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Laura Härkönen

SEASONAL VARIATION IN THE LIFE HISTORIES OF A VIVIPAROUS ECTOPARASITE, THE DEER KED

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Professor Arja Kaitala
Docent Jouni Aspi

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Docent Tero Klemola
Doctor Tiit Teder

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University of Oulu Graduate School; University of Oulu, Faculty of Science, Department of Biology, P.O. Box 3000, FI-90014 University of Oulu, Finland
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Abstract

The life histories of ectoparasites are shaped by both host and off-host environment. A suitable host is primarily needed during reproduction, whereas juvenile stages outside the host are directly exposed to environmental variability. Viviparity, i.e. the development of an embryo inside the body of the mother resulting in large offspring size, increases offspring survival. The production of large offspring has its consequences in terms of high variation in offspring age and in the environment that each young individual will face. I used a viviparous ectoparasite, the deer ked (*Lipoptena cervi*), to investigate the consequences of long reproductive lifespan and varying offspring environment on offspring life-histories and seasonal adaptations.

Offspring life-histories varied seasonally. I showed that the resources provided by the deer ked females determine offspring performance throughout its off-host period. Offspring size increased towards the spring and the end of the reproductive period, and simultaneously offspring survival and cold tolerance increased. Seasonal variation in offspring size did not reflect the resources that would guarantee offspring survival during the longest diapause or the highest cold tolerance during the harshest winter period. Diapause intensity varies with birth time according to the expected length of the winter ahead. However, the deer ked pupae, regardless of their age, overwinter at an opportunistic diapause, which may be terminated rapidly only by an exposure to high temperature. Contrary to general observations, photoperiod has no role in regulating the seasonal shifts of the deer ked. Neither is high cold tolerance associated only with diapause, but it remains high through four seasons, also in the active developmental and adult stages. I also evaluated the effects of life-history variation on the invasion potential of the deer ked. I conducted a large-scale transplant experiment to test the survival and pupal development at and beyond the current range. I found that the lower spring and summer temperatures and the shorter growth season in the north cause a deterioration in pupal performance and shorten the flight period. However, the colder climate may not totally prevent further spread. A more important factor that will affect deer ked invasion is host availability, and especially in Finland, the density of the moose population.

Seasonal variation in offspring life histories in viviparous ectoparasites differs from the variation patterns reported in most invertebrates. This may be due to the extremely large offspring size and to the fact that maternally derived resources determine offspring performance through the entire off-host period. Variation in offspring performance is thus determined by maternal resources and seasonal variation in the condition of the moose.

Keywords: cold tolerance, diapause, ectoparasite, invasive species, moose louse fly, offspring size, seasonality, viviparity

Härkönen, Laura, Jälkeläisten elinkierto-ominaisuuksien vuodenaikaisvaihtelu hirvikärpäsellä
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Tiivistelmä

Useimpien ulkoloisten elinkierto on riippuvainen isännästä ja isännän ulkopuolella kasvavien jälkeläisten kohtaamista ympäristöoloista. Viviparia eli jälkeläisen kehitys naaraan sisällä ja siitä usein seuraava suuri jälkeläiskoko parantavat jälkeläisten selviytymistä. Suurten jälkeläisten tuottaminen pitkällä aikavälillä johtaa siihen, että eri-ikäiset jälkeläiset kohtaavat vuodenajasta riippuen hyvin erilaiset olosuhteet. Väitöstyössäni tarkastelin Suomessa nopeasti yleistyneen hirvieläinten ulkoloisen, hirvikärpäsen (*Lipoptena cervi*), avulla, mitä seurauksia vivipariallа, pitkällä lisääntymiskaudella ja ympäristön vuodenaikaisvaihtelulla on jälkeläisten elinkierto-ominaisuuksiin.

