caution.

Variances of all estimates increased after correction for blind areas, in particular for HL and KA (Tab. 2).

Table 2

Count area	N	σ^2	$s(\sigma^2)$	log (L)
Uncorrected estimates				
HL	57	8.3337	1.1694	-111.8316
KA	57	7.1851	0.9862	-110.6666
LO1	63	5.9848	0.7710	-113.9844
LO2	52	5.8632	0.8308	-92.7808
HL+KA	114	7.7526	0.7598	-222.7840
LO1+LO2	115	5.9298	0.5652	-206.7709
HL+KA+LO1+LO2	229	6.8251	0.4650	-431.4815
Corrected estimates				
HL	57	15.6702	4.0271	-111.9335
KA	57	12.1326	2.6557	-110.0351
LO1	63	6.5405	0.9301	-113.0635
LO2	52	6.7237	1.0942	-92.2307
HL+KA	114	13.7247	2.2817	-222.2618
LO1+LO2	115	6.6200	0.7091	-205.3024
HL+KA+LO1+LO2	229	9.0370	0.8027	-435.3516

Table 2. Sample sizes, estimates and log Likehood values resulting from applying the standard and the corrected model to various poolings of data. Estimates are scaled to units of 30 m (i.e. distances having values 1, 2, .. , 10) and should be multiplied by 900 (and the standard deviation with 30) to match a metric scale.

POOLING OF DATA SETS

Given the sparse data sets from individual count areas, an obvious option was to consider pooling of data. Indeed, at the outset the intention was to pool measurements from count areas. While counts were only planned at HL and KA, the two additional areas on Lolland were included to augment the data when the first counts showed that data sets from HL and KA would be sparse despite the effort invested.

Including the model treating data as four separate sets, there are 15 possible ways of pooling data from four count areas. We used a model fitting framework (Burnham & Anderson 2001) for assessing the relative weight of the evidence in support of each of these.

For uncorrected estimates, the top model was pooling of all four data sets, while pooling of data sets two by two, respectively (HL+KA) and (LO1+LO2) (Tab. 3). With Akaike weights of 0.25 and 0.22, there was little to indicate any preference between the two (Tab. 3).

Table 3

Model	K	AIC_c	$\Delta QAIC_c$	w_i
Standard model				
HL+KA+LO1+LO2	1	433.4991	0.0000	0.2491
HL+KA,LO1+LO2	2	433.6080	0.1089	0.2234
HL,KA+LO1+LO2	2	433.9992	0.5001	0.1511
HL+KA+LO2,LO1	2	434.8471	1.3480	0.0647
Corrected model				
HL+KA, LO1+LO2	2	431.6173	0.0000	0.7151
HL,KA,LO1+LO2	3	433.3777	1.7604	0.1230
HL+KA, LO1,LO2	3	433.6627	2.0454	0.0925
HL,KA+LO1+LO2	2	435.0321	3.4148	0.0235

Table 3. The four top models resulting from comparing the 15 candidate models for pooling data sets in a model fitting framework for respectively uncorrected and corrected estimates. Pooling is indicated by +. N=229 for all cases, K = number of parameters in model. Models are ranked from the top model down, and for each the Akaike Criterion (corrected for sample size), its relative difference to other models and the Akaike weight is given.

For the corrected estimates, the top model was pooling of data sets two by two, respectively (HL+KA) versus (LO1+LO2). After correction for blind areas, however, support for this model was considerably stronger than in uncorrected estimates, with an Akaike weight of 0.72. Compared to the second best model, the support in the data for this pooling is nearly 7 times larger (Tab. 3).

We therefore continued the analyses based on this pooling. Inspection of the pooled data for the two regions (Fig. 4) shows that even if the two data sets are almost equally sized (resp. N = 114 and 115), there are fewer observations under 60 m for HL+KA (20 against 30), almost equal numbers for distances between 61 and 150 m (76 against 72), and 18 against 13 for distances over 150 m. This distribution is contrary to what would be expected from the distribution of visible area over distance (Fig. 3), and undoubtedly this is the reason why correction for blind areas makes the scale parameter estimates highly significantly different.

Notably, the corrected and uncorrected models resulted in almost identical expected distributions of observed distances (f(r)) in spite of the different estimates of σ^2 (Fig. 4). Also, comparison of corrected and uncorrected models by model fitting (not shown) would have indicated the corrected model to be only a marginally better choice (cf. the Likelihoods given in Tab. 2). Pooling resulted in acceptance by Goodness-of-fit tests for both sets, and for both models. For the corrected model, test statistics were (VH+KA): $\chi^2 = 6.09$, df = 7, $0.50 < P < 0.60$ and (LO1+LO2): $\chi^2 = 2.31$, df = 6, $0.80 < P < 0.90$. Test statistics for the uncorrected model were nearly identical (cf. Fig. 4).

COMPARISON OF MODELS

In terms of estimated proportions seen, P_a , correction for blind areas had considerable impacts, naturally depending on the choice of truncation distance *w*. For *w*

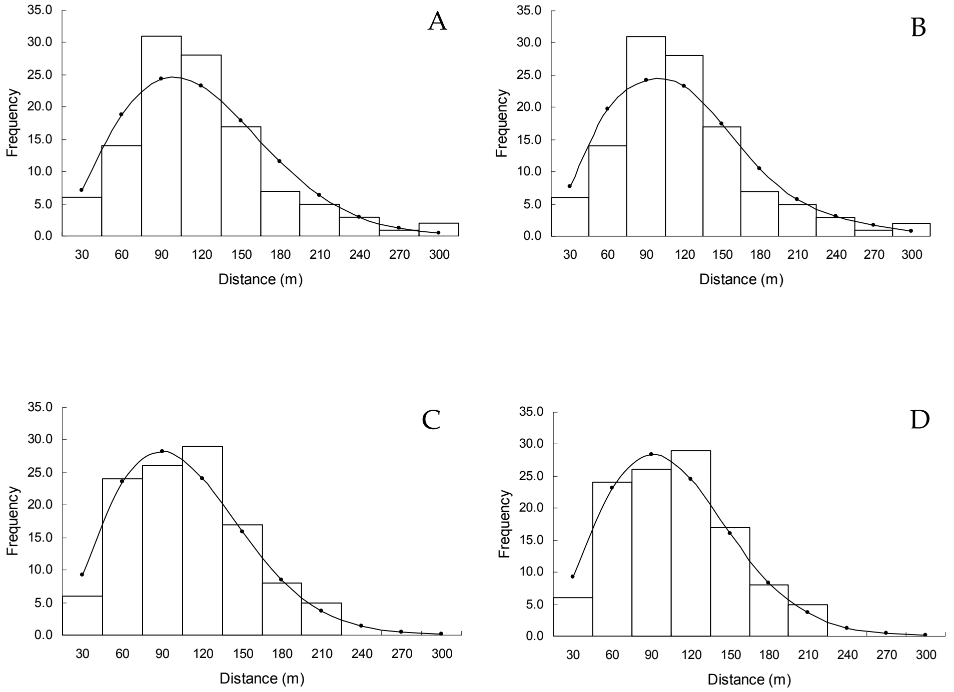

Fig. 4. Observed (histograms) and expected (curves) distributions of distances for pooled data sets with distance intervals of 30 m. A and B: (VH+KA), expected distributions derived from respectively uncorrected and corrected models. C and D: (LO1+LO2), expected distributions derived from respectively uncorrected and corrected models. Notably, the two models predicted nearly identical distributions in both cases.

= 300 m, the uncorrected model led to estimates of resp. c. 17% in HL and KA and 11-12% on Lolland (Tab. 4). In contrast, correction for blind areas led to estimates of c. 40% seen in HL and KA, and 18% at LO1 and LO2. It should be noted that part of this difference was due to the more open terrain at Lolland, meaning that hares present at count points in those areas were farther from the observer (cf. Fig. 3). For the detection function for HL+KA, c. 33% would have been detected in the count areas at Lolland.

When corrected for blind areas, density estimates increased 19% for HL, 37% for KA, 10% for LO1 and 70% for LO2 (Tab. 4). In all cases, standard deviations of density estimates were larger for the corrected model (Tab.4). Coefficients of variation, however, were essentially unchanged.

Table 4

Count area	P_a	Sdev(P_a)	D	Sdev(D)
Uncorrected estimates				
HL	0.1675	0.0162	2.5032	0.4725
KA	0.1695	0.0164	3.9058	0.8172
LO1	0.1169	0.0112	40.0848	11.5978
LO2	0.1117	0.0107	51.9690	12.0075
Corrected estimates				
HL	0.4157	0.0210	2.9803	0.5056
KA	0.4076	0.0225	5.3771	1.0402
LO1	0.1827	0.0307	44.1795	14.3739
LO2	0.1852	0.0358	88.4328	25.8363

Table 4. Mean estimated proportions seen (P_a) and densities (D) resulting from 3,000 Monte-Carlo simulations of data sets in the four count areas.

Discussion

CHOICE OF DETECTION FUNCTION

The estimate of P_a depends on the detection function, and it is generally recommended that more than one socalled key-function is fitted before a final choice of model is made (Buckland *et al.* 2001).

Because of the rather complicated calculations involved with corrections for blind areas, we fitted only one type of key-function to our data. The choice of a half-normal detection function was perhaps rather incidental, but it has been found to fit other data sets for hares quite well (Hutchings & Harris 1996, Péroux *et al.* 1997, Reid *et al.* 2007), although a slightly better fit was achieved by Fourier-series expansion in some of these cases. Other key-functions, e.g. the cosine-key, might have fitted our data equally well, but in terms of a correction for blind areas it is considered unlikely that use of a cosine-key would have changed the conclusions much.

The two fundamental assumptions underlying the analysis of distance sampling data are 1) that the detection probability is 1.0 at distance 0 and decreases with increasing distance, and 2) that individuals distribute randomly over the observed habitat. The former of these has attracted much attention. For very good reasons, it is generally recommended to consider the selected key-function only as a starting point for further refinement by superimposing second-order functions, in order to improve the fit to the data (Buckland *et al.* 2001). On the other hand, the second assumption has attracted less attention.

For two reasons we did not attempt further refinement of the choice of detection function. First, the data were too sparse to substantiate such attempts (Alldredge *et al.* (2007) have shown that simultaneous observations of multiple species at count points lead to improved estimation of single species, but that idea occurred too late for the present work). Second, terrestrial habitats are heterogeneous, and it is a distinct possibility that animals do not distribute randomly over the habitat under study, as has indeed been noted for red foxes *Vulpes vulpes* and hares in a number of cases (Ruette *et al.* 2003, Reid *et al.* 2007, Roedenbeck & Voser 2008). In our case, the visible subareas may well include at least some heterogeneity. If hares do not forage too close to roads (see below), buildings or hedgerows, the distribution over habitat will not be random, and particularly when the number of count points is small – or line transects short - the distribution of distances to the observer could be affected. If so, fitting of a detection function could potentially be affected by habitat heterogeneity.

COMPARISONS OF MODELS

Though leading to distinctly different estimates of σ^2 and the distribution u(r) of individuals over distances (cf. Tab. 2 and Fig. 3), the two models predicted almost identical distributions of observations over distance (Fig. 4). In this respect, there was no consequence of correcting for blind areas.

Instead, the consequences of correcting for blind areas were 1) detection functions for the four count areas proved significantly different, 2) estimated proportions seen increased radically, by c. 250% in HL and KA and by c. 60% in LO1 and LO2 (cf. Tab. 4), 3) variances increased of all estimates (though CV's were nearly the same), and 4) estimated densities increased by 10-70%.

Taking the discussion of Buckland *et al.* (2001) literally, and carrying out comparisons between a fully uncorrected standard model and a corrected one, might be considered provocative. In a more realistic analysis of these data, angular sections ψ would presumably have been omitted for some points, thereby decreasing the differences between corrected and uncorrected density estimates. Reid *et al.* (2007) corrected for blind areas by omitting angular sections of 20° where detectability was hindered. Their density estimates were somewhat higher than those resulting from the uncorrected model, too, but since the figures given also include a correction for road avoidance it is not possible to compare them with our results.

We note, however, that for habitats with partial observability, quite considerable systematic errors may be introduced if results are not corrected for blind areas. In cases where larger proportions can be counted, the standard model might lead to estimates closer to the corrected ones, but even in that case fitting a general model that produces roughly correct results by committing two oppositely directed errors - underestimating the proportion seen and overestimating the counted area - will not be a very satisfactory solution.

We therefore conclude that for point transect counts – in our case the only realistic possibility – in this type of landscape, correction for blind areas will be necessary for reliable estimation of P_a and D.

In extending the standard model, we did not change the basic assumptions of distance sampling models: that objects are randomly distributed over the observed area, that individuals are detected with certainty at zero distance, and that the half-normal detection probability fitted the data. Since the basic assumptions are the same, the model used here should thus be applicable to other types of habitat, too.

DIFFERENCES IN DETECTION PROBABILITIES

No immediately convincing explanation for the differences in detection probabilities could be found in observational circumstances. Observers, equipment, times of year and overall meteorological conditions were the same for all four areas (Wincentz & Noer *unpublished*). Instead, our attention was drawn to non-random distributions of animals over the observed habitat.

Recently, road avoidance has been reported for hares by Reid *et al.* (2007) and Roedenbeck & Voser (2008). All points in our investigation were accessible by car, and some differences existed between count areas. Some of the points in HL and at KA were placed on larger roads, but omission of larger roads from the data did not change the resulting estimates (Wincentz & Noer *unpublished*). However, even for comparable road types, traffic density could be assumed to be higher in the two areas in Jutland.

Wincentz & Noer (*unpublished*) studied the effect of road avoidance on the estimated densities by computer simulations. While the estimate of σ^2 for LO1 and LO2 given in this paper (6.6200) is probably comparable to the detection functions fitted by Reid *et al.* (2007), the simulations showed that the much larger estimate for HL and KA could at least partly result from a similar value of σ^2 when combined with strong road avoidance. However, equally good fits to the data could be achieved by assuming a somewhat larger value of σ^2, combined with somewhat weaker reactions to roads. The most notable difference between these two possibilities were that while a value of $\sigma^2 = 6.6200$ and strong avoidance behaviour fitted the data for distances up to 120 m best, larger values produced a better fit to the distances over 150 m.

When choosing between such options, the recommended standard procedure is to leave out the 10-15% largest distances measured by truncation, since they will influence estimates of σ^2 strongly (Buckland *et al.* 2001). For this reason, Reid *et al.* (2007) truncated their measurements at a distance of $w = 250$ m. For our data, we consider that if detection probabilities were indeed identical for the four areas this would be the correct procedure, but we also consider that if not correct, truncation would mean discarding valuable data. For this reason, we have maintained a truncation distance of 300 m throughout.

Even if the difference in detected proportions between the two regions were fully explained by road avoidance, the implication is that it is not merely a result of placing count points on roads. A difference between regions in strength of reactions must be inferred as well. Thus, pooling of data sets in distance sampling analyses in terrestrial habitat types should be done very carefully, because there may be heterogeneity in the distribution of animals over habitat and/or in detection probabilities. Both may have important implications for the interpretation of the data collected.

IMPLICATIONS FOR MANAGEMENT

The brown hare is a popular hunting object, and given the declines that have taken place, complete or at least partial protections have been proposed repeatedly in Denmark. Such issues, of course, can not be addressed convincingly without firm estimates of densities.

Capture-recapture analyses of hare densities have been carried out in some cases (Abildgaard, Andersen & Barndorff-Nielsen 1972, Marboutin & Peroux 1995). These investigations covered periods of respectively 13 and 3 years. Clearly, attempts to assess hare-densities by capture-recapture studies – though providing sound estimates – would be resource-heavy, and it would take some years before estimates would be available. In contrast, spotlight counts can provide 'rough-and-ready' estimates that can serve as basis for further management decisions.

The estimated distances at which there would be a 50% chance of detecting a hare were 90-100 m for LO and 130-140 m for HL and KA. Thus, distance sampling methods are clearly a necessity if numbers counted should be reliably converted to density estimates. Even within a truncation distance of 150 m (which will leave out valuable information for estimating densities), less than 50% of the hares present at a count point were detected at LO1 and LO2. According to Wincentz & Noer (*unpublished*), road avoidance may have deflated estimated densities at HL and KA, but at least distance sampling will provide insight into sources of error that would not have been revealed by traditional counting methods.

In terrains such as those studied here, correction for blind areas is necessary – even if the mapping of visible areas is tedious. We note that twice as much area was covered per count point on LO1 than at HL and KA, which will invalidate comparisons of numbers counted even before introduction of detection probabilities. For the purposes of this study the original mapping of visible proportions in 10 m intervals (cf. Fig. 2) might as well have been done in 30 m segments. With hindsight, this choice might have been more profitably made after inspection of distance measurements.

One further point will be that many monitoring programmes are based on socalled index counts. The results presented above strongly suggest that even if the detection function may be of the same basic type, estimated proportions seen may vary considerably between localities. In addition to the geographic variation in estimated detection probabilities found in this study, there might be temporal changes as well – e.g. because of changes in traffic volume. If that were the case, the use of simple counts for detecting population trends might be invalidated, simply because of changes in the detected proportions. Obviously, therefore, index counts need to be supported by estimation of detection probabilities based on distance sampling.

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SUPPLEMENTARY MATERIAL

Appendix S1: Maximum Likelihood estimation of σ^2 .

Notation below follows that of Buckland *et al.* (2001) as closely as possible. Data consist of N measurements, made at N_p points, with distances truncated at *w* and grouped into *w* intervals. Indexing could be made after either of these. For the hare data, 229 measurements were made at 101 points, each with 30 10m distance intervals, i.e. the matrix of visible fractions was 101x30. We therefore preferred to index after distance measurements, i.e. i = 1, 2, …, N, where the i'th observation is made at point j(i) and at distance h(i).