Väitöskirjatyössäni havaitsin, että hirvikärpäsjälkeläisten elinkiertopiirteet vaihtelevat jälkeläisen syntymäajan mukaan. Osoitin myös, että hirvikärpäsnaaraan jälkeläisilleen tarjoamat ravintovarat määrittelevät jälkeläisten isännästä riippumattoman elinkierron aikaisen menestyksen. Jälkeläisten keskimääräinen koko kasvoi lisääntymiskauden edetessä talvesta kohti kevättä, jolloin myös selviytyminen ja kylmänsietokyky paranivat. Jälkeläiskoon vuodenaikaisvaihtelu ei näin ollen vastaa jälkeläisten tarvitsemia resursseja suhteessa talvehtimisen pituuteen eikä korreloi koville talvipakkasille altistumisen todennäköisyyden kanssa. Lepotilan keston määrittelevä diapaussin syvyys vaihteli syntymävuodenaikaa vastaavasti. Diapaussin havaitsin kuitenkin olevan ensisijaisesti opportunistinen, jolloin pelkästään korkea lämpötila voi nopeasti päättää lepotilan kaikenikäisillä yksilöillä. Vastoin yleisiä käsityksiä valorytmi ei vaikuta diapaussin säätelyyn. Vastoin ennakko-odotuksia kylmänsietokyky säilyy korkeana vuoden ympäri ja kaikissa tutkituissa elinkierron vaiheissa. Sovelsin tutkimieni elinkiertopiirteiden vaikutusta myös lajin invaasiokykyyn, ja tutkin istutuskokeen avulla koteloiden selviytymistä ja kehitystä nykyisellä esiintymisalueella ja sen pohjoispuolella. Alhaisemmat kevät- ja kesälämpötilat sekä lyhyempi kasvukausi vähentävät aikuiseksi selviytymistä ja lyhentävät lentoaikaa syksyllä. Ilmastotekijöiden suhteen hirvikärpänen voisi esiintyä nykyistä pohjoisempana. Tärkein tekijä hirvikärpäsen leviämistä tarkasteltaessa on kuitenkin sopivien isäntäeläi-mien saatavuus ja Suomen oloissa erityisesti hirven eli hirvikärpäsen pääisännän kannan tiheys.

Tutkimukseni perusteella ulkoloisten vivipariasta seuraava jälkeläisten elinkiertopiirteiden ajallinen vaihtelu eroaa muiden selkärangattomien vastaavasta vaihtelusta. Yhtenä syynä eroihin lienee se, että hirvikärpäsnaaras tuottaa erityisen suuria jälkeläisiä ja että jälkeläiset puolestaan ovat täysin riippuvaisia emon antamista resursseista. Emon lisääntymisresurssit ja hirven kunnon vuodenaikaisvaihtelu vaikuttavat mahdollisesti siihen, minkälaisia jälkeläisiä hirvikärpänen milloinkin kykenee tuottamaan.

Asiasanat: hirven täikärpänen, invaasiolaji, jälkeläiskoko, kylmänkestävyys, lepotila, vivipaarinen lisääntyminen, vuodenaikaisuus

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List of original articles

This thesis is based on the following papers, which are referred to by their Roman numerals.

I Härkönen L, Hurme E & Kaitala A. Unexpected seasonal variation in offspring size and performance in a viviparous ectoparasite. Manuscript

II Härkönen L, Kaitala A, Kaunisto S & Repo T (2012) High cold tolerance through four seasons and all free-living stages in an ectoparasite. Parasitology 139: 926-935.

III Härkönen L & Kaitala A. Seasonal variation in offspring age and diapause in a viviparous ectoparasite. Manuscript.

IV Härkönen L, Härkönen S, Kaitala A, Kaunisto S, Kortet R, Laaksonen S & Ylönen H (2010) Predicting range expansion of an ectoparasite – the effect of spring and summer temperatures on deer ked (*Lipoptena cervi*) performance along a latitudinal gradient. Ecography 33: 906-912.

Contents

1 Introduction

Ectoparasite life-histories are shaped by both the host and the off-host environment. A suitable host is primarily needed for food and reproduction, and parasites are often specialised to use only a few host species (Poulin 2007). Most ectoparasites live permanently attached to the surface of one host individual. Some ectoparasite species need a host throughout their lifespan (e.g. lice), whereas some spend a part of their life cycle outside the host (e.g. fleas, ticks and louse-flies) (Lehane 2005, Krasnov 2008, Kaitala *et al.* 2009). Off-host stages are directly exposed to similar abiotic and biotic mortality factors as are free-living organisms. Viviparity increases offspring survival, but it is often associated with a long reproductive period (Stearns 1992). However, the parasitic life cycle also includes a period of host location and thus host availability, i.e. the density and movements of suitable hosts, has an important role in the ability of a parasite to complete its off-host period. There are surprisingly few studies of the consequences of asynchronous offspring production on offspring life histories in viviparous invertebrates. This thesis aims to increase our understanding of life histories in viviparous ectoparasites and their adaptations to seasonal off-host environments.

1.1 Life histories of viviparous ectoparasites

Viviparity, i.e. prolonged maternal provision and offspring development inside the body of the mother, increases offspring survival by increasing offspring size and shortening the vulnerable juvenile time (‘safe harbour hypothesis’; Clutton-Brock 1991, Stearns 1992). Endothermic mammals offer a safe breeding habitat with continuously available food for parasites all year around (Tinsley 1999, Krasnov *et al.* 2002). Meier *et al.* (1999) suggest that due to the constant environment, a viviparous reproduction strategy has evolved in a group of blood-feeding ectoparasites (Glossinidae, Hippoboscidae, Nycteribiidae, and Streblidae). In these ectoparasites, all juvenile development occurs with the resources provided by their mother (e.g. Langley & Clutton-Brock 1998).

Large offspring tend to perform better than small ones in a given environment: large propagules survive better and develop faster (Smith & Fretwell 1974, Parker & Begon 1986, Roff 1992, Fox 1994). In ectoparasites, large size may also enhance offspring performance by increasing starvation resistance during the non-feeding period (Langley & Clutton-Brock 1998). Thus, the performance of non-feeding ectoparasite stages outside the host is largely determined by maternal ability to provision each offspring. However, the high investment of time and resources in each offspring decreases the total number of offspring that a female can produce during her reproductive lifespan (i.e. fecundity costs; Parker & Begon 1986, Fischer *et al.* 2006). As a result of high maternal investment, the period of offspring production is often asynchronous, and the age of the offspring varies (Clutton-Brock 1991, Stearns 1992).

Viviparity is associated with a prolonged reproductive period because only a few large offspring can be produced at once (Clutton-Brock 1991). Offspring size often varies within females as a result of ageing: in invertebrates, young females in good condition and with high energy reserves often produce large high-quality offspring characterised by high survival and early emergence (Mousseau & Dingle 1991, Roff 1992). Offspring size then decreases, mainly because the maternal resources available for reproduction decline (Roff, 1992, Mousseau & Dingle 1991, Fox, 1993). The size of an offspring may also vary within females as a result of trade-offs associated with offspring size. First, an increase in the size of earlier produced offspring often negatively affects the size of offspring produced at an older age (i.e. reproductive costs; Stearns 1992, Kindsvater *et al.* 2010). Second, high reproductive effort early in life is likely to decrease maternal survival and thus shorten reproductive lifespan (i.e. survival costs; Stearns 1992). Altogether, the maternal provision per offspring should be balanced so that the number of offspring surviving to reproduction in the life cycle is maximal (Stearns 1992).

1.2 Seasonal adaptations of ectoparasites

Temperate zone ectoparasites are mainly protected from unfavourable temperatures by the thermoregulatory abilities of vertebrate hosts (Wharton 1999, but see Moyer *et al.* 2002). The long reproductive lifespan in viviparous ectoparasites means that the offspring, once shed from the host, will encounter a variable seasonal environment. It is expected that the relationship between maternal provision and offspring environment determines offspring performance through the free-living stages (Langley & Clutton-Brock 1998). So far, the effects of high seasonal asynchrony in offspring life cycles in relation to the respective seasonal variation in their environments have not been investigated in ectoparasites.