Standard point transect counts

For a half-normal detection function g(x), the probability that an observed individual is detected when its distance to the observer is in the interval [a ; b [will be

$$P\{ a \leq r < b \} = 2 \pi \sigma^2 [\exp\{-a^2 /(2\sigma^2)\} - \exp\{-b^2 /(2\sigma^2)\}] / \nu \quad ,$$

where the constant ν corrects for area left out by truncation. For convenience, we write g(x) instead of $\exp\{-x^2 /(2\sigma^2)\}$ in the following. Then
$\nu = 2 \pi \sigma^2 (1-g(w))$, and

$$P\{ a \leq r < b \} = (g(a) - g(b)) / (1 - g(w)) \quad .$$

Disregarding multinomial coefficients, the Likelihood-function for N observations can be written as

$$L(\sigma^2) = \prod_{i=1}^{N} [(g(h(i)-1) - g(h(i))) / (1 - g(w))] \quad , \text{and}$$

$$\log (L(\sigma^2)) = \sum_{i=1}^{N} \log (g(h(i)-1) - g(h(i))) - N \log (1 - g(w))$$

Point transects with blind areas

For standard point transects, the area of the h'th interval is $\pi (h^2 - (h-1)^2)$. If for point j only a fraction $R_{j,h}$ can be observed in the interval [h-1; h [,

$$P\{ h-1 \leq r < h \} = 2 \pi \sigma^2 R_{j,h} (g(h-1) - g(h)) / \nu_j \quad ,$$

where for j = 1, 2, …, n_p

$$\nu_j = \sum_{h=1}^{w} 2 \pi \sigma^2 R_{j,h} (g(h-1) - g(h))$$

If the i'th observation is made at point j(i) and distance h(i), the Likelihood-function (omitting multinomial coefficients) can be written as

$$L(\sigma^2) \quad = \quad \prod_{i=1}^{N} 2\,\pi\,\sigma^2\, R_{j(i),h(i)}\, (g(h(i)-1) - g(h(i))/\, (\nu_{j(i)}\,)$$

and the logarithm of the Likelihood-function is

$$\log\,(\,L(\sigma^2)) \quad = \quad \sum_{i=1}^{N} \log\,[2\,\pi\,\sigma^2\, R_{j(i),h(i)}\, (g(h(i)-1) - g(h(i)))\, /\, \nu_{j(i)}\,]$$

$$= \quad \sum_{i=1}^{N} \log\,(R_{j(i),h(i)}) \; + \; \sum_{i=1}^{N} \log\,(g(h(i)-1) - g(h(i))) \; - \; \sum_{i=1}^{N} \log\,(\nu_{j(i)}) \; + \; N \log\,(2\,\pi\,\sigma^2\,)$$

Notation

Noting that the factor $2\,\pi\,\sigma^2$ is also part of the expression for ν, we substitute μ for $\nu/2\,\pi\,\sigma^2$. Then

$$\text{Log}\,(L(\sigma^2)) \quad = \quad \sum_{i=1}^{N} \log\,(R_{j(i),h(i)}) \; + \; \sum_{i=1}^{N} \log\,(g(h(i)-1) - g(h(i))) \; - \; \sum_{i=1}^{N} \log\,(\mu_{j(i)})$$

To simplify notation further, we write $\xi = \sigma^2$. Then

$$g(h) \quad = \quad \exp\,\{-h^2\,/(2\xi)\} \; ,$$

$$d\,(g(h))\,/\,d\xi \quad = \quad [\,h^2/(2\xi^2\,)]\; g(h) \; ,$$

and

$$d^2\,(g(h))\,/\,d\xi^2 \quad = \quad [\,(h^2/(2\xi^2))^2 \; - \; (h^2/\xi^3)\,]\; g(h)$$

Differentiation of log L

The Likelihood-function is the sum of terms that can be differentiated separately and then added. If $\log L(\xi) = A + B + C$, A is a constant not depending on ξ. Therefore, since $d(A)\,/\,d\xi = 0$ and $d^2(A)\,/\,d\xi^2 = 0$,

$$d\,(\log\,(L(\xi)))\,/\,d\xi \quad = \quad d(B)/d\xi \; - \; d(C)/d\xi$$

and

$$d^2\,(\log\,(L(\xi)))/d\xi^2 \quad = \quad d^2(B)/d\xi^2 \; - \; d^2(C)/d\xi^2$$

Differentiation of B

The function B is identical for the standard and corrected model.

$$d(B) / d\xi \quad = \quad d\,(\sum_{i=1}^{N} \log (g(h-1) - g(h))) / d\xi$$

$$= \quad \sum_{i=1}^{N} \{ [((h(i) -1)^2/(2\xi^2)) g(h-1) - (h(i)^2/(2\xi^2)) g(h)] / (g(h-1) - g(h)) \}$$

and

$$d^2(B)/ d\xi^2 \quad = \quad d^2 (\Sigma \log (g(h-1) - g(h))) / d\xi^2$$

$$= \quad \sum_{i=1}^{N} \{ [((((h(i)-1)^2/(2\xi^2))^2 - ((h(i)-1)^2/\xi^3)) g(h-1) - (((h(i)^2/(2\xi^2))^2 - (h(i)^2/\xi^3)) g(h)) (g(h-1) - g(h))$$

$$- (((h(i)-1)^2/(2\xi^2)) g(h-1) - (h(i)^2/(2\xi^2)) g(h))^2] / (g(h-1) - g(h)) \}$$

Differentiation of C

The difference between the standard and corrected model lies in the term C. For the standard model,

$$C \quad = \quad N \log (1 - g(w)) \quad ,$$

$$dC / d\xi \quad = \quad - N (w^2/2\xi^2) g(w) / (1 - g(w)) \quad , \quad \text{and}$$

$$d^2 C / d\xi^2 \quad = \quad - N (w^2/2\xi^2)^2 g(w) / (1 - g(w))^2 \quad .$$

For the corrected model, C is a function of $\mu_{j(i)}$:

$$C \quad = \quad f(\mu_{j(1)} ,\mu_{j(2)} , \ldots , \mu_{j(N)}) \quad = \quad \sum_{i=1}^{N} \log (\mu_{j(i)})$$

Hence,

$$d(C)/ d\xi \quad = \quad \sum_{i=1}^{N} d (\log(\mu_{j(i)})/d\xi \quad = \quad \sum_{i=1}^{N} ((d (\mu_{j(i)})/d\xi))/ \mu_{j(i)}$$

and

$$d^2 (C)/ d\xi^2 = \sum_{i=1}^{N} ((d^2(\mu_{j(i)})/d\xi^2)\, \mu_{j(i)} - (d(\mu_{j(i)})/d\xi)^2) / (\mu_{j(i)})^2$$

where

$$d\mu_i / d\xi = \sum_{h=1}^{w} R_{j(i),h} [((h-1)^2/(2\xi^2))\, g(h-1) - (h^2/(2\xi^2))\, g(h)] \qquad ,$$

and

$$d^2(\mu_{j(i)}) / d\xi^2 = \sum_{h=1}^{w} R_{j(i),h} \left\{ [((((h-1)^2/(2\xi^2))^2 - (h-1)^2/\xi^3)\, g(h-1) - ((h^2/(2\xi^2))^2 - h^2/\xi^3)\, g(h)) \right\}$$

<u>Numerical solutions</u>

Solutions to the equation $d (\log L(\xi)) / d\xi = 0$ were found by Newton-Raphson iteration, i.e.

$$\xi_{i+1} = \xi_i - d^2 (\log (L(\xi)))/d\xi^2 / d(\log (L(\xi)))/d\xi \,\Big|_{\xi = \xi i}$$

(i = 1,2,...)

The variance of ξ was estimated by $\mathrm{Var}(\xi) = -1/(d^2 (\log (L(\xi)))/d\xi^2)$. These derived values could be compared to the results of Monte-Carlo simulations (Appendix S2). In all cases, the two values were close.

Programme Distance does not offer solutions for these modifications. Instead, estimation and simulations were done by user-written programmes (Borland Pascal v. 7.0). For the standard method as used in this paper, solutions were compared to those resulting from Distance, results being nearly identical.

Appendix S2: Statistical properties

The statistical properties concern the distribution of estimates, mainly 1) bias (i.e. if the expected value of an estimate corresponds to the true value of the parameter, and 2) asymptotic shape and variance (the term asymptotic meaning the behaviour for large and very large sample sizes). Emphasis was placed on small and medium sample sizes.

Properties were examined by Monte-Carlo simulations, i.e. by simulating data sets having known scale parameters and detection probabilities. This means that simulated distributions fulfill the basic assumptions of the model, both with respect to the shape of the detection function (Half-normal, and distribution of individuals (even). Checking whether assumptions are met for actual data sets should therefore be done by the usual methods of model control, before the following results are assumed to be valid.

Since σ^2 is a scale parameter, it is only necessary to evaluate its distribution for one specific value. The distribution should match any other value after proper rescaling. Basically, we chose to do these simulations for $\sigma^2 = 122.5$, approximately matching the largest values found in the hare data, and for sample sizes of 50, 75, 100, 125, and 150, spanning what was found for hares. Of the latter, we only show results for N=50, N=100, and N=150. In each case, 10,000 data sets were simulated, and σ^2 and its variance were estimated from these.

For the standard model with unrestricted sightability, these simulations were straightforward and done by random sampling from the distribution f(r) of observed distances. For the model correcting for blind areas, we simulated outcomes corresponding to the scenario at Lolland (40% of the potential area visible at 300 m) and in Himmerland and Kalø (10% of the potential area visible at 300 m). Matching these, the Lolland-simulations inevitably were based on only 20 count points, while the Himmerland and Kalø simulations were based on 54+27 = 81. Noting that in fact many points were quite similar in visible proportions (though different in configuration), this would probably not reduce the generality of the Lolland scenario. In each case, the simulation of a data set comprised two steps, viz. 1) random selection of a specific point, and 2) random allocation of a measured distance given which point it was made at. Points were selected with probabilities matching their visible areas. This means that if one point had twice the observable area than another, it would also be represented with twice as many observation in the data sets.

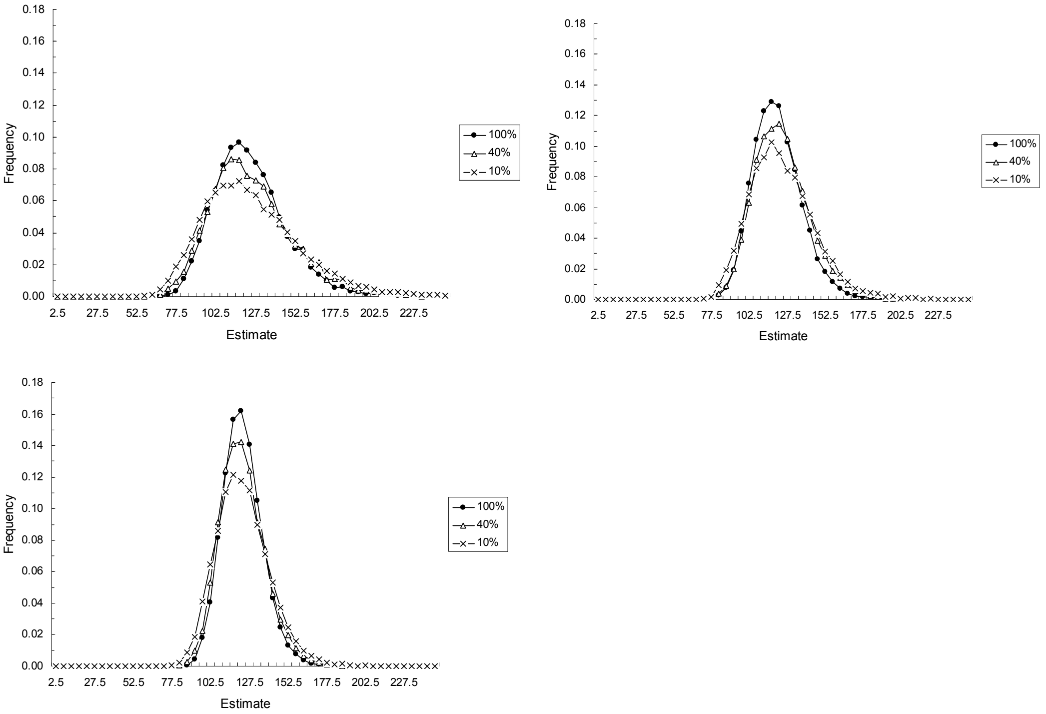

Fig. 1. Sampling distributions of estimates of σ^2 resulting from 10,000 data sets with respectively N=50, N=100, and N=150. The standard distance sampling model is denoted 100% (of the visible area within 300 m).

In all cases, means were close to the true value of $\sigma^2 = 122.5$, indicating that estimators are basically unbiased. Sampling distributions were unimodal, but skewed for small sample sizes (Fig. 1). This skewness is also found for the standard model, though becoming more pronounced when correction for blind areas is introduced. For both corrected and uncorrected estimates increasing sample sizes made the sampling distribution of σ^2 increasingly symmetric (normal, see below), but the standard model converges faster, and so do corrected estimates when 40% of the area can be seen. Accordingly, care should be taken when applying Likelihood-ratio tests to sample sizes under c. 75 for corrected models, especially when visible proportions are small.

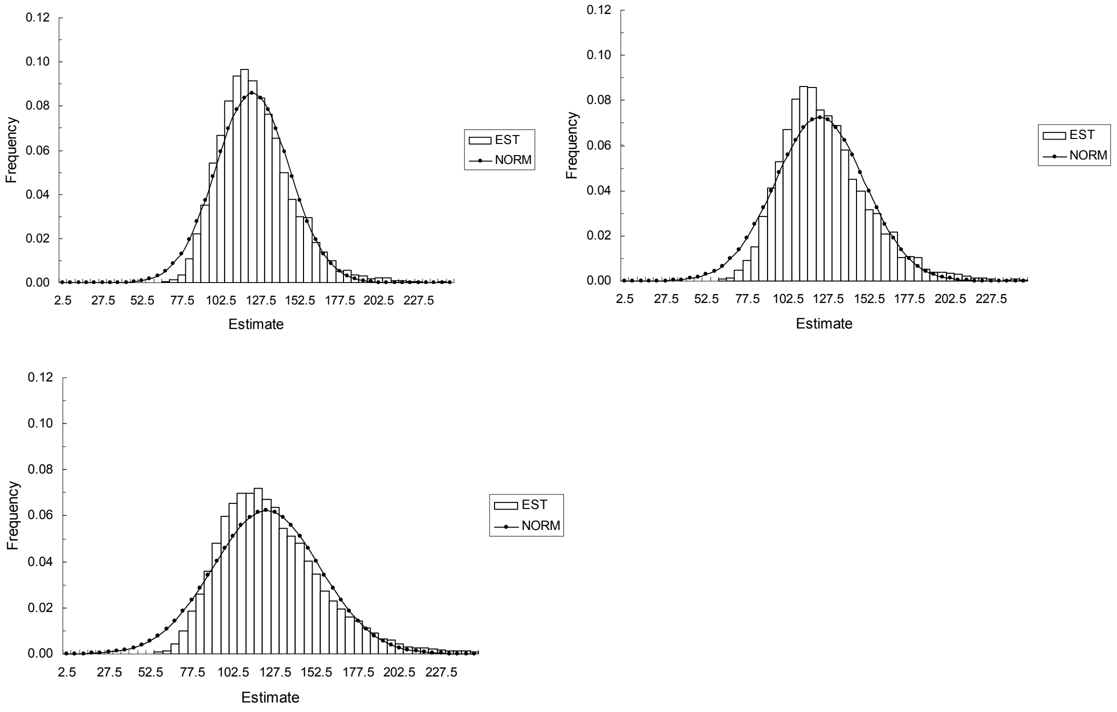

Fig. 2. Sampling distributions of 10,000 estimates of σ^2 when N=50, for respectively the standard model (left), 40% visible at 300 m (middle), and 10% visible at 300 m (right), compared to theoretical normal distributions having the same mean and variance.

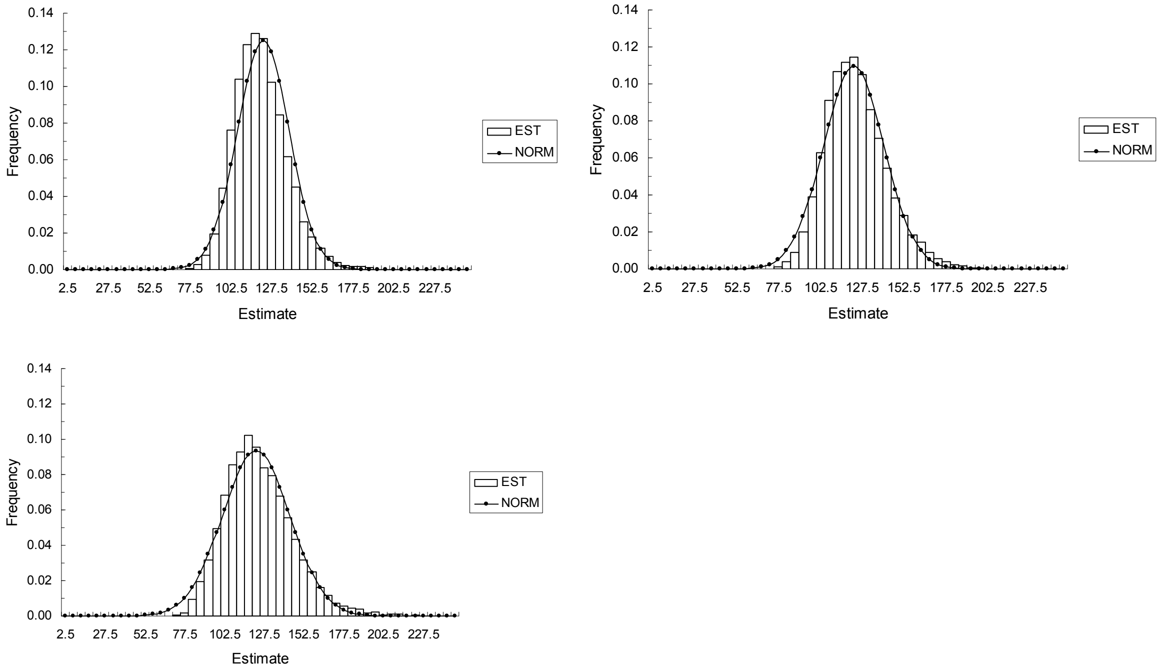

Fig. 3. Sampling distributions of 10,000 estimates of σ^2 when N=100, for respectively the standard model (left), 40% visible at 300 m (middle), and 10% visible at 300 m (right), compared to theoretical normal distributions having the same mean and variance.

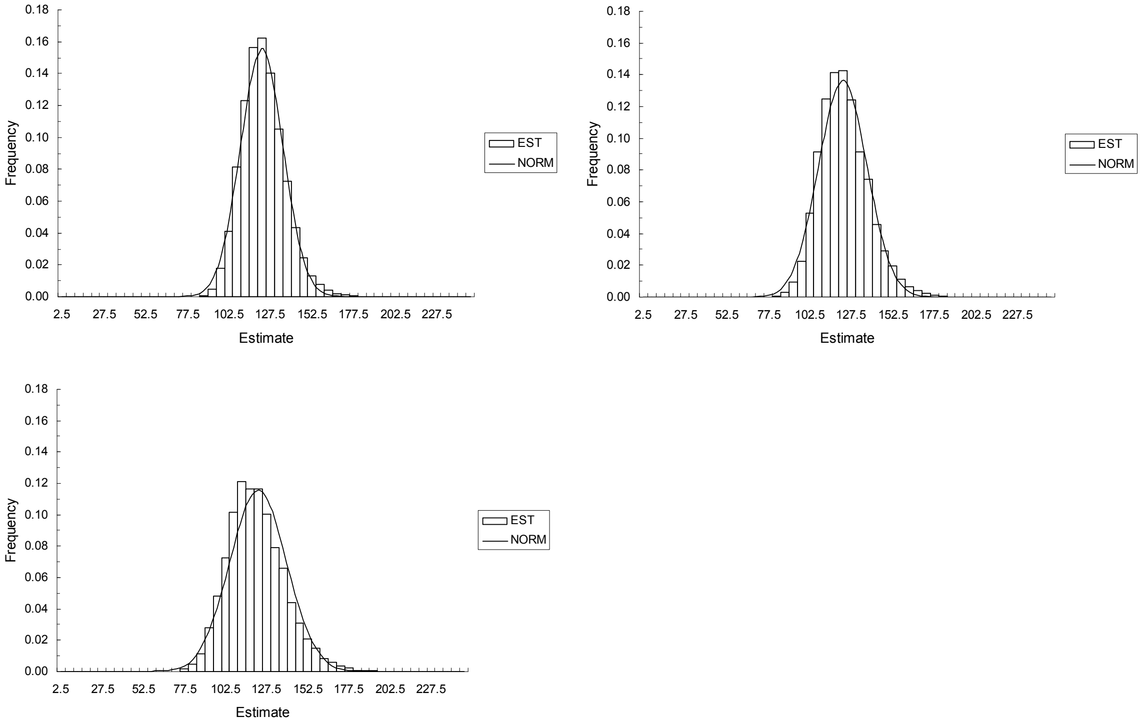

Fig. 4. Sampling distributions of 10,000 estimates of σ^2 when N=150, for respectively the standard model (left), 40% visible at 300 m (middle), and 10% visible at 300 m (right), compared to theoretical normal distributions having the same mean and variance.

To check asymptotic properties we compared the simulated distributions to normal distributions having a mean of 122.5 and a variance calculated from the 10,000 resulting values of σ^2. Nothe that the estimate of σ^2 is scaled to observations grouped into 10 m intervals in order to improve resolution of graphs, and so deviates from the estimates for 30 m intervals given in the main text.

All simulations resulted in a few very large values of σ^2. Especially for data sets of N=50 (Fig. 2) and 10% of the area visible at 300 m, the simulated distributions were asymmetric, and a small proportion of very high values of σ^2 increased the varince of the normal distribution shown for comparison, making the latter seem a bit off.seem to be asymptotically normal, and hardly any problem with using Likelihood-ratio tests in the present case, though one should be slightly cautios with N=50. Note that some of the plots appear almost counterintuitive with respect to the two distributions having the same mean. The reason is that a few very high estimated values of σ^2 (not detectable in the plots) ensured that the mean of the simulations indeed was close to the theoretical value of 122.5.