Offspring size may vary as a result of seasonal phenotypic plasticity. Females may adjust their offspring size in response to predictable environmental cues that signalise future environmental conditions for the offspring (i.e. anticipatory or cued plasticity) (McGinley *et al.* 1987, Schultz 1991, Landa 1992, Mousseau & Fox 1998). For example, an increase in offspring energy reserves increases survival at prolonged low temperatures (Colinet *et al.* 2006). Females may produce larger propagules if cold or otherwise poor conditions are expected for their offspring, or the size may vary according to the duration of unfavourable conditions (Landa 1992, Fischer *et al.* 2003, Bownds *et al.* 2010). Alternatively, the seasonal variation in offspring size may be an immediate and unavoidable side effect of environmental heterogeneity for females (i.e. responsive or direct phenotypic plasticity). Blood-feeding ectoparasites often feed concurrently during their reproduction. Therefore, the quality of the resources, i.e. blood, acquired for reproduction may vary seasonally and directly affect the ability to provision the offspring (Langley & Clutton-Brock 1998, Tschirren *et al.* 2007).

Off-host survival in temperate ectoparasites depends on the ability to tolerate environmental stress and to time the life cycle correctly with seasonality and host availability. Ectothermic organisms have evolved a wide range of behavioural and physiological adaptations in order to survive through the unfavourable seasons (Danks 1987, Tauber *et al.* 1986, Leather *et al.* 1993). Like most invertebrates, ectoparasites use diapause-mediated dormancy to survive through the winter and to persist in an area over those times when suitable hosts are not available (Kennedy *et al.* 1975, Wharton 1999). In the definition by Tauber *et al.* (1987), diapause is a dynamic state of low metabolic activity which is associated with suppressed development and increased resistance to environmental stressors, such as frosts and drought. Once diapause has begun, metabolic activity remains suppressed, even if the conditions are favourable for development. Diapause is considered irreversible until a series of physiological changes have occurred (i.e. completion of diapause development).

One consequence of the long reproductive period is that offspring enter diapause asynchronously. Correct timing of the seasonal shifts, especially from diapause to the active phase, helps in the synchronisation of the life cycles (Tauber *et al.* 1986). Time to diapause termination is primarily determined by diapause intensity and, secondarily, it is regulated by seasonal cues (Masaki 2002). Diapause intensity, or diapause depth, is a physiological trait that determines the diapause duration of an individual under given conditions: the earlier diapause is entered, i.e. the longer it should last, the deeper the diapause is at the time of induction (Masaki 2002, Dambroski & Feder 2007). Diapause intensity then decreases with the increasing number of days at low temperature, when responsiveness to diapause terminating cues increases correspondingly (Tauber *et al.* 1986). This usually means that the length of experienced cold, often referred as chilling, determines when the diapause can be terminated at earliest. The required cold period may vary from a few weeks to several months (Masaki 2002). Insects also use seasonal changes in external signals, such as photoperiod, temperature or moisture, to time the period of dormancy correctly with current and future conditions (Tauber *et al.* 1986, Danks 1987, Leather *et al.* 1992). For example, diapause is often induced and maintained under short day-length, which predicts winter, whereas long day-length predicts approaching spring and directs the diapause towards its termination (Bradshaw & Holzapfel 2007). Diapause is terminated when diapause intensity has decreased sufficiently, and it is no longer maintained by token environmental stimuli. Further growth and development begin once the ambient temperature rises above the threshold.

Temperate ectoparasites must cope with low seasonal temperatures, and preparing for winter begins well in advance before adverse conditions arise (Tauber *et al.* 1986). In addition to diapause as such, winter survival also depends on other physiological adjustments. Freeze-intolerant insects may increase their tolerance to harsh frosts by lowering their freezing point, i.e. by supercooling (Leather *et al.* 1993). Diapause-mediated cold-hardening often requires weeks of acclimation: e.g. a shortening photoperiod and decreasing temperatures in autumn stimulate the production of cryoprotectants, which increase the cold tolerance towards winter (Tauber *et al.* 1986).