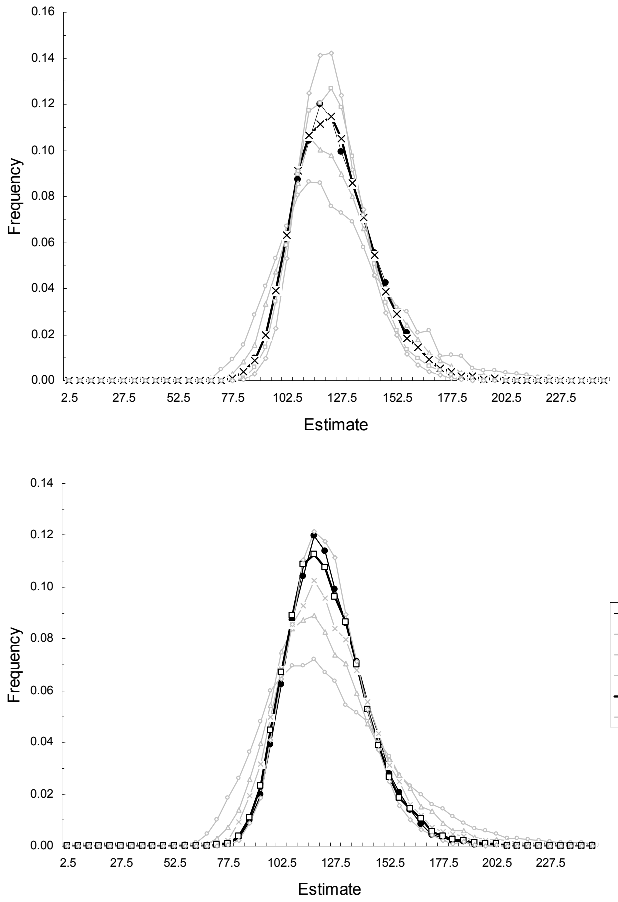

Fig. 5. Comparisons between the standard and corrected models for 40% (left) and 10% (right). For the standard model (here denoted 100% after the proportion of the area that could be counted, the distribution of estimates when N=75 is shown, while for the corrected model graphs for N=50, 75, 100, 125, and 150 are given. The graphs for the sample sizes giving the closest approximation to the standard situation are shown in solid.

Comparing results for the standard model and the corrected models, sampling distributions of σ are shown in Fig. 5. When 40% of the potential area could be counted at 300 m., a sample size of 100 resulted in a distribution closely matching the standard model for N=75. In such a scenario therefore, the loss of precision caused by reduced visibility could be compensated by increasing sample size one third (left diagram). When only 10% of the area could be seen at 300 m., an increase in sample size of 70-100% would be necessary (right diagram).

IV


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Distance sampling estimates of densities of brown hares

Wincentz, T. & Noer, H.

Manuscript

Distance sampling estimates of densities of brown hares

Trine-Lee Wincentz & Henning Noer

Distance sampling was applied to point spotlight counts of brown hares in four areas in Denmark during 2005-06. Estimated spring densities (3, 6, 65 and 111 individuals/km^2) varied significantly between areas, but could potentially have been affected by avoidance of habitat in proximity to roads. To assess the sensitivity of detection probabilities and density estimates to road avoidance, we simulated five scenarios of increasingly strong avoidance reactions across four values of increasing detection probabilities. The results showed that the data for the two areas having the lowest densities were equally well described by models having a rapid fall-off in detectability for increasing distance plus strong road avoidance, and models with higher detectability plus more moderate road avoidance. Thus, neither explanation could be ruled out. Over the range of avoidance behaviour and detection probabilities investigated, road avoidance inflated estimated proportions seen by 6-85% and decreased density estimates by 16-45%. The estimates implied that even within distances of 150 m less than 50% of the hares are detected by spotlight counts, thus invalidating use of uncorrected count figures for estimating densities. Overall, in spite of the uncertainties distance sampling provided what can be assumed to be more realistic density estimates, but when applying it care must be taken because the assumption that individuals are evenly distributed over habitat may be violated.

Key words:Brown hare, Lepus europaeus, density, distance sampling, road avoidance

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Populations of brown hares *Lepus europaeus* in Western and Northern Europe have been declining during the past 50 years (e.g. Edwards *et al.* 2000, Smith *et al.* 2005). Most evidence for these declines, however, derives from bag statistics, and only a limited number of studies provide actual estimates of numbers or densities.

Collection of data that can support firm density estimates of hares is not a simple task. While two older studies (Abildgaard *et al.* 1972, Marboutin & Peroux 1995) estimated population sizes from capture-recapture data, since 1990 visual counts – in most cases conducted during the period of nocturnal activity by means of spotlights - have attracted more attention (Verheyden 1991, Hutchings and Harris 1996, Péroux et al. 1997, Roedenbeck & Voser 2008, Strauß et al. 2008). Undoubtedly, visual counts are less resource demanding than capture-recapture studies in terms of both time and manpower, and so may be preferable for many purposes.

One fundamental problem for visual counts, however, is that not all individuals are necessarily detected. Spotlight counts, in particular, may suffer from substantial undetected proportions since the quantity of light that an illuminated object returns to an observers eye decrease with the fourth power of distance (e.g. Eastwood 1967). Thus, for each time the distance to a hare is doubled - whether from 50 to 100 m or from 100 to 200 m - the amount of light returned will be reduced by c. 94%.

Distance sampling (Buckland *et al.* 2001) offers a method for estimating the proportion seen, and hence for correcting counted numbers for undetected individuals. As indicated by the name, distances to all detected individuals are measured, and the proportions of the counted area at various distances from the observer are calculated or mapped. If it is assumed that animals distribute evenly over the visible habitat, a function describing the proportion detected at various distances from the observer - the so-called detection function - can be fitted to the data, and from this the proportion detected, say P_a , can be estimated.

Though use of distance sampling is in need, so far only Peroux *et al.* (1997) and Reid *et al.* (2007, in a study of the Irish hare (*L. timidus hibernicus*)) have applied the method to spotlight counts of hares. Hutchings & Harris (1996) applied the method, too, but their study was based on daytime transect counts.

The two typical protocols for counting animals are transect and point counts. For the latter, Buckland *et al.* (2001) proposed the term point transect counts for point counts when distance sampling is applied. In order to assess densities of hares in Danish farmlands, we conducted point transect spotlight counts in four areas during 2005 and 2006. Part of the purpose being to evaluate the use of distance sampling for hare counts, the study might be viewed partly as a pilot study. Corrected for unseen areas (Noer & Wincentz *unpublished*), overall estimates clearly showed that substantial proportions were not detected, and that - possibly even more problematic - highly significant differences in detection probabilities between count areas were found, two areas in Jutland differing significantly from two at the island of Lolland, south-eastern Denmark. To a distance of 150 m, estimated proportions seen were 69% in Jutland and 47% on Lolland, while within a distance of 300 m estimates were 40% and 18%.

Point spotlight counts from cars are conducted from roads, and thus points are not necessarily randomly placed with respect to habitat. Recently, evidence that hares avoid habitat in close proximity to roads has been presented by Reid *et al.* (2007) and Roedenbeck & Voser (2008). In our count areas there was a preponderance of larger roads with heavier traffic in the two areas censused in Jutland. This, of course, leaves open the question whether the different detection probabilities might actually be caused by road avoidance, and whether and to what extent the resulting density estimates may have been influenced by avoidance of roads.

The purpose of this paper is 1) to present density estimates obtained from distance sampling for four Danish areas, 2) to carry out a sensitivity analysis, assessing to what extent avoidance of habitat in proximity to roads will affect estimated proportions detected and densities, and 3) to evaluate the use of distance sampling for hare counts by spotlight. In particular, we examine the specific question whether the differences between regions in detectability can be explained by avoidance of roads.

Material and methods

Count areas

Two areas in Jutland, respectively a 100 km^2 area in Himmerland (HL, center 56°52′ N, 9°20′ E) and a 50 km^2 area around the estate of Kalø (KA, center 56°20′ N, 10°30′ E) were originally selected for counts. However, since few hares were observed, sample sizes were augmented by inclusion of two additional areas, of respectively 20 and 10 km^2, centred on local estates on the island of Lolland (LO1, 54°55′ N, 11°05′ E, and LO2, 54°42′ N, 11°20′ E) during autumn 2005 and spring 2006. These areas were chosen because densities of hares reportedly were high. Thus, while the two areas in Jutland represent densities in randomly chosen farmlands, the two areas on Lolland should rather be interpreted to represent maximum densities.

Habitats consisted mainly of arable land, with crops typical for NW-Europe. All areas are heavily infrastructured, and most of the habitat is less than 500 m from the nearest road. Roads comprise 1) tracks, 2) small, unpaved backcountry roads (width 2-4 m), 3) small, paved roads (width 2-4 m) with limited traffic density, and 4) larger, paved roads (width > 4m) with considerably higher traffic density. These are termed categories 1-4 below.

Counting technique

Counts were made from pick-ups with a roof-mounted, hand-rotable spotlight (Hella, 2x100W). All counts were conducted from within the car, i.e. the eye-height of observers was constant, c. 150 cm agl. Counts were conducted by two observers, each covering a 180° sector. All distances were measured to the nearest meter by binocular laser range finders (Leica GEOVID 7x42 BDA).

Table 1

Count area	No. of counts	N_p	N_{tot}	H_{spring}	N_m
VH	6 (9)	54	324	41	57
KA	6 (9)	27	162	34	57*
LO1	2 (4)	12	24	47	63
LO2	2 (4)	8	16	33	52
Total	16 (26)	101	809	155	229

Table 1. Survey of the material collected for each count area, with respectively number of counts during spring (total number of counts given in parentheses), number of count points (N_p), total number of points counted during spring surveys (N_{tot}), number of hares counted during spring surveys (H_{spring}) and total number of distances included in the estimation of σ^2 (N_m). * 12 additional measurements made during the spring of 2007 included.

Excepting a few individuals very close to the observers, all individuals were detected from reflection of light from eyes. Detected individuals usually froze in a crouching posture when illuminated. A few individuals moved slowly. For these, the distance to the point where they were first seen was used. None were seen running, and no movements that were obviously caused by disturbance from the car were observed.

At HL and KA, all observations were of single individuals. At LO1 and LO2, some observations were made either of females with leverets (autumn counts) or of adult individuals spaced so close that gregariousness could not be completely ruled out. For the purpose of this paper, these have all been treated as a single observation (= the individual first detected). In this way, the sample size was reduced from 136 to 115.

In all four counts areas, however, large parts of the terrain were screened from observation by buildings, hedgerows, copses, or terrain undulations. While theoretically 28.27 ha will be visible within a radius of 300 m from a point, in practice, a mean of 9.79 ha (31%) per point could be covered in HL, 8.46 ha (30%) at KA, 16.40 ha (59%) in LO1 and 10.74 ha (38%) in LO2. Noer & Wincentz (*unpublished*) extended the standard method for statistical analysis of distance sampling data to include correction for unseen areas. We use that method for all calculations presented in this paper.

Sampling protocol

Three counts were conducted in HL and KA during each of the springs of 2005 and 2006, while three were made in autumn 2005 (Table 1). At LO1 and LO2, two counts were made during the autumn of 2005, and a further two during the spring of 2006 (Tab. 1). Thus, for LO1 and LO2 density estimates are for the spring of 2006, while for HL and KA they represent the mean density over these two years. Since there was little difference in numbers counted in the two years (21 and 20 in HL and 20 and 13 at KA), this pooling seems to imply little loss of generality.

Spring counts were made late March to early May, while autumn counts were made in September. The latter period, however, turned out to coincide with autumn ploughing, and a considerable proportion of the points counted were void of hares, either because of the presence of tractors doing nocturnal ploughing, or because fields were newly ploughed and so offered no food for hares. For this reason, autumn data were too sparse for density estimation, and only spring densities are presented here. Distance measurements made at undisturbed points during autumn were, however, included in the estimation of detection functions, after verification that they were comparable to measurements made during spring counts.

All counts were made from 30 minutes to 4 hours after sunset, when foraging activity in fields is assumed to be highest (cf. Verheyden 1991). Counts were only conducted on nights with suitable weather conditions (relatively calm, no precipitation, and no snow cover).

Detection functions and density estimates

Data sets were sparse, especially in Jutland, where 9 counts only resulted in totals of 57 and 45 observations for the two areas. To augment the data, a further 12 measurements obtained in spring 2007 were included in the Kalø data (Tab. 1).

Half-normal detection functions were used in all cases. Data were pooled into 30 m intervals, and a truncation distance of 300 m was used (Noer & Wincentz *unpublished*). In the fitting of half-normal detection functions, data from respectively the two areas on Lolland and the two in Jutland were pooled, resulting in significantly different estimates of the scale parameter σ^2 of respectively 6.6200 and 13.7247 (Noer & Wincentz *unpublished*).

Though perhaps not very different at first inspection (Fig. 1), these two detection functions lead to substantial differences in overall proportions detected within 300 m, respectively c. 18% on Lolland and c. 40% in Jutland. This is so because for point counts only a small proportion of the area covered is close to the observer (Fig. 2).

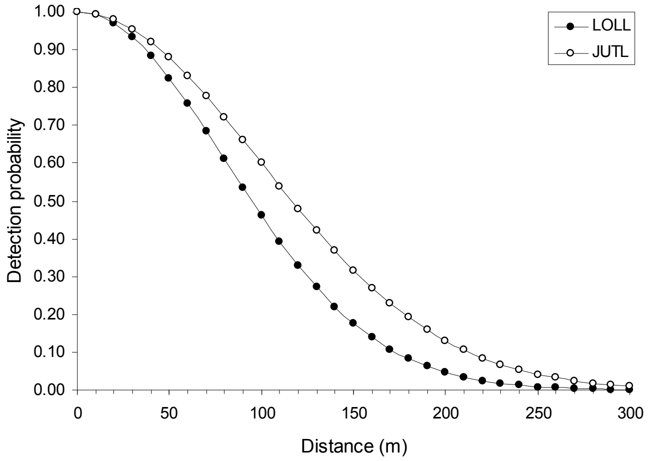

Fig. 1. The estimated half-normal detection functions for count areas on Lolland (scale parameter σ^2 = 6.6200) and in Jutland (scale parameter σ^2 = 13.7247) .

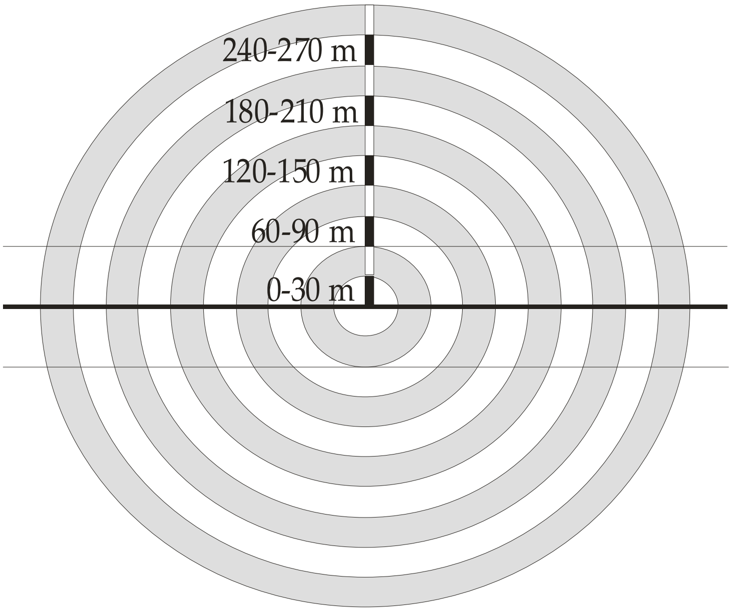

Fig. 2. The basic outlines of a count point truncated at 300 m and with observations grouped into ten 30 m intervals. For point counts truncated at 300 m, 75% of the area lies more than 150 m from the observer. The point is situated on a road (horizontal solid line), and +/- 60 m zones are indicated by thin lines.

Densities were estimated from the basic formula $D = N/(AP_a)$ (Buckland *et al* . 2001), where N is the number of individuals counted, A the total area covered, and P_a the proportion of individuals that are detected. For this estimate of D, an approximate expression for the variance is $\mathrm{var}(D) \approx D^2\ [\mathrm{var}(N)/N^2 + \mathrm{var}(P_a)/P_a^2]$ (Buckland *et al.* 2001).

Var(N) – the variance of the total number counted – was estimated from the pooled sets of points (Table 1), with $\mathrm{var}(N) = C\ N_p\ \Sigma\ (n_i - n_{mean})/(CN_p - 1)$, where C is the number of counts, N_p the number of counted points, n_i the number of hares detected at point i ($i = 1, 2, 3, \ldots CN_P$), and n_{mean} the average number of hares that were seen at a point. An explicit expression for $\mathrm{var}(P_a)$ is given by Noer & Wincentz (*unpublished*).

Pairwise comparisons of density estimates were done by means of z-tests. Under the hypothesis $H_0 : D_1 = D_2$, the distribution of the test-statistic $z = (D_1 - D_2)/[\mathrm{var}(D_1) + \mathrm{var}(D_2)]^{½}$ will be approximately standard normal (i.e. normal with expectation 0 and variance 1.0).

Simulations of avoidance reactions

Count point geometry

An ideal point, placed on a straight road, is shown in Fig. 2, with distances grouped into 30 m intervals. If hares avoid being nearer than 60 m from a road, obviously there will be no observations in the intervals 0-30 m and 30-60 m from the observer. Since for point counts most of the area counted is relatively far from the observer, the proportion of the counted area that is affected will decrease for increasing distance (Fig. 2). Note, however, that even if avoidance is restricted to 60 m from roads, for points with a truncation distance of 300 m densities will be decreased in 28% of the counted area.

Avoidance reactions

Depending on the specific stimuli that trigger avoidance behaviour, the resulting distribution of hares may take many forms. If, for example, hares react to passing vehicles, they may initially distribute randomly when moving out to forage at night, and then gradually be displaced by moving away when vehicles pass. In this case, the distribution of measured distances will have a deficit of observations close to the road (and the observers), and a surplus at some distance, where hares gradually accumulate (Fig. 3). Conversely, if hares avoid habitat close to roads (i.e. the road *per se* is the stimulus) when moving out to forage, few hares would still be seen close to roads, but there would expectedly be no tendency for increased numbers at certain distances (Fig. 3).

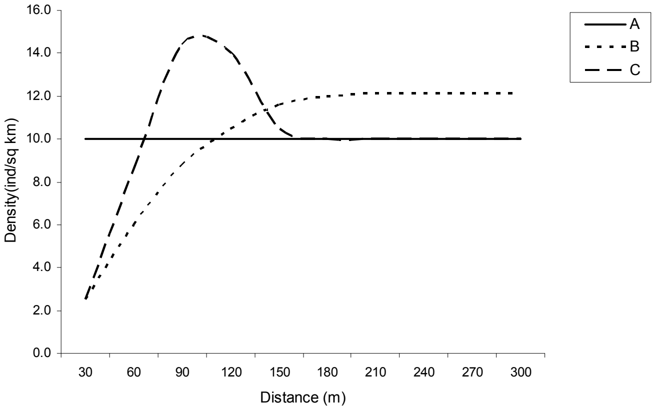

Fig. 3. Hypothetical effects of road avoidance on density at different distances to a road. A: No road avoidance. B: The hares react to the road *per se*, avoiding habitat in proximity to the road when moving out to forage at night. C: Hares react to passing cars, and following initial settling like situation A, they are gradually displaced as cars pass.

These two patterns are not mutually exclusive. In areas with high densities of hares, scenario 1 might presumably lead to 2, if accumulation of hares at certain distances eventually results in redistribution further away from the road. In the data sets studied here, however, densities were low, and most hares were seen alone on the observation points, the maximum number observed at a single point in Jutland being three.

All scenarios described below were simulated for the two behavioural patterns outlined above. However, the simulations showed that under the scenarios studied, when combined with the detection functions, the differences were too small to influence the results, given the sparse sample sizes. Therefore, only results from scenario 1 are presented below.

Simulations were run for all four count areas. Since one purpose was to investigate whether the detection function for HL and KA might have been inflated by road avoidance, all results shown below were based on the 54 points counted in HL. Since a common detection function was estimated for HL and KA, results based on the 27 points counted at KA were nearly identical.

All simulations were based on half-normal detection probabilities. Basically, simulations followed these steps:

1. The point at which a hare would be present was selected by the programme. Points were chosen with probability a_p /A (i = 1, 2, .., n_p), where a_p = visible area at point p, A = total area covered in each count, and n_p = total number of points).
2. A hare was placed randomly within a 300 m circle, i.e. the basic assumption of an even distribution over habitat was fulfilled.
3. Given the count point p, the distance from and direction to the observer, it was determined whether the hare would be visible. This would be the case with probability R_{pi} , where R_{pi} was the fraction of habitat visible at point p and distance i. If the hare was not present in the visible part of the habitat, the process returned to step 1.
4. If potentially visible, the hare was 'detected' with a probability determined from the true value of the scale parameter σ^2 (set in the programme before each run) and the distance from the observer. If not 'detected', Step 1 was resumed.
5. If 'detected', the hare was counted as observation i (i = 1,2,3,..., N). Note that one advantage of such simulations is that the true proportion that is detected is readily calculated.
6. When N = 114 (i.e. corresponding to the data set collected at HL+KA), Maximum-Likelihood estimation was done as described by Noer & Wincentz (*unpublished)*.

Avoidance of habitat in proximity to roads was simulated from the basic scenario. Provided a hare was closer than 0-30, 30-60, 60-90 or 90-120 m from the road at the outset, it moved 30 or 60 m directly away from the road with a probability depending on the initial distance. If this process caused the individual to either disappear from the visible part of the habitat or to transgress the truncation distance *w*, step 1 described above was resumed.

In addition to the basic scenario with no avoidance, we considered four scenarios of increasingly strong reactions to roads:

1. <u>No avoidance reactions</u> (introduced for the sake of comparison). Simulated by hares distributing freely over all observed habitat. This is termed Scenario A in the following.
2. <u>Weak avoidance behaviour</u>. 50% of the hares initially placed less than 30 m from the road, 25% of those 30-60 m, and 10% of those 60-90 m moved 30 m directly away from the road. Termed Scenario B.

3. Moderate avoidance behaviour. As in Scenario B, except that hares reacting moved 60 m. Termed Scenario C.
4. Strong avoidance behaviour. 75% of the hares closer than 30 m, 50% of those 30-60 m , 25% of those 60-90 m and 10% of those 90-120 m moved 30 m. Termed Scenario D.
5. Very strong avoidance behaviour. 75% of the hares closer than 30 m, 50% of those 30-60 m , 25% of those 60-90 m and 10% of those 90-120 m moved 60 m. Termed Scenario E.

The choice of distances moved (30 or 60 m) was based on results presented by Reid *et al.* (2007). Their results were consistent with our own material. Roedenbeck & Voser suspected depressed densities up to 300 m from roads, but densities estimated at different distances had large variances and were not significantly different. With respect to the choice of proportions moving, we note that in reality 47 of 115 recorded distances in Jutland were measured from roads of categories 3 and 4, while the remainder were made from unpaved tracks (categories 1 and 2). Thus, assuming 50% of the individuals to move should correspond to a situation where all roads of categories 3 and 4 release strong or very strong avoidance behaviour.

In addition to the distortion of the distribution over habitat that would result from road avoidance, the observed distribution will also depend on the detection function. If, for example, hares move more than 100 m away from roads, and the probability of detection is low at distances over 100 m, the outcome might be merely a too low estimated density within the observable area, while the model might fit the observed distribution of measured distances quite well. In order to assess the impact of detection probabilities, all scenarios were run for four values of the scale parameter, respectively $\sigma^2 = 6.6200$ (the estimate for the pooled Lolland observations), $\sigma^2 = 8.6200$, $\sigma^2 = 10.6200$, and $\sigma^2 = 12.6200$. The corresponding detection functions were intermediate to those shown in Fig. 1.

To ensure maximum comparability, all calculations were based on a series of 3,000 initially identical data sets that only differed in strength of reactions and detection probabilities.

Expected numbers of observed hares were calculated for each 30 m interval as means of the 3,000 resulting data sets. To assess the realism of the varying degrees of avoidance, these means were compared to the actually observed numbers. For these comparisons, we used the standard term for χ^2 Goodness-of-fit tests, $\Sigma (O_i - E_i)^2 / E_i$. In order to facilitate cross-comparisons distances over 180 m were always pooled, i.e. the χ^2-statistic was always the sum of 7 terms (df = 6).

Results

Density estimates

Use of distance sampling resulted in substantial differences in estimated proportions seen within a distance of 300 m (P_a), respectively c. 40% in HL and KA and c. 18% at LO1 and LO2 (Noer & Wincentz *unpublished*). Since count areas were pooled for estimates of σ2, the minor differences between respectively HL and KA and LO1 and LO2 derived from terrain differences (Noer & Wincentz *unpublished*).

Based on these values of P_a, estimated densities showed large variation across the four counted areas, ranging from c. 3 to c. 111 individuals/km^2 habitat (Table 2). All estimates differed significantly (e.g. KA and LO1: $z = -4.06$, $P < 0.001$, LO1 and LO2: $z = - 2.97$, $0.005 < P < 0.010$), except HL and KA ($z = -0.27$, $0.300 < P < = 0.400$).

Table 2

Count area	P_a	95% c.l.	D	95% c.l.
HL	0.4115	0.3302 - 0.4927	3.1412	1.8578-4.4247
KA	0.4041	0.3234 - 0.4847	5.9636	3.2315-8.6958
LO1	0.1828	0.1488 - 0.2168	65.2396	28.5811-101.8981
LO2	0.1853	0.1524 - 0.2183	111.1745	49.6712-172.6777

Table 2. Estimated proportions seen (P_a) and densities for spring counts, with 95% confidence limits.

95% confidence limits comprised 40-55% of estimated densities (Table 2). Of the two components contributing to the variance estimate, by far the largest part (77-90%)

derived from the variance of total numbers counted. Note, however, that apart from the statistical uncertainty - which is calculated on the assumption that the correct detection function was fitted to the data - there is a systematic influence of the fitted detection function on P_a, since fitting of an incorrect detection function will result in biased estimates.

Evidence for road avoidance

Plots of measured distances against road type suggested avoidance of habitat closer than 50-60 m to roads of categories 3 and 4 in HL and at KA (Fig. 4A). On Lolland, no counts were made from large paved roads (category 4), and no similar tendency could be discerned in the measured distances. Indeed, hares observed from category 3 roads tended to be closer to the observer than hares observed from roads of category 1 and 2 (Fig. 4B).

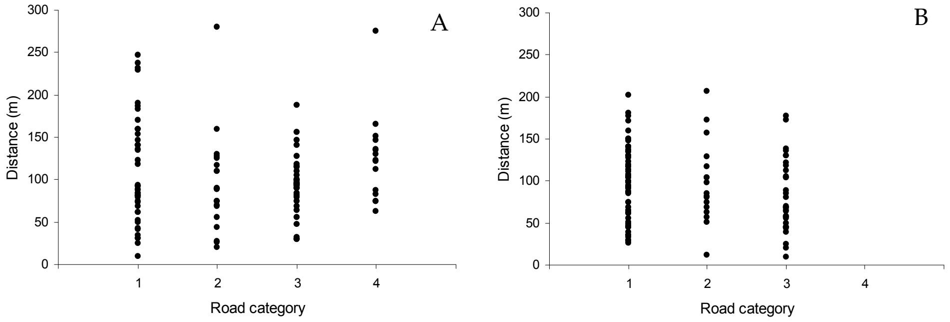

Fig. 4. Distances to detected hares measured for different road categories. Pooled data for HL and KA (A, N = 114) and LO1 and LO2 (B, N = 115)

Though no figures for traffic volume are available, undoubtedly larger roads (categories 3 and 4) carried considerably more traffic in HL and at KA. The difference between Jutland and Lolland in distances measured from category 3 roads may therefore be an indication that avoidance reactions are triggered by traffic rather than by roads *per se*.

Differences between road categories in distances measured were not significant for either of the two regions (Spearman rank correlation coefficient between road category and distance, Jutland: $r_s = 0.0532$, $t = 0.5638$, $df = 112$, $0.600 < P < 0.700$, Lolland: $r_s =$

-0.0374, t = -0.3978, df = 113, 0.300 < P < 0.400).

For the data from Jutland, we repeated the estimation of detection functions by excluding the 15 observations from category 4 roads in the first step, and all 46 distances measured from paved roads (categories 3 and 4) in the second. The first exclusions only decreased the estimated scale parameter very slightly ($\sigma^2 = 12.6827$ instead of 13.7247, N = 99), while the second actually resulted in an increase ($\sigma^2 = 16.5228$, N = 68). For Lolland, exclusion of roads of Category 3 increased the scale parameter estimate slightly ($\sigma^2 = 7.3969$ instead of 6.6200, N = 81).

Thus, there was no strong support for road avoidance having affected estimates in the actual results. However, the tendency for road avoidance suggested by the data in Fig. 3A is further supported by the avoidance of habitat in proximity to roads documented by Reid *et al.* (2007). Further, although the differences in our material were not statistically significant, the plots in Fig. 3 still suffice to make assumptions of unaffected distributions of distances to hares observed from count points placed on roads questionable.

Effect of road avoidance on estimates of σ^2

For all values of σ^2, the mean of 3,000 simulations without road avoidance (scenario A) was slightly higher than the actual value. This is a result of the slightly skewed distributions of estimates for a sample size of 114 (Noer & Wincentz *unpublished*).

For $\sigma^2 = 6.6200$ (the estimate for LO1 and LO2) and no road avoidance, the model predicted far too many observations at distances 0-30 and 30-60 m and too few over 120 m to fit the data for HL and KA (Fig. 5A, GoF = 26.48). These discrepancies, however, gradually decreased for stronger avoidance reactions. Of the simulated scenarios, very strong avoidance produced the best fit to the actual data (Fig. 5E). Increasing the strength of reactions further (not shown) resulted in increasing GoF-values. Corresponding to the increase in numbers reacting and strength of reactions, the estimate of σ^2 was gradually inflated, to 9.4836 for very strong avoidance (Table 3, scenario E). Since according to the estimates given by Noer & Wincentz (*unpublished*) the 95% confidence limits were [$5.2302 < \sigma^2 < 8.0098$] for LO1 and LO2, and [$9.2526 < \sigma^2 < 18.1968$] for HL and KA, this can be taken to indicate that the differences between

Jutland and Lolland in detection probabilities might potentially be explained by road avoidance in HL and KA (but see discussion below).

Increasing σ^2 to 8.6200 and 10.6200 - i.e. increasing detectability - produced acceptable fits to the observations as well (Fig.s 6 and 7). In both cases, weak avoidance produced a marginally better fit than moderate avoidance (Fig.s 6 and 7 B and C). The resulting scale parameter estimates ranged between 9.7730 and 10.2910 for σ^2 = 8.6200 and between 12.0222 and 12.6639 for σ^2 = 10.6200 (Table 3). Thus, road avoidance could inflate the scale parameter estimate into levels approaching what was actually observed in Jutland.

For σ^2 = 12.6200, the fit decreased again, and GoF-values were above 7 for all scenarios. In fact, the best fit was obtained for Scenario A, i.e. no avoidance reactions (Fig. 8).

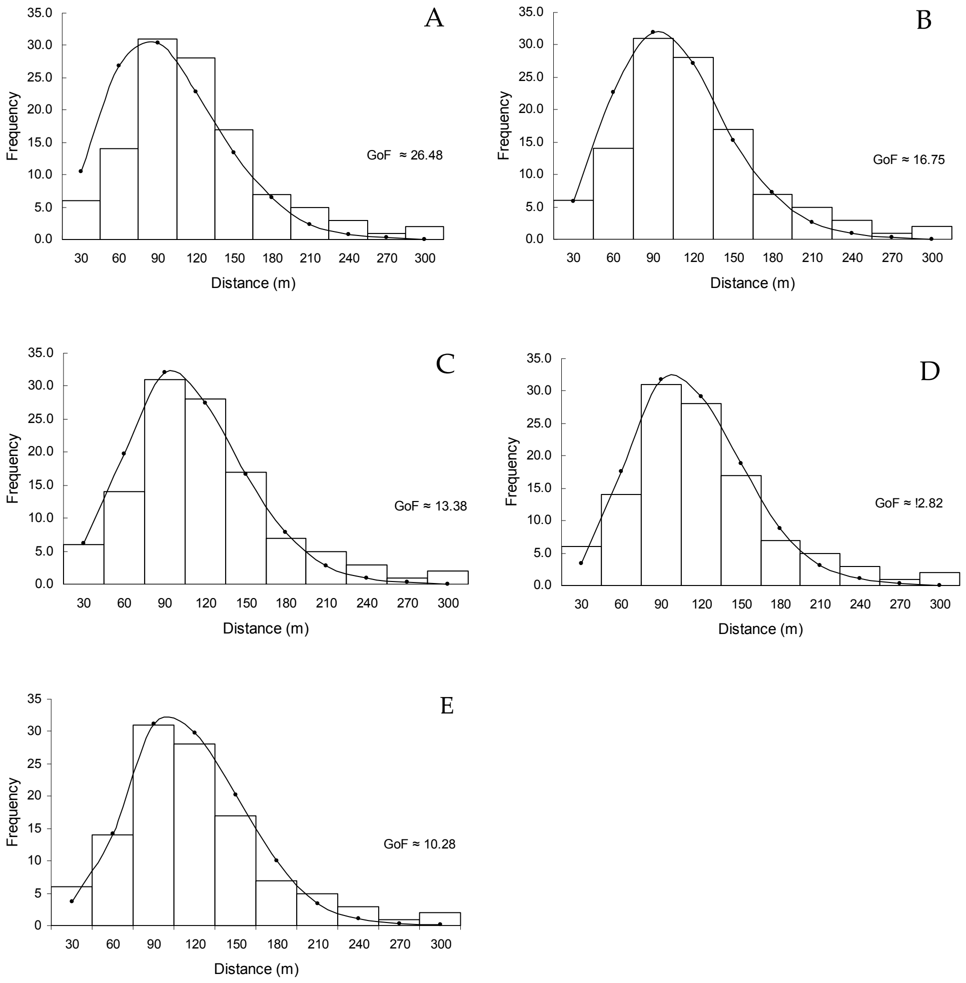

Fig.5. Observed (histograms) and expected (curves, means of 3,000 simulated data sets) distributions of distances in the 5 scenarios of increasingly stronger avoindance of habitat close to roads discussed in the text, for a Half-normal detection function with scale parameter $\sigma^2 = 6.6200$.

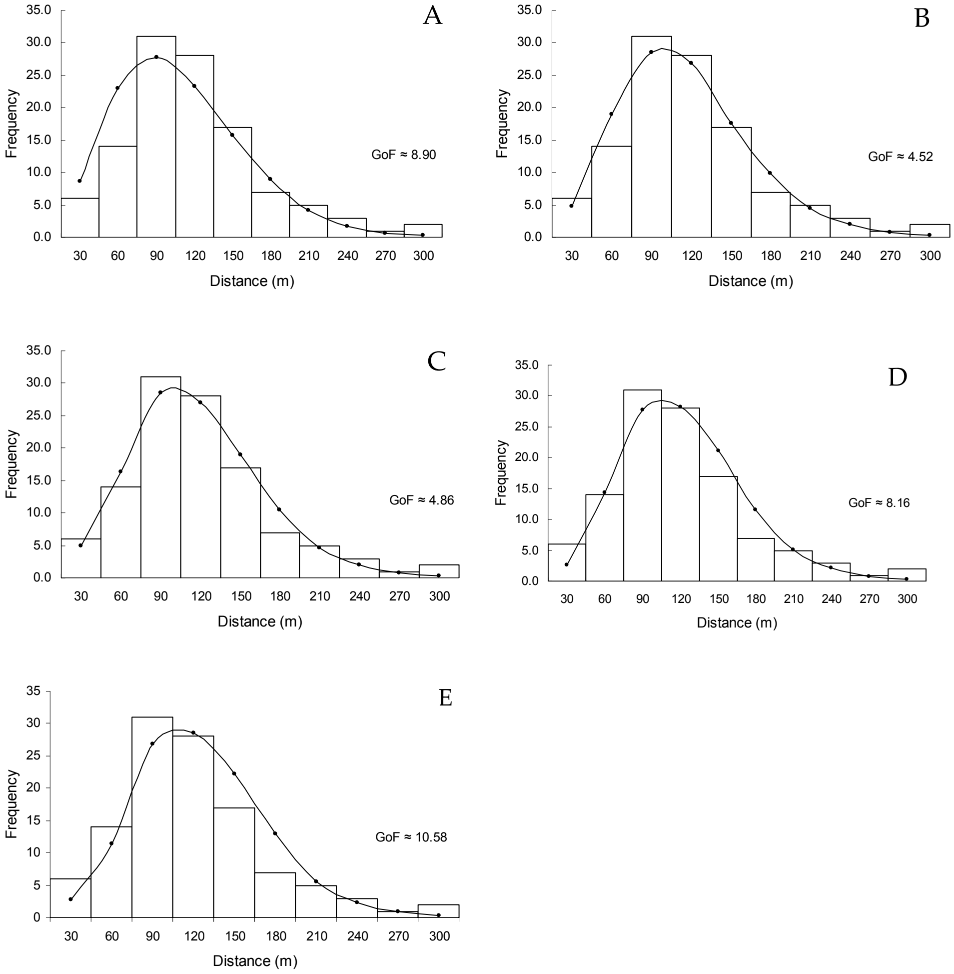

Fig. 6. Observed (histograms) and expected (curves, means of 3,000 simulated data sets) distributions of distances in the 5 scenarios of increasingly stronger avoidance of habitat close to roads discussed in the text, for a Half-normal detection function with scale parameter $\sigma^2 = 8.6200$.

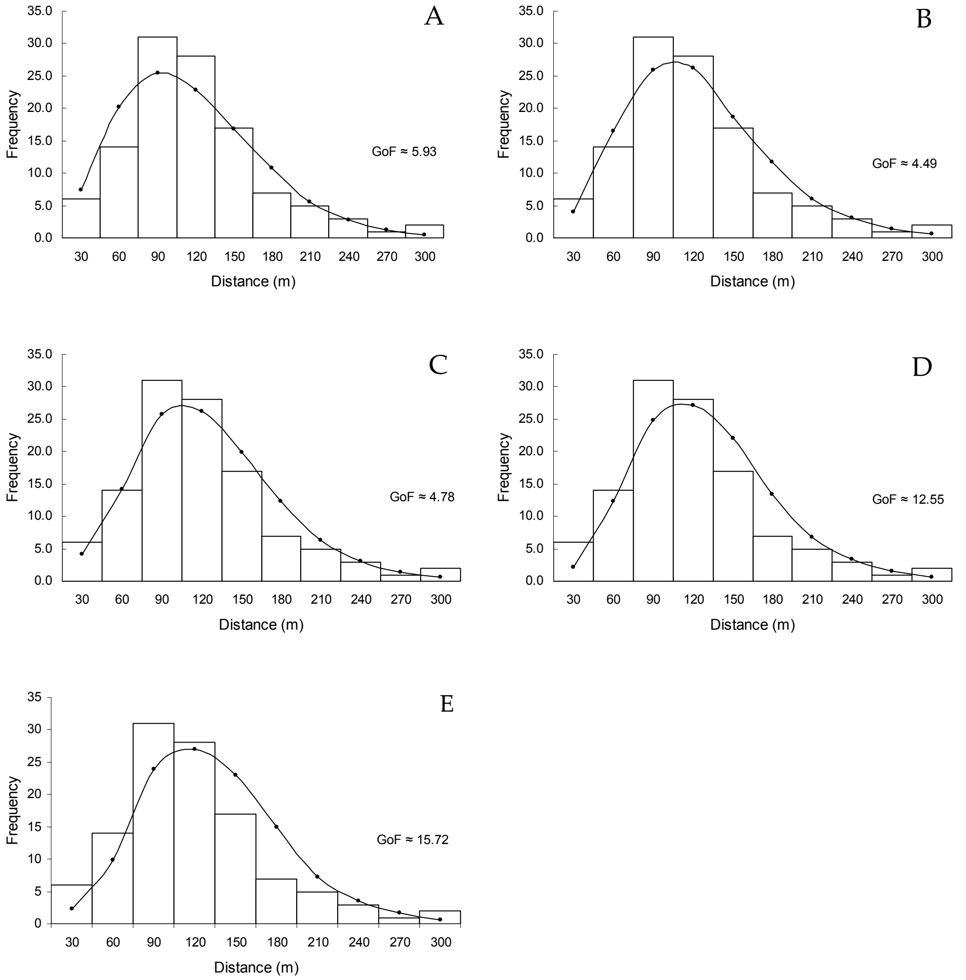

Fig. 7. Observed (histograms) and expected (curves, means of 3,000 simulated data sets) distributions of distances in the 5 scenarios of increasingly stronger avoidance of habitat close to roads discussed in the text, for a Half-normal detection function with scale parameter $\sigma^2 = 10.6200$.