Large size increases tolerance to prolonged low temperatures (Colinet *et al.* 2006): besides the fact that large individuals have greater energy reserves, the formation of lethal body ice may be slower in these large individuals (Ansart & Vernon 2004), but the opposite pattern has also been reported by e.g. Hahn *et al.* (2008). Metabolic activity during diapause is low but body maintenance and physiological adjustments, such as supercooling, consume energy reserves (Leather *et al.* 1993, Colinet *et al.* 2006, Matsuo 2006). The diapause has been considered costly if it is associated with loss of metabolic resources, which increases mortality and reduces reproductive opportunities. These costs are often associated with extended diapause (e.g. for several years; Matsuo 2006). In ectoparasites, the non-feeding period may continue diapause: the loss of energetic reserves and its effects on performance during the further off-host lifespan are unknown.

1.3 Invasion process in ectoparasites

Invasive species have recently been a focus of interest (e.g. Sakai *et al.* 2001, Davis 2009). The terminology used in connection with species invasions originates from alien species that are introduced to new geographical areas, mainly by human activity. After introduction they expand rapidly having negative effects on the colonised ecosystem (Mooney & Cleland 200). Here, I use the term invasion as defined by Reise *et al.* (2006), since it applies to all species and any process of rapid spread and establishment beyond the original range (also recommended by Davis 2009). The majority of past research done with the life histories of invasive species is not generally applicable but restricted to a narrow range of taxa, and mainly to free-living organisms (Carroll & Dingle 1996). So far, the life histories of invasive ectoparasites have been poorly studied (but see Samuel *et al.* 2000).

The invasion process occurs through three main steps, namely introduction, establishment and further spread (Reise *et al.* 2006, Davis 2009). In parasites, this process is complicated because it depends on both host and parasite ecology. The most important factors determining the parasites' range are the distribution and density of suitable hosts, and their ability to resist parasites (Wild *et al.* 2009). The parasites' own active dispersal capacity, i.e. ability to fly and migrate long distances, is often poor (Samuel *et al.* 2000). For arthropods that parasitise larger vertebrates, the host individuals are often the carriers of a founder population into novel environments (Boulinier *et al.* 2001). When on the host, food and favourable conditions are secured, regardless of external environment, whereas off-host stages must cope with the new climatic conditions.

Factors that fundamentally determine the species range limits derive from interactions between individual life-history characteristics and the experienced environment. In ectoparasites, off-host life-history stages may constrain distribution because they are directly exposed to abiotic and biotic factors (Samuel *et al.* 2000). The population at the invasion frontier more frequently experiences strong, limiting environmental stressors, and therefore individuals at range-margin are likely to express those characteristics that determine the invasion potential of a species or a population (Hill *et al.* 1999, Hill *et al.* 2011). For instance, the amount of variation in individual life histories often determines the evolutionary potential of a species to adapt to novel environments beyond their current range (Stearns & Hoekstra 2005). Phenotypic plasticity is believed to be the primary strategy for invasive species because it allows populations to tolerate and establish at and beyond the range limit without adapting to the local environment first (Agrawal 2001, Yeh & Price 2004, Richards *et al.* 2006). Furthermore, pre-adaptations originating from native areas, such as high physiological tolerance to a varying range of environmental stressors, may also be important in helping the invasive population to establish and persist in variable environments (Davis 2009, Sexton *et al.* 2009).

The relationship between ambient temperature and ecological requirements is often used to forecast species invasions (Crozier 2004a, 2000b, Thuiller *et al.* 2005) or the effects of climate change on geographical distribution (Cammell & Knight 1992, Thuiller 2003, Bale & Hayward 2010). Towards the north, ectoparasites outside the host are faced with a severer climate. Successful establishment in the north depends on the ability to withstand longer winters, shorter summers and lower seasonal temperatures. Juvenile growth and development rates are often sensitive to temperature, and flight capacity depends on the environmental temperature (Meats 1989, Angilletta 2009). Timing the emergence time of the host-seeking stage to match host movements is particularly important in northern habitats, where the transmission period is shorter due to earlier arrival of winter (Samuel *et al.* 2000). However, the interaction between photoperiod and temperature varies along a latitudinal cline. The shifts between diapause and active phases are often adapted to seasonal rhythm at the native range. Thus during rapid range expansion, diapause in the new northern environment may be induced and/or terminated at a locally inappropriate time of the season (Bale and Hayward 2010).