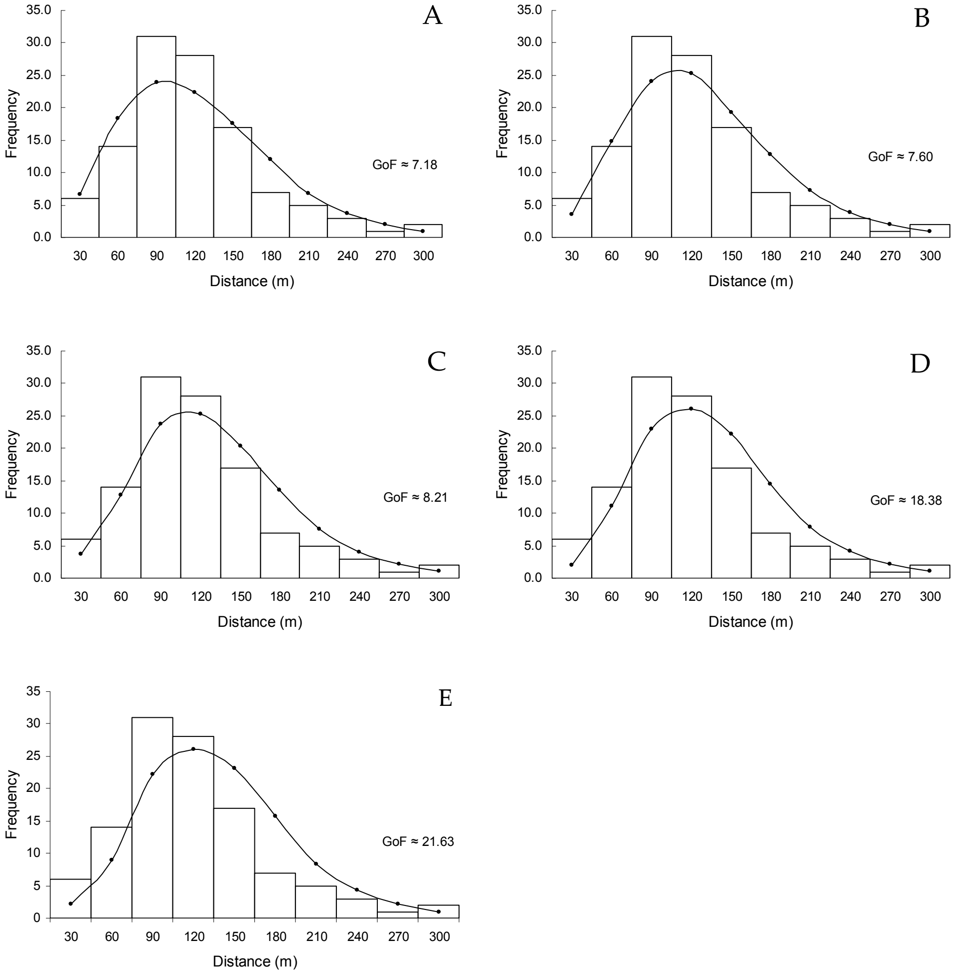

Fig. 8. Observed (histograms) and expected (curves, means of 3,000 simulated data sets) distributions of distances in the 5 scenarios of increasingly stronger avoidance of habitat close to roads discussed in the text, for a Half-normal detection function with scale parameter $\sigma^2 = 12.6200$.

Table 3

Scenario	True σ^2	Mean estimated σ^2	True P_a	Mean estimated P_a	True Density	Mean estimated density	Unconverged data sets*
A	6.6200	6.6144	0.2299	0.2498	5.6240	5.2185	0
B	6.6200	7.5737	0.2109	0.2760	6.1307	4.7169	0
C	6.6200	7.9727	0.2023	0.2864	6.3913	4.5448	0
D	6.6200	8.8110	0.1877	0.3073	6.8884	4.2316	0
E	**6.6200**	**9.4780**	**0.1751**	**0.3232**	**7.3841**	**4.0216**	0
A	8.6200	8.6553	0.2810	0.3033	4.6013	4.2992	0
B	**8.6200**	**9.7730**	**0.2632**	**0.3298**	**4.9125**	**3.9465**	0
C	8.6200	10.2910	0.2546	0.3414	5.0784	3.8112	0
D	8.6200	11.3168	0.2401	0.3634	5.3851	3.5790	0
E	8.6200	12.2213	0.2269	0.3817	5.6984	3.4066	0
A	10.6200	10.7393	0.3263	0.3509	3.9625	3.7142	0
B	**10.6200**	**12.0222**	**0.3095**	**0.3776**	**4.1776**	**3.4474**	1
C	10.6200	12.6639	0.3012	0.3901	4.2927	3.3374	2
D	10.6200	13.8565	0.2875	0.4122	4.4973	3.1557	12
E	10.6200	14.9602	0.2743	0.4316	4.7137	3.0117	43
A	**12.6200**	**12.7979**	**0.3670**	**0.3924**	**3.5231**	**3.3214**	3
B	12.6200	14.2115	0.3515	0.4183	3.6784	3.1135	23
C	12.6200	14.8976	0.3432	0.4303	3.7674	3.0205	56
D	12.6200	16.0409	0.3301	0.4494	3.9169	2.8914	210
E	12.6200	16.9429	0.3172	0.4639	4.0762	2.7973	605

Table 3. Influence of road avoidance on estimates of σ^2, proportion seen and density. Avoidance scenarios giving the best fit to the observed numbers are shown in bold. In all scenarios, densities result from 41 counted individuals, and the difference in densities reflect the differences in detection probabilities.

* Note: The term 'unconverged' implies that a Maximum-Likelihood solution for σ^2 could not be found. The reason why an increasing number of data sets have no solution as road avoidance increases is that the distribution of observed distances becomes increasingly distorted. For very strong avoidance behaviour (Scenario E), even the number of hares present in the interval 150-180 m is affected. While this has small implications when the probability of detection at these distances is small ($\sigma^2 = 6.6200$ or 8.6200) – simply because the observer only detects a small fraction of the hares present at these distance (cp. Fig. 5E and 6E) - increasing numbers are detected at these distances when σ^2 assumes larger values (10.6200 and 12.6200). In that case, many of the simulated data sets have far higher numbers of observations over 150 m than can be fitted by a Half-normal detection function (Fig. 7E and 8E).

Effects on estimates of proportions seen and density

Compared to the actual observations made in HL and at KA, all values of σ^2 predicted more observations than were actually made in the intervals of 0-30 and 30-60 m in scenario A (no road avoidance). Increasingly strong road avoidance gradually decreased all ‘true’ proportions seen by c. 5%, regardless of the true value of σ^2 (Tab. 3). This would be the true effect of road avoidance, resulting from hares distributing at slightly larger distances from the observer when avoiding roads.

Estimated values of P_a , on the other hand, increased c. 8% from scenario A to scenario E for all values of σ^2. This could be termed the ‘observed’ effect of road avoidance. Thus, in relative terms the influence of road avoidance on the estimated P_a decreased for increasing detection probabilities, from a 33% increase for $\sigma^2 = 6.6200$ to a 15% increase for $\sigma^2 = 12.6200$ (Tab. 3).

This net outcome - to inflate the estimated proportion seen - resulted in deflated density estimates. Since densities are estimated by division of the number counted by P_a, the relative impact on estimated density was far larger when σ^2 was small and the estimated detected proportion correspondingly low. The 'worst possible case' was thus strong avoidance (scenario E) when σ^2 was 6.6200 (Tab. 3), resulting in an estimated D of only 54% of the actual value, while for $\sigma^2 = 12.6200$ very strong avoidance (scenario E) the resulting estimate was 69% of the actual density. For intermediate values ($\sigma^2 =$ 8.6200 and 10.6200 and weak or moderate avoidance (scenarios B and C), estimated densities were c. 80% of the actual values (Tab.3).

Discussion

Observed and expected distributions

The present status of the brown hare in the two count areas in Jutland has been a natural focus of attention in this paper. With estimated spring densities of 3-6 individuals per km^2 habitat, a still declining population and low recruitment rates (Wincentz & Sunde *unpublished*, Wincentz *et al. unpublished*), local extinctions within a foreseeable future seem a distinct possibility. Moreover, the hare is a popular game species, and for densities this low the present take of hares by hunters in the two regions may approach - or even transgress - the c. 20% of the population that modelling predicts to be a maximum sustainable harvest rate (Marboutin *et al.* 2003).

For this reason, and because it is the results from HL and KA that may have been strongest affected by road avoidance, the observations made in Jutland have been used as a 'benchmark' throughout this paper. The purpose being to assess the range of possible interpretations that the data can be given if road avoidance influences the results, for all simulations presented here, the resulting predicted distributions of distances have been compared to this set of observations.

It should be noted, however, that in this approach it has tacitly been assumed that there is no road avoidance at LO1 and LO2. The data in Fig. 4 do not suggest this, but on the other hand detection probabilities for LO1 and LO2 are slightly larger than those given by Reid *et al.* (2007), where road avoidance affected estimates. Road avoidance, of course, would have inflated values for P_a and deflated density estimates in that region as well. If road avoidance affected the estimates for Lolland, however, it is rather certain that this influence was far weaker than in Jutland, as evidenced by the differences in estimated detection probabilities.

Differences in estimated detection probabilities

From the outset, we expected detection probabilities to be essentially constant across count areas and seasons. However, when corrected for blind areas, the differences between scale parameter estimates became highly significant, and the support for pooling data from count areas pairwise (HL+KA vs. LO1+LO2) surprisingly strong. Basically, the two sets of data collected in Jutland had fewer observations than predicted by the half-normal detection function at distances under 60 m, a surplus of observations at distances between 60 and 120 m (that might indicate the displacement due to reactions to traffic outlined above, cf. Fig. 3), and a surplus of distance measurements over 150 m. Since larger parts of the visible terrain were closer to the observer in Jutland this distribution was in contrast to what would be expected after correction for blind areas (Noer & Wincentz *unpublished*).

The results presented in this paper show that the differences between count areas in detection probabilities might indeed by explained by road avoidance - if only by assuming that it was very strong (Table 3 and Fig. 5E). On the other hand, strong avoidance did only result in a scale parameter estimate of $\sigma^2 = 9.3760$ (Table 3) while the actual estimate for HL+KA was $\sigma^2 = 13.7247$, and other simulations indeed indicated that models assuming higher values of σ^2 than 6.6200 (i.e. combinations of higher detectability and more moderate road avoidance) could fit the data equally well.

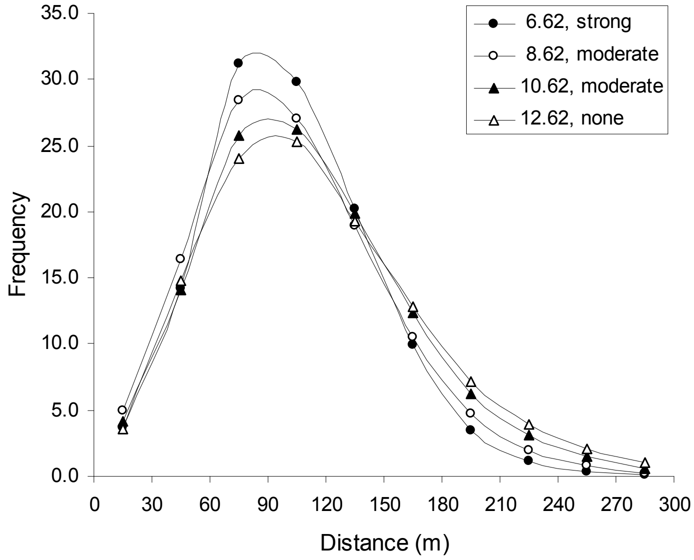

Fig. 9. Comparison of predicted distributions (means of 3,000 simulations) of 114 distance measurements for respectively $\sigma^2 = 6.6200$ and strong avoidance behaviour, $\sigma^2 = 8.6200$ and moderate avoidance, $\sigma^2 = 10.6200$ and moderate avoidance, and $\sigma^2 = 12.6200$ and no avoidance.

Comparison between predictions resulting from the four different values of σ^2 input to the simulations show that they basically do not differ very much (Fig. 9). Compared to the actual observations, $\sigma^2 = 6.6200$ when combined with very strong avoidance could predict the actually observed numbers very well up to distances below 150 m, and the GoF-value of 10.28 in Fig. 5E mainly derives from a deficit of distances over 200 m. On the other hand, higher values of σ^2 in combination with weak or moderate avoidance - or even the absence of avoidance - resulted in a slightly poorer fit to the surplus of observations at 60-120 m, though because of a better fit to the number of observations over 180 m (Fig. 9) the Goodness-of-fit was comparable or even better (cf. Fig. 6C, Fig. 7C, and Fig. 8A).

Thus, the various scenarios we investigated predict distributions that are so similar that very large data sets would be necessary to settle this issue.

Usually, it is recommended that the 10-15% largest distances measured are discarded (by truncation) before model fitting (Buckland *et al.* 2001). Since a few large distances can affect estimates considerably, the rationale for doing so is quite sound. On the other hand, truncation did not change the scale parameter estimates much in our data sets (Noer & Wincentz *unpublished*), and in this paper we showed that omission of data collected from large and more heavily trafficked roads in Jutland only changed estimates very little. If the difference in detection probabilities in our measurements reflects reality, truncation would actually be throwing away what perhaps could be the most valuable information in these data sets. For these reasons, we conclude that the differences between count areas in detection probabilities may have been caused by road avoidance, but that they may also have been real, though possibly more or less inflated by road avoidance.

For the latter case, a list of other factors that might conceivably affect detection probabilities would be comprehensive, including both meteorological conditions and biological factors. Background light, temperature, and relative humidity might all both differ between areas and interfere with the probability of detecting hares at a distance. A biological factor of potential influence was that foxes were abundant in both areas in Jutland, but not on Lolland. The presence of foxes will presumably make hares more wary, and so could increase the probability that a hare looks in the direction of the disturbance when illuminated – and by doing so is revealed by the light reflected from its eyes.

Methods for estimating hare densities

Methods applied in order to estimate densities of hares range from capture-recapture studies (Abildgaard *et al.* 1972, Marboutin & Peroux 1995) to uncorrected count numbers (Strauss *et al.* 2008). Of these, the former undoubtedly will provide the more reliable estimates, but it is very resource-demanding in terms of time and man-power and must at least comprise three study years. The latter, on the other hand, will be even more problematic than the results discussed here. For LO1 and LO2, even for a truncation distance of 150 m only an estimated 47% of the hares present are detected by spotlight counts. For HL, the spring density estimated from uncorrected numbers counted within this distance would have been 36/(324x0.0456) = 2.44 individuals per km^2 habitat – even lower than the 3.14 found by application of distance sampling without considering the influence of road avoidance (Tab. 2). The corresponding figures would have been 3.61 at Kalø, 32.11 at LO1 and 50.05 at LO2 (cf. estimates given in Tab. 2).

Transect counts from cars were ruled out in this case, and we discarded cross-country transects with handheld spotlights because hares might react to an observer well before detected. Thus, we do not have the data to evaluate densities at various distances to roads provided by Reid *et al.* (2007). We do notice however, that their estimates resembled ours, including density estimates that approximately doubled when corrected for road avoidance (cf. $\sigma^2 = 6.6200$ and strong avoidance behaviour in Tab. 3). Thus, both studies confirm that uncorrected count data will in all probability be far lower than what results from distance sampling. Note also that the difficulties in the interpretation discussed here would never have been realised if only count data were available.

In our case, estimated spring densities were widely different for the four counted areas. In Jutland, estimates were 3-6 hares per km^2 habitat, while at Lolland values of 65 and 111 were found (Tab. 2). Regardless of the interpretation of the data, these differences are far too large to have been caused by violation of assumptions of the method. For the data from HL and KA, estimated densities were down to 3.14 and 5.96 individuals per km^2 of habitat. The worst possible cases studied here suggest that they may have been as high as respectively 7.4 and 14.8 ind./km^2, but even in that case - given that the population is still declining and the proportion of young hares in the population in autumn is too low to sustain the population, evidently the focus of future management concerns must the densities in Jutland. Obviously, a mere two count areas can not be considered representative, but given the results presented here, distance sampling of hares in more count areas, comprising studies designed to provide more insight into the problems of distribution of hares over habitat, are undoubtedly in demand.

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Brown hares on the edge: Genetic population structure of the Danish brown hare

Andersen, L.W., Fredsted, T., Wincentz, T. & Pertoldi, C.

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Brown hares on the edge: Genetic population structure of the Danish brown hare

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Andersen L. W., Fredsted T., Wincentz T. and Pertoldi C. 2009. Brown hares on the edge: Genetic population structure of the Danish brown hare. Acta Theriologica 54: 97–110.

Denmark lies on the edge of the distributional range of the brown hare *Lepus europaeus* Pallas, 1778, where population differentiation is most likely to occur. A total of 369 brown hares from eight geographically distinct Danish European brown hare populations were used to study the genetic population structure. In all, 480bp of the mitochondrial D-loop were sequenced in both directions. Observed genetic diversity (π) was relatively low ($\pi = 0.41\%$) while haplotype diversity (h = 0.808) and the number of unique haplotypes (19) were similar to levels found in other European brown hare populations. The observed population structure was pronounced (pairwise conventional F_{ST} and ϕ_{st} ranged between 6.9–57% and 5–69.8%, respectively). There was no correlation between the geographic and the genetic distance. Population structure was influenced by genetic drift, anthropogenic effects (eg translocation and escapes from hare-farms) and by post-glacial recolonization from southern refuges or refuges north east of the Black Sea. Analysis of historical population expansion/fluctuation events indicated that the populations have experienced different demographic events in the recent past. Relatively high sequence divergence between some populations might be explained by multiple recolonization events after the last Pleistocene glaciations or by stocking effects. Colonization from southern refuges was supported by the observation that haplotype 2 in the Danish brown hare was identical to the central European ancestral haplotype c07.

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Key words: *Lepus europaeus*, mitochondrial DNA, habitat fragmentation

Introduction

Species at the edge of their distributional range are more likely to be subject to population fluctuations and low periodic populations sizes, causing bottleneck effects and genetic drift (Brown 1984, Schwartz *et al.* 2003), features likely to cause greater levels of population differentiation than in core areas. However, depending on dispersal pattern and social structure, the genetic diversity at the edge of the population range will also be greatly influenced by immigrants from more abundant adjacent populations (Sagarin and Gaines 2002) or from translocations or escapes of captive-reared individuals.

Denmark lies on the northern edge of the distribution range of the brown hare *Lepus europaeus* Pallas, 1778, and so may be expected to show some degree of population structure which might be influenced by many of the above mentioned factors. The brown hare population has also been declining since 1960 here, as elsewhere in Europe (eg England: Tapper and Parsons 1984, Poland: Wasilewski 1991, Panek and Kamieniarz 1999, Germany: Fickel *et al.* 2005), reflected in a drop in the Danish annual hunting bag statistics [(> 400 000 before 1960 to 67 600 in 2004/2005 (http://vildtudbytte.dmu.dk/)].

The primary cause of decline is habitat changes associated with agricultural intensification (Smith *et al.* 2004, Smith *et al.* 2005). Habitat fragmentation can reduce genetic variability as a result of reductions in effective population size (N_e), and increase genetic differentiation between remaining fragments. Population subdivision may reduce adaptive potential in response to environmental changes which together with the demographic consequences of habitat loss, enhance the risk of reduction in population size (Bijlsma *et al.* 2000).

However, genetic consequences of translocation between two wild populations may likewise reduce N_e due to outbreeding depression in the stocked population. This will reduce overall fitness, increase inbreeding (due to reduced successful mating) and cause loss of genetic variability in the stocked population (Ryman *et al.* 1995). Introgression caused by the translocated (or released/escaped) individuals into a locally adapted population may also result in degradation of co-adapted gene complexes (Thulin *et al.* 2002, Thulin 2003, Bekkevold *et al.* 2006).

The brown hares have been translocated in and between many countries, potentially masking phylogeographical events and population structure. Brown hares reared in captivity (often of unknown origin) have been (and still are) translocated to restock populations that are locally extinct or over-exploited by hunting (Kasapidis *et al.* 2005). For instance in France, the indigenous hares have been replaced by hares introduced from Eastern Europe (Flux 1983). In Italy, the brown hare has been replaced by other introduced non-indigenous hares (Pierpaoli *et al.* 1999) and in Greece the genetic population structure has been influenced by introduction of captive-reared individuals from Italy, Bulgaria and former Yugoslavia (Mamuris *et al.* 2001).

In addition to the effects of habitat fragmentation and translocation/escapes, genetic structure is influenced by complex interactions between social organization, dispersal tendencies, population history and environmental factors (Chepko-Sade and Halpin 1987).

The social organization of the brown hare in Denmark is characterized by seasonal breeding and polygamous mating system. Young hares of both sexes are highly sedentary, 55% stay within two km of their birth-place, and only 11% move more than five km before their first reproductive season (Bray *et al.* 2004). Males disperse more frequently than are highly philopatric females (Bray *et al.* 2004). Once the home range is established, brown hares show high site fidelity (Bray *et al.* 2007).

Quaternary climatic fluctuations in the Palaearctic caused repeated local extinctions and shifts in distribution range and population size of animals, which may lead to losses of genetic diversity and inter-population divergence (Hewitt 1999). This complex dynamics in the late Pleistocene/Holocene make it difficult to disentangle the genetic consequences of natural climatic and habitat changes from anthropogenic influences. Furthermore, Denmark consists of numerous small islands and natural geographical barriers which also have shaped population structure. It is therefore important to determine the timing of genetic changes to differentiate those which have occurred recently from those which occurred before anthropogenic influence.

Based on variance in the d-loop region in the mtDNA the aims of the study were, (1) to analyse the population structure of the Danish brown hares from eight geographical localities testing the hypothesis that the Danish brown hares on the different islands constitute separate genetic populations and (2) to establish whether the pattern of genetic variability between populations results from genetic drift or translocation/escape effects or historical events. It was hypothesised that if anthropogenic impacts have no effects on the ancient population history, the clustering of the eight populations based on genetic drift alone will be identical to the clustering based on an evolutionary model. Furthermore, the results of hierarchical F_{ST} and ϕ_{ST} analysis are expected to explain a higher percentage of the genetic variance when structuring according to islands compared to single localities.

Finally, the phylogeographic relationship between Danish and European brown hare populations was investigated.

Material and methods

Sampling

During 2003–2005 The National Environmental Research Institute of Denmark collected wild hares from several locations in Denmark (Asferg, pers. comm.), covering most of the distribution range of the brown hare in Denmark. A total of 369 hares from eight localities were examined in the present study (Table 1, Fig. 1).

DNA extraction and sequencing

DNA was extracted from hare muscle tissue using a slight modification of the Chelex protocol (Walsh *et al.* 1991). A 480 bp fragment of the mtDNA D-loop (control region) was PCR amplified using the primers L15997 5'-CACCATTAGCACCCAAAGCT-3' located in the tRNA gene and H16498 5'-CCTGAAGTAGGAACCAGATG-3' (Gerloff *et al.* 1999). PCR was performed in 10 µl (1 µl buffer (1.5 mM $MgCl_2$), 1.6 µl dNTP , 0.5 µl primer (10 pmol/µl), 0.1 µl *Taq* polymerase (Amersham Pharmacia Biotech). Amplification conditions were 94°C for 3 min, and 35 cycles of 94°C for 30 s, 50°C for 40 s and 72°C for 60 s and a final extension step of 72°C for 7 min. Sequencing was conducted using an Automatic Sequencer ABI 3730xl. Both strands were sequenced in all samples.

Haplotype diversity and nucleotide diversity

Sequences were analyzed using Bioedit version 7.0.0 (Hall 1999). Identical haplotypes among the 369 sequences were found using the Collapse tool in FaBox 1.2 (http://www.birc.au.dk/fabox/) (Villesen 2007). Variation in the D-loop was estimated as haplotype diversity (h) and nucleotide diversity (π) (Nei 1978) using ARLEQUIN (http://cmpg.unibe.ch/software/arlequin3/; Schneider *et al.* 2000).

Population structure

To quantify the genetic differentiation among localities caused by genetic drift (F_{ST}; Wright 1951) and taking evolutionary divergence into account, conventional F_{ST} statistics based on haplotype frequencies and ϕ_{ST} based on Tamura and Nei (1993) evolutionary genetic distance (nucleotide substitutions, $\alpha = 0.17$ as recommended by Fickel *et al.* (2005) were calculated pairwise amongst the eight localities (ARLEQUIN v. 3.01, Schneider *et al.* 2000). Furthermore, AMOVA analysis was performed (ARLEQUIN v. 3.01,

Table 1. Genetic diversity and population expansion indices for the total samples of brown hare [*n* – sample size, h – haplotype diversity ± standard error, π – nucleotide diversity ± standard error, Tajima's *D* (see txt), Fu's F_S (see text), SSD – sum of squares deviations between observed and expected mismatch, Rag. Id. – Raggedness Index of the mismatch distribution]. Bold – significant at the 5% level.

No.	Locality	*n*	h	π (%)	Tajima's *D*	Fu's F_S	SSD	Rag. Id
1	Magrethe Kog, S. Jutland	28	0.78 ± 0.003	0.26 ± 0.2	–0.572	–2.11	0.01	0.1
2	Hjelm Island	33	Only one haplotype					
3	Giesegaard, Zealand	29	0.26 ± 0.10	0.18 ± 0.14	–1.29	–0.04	0.05	0.54
4	Borrebygaard, Zealand	46	0.70 ± 0.03	0.22 ± 0.17	–0.15	–0.02	0.01	0.08
5	Vennerslund, Falster	78	0.82 ± 0.02	0.55 ± 0.33	1.58	1.3	0.05	0.18
6	Pandebjerg, Falster	58	0.79 ± 0.03	0.53 ± 0.32	1.22	0.65	**0.1**	**0.23**
7	Orebygaard, Lolland	42	0.67 ± 0.05	0.32 ± 0.22	0.27	0.05	0.04	0.14
8	Bornholm	55	0.62 ± 0.06	0.18 ± 0.14	–0.54	–2.46	0.01	0.09

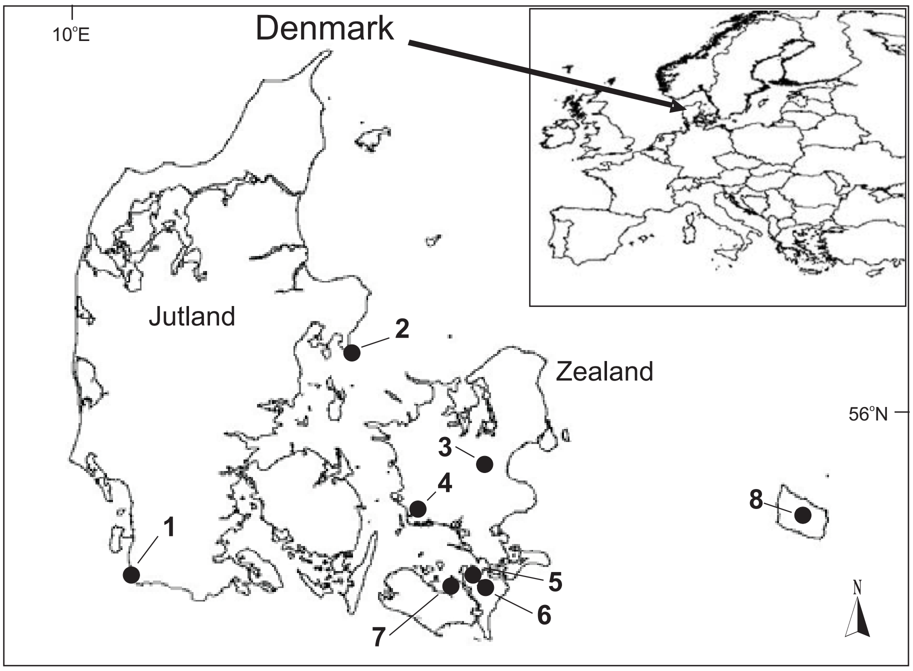


Fig. 1. Map of the eight sampling locations (populations) in Denmark. 1 – Margrethe Kog Estate, 2 – Hjelm Island, 3 – Giesegaard Estate, 4 – Borreby Estate, 5 – Vennerslund Estate, 6 – Pandebjerg Estate, 7 – Orebygaard Estate, 8 – Bornholm Island.

Schneider *et al.* 2000) when separating males and females due to the phylopatric behavior of the brown hare.

Isolation by distance was estimated using geographical distances measured between the cores of the distribution areas of the populations in question. The relationships between the genetic and geographical distances amongst the populations were estimated using a Mantel test implemented in ARLEQUIN v. 2.0 (Schneider *et al.* 2000).

Genetic drift and anthropogenic induced causes or population history (local phylogeograhy)

This was analysed estimating the genetic relationship based on pairwise genetic distances using conventional F-statistic and ϕ_{ST} with the evolutionary model of Tamura and Nei (1993) performed in ARLEQUIN (v 3.01, Schneider *et al.* 2000). An unrooted tree based on both pairwise genetic distance and evolutionary genetic distance were constructed using the Neighbour-Joining algorithm implemented in the PHYLIP v. 3.6 package (Felsenstein 2004).

The hierarchical F_{ST} and ϕ_{ST} analysis were performed as an AMOVA analysis in ARLEQUIN v. 3.01 without grouping data and grouping the data according to the islands Jutland (locality 1), Helms Island (locality 2), Zealand (locality 3, 4), Lolland (locality 7), Falster (locality 5, 6) and Bornholm Island (locality 8) (Schneider *et al.* 2000) (Fig. 1).

Furthermore, historical population expansion/fluctuation events of the Danish brown hares was analysed using Tajima's D test of selective neutrality (Tajima 1989) and Fu and Li's, F_S (Fu and Li 1993) and mismatch distributions (Rogers and Harpending 1992). Significant negative D values can be indicative of changes in population size or linkage to a locus under selection while positive values can be indicative for a population bottleneck (Johnson *et al.* 2007). Significant large negative F_S values can be indicative of an excess of recent mutations, rejecting population equilibrium (Fu 1997). The significance of Tajima's D and Fu and Li's, F_S (Fu and Li 1993) were evaluated after 1000 permutations in ARLEQUIN.

Declining populations or populations at demographic equilibrium are expected to show a multimodal, or ragged, distribution of pairwise nucleotide differences (mismatch distribution), while sudden demographic expansions of a population should display a smooth unimodal distribution and star-like phylogeny (Rogers and Harpending 1992). The patterns (unimodal or multimodal) of the mismatch distributions were analysed, testing the goodness of fit between observed and expected mismatch distributions under the sudden expansion model using the parametric bootstrap (1000) approach in ARLEQUIN. The test statistic used was the sum of square deviations (SSD) between observed and expected distributions, calculating P-values as the proportion of simulations producing a larger SSD than the observed SSD. The raggedness index was calculated in order to quantify the smoothness of the mismatch distribution and the significance was evaluated in ARLEQUIN similar to SSD. These tests were applied at the total samples representing the eight different localities and when partitioning according to sex.

The sequential Bonferroni procedure was applied in the table wide test of population structure at the 5% significance level (Rice 1989).

Phylogeographic analyses

The phylogeographic relationship of the Danish brown hare and the brown hares from Germany, Italy, Hungary and Europe were analysed (GenBank Acc. no DQ 645432-50, AY103497-531, AY149725, AY149727, AU435387-411, AF157435, AF157438, AF157440, AF157443, DQ469654-69) using 81 brown hare sequences from GenBank. The areas represented countries from which brown hares were known to have been imported. The sequences were aligned in SEQUENCHER 4.2 and cropped to the shortest sequence (244bp).

Phylogeographic relationships amongst the unique haplotypes were estimated using the software TCS (Clement *et al*. 2000), which is based on the statistical parsimony method of Templeton *et al*. (1992). Gaps were set as the fifth state and the parsimony criterion at 95%. This method links the haplotypes according to the smaller number of differences (mutations) defined by the 95% criterion and identifies the most probable ancestral haplotype.

Results

Genetic variation

Alignment of DNA sequences was unambiguous regarding insertions and deletions. However, three sites were excluded from all analyses (leaving 477 bp) because of inconsistent traces. The area contained many C's and T's which can be difficult to sequence.

A total of 16 segregating sites (13 informative sites, 3 singletons), defining 19 different haplotypes were found in the 369 sequenced individuals. The nucleotide diversity, π and haplotype diversity, h, ranged between 0–0.55% and 0–0.82, respectively (Table 1) amongst the eight localities.

Five of the eight localities (locality 1, 5, 6, 7, 8; Fig. 1) had private haplotypes, while 11 of the 19 haplotypes were private (haplotype: 3, 6, 8, 11, 12, 13, 14, 15, 17, 18, 19) (Table 2). Halotype 5 was the only haplotype shared amongst all localities, while the private haplotype 11 was the haplotype observed with the highest frequency (0.357). Of the private haplotypes one (H14, Table 2) was found in a single male on Bornholm.

Population structure

Population pairwise conventional F_{ST} values ranged from 6.7–62.9% where two pairwise comparisons were not significant after application of the sequential Bonferroni correction (locality pairs: 1–4 and 2–3) (Table 3, below diagonal). Average population differentiation estimate was $F_{ST} = 0.24$, ~24%, ($p < 0.05$). Taking the evolutionary distance based on nucleotide substitutions (Tamura and Nei 1993) into account, the pairwise ϕ_{ST} ranged from 5–69.8% (Table 3 above diagonal) with fewer significantly genetic different locality pairs. The four locality pairs 1–6, 2–3, 3–7 and 5–6 were not significant after sequential Bonferroni correction. Overall sex specific F_{ST} estimates did not reveal any differences between sexes ($F_{ST\,(\mathrm{males})} = 0.24$; $F_{ST\,(\mathrm{females})} = 0.23$, $p > 0.05$). However, when partitioning according to populations and sex (Table 3) taking the evolutionary distance into account and counting the number of times the female ϕ_{ST} values were significant when the male ϕ_{ST} values were non-significant and vise versa (female ϕ_{ST} values non-significant and male ϕ_{ST} values significant) showed that 6/28 female ϕ_{ST} values were significant in the first case and 1/28 male ϕ_{ST} values in the second case. This higher number of pairwise female ϕ_{ST} estimates (6/28) was more than expected just by chance at the 5% significance level.

No isolation by distance was observed linking the geographical distance with the genetic distance between the localities (data not shown/regression coefficient $r = 0.000845$, $p = 0.0547$).

Anthropogenic influences and population history

Dividing the localities into six groups representing Jutland (locality 1), Hjelm Island (locality 2), Zealand (locality 3, 4), Falster (locality 5, 6), Lolland (locality 7) and Bornholm (locality 8) gave an overall F_{CT} (amongst groups) = 12.9% (p=0.096) based on haplotype frequencies, and ϕ_{ct} = 15.2% ($p = 0.045$).

The genetic relationships among the localities assuming different influences of genetic drift and mutation (Fig. 2a, b) both indicated a

Table 2. Haplotype frequencies in eight populations of brown hares in Denmark defined in Fig. 1 and Table1. Haplotypes in *italics* are found in German brown hares (Fickel *et al*. 2005). F– females, M– males.

Locality	1	F	M	2	F	M	3	F	M	4	F	M	5	F	M	6	F	M	7	F	M	8	F	M
H1	0.4	0.14	0.29							0.28	0.15	0.13	0.19	0.12	0.08	0.02	0.02					0.13	0.06	0.07
H2	0.1	0.04	0.04				0.04		0.03				0.03	0.03		0.03		0.03				0.58	0.36	0.22
H3													0.14	0.03	0.01									
H4	0.04		0.04							0.02	0.02		0.06	0.01	0.05	0.24	0.16	0.09				0.07	0.07	
H5	0.1	0.04	0.11	1	0.36	0.64	0.86	0.45	0.41	0.39	0.3	0.09	0.32	0.14	0.18	0.33	0.22	0.1	0.45	0.33	0.12	0.02		
H6													0.12	0.05	0.07									
H7							0.07	0.04	0.04	0.02	0.02		0.13	0.09	0.04	0.1	0.05	0.05						
H8																						0.16	0.09	0.07
H9							0.04	0.04								0.21	0.14	0.07	0.02		0.02			
H10										0.28	0.2	0.09	0.01		0.01				0.12	0.01	0.02			
H11																			0.36	0.19	0.17			
H12																0.03	0.03							
H13																			0.02	0.02				
H14																						0.02		0.02
H15	0.11	0.04	0.07																					
H16																0.03	0.03		0.02		0.02			
H17	0.14	0.04	0.1																					
H18	0.04	0.04																						
H19		.																				0.02	0.02	

Table 3. Pairwise F_{ST} values in the total population and after stratification according to sex, based on conventional *Fst* (below diagonal) and on evolutionary distance, ϕ_{ST} (Tamura and Nei 1993 above diagonal). Numbers 1–8 represents the eight localities defined in Fig. 1 and Table 1. Values in italics are not significant after sequential Bonferroni correction.

Locality	1	2	3	4	5	6	7	8
1		0.7	0.47	0.21	0.09	*0.06*	0.3	0.27
2	0.57		*0.07*	0.43	0.37	0.37	0.29	0.65
3	0.41	*0.07*		0.22	0.25	0.24	*0.11*	0.46
4	*0.10*	0.38	0.25		0.12	0.12	0.18	0.39
5	0.08	0.32	0.21	0.07		*0.05*	0.16	0.20
6	0.16	0.35	0.22	0.13	0.07		0.14	0.19
7	0.23	0.36	0.22	0.13	0.13	0.14		0.28
8	0.21	0.63	0.51	0.31	0.24	0.26	0.35	
Females								
1		0.72	0.45	0.24	*0.08*	*0.04*	0.32	0.26
2	0.58		*0.02*	0.3	0.38	0.31	0.15	0.6
3	0.44	*0.02*		0.13	0.28	0.19	*0.03*	0.44
4	*0.11*	0.27	0.19		0.18	0.12	0.12	0.38
5	*0.05*	0.32	0.24	0.09		0.07	0.23	0.22
6	0.15	0.28	0.19	0.11	0.09		0.15	0.17
7	0.23	0.24	*0.16*	*0.08*	0.15	0.13		0.31
8	0.22	0.60	0.53	0.33	0.24	0.28	0.38	
Males								
1		0.68	0.47	*0.11*	*0.08*	*0.03*	0.28	0.26
2	0.55		*0.07*	0.64	0.32	0.44	0.49	0.69
3	0.36	*0.08*		0.35	0.18	0.26	0.23	0.46
4	*0.04*	0.57	0.35		*0.04*	*0.08*	0.27	0.39
5	*0.08*	0.29	0.14	*0.07*		*0.03*	*0.09*	0.18
6	0.14	0.42	0.23	0.17	*0.05*		*0.13*	0.18
7	0.21	0.53	0.31	0.21	0.14	0.14		0.24
8	0.16	0.65	0.47	0.25	0.24	0.21	0.31	

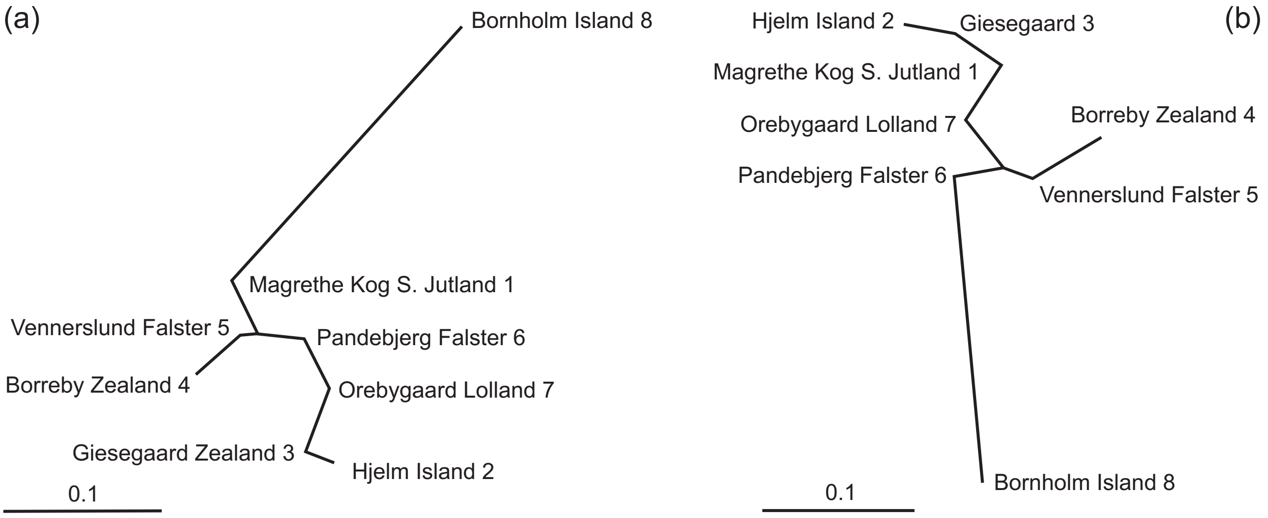
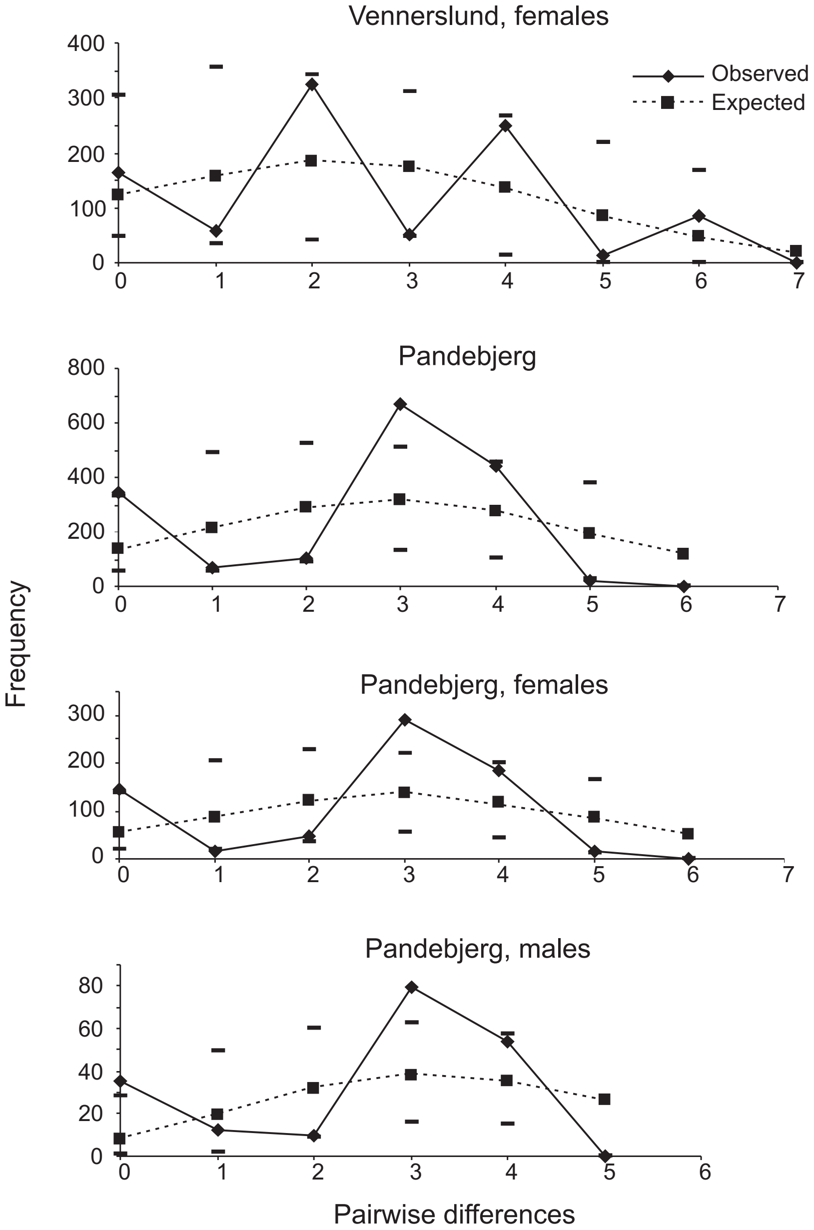