1.4 The aims of the study

This thesis aims to explore life-history variation in a viviparous ectoparasite. I use a viviparous ectoparasite, the deer ked (*Lipoptena cervi*, Hippoboscidae), to investigate the consequences of a long reproductive lifespan and seasonally varying off-host environment on offspring life histories and seasonal adaptations. Using field-collected data and manipulative experiments I test theories from the fields of evolutionary ecological and ecological physiology. I will also evaluate the effects of life-history variation on the invasion potential of the deer ked.

The deer ked is a highly prevalent ectoparasitic fly on boreal cervids, especially on moose (*Alces alces*). Throughout the reproductive lifespan, the adult deer ked is attached to the same host individual and feeds on blood concurrently (Haarløv 1964). Since viviparous females produce one large prepupa at a time, offspring production is highly asynchronous. The reproductive period of a female extends through at least three seasons, from autumn to the following spring (Kaitala *et al.* 2009). Newborn pupae drop off the host, often onto host bedding sites. A long reproductive period means that offspring age varies, and that they experience very different seasonal conditions outside the host depending on their birth time. The off-host stages depend totally on the nutritional resources that the mother is able to transfer from their host to the developing offspring. So far, there is no empirical evidence as to whether ectoparasite females produce different offspring according to predictable seasonal variation outside the host.

The first part of this thesis concentrates on the effects of varying offspring age on offspring provisioning and regulation of offspring diapause, and the effects of both birth time and offspring size on overwintering survival and cold tolerance. First, I tested whether offspring size varies according to the seasonal conditions that a young individual will face outside the host (I). I expect that young females in autumn and early winter would increase the size of the offspring, when both the expected diapause duration for the newborn offspring (I) and the length of exposure to harsh winter temperatures (II) are long. I also explored in detail how the maternally-derived resources and diapause duration affect survival through the entire pupal period (I) and the tolerance to environmental stress during all off-host stages (II). Second, I test the effects of both environmental cues and a physiological switch mechanism determining the timing of diapause termination in pupae of varying age (III). I expect that age-dependent features affect offspring diapause regulation and its duration differently. I also discuss the role of diapause in synchronising offspring life cycles

In the second part of this thesis, I aim to find factors that may facilitate rapid invasion by the Finnish deer ked population. The invasion of this ectoparasite has previously been explained by an increase in host numbers (Hackman *et al.* 1983, Välimäki *et al.* 2010, Meier 2012), but the effects of abiotic factors on off-host performance have not been studied before. To evaluate the invasiveness of the deer ked against this background, I conducted the life-history studies (I-III) using a population from the northern range limit, in which the individuals are likely to express the characteristics determining population invasiveness (Hill *et al.* 2011). I also conducted a common garden experiment along a latitudinal gradient (including areas in present range as well as to the north of the current range), to test how pupal performance is affected by spring and summer temperatures (IV).

The detailed study questions, experiments and predictions for the hypotheses are summarised in Table 1.

Table 1. Hypothesis tested for seasonal variation in offspring characteristics and range expansion potential, and experiments conducted with their predictions.

Study	Hypothesis	Experiments	Predictions
I	Asynchronous reproduction causes seasonal variation in offspring characteristics (Roff 1992, Stearns 1992, Marshall *et al.* 2010).	Pupal mass measurements after collection or diapause. The effects of varying diapause duration and weight loss on survival and development time.	Offspring size and survival decreases with maternal age. Offspring size variation reflects resources for varying diapause duration.
II	Females produce offspring with varying cold tolerance according to season. Cold tolerance decreases after diapause (Tauber *et al.* 1986).	Measurements of cold-hardening capacity in relation to size and life stage assessed as survival at three-day frost exposure and freezing point (SCP).	Most tolerant offspring produced in winter Large size increases cold-hardening capacity. Cold-hardening capacity high in diapausing pupae and low in active summer stages.
III	Offspring diapause determination varies with birth season. Time to diapause termination determined by varying diapause intensity and predictable seasonal cues (Tauber *et al.* 1986, Masaki 2002).	Seasonal variation in ecophysiological diapause in pupae of varying age: effect of photoperiod and chilling on diapause termination and adult emergence.	Short day-length always maintains diapause chilling obligatory to start post-diapause development. Autumn pupae have deeper diapause and require longer chilling than pupae produced later in winter and spring.
IV	Relationship between temperature and ecological requirements predicts invasion potential (Thuiller 2005, Davis 2009).	Transplant experiment: effects of spring and summer temperatures on offspring performance.	Decreasing temperature decrease pupal performance along the latitudinal gradient.