Fig. 2. Neighbour joining tree of the pairwise conventional F_{ST}-estimates (a) and Tamura and Nei (1993), (b) genetic distance showing the genetic relationships among the localities assuming different evolutionary influences of genetic drift and mutation.

closer relationship between 2 and 3 compared to the other localities. Furthermore, locality 4 and 8 clustered separately from the rest under both assumptions. The major difference between the two estimates was that, assuming genetic drift as the major force, locality 1 was closely related to 5 and 6 on Falster, while assuming mutation as the evolutionary force, 1 was closely related to 3 an 7 on Zealand and Lolland, respectively.

The tests of Tajima (1989) and Fu and Li (1993) were non-significant except when partitioning according to sex. A significantly negative F_S (–3.46) was observed in the female sample from Magrethe Kog suggesting a population ex-


Vennerslund, females

Pandebjerg

Pandebjerg, females

Pandebjerg, males

Fig. 3. Observed and expected mismatch distributions of D-loop sequences of the two brown hare populations, Vennerslund and Pandebjerg, based on nucleotide differences. The square dots show the theoretical expected distribution under a population expansion model. Bars (–) are 95% confidence intervals estimates based on the observed values.'

pansion which was supported by the observed mismatch distribution (SSD = 0.02, p = 0.39). The mismatch distributions generated unimodal patterns characteristic for population expansion or bottleneck for most of the populations which was supported by the statistical fit (Table 1, SSD and Rag. Id.). For two populations, Venners- lund [Vennerslund female (n = 44), SSD = 0.07, p = 0.03, Rag.Id = 0.3, p = 0.003] and Pandebjerg, we could reject population expansion since both mismatch and raggedness p-values were significant (Table 1, Fig. 3), and the mismatch distribution's multimodality suggested that these might be in mutation-drift equilibrium or declining.

Phylogeographic relationship

After cropping the sequences to 244bp, a total of 53 different haplotypes were observed amongst the 81 downloaded sequences and the Danish brown hare sequences from the present study (Fig. 4a, b). Cropping the original 477 bp in the present study to the 244bp unfortunately excluded some polymorphic sites which resulted in a lack of differentiation amongst some of the haplotypes. This was observed between the Danish H1 and the Italian i2 haplotypes, and H16 and i1. Aligning those pairs separately using a longer sequence (350 bp) showed that they differed from each other pairwise by two mutations at least. This was however not observed when aligning larger sequences of c07 and H2. These two haplotypes were identical. Aligning the largest number of basepairs, five of the 19 Danish haplotypes (haplotypes 1, 2, 5, 7, 16) were identical to German haplotypes (Fickel *et al*. 2008).

The parsimony network obtained in the phylogeographic analysis (Fig. 4a) revealed that a few Danish haplotypes were unique but closely related to German and Italian haplotypes (Fickel *et al.* 2008) showing a star like phylogeny suggesting haplotype c07 to be ancestral. To increase legibility a sub-network based on the 477bp sequence of the Danish brown hare haplotypes was constructed using TCS (Fig. 4b). This network suggested that haplotype H1 was ancestral, but it was closely connected to H2 or the c07 haplotype. There was no straightforward connection between number of mutations separating the different haplotypes and the haplotypes found in most of the populations except for Bornholm, where the haplotypes observed were separated by one mutation.

Discussion

The results from this study indicated that the hare population in Denmark was not panmictic. The observed genetic divergence of local populations was probably a result of ancient population genetic history and drift combined with several years of translocation/escapes between different localities.

Population structure

A pronounced genetic structure and low migration was revealed between most of the Danish brown hare localities. The very high, significant F_{ST} and ϕ_{ST} values between many localities were probably influenced by factors such as the philopatry displayed by female hares (illustrated by the higher number of pairwise significant ϕ_{ST} values between female samples when partitioning according to sex) and the geographical distance separating the localities all enhancing the effect of drift. This is supported by a study of Fickel *et al.* (2005) on the German brown hare in North-Rhine Westphalia using both mtDNA and microsatellite variation. They found that amongst the 21 sampling locations 51% of the pairwise F_{ST} estimates were significant (average F_{ST} = 31.5%, $p < 0.001$) despite the small geographical area (mean distance = 71.2 ± 36.4 km) covered.

Anthropogenic influences and population history

Translocations

In Denmark captive breeding of brown hares was initiated in mid 1980s with hares imported from Italy, France, Hungary and Sweden (Hansen *et al.* 1990). Hence, the genetic makeup of hares from these countries could have been either introgressed with mountain hare *Lepus timidus*

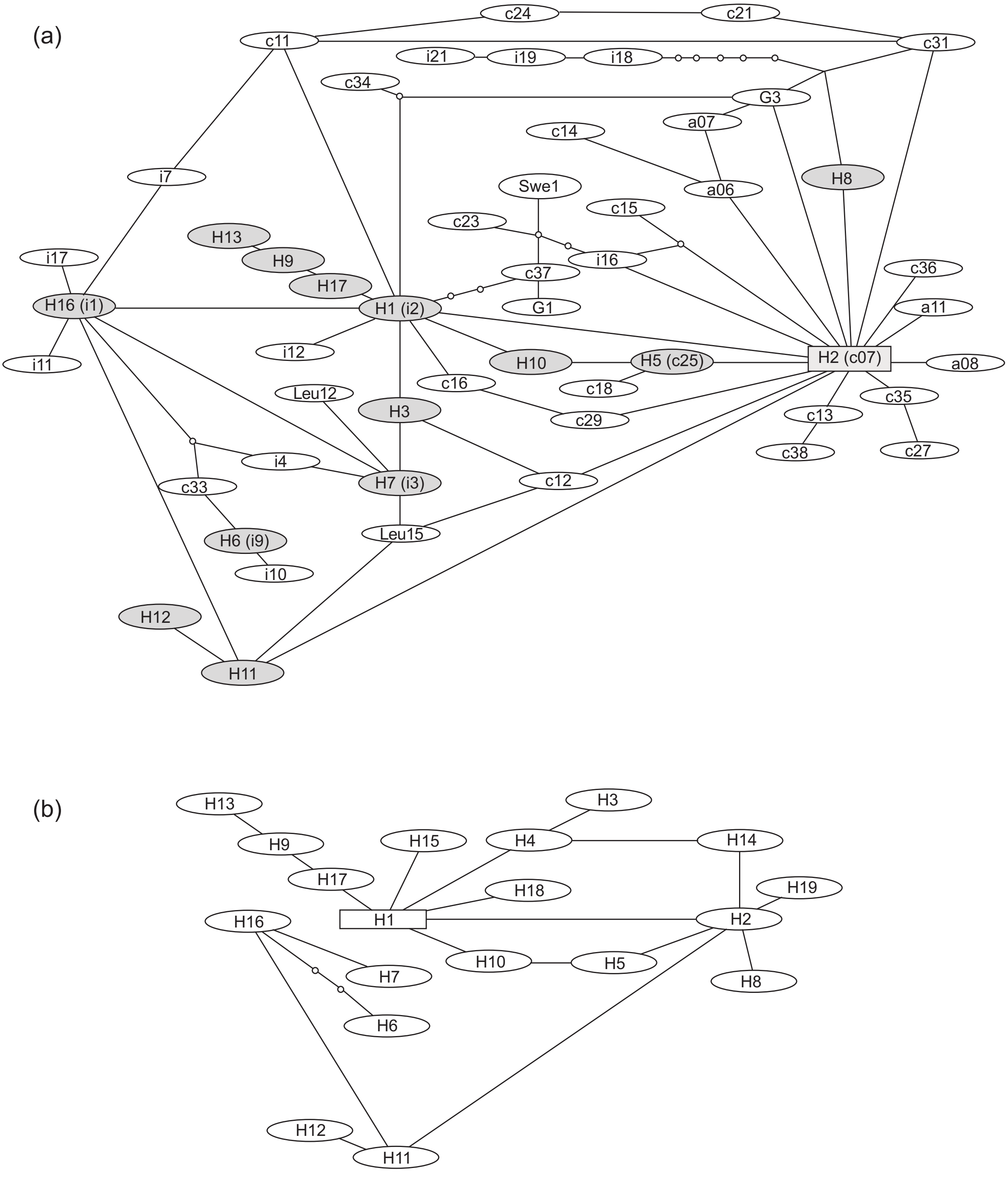


Fig. 4. Parsimony network showing the haplotype relationship amongst the 81 downloaded brown hare haplotypes and the 19 haplotypes from this study (a) and for legibility the relationship amongst the Danish haplotypes are shown in (b) using the 477bp sequences. The grey shaded haplotypes in (a) are the Danish haplotypes. The small circles show the minimum number of steps separating the haplotypes. No circle indicates one step. i – Italian haplotypes (Fickel *et al.* 2008), G – German haplotype (Kasapidis *et al.* 2005), Leu – Hungarian haplotypes (Kasapidis *et al.* 2005), c – Central European haplotypes (Fickel *et al.* 2008), SWE – Swedish haplotype (Thulin *et al.* 1997), H – Danish haplotypes (Fredsted *et al.* 2006).

haplotypes (Sweden, Thulin *et al.* 1997, Thulin 2003) or from other sub-species (Italy, Pierpaoli *et al.* 1999) and would have influenced the genetic population structure of the Danish brown hare if individuals escaped from captivity as suggested by Fredsted *et al.* (2006). In the early years following establishment of the hare-farms, captive-reared brown hares were predominately used in the breeding program (Kleist 1995), reducing the genetic diversity amongst farmed hares. Hence, escapes or releases from farms would have been expected to affect the genetic makeup of the Danish wild brown hare population. Brown hares captured from southern Danish islands have been released elsewhere in Denmark to enhance existing populations (Hansen *et al.* 1990), hence translocating genes from one population to another. Unfortunately, numbers of hares used in re-stocking, the origin of the stocked hares and the location of re-stocking areas have not been recorded making interpretation of the data difficult. The effect of translocation could be assessed using different approaches to estimate genetic relationships. The geographical origin of locality 1 was in the extreme south of Denmark, close to the German border; hence a close relationship to locality 5 or 6 is not expected as indicated in Fig. 2a based on genetic drift. When taking the evolutionary distance into account (Fig. 2b) this locality was more closely related to locality 2, 3 and 7, representing three different islands, which could indicate a translocation effect. Alternatively, all three localities have been stocked from the same unknown population or hares could have been translocated between the localities. Locality 2 is known to have been founded by brown hares from another island (Samsø) that unfortunately was not represented in the present dataset.

Population history

As expected, hare populations situated on islands well away from the mainland have restricted gene flow, and were locally subjected to genetic drift. This is illustrated by the result of the hierarchical ϕ analyses, where the localities were grouped hierarchical according to the island they were located. Here, the marginally significant island effect (ϕ_{CT} = 16%, p = 0.045) probably reflected the ancient population history of the autochthonous Danish Brown hare. This was also illustrated in both trees where locality 8 (Bornholm Island) and locality 2 (Helms Island) separated out.

If the observed differentiation reflects the results of recent fragmentation, we would expect samples from localities close to each other to be more closely related than those further apart (isolation by distance) reflecting geographical distance. The genetic relationship between the localities based on drift alone, indicated no isolation by distance, suggesting that translocation/ escapes and/or philopatry have influenced the observed genetic patterns. This was confirmed by the close genetic relationship between the known translocated localities 2 and 3, due to the high frequency of haplotype 5 in locality 3, the only one found in locality 2. Localities 2 and 3 were on totally different islands showing how translocation can distort genetic relationships between populations.

Alternatively, the observed differentiation could reflect historical fragmentation, where populations have diverged from each other in the past and currently exist in a certain equilibrium state. The two hypotheses are not mutually exclusive, and the observed genetic structure is probably due to both ancient history and recent stocking processes, ie the different analysed populations have experienced different histories. This was confirmed by analyses of population demography. The combination of low nucleotide diversity and high haplotype diversity as observed in this study has often been suggested to result from a recent expansion process following a period of reduced population size (Trizio *et al.* 2005). This hypothesis is supported by the mismatch distributions of the pair wise sequence differences, which showed positively skewed distributions (distributions close to zero, ‘L-shaped’) patterns typical of populations showing expansion following a genetic signature of reduction in population size (Rogers and Harpending 1992, Luikart *et al.* 2001). This pattern was evident in all populations except those from Vennerslund and Pandebjerg, two nearby populations on the island Falster. The observed multimodal mismatch distribution in these populations indicates long term demographic stability (Slatkin and Hudson 1991, Matocq *et al*. 2000) suggesting that the Danish brown hare populations have experienced different demographic events in the recent past. The apparent "population growth" in some of the populations of the Danish brown hare might be attributed to the stocking process that has taken place explaining the observed genetic variability pattern.

Colonization of Denmark

Brown hares have colonized Denmark around 10 000 years ago, following Holocene climate warming (Hewitt 1999). It can be postulated that hares, which colonized northern temperate zones by dispersal from southern refuges arrived with low levels of genetic variability due to recurrent population decline and bottlenecks during Pleistocene climatic changes (Fickel *et al.* 2005).

The phylogeographic analysis based on the most parsimonious network of the haplotype relationships, including haplotypes from those countries from where brown hares had been imported, indentified the c07 haplotype as the ancestral haplotype which Fickel *et al*. (2008) recognised to be the ancestral haplotype of central European brown hares. This confirmed the colonisation from southern refugees. Fickel *et al.* (2008) suggested that the most probable source population for the central European brown hares was the Italian brown hares. This hypothesis was partly supported by the present analysis where close relationships were observed between some of the Danish and Italian haplotypes.

The five haplotypes shared between the German and Danish brown hares did not represent private haplotypes which conformed to expectations because old haplotypes (coming from Germany) would be more frequent.

Implications

Hunting can cause extinction of small local populations and result in loss of genetic variation (Fickel *et al.* 2005). In general such local extinctions have not been assumed to have major population impacts since vacant habitats were assumed to be re-colonized by migrants from neighboring, presumed genetically similar populations (Ray 2001). However, Danish brown hare populations might not re-colonize areas of local extinctions immediately as indicated by the high genetic differentiation and restricted gene flow between nearby localities (eg locality 3 and 4). This may be the result of a combination of agricultural habitat fragmentation, inherent low dispersal distances and the philopatric behaviour of the brown hare. Hence, loss of genetic diversity (ie haplotypes) accumulates and persists even if numbers of individuals do recover.

Increased connectivity between scattered populations would be of great value, increasing the effective population size and reducing the impact of demographic and genetic stochasticity in several ways. Immigrants have an impact on population size by increasing numerical abundance: the so-called (ecological) 'rescue effect' (Brown and Kodric-Brown 1977), and population growth rate may be enhanced by immigrant genomes reducing the effects of inbreeding and maintaining or even increasing genetic variability (Saccheri and Brakefield 2002), a kind of genetic rescue effect' (Richards 2000). But creating an artificial gene flow between the different populations such as those in the present study may hinder local adaptation and decrease fitness in recipient populations (Kirkpatrick and Barton 1997).

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Research article

Introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into wild brown hares (*Lepus europaeus*) in Denmark

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Abstract

Background: In Europe the mountain hare (*Lepus timidus*) exists in Great Britain, Norway, Sweden, Finland, parts of the Alps and in Eastern Europe, but not in Denmark. Interspecific hybridization has been demonstrated between native Swedish mountain hares and introduced brown hares (*Lepus europaeus*). During the data collection in a study concerning Danish brown hares we identified 16 hares with a single very divergent haplotype.

Results: Phylogenetic analysis shows that the divergent Danish haplotype is most closely related to the Swedish mountain hare. The frequency of *Lepus timidus* mtDNA haplotype in the Eastern Danish hare populations is estimated to 6%.

Conclusion: In contrast to what is known, the Danish hare populations are not pure *L. europaeus* populations but include introgressed brown hares with Swedish *L. timidus* mtDNA. The most probable explanation of this is natural migration or translocation of introgressed brown hares from Sweden. The impurity of hare populations has implications for conservation and population genetics.

Background

The brown hare (*Lepus europaeus* Pallas, 1778) is widely distributed throughout Europe, up to 60°N, in Asia Minor and probably south to Israel. It is a popular game animal and its range has expanded to the east both by natural dispersion and by translocations to central and far-east Siberia [1]. Worldwide it has also been imported to many other countries, outside its natural distribution range, e.g. South and North America, Australia and New Zealand.

The brown hare is common all over Denmark and is the only *Lepus* species that exists in the country. Since 1960 the hare population has declined drastically in Europe and in Denmark. The primary cause of the decline is habitat changes in relation to agricultural intensification, whereas effects of climatic changes and predator abundance have increased by loss of year-round access to high-quality food and cover [2]. Additional factors like predators (especially foxes), birds of prey and traffic may have influenced the population locally [3]. In Denmark this is reflected by a large drop in annual number of hares shot from more than 400,000 before 1960 to 67,600 in 2004/2005 [4].

The general range of the mountain hare (*Lepus timidus* Linnaeus, 1758) covers much of the Palaearctic. In Europe it exists in Great Britain, Norway, Sweden, Finland, parts of the Alps and in Eastern Europe but it does not exist in Denmark [5]. During the late nineteenth century the brown hare was introduced in southern Sweden as a game animal [6]. In Scandinavia the mountain hare populations have been retreating northwards since the introduction of the brown hare, presumably as a result of a gradual competitive exclusion by the latter [7]. Mountain hares supposedly colonized Scandinavia through repeated immigration waves over two different post-glacial colonization routes; one from the south and one from the northeast [8].

Given the sympatric distribution of several hare species, e.g. in Sweden, Italy, and Spain, a number of studies focused on the possibility of interspecific hybridization. The first evidence of interspecific hybridization in hares concerns *L. timidus* and *L. europaeus* in Sweden [9-11] where unidirectional introgression of the native *L. timidus* mtDNA occurs in the introduced *L. europaeus*. However, introgression of mitochondrial DNA into other hare species has been demonstrated, e.g. the introgression of *L. timidus* mtDNA into *L. granatensis*, *L. europaeus* and *L. castroviejoi* in northern Iberia [12] and introgression of foreign mtDNA is likely to occur in several hare species from Asia [13]. These findings have lead to the conclusion that the genetic integrity of many European species largely depends on differences in behaviour and ecology which, at best, offer semi-permeable isolation [14]. In captivity, mountain hare females mate with brown hare males and produce viable offspring, whereas the reverse crosses do not happen spontaneously but can be performed successfully with insemination [15]. The F_1 hybrids are morphological intermediates between the two species [16].

During the data analysis in a study concerning Danish brown hares we identified 16 hares with a very divergent haplotype. Here we show that these 16 individuals from the Danish wild brown hare populations carry mountain hare mtDNA haplotypes.

Results

During alignment of 385 hare sequences, 16 sequences (6 males, 10 females) were clearly different from the rest and collapsed into a single haplotype. The 16 individuals were obtained from five of the eight populations sampled, and these five populations are all located on islands in the eastern part of Denmark (Figure 1). The frequency of sampled individuals with *L. timidus* mtDNA in those five populations averaged 6% (a crude estimate across all eight populations is 4.16%, Figure 1).

A BLAST search against GenBank with the divergent haplotype mtDNA returned *L. timidus* as the best hit (99% similarity), but not a single *L. europaeus* sequence in the first 50 hits. The subsequent phylogenetic analysis (Figure 2) revealed that the divergent Danish haplotype is grouped with *L. timidus* samples from across Europe (100% posterior probability in all three independent runs) and most closely related to the Swedish *L. timidus* haplotypes (100% posterior probability in all three independent runs, green clade in figure 2). The other Danish haplotypes included in the phylogenetic analysis, the 19 *L. europaeus* haplotypes, are grouped with the *L. europaeus* clade as expected, and they are most closely related to German haplotypes (100% posterior probability from three independent runs, red clade in figure 2). Hence, the Danish haplotypes clearly originate from two different species.

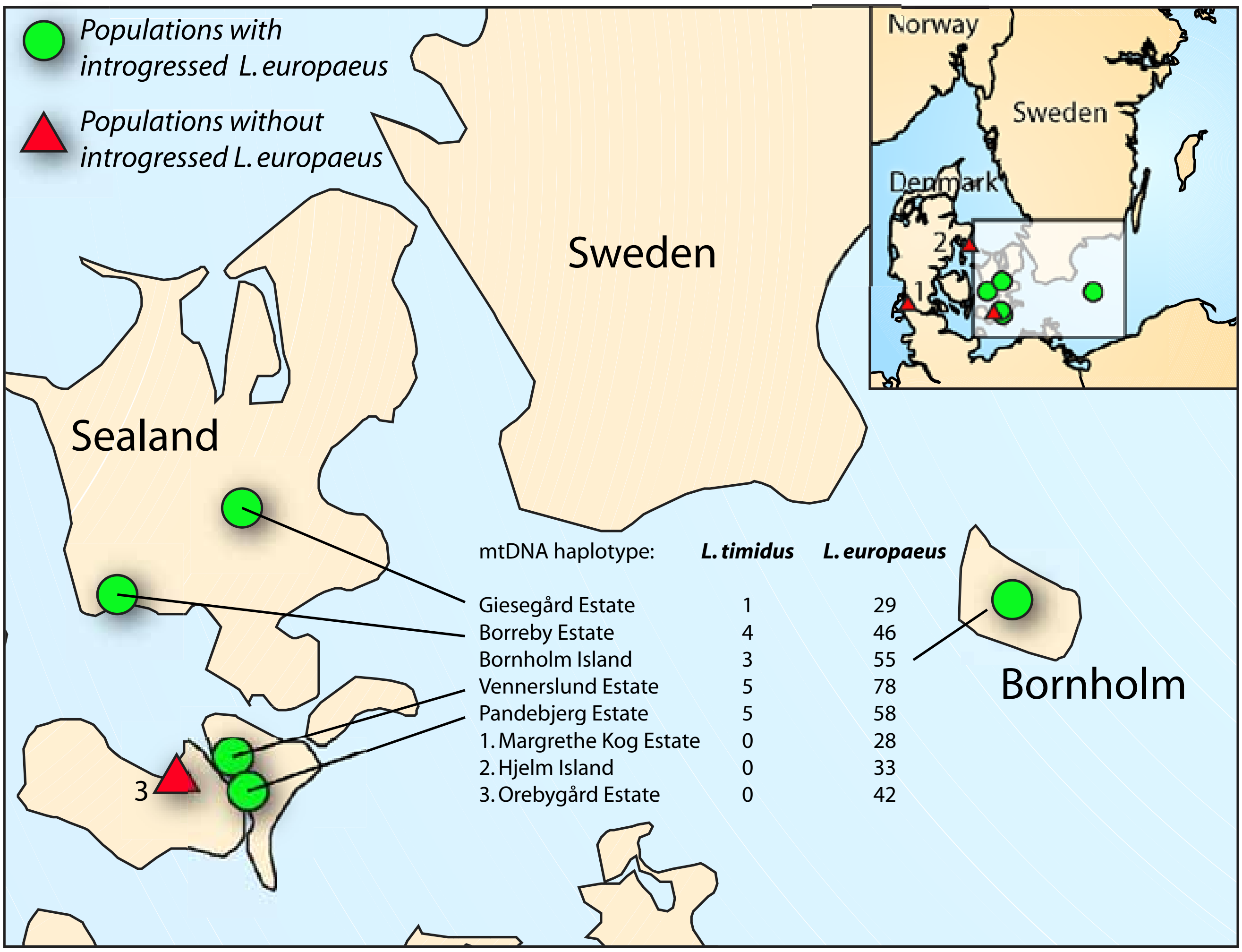
Discussion

The results show the presence of mountain hare mtDNA in Danish brown hare populations. From mtDNA alone we cannot decide whether these 16 individuals are true mountain hares or introgressed brown hares. This would require further analysis using nuclear gene sequences (e.g. *transferrin*) (there are no differential diagnostic microsatellite loci for the two species [17]). However, given a) the frequency of the *L. timidus* haplotype where it is present (6% in the five populations), b) that *L. timidus* is not reported to be a native breeding species in Denmark [5], and c) that the two species are able to hybridize [9], these individuals most likely represent introgressed brown hares.

The most likely origins of introgression are; 1) natural migration of mountain hares from Sweden followed by introgression, or 2) natural migration or translocation of introgressed brown hares from Sweden. However, since the documented distribution of mountain hares in Sweden does not include southern Sweden [9,10], natural migration of mountain hares is less probable than introduction of introgressed brown hares (carrying mountain hare mtDNA) from Sweden.

The fact that The Baltic Sea occasionally freezes over in very severe winters facilitates natural migration of introgressed brown hares from southern Sweden across the sea barrier to the Eastern Danish islands (Figure 1). This is also supported by the results from the genetic analyses (Figure 2).

During the mid 1980'ies a network of hare breeding facilities was established in Denmark, and it was initiated with imported hares primarily from Italy, France, Hungary and Sweden [18]. No combined record is kept of the amount and origin of hares imported to Denmark. In 1993, when farming hares were prohibited in Denmark, there were 100 hare-farms in Denmark, with an annual export of up to 5,500 hares [19]. As approximately 15% of Swedish *L. europaeus* specimens from sympatric areas carried trans-



mtDNA haplotype:	***L. timidus***	***L. europaeus***
Giesegård Estate	1	29
Borreby Estate	4	46
Bornholm Island	3	55
Vennerslund Estate	5	78
Pandebjerg Estate	5	58
1. Margrethe Kog Estate	0	28
2. Hjelm Island	0	33
3. Orebygård Estate	0	42

Figure 1
Location and frequency of the *Lepus timidus* haplotype in Denmark. *Lepus timidus* was found in five (large map) of the eight (small map) sampling locations.

mitted *L. timidus* mtDNA [10], it is possible that phenotypic brown hare specimens imported from Sweden for breeding purposes have carried the *L. timidus* mtDNA and later escaped to the natural hare population.

The detection of only one haplotype in the 16 individuals shows low mtDNA diversity in the total population of hybrids in Denmark. The geographical distribution of the 16 intogressed brown hares (Figure 1) and the lack of haplotype diversity support a common origin in southern Sweden.

The implications of introgressed brown hares in the Danish hare populations are currently unclear. It has been suggested that hares with an alien mtDNA have a lowered fitness as a result of a functional incompatibility between the cytoplasmatic mitochondrial genes and the cell *nucleus* [10]. In opposition to this, due to its observed high frequency in brown hares (93%) Melo-Ferreira *et al.* [20] suggested, that the ancient *L. timidus* mtDNA observed in the Iberian Peninsular might have some selective advantage depending on the nuclear background.

Thulin *et al.* [17] raised the question as to whether any 'pure' population of brown hares exists anywhere. Our findings give further support to this statement. This has important consequences for conservation and population genetics, e.g. problematic definition of management units, unclear fitness effects, genetically mixed populations and the inclusion of nuclear markers will be necessary for future hare studies.

Conclusion

Contrary to what is known, the Danish hare populations are not pure *L. europaeus* populations but include introgressed brown hares with Swedish *L. timidus* mtDNA. The

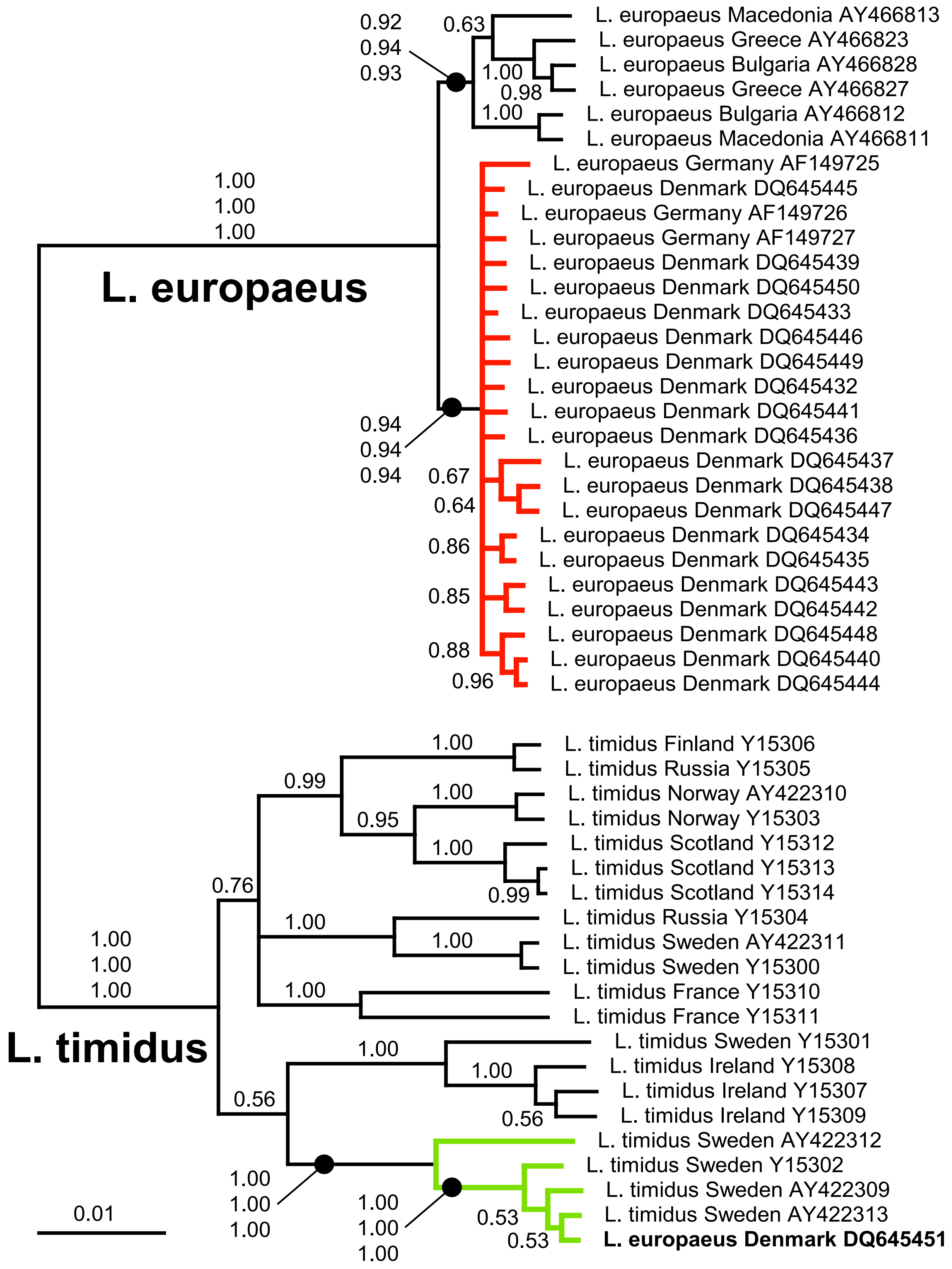


Figure 2

Bayesian phylogeny of 370 bp from the mitochondrial D-loop of 20 *Lepus timidus* and 28 *Lepus europaeus* haplotypes from Europe, including the 19 Danish *Lepus europaeus* haplotypes, and the divergent Danish haplotype. The numbers show nodal support, given as posterior probabilities (for selected nodes, posterior probabilities from three independent runs are shown).

most probable explanation is natural migration or translocation of introgressed brown hares from Sweden. The impurity of hare populations has implications for conservation and population genetics.

Methods

Sample collection

During 2003–2006 The National Environmental Research Institute of Denmark collected tissue samples from shot, wild hares at eight locations in Denmark (Figure 1). The hares were autopsied and several organs and muscle tissue samples were taken out for later analyses.

Sequencing

DNA was extracted from hare muscle tissue using a slight modification of the Chelex protocol [21]. The tissue was placed into 1.5 mL Eppendorf tubes containing 200 µl of 20% Chelex resin solution (BIO-Rad, Hercules, CA, USA). Tubes were vortexed briefly, boiled at 100°C for 20 min in a heating block, centrifuged 3 min at 13000 rpm and stored at minus 20°C.

A 494 bp fragment of the mtDNA D-loop (control region) was amplified via PCR, using the mammalian control region primers L15997 5'-CACCATTAGCACCCAAAGCT-3' located in the tRNA gene and H16498 5'-CCTGAAGTAGGAACCAGATG-3' [22]. PCR was performed in a total volume of 10 µl and contained 1 µl buffer (1.5 mM $MgCl_2$), 1.6 µl dNTP (1.25 mM of A, C, G, T, respectively), 0.5 µl of each primer (10 pmol/µl), 0.1 µl *Taq* polymerase (Amersham Pharmacia Biotech), topped up with distilled water to 9 µl and 1 µl template DNA (40–60 ng/µl) was added. Cycling conditions were 94°C for 3 min, and 35 cycles of 94°C for 30 s, 50°C for 40 s and 72°C for 60 s and a final extension step of 72°C for 7 min. Sequencing was conducted under BigDye™ terminator cycling conditions, the reacted products purified using ethanol precipitation and run using an Automatic Sequencer ABI 3730xl. Both strands were sequenced in all samples.

Haplotype editing and collapsing

Sequences were edited using Bioedit version 7.0.0 [23]. Identical haplotypes among the 385 sequences were found using DNAcollapser [24].

Phylogenetic analyses

Nine *L. europaeus* and 20 *L. timidus* sequences were downloaded from GenBank. Sequences with geographical information were selected to represent haplotypes from all over Europe and Russia. Together with the 19 Danish *L. europaeus* and the divergent haplotype, these 49 sequences (table 1) were aligned using Muscle [25] and cropped to the shortest sequence (370 bp). Phylogenies and nodal support were estimated using MrBayes, version 3.0b4 [26] under a Bayesian framework [27,28] using a general time reversible substitution model (GTR + γ) and the coalescent branch length model. Bayesian analysis was initiated with random starting trees, run for 5×10^6 generations, and the Markov chain was sampled every 1000 generations. Model parameters were estimated

Table 1: Sampling localities and accession numbers of D-loop sequences used in the phylogenetic analysis (figure 2).

Species	Sampling locality	Accession #
L. europaeus	Bulgaria	AY466812
L. europaeus	Bulgaria	AY466828
L. europaeus	Macedonia	AY466811
L. europaeus	Macedonia	AY466813
L. europaeus	Greece	AY466823
L. europaeus	Greece	AY466827
L. europaeus	Germany	AF149725
L. europaeus	Germany	AF149726
L. europaeus	Germany	AF149727
L. timidus	Sweden, Grimso	AY422312
L. timidus	Sweden, Kalix	AY422311
L. timidus	Norway, Ringebu	AY422310
L. timidus	Sweden, Salsta	AY422313
L. timidus	Sweden, Vaxvik	AY422309
L. timidus	Finland	Y15306
L. timidus	France	Y15310
L. timidus	France	Y15311
L. timidus	Ireland	Y15307
L. timidus	Ireland	Y15308
L. timidus	Ireland	Y15309
L. timidus	Norway	Y15303
L. timidus	Russia	Y15304
L. timidus	Russia	Y15305
L. timidus	Scotland	Y15312
L. timidus	Scotland	Y15313
L. timidus	Scotland	Y15314
L. timidus	Sweden	Y15300
L. timidus	Sweden	Y15301
L. timidus	Sweden	Y15302
L. europaeus	Denmark	DQ645432
L. europaeus	Denmark	DQ645433
L. europaeus	Denmark	DQ645434
L. europaeus	Denmark	DQ645435
L. europaeus	Denmark	DQ645436
L. europaeus	Denmark	DQ645437
L. europaeus	Denmark	DQ645438
L. europaeus	Denmark	DQ645439
L. europaeus	Denmark	DQ645440
L. europaeus	Denmark	DQ645441
L. europaeus	Denmark	DQ645442
L. europaeus	Denmark	DQ645443
L. europaeus	Denmark	DQ645444
L. europaeus	Denmark	DQ645445
L. europaeus	Denmark	DQ645446
L. europaeus	Denmark	DQ645447
L. europaeus	Denmark	DQ645448
L. europaeus	Denmark	DQ645449
L. europaeus	Denmark	DQ645450
L. europaeus, introgressed	Denmark	DQ645451

directly from the data and three independent replicates were conducted to avoid entrapment in local optima [29]. The initial 1,250 trees were discarded as "burn-in" and the remaining 3,750 trees were used to estimate nodal support as posterior probabilities.

Authors' contributions

TF carried out all the lab-work, edited and analyzed the sequence data and drafted the manuscript. PV carried out the phylogenetic analysis, prepared the figures and drafted the manuscript. TW organized the samples and drafted the manuscript. All authors have read and approved the final manuscript.

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IDENTIFYING CAUSES FOR POPULATION DECLINE OF THE BROWN HARE *(LEPUS EUROPAEUS)* IN AGRICULTURAL LANDSCAPES IN DENMARK

In recent decades the brown hare (*Lepus europaeus*) in Denmark has undergone a substantial decline, but proximate causes are unknown, and little is known about actual densities. In this thesis, hare populations are investigated with respect to age composition and reproductive parameters in relation to habitat and phenotype, and point transect counts are evaluated in assessing hare densities. Data from culled individuals suggest that contemporary reproductive parameters and juvenile recruitment vary across Denmark, while adult survival seems constant across populations. Juvenile recruitment (indexed as the proportion of hares culled in autumn comprised by individuals < 1 year) is lowest in areas with low densities, but unrelated to the reproductive output of females indexed as the mean number of placental scars, indicating high spatial variation in post-natal survival of offspring. Some variation in the demographic and phenotypic (size, weight) parameters is attributable to habitat composition. In a historical perspective, the proportions of juveniles in game bags dropped significantly between the 1950ies when hare populations where stable and the 1980ies and later, when hare populations according to bag size decreased with 5% annually. Simple matrix population models based on the estimated annual survival for adult females at present and the estimated fecundity for the 1950ies, 1980ies, 1990ies and since 2000, predicted the same population growth rates for each decade as was actually observed in the annual bag records. The model substantiates the supposition that declines in the Danish hare population are caused by reduced juvenile recruitment, and moreover, the model predicts further population decline. Point transect counts are suitable and corrections for detection necessary, when monitoring hare populations, but work is still needed, before the effects of e.g. road avoidance on density estimates are fully clarified. The genetic variation of hares reveals that the population is subdivided and gene flow restricted even between close populations. The genetic differences are caused by a combination of genetic drift, ancient history and translocations. Future research should focus on determining causes of juvenile mortality, and reasons for variation in female fecundity, as well as the genetic consequences of the low densities on the mainland, along with the sustainability of hunting in low-density areas.

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