2 Materials and Methods

2.1 Study species

The deer ked is a univoltine ectoparasite on boreal cervids. In Finland the deer ked is most abundant on moose (*Alces alces*) (Kaunisto 2009, Välimäki *et al.* 2011): a single moose has been observed to host over 17,000 flies (Paakkonen *et al.*, 2010). In Northern Europe, the deer ked also parasitises the roe deer (*Capreolus capreolus*), the wild forest reindeer (*Rangifer tarandus fennicus*) and the semi-domesticated reindeer (*Rangifer tarandus tarandus*) (Kaunisto *et al.* 2009, Välimäki *et al.* 2011). Deer keds reproduce only on cervids, but they also attack other large mammals, e.g. human beings, causing mainly nuisance and allergic reactions (Laukkanen *et al.* 2005, Reunala *et al.* 2008, Kortet *et al.* 2010).

The lifespan of a deer ked may extend as long as two years: the first year is spent outside the host and the second on the host. Thus, the deer ked spend the winter in two separate stages: as reproducing adults they remain active throughout the winter on the host while the next generation overwinters on the ground at pupal diapause (Haarløv 1964).

After pupal diapause and development on the ground, adult deer keds emerge synchronously in late summer (Kaitala *et al.* 2009). The adults ambush hosts and attack them at close quarters (reviewed in Hackman *et al.* 1983). After accepting the host, the adults drop off their wings. Females fertilise only one egg at a time and retain each egg within their uterus. Offspring develop internally through all the larval stages, a strategy called adenotrophic viviparity (Imms 1957) or pupiparity (Meier *et al.* 1999). The total number of offspring is unknown, but a closely related species, the sheep ked (Melophagus ovinus), produces a total of a few dozen pupae (Small 2005). According to Ivanov (1981), deer keds start producing pupae a month after they have attached to the host. The main reproductive period in Finland was not known before. I have observed the first pupae at the beginning of October. In mid-April the number of deer ked adults on moose and pupae on moose bedding sites are still high. I have found some adults and pupae on moose in June, but they have also been found as late as in early July (Sauli Laaksonen, pers. comm.).

After birth, an immobile pupa falls from the host, often onto host bedding sites, where they overwinter at pupal diapause (Haarløv 1964). Early-born pupae in autumn will experience a long winter, whereas late-born pupae in late winter may overwinter only shortly before developmental period. Adults emerge in late summer after approximately three months of pupal development (Kaunisto *et al.* 2011). Thus, depending on birth time, the entire non-feeding period outside the host differs from a few months to as much as a year. Moreover, moose are highly mobile and may move for hundreds of kilometres during the deer ked's reproductive life span (Heikkinen 2000). Thus the offspring of each female may be dispersed over a wide range of habitats and geographical areas.

The geographical distribution of the deer ked covers large parts of Eurasia: from the British Isles to Central Europe, Fennoscandia, Siberia, Northern China and Korea (e.g. Haarløv 1964, Maa 1969, Kim *et al.* 2010, Välimäki *et al.* 2010). The outbreak of deer ked in the Finnish moose population began from South-Eastern Finland (60°N) in the 1960s and nowadays the current range limit is near the northern edge of Ostrobothnia (ca. 65°N) (Hackman 1977, Välimäki *et al.* 2010). Before the arrival of the deer ked in Finland, the moose was almost extinct for a few decades (during the 1940–50s). The rapid range expansion has been associated with the growing moose population (Hackman *et al.* 1983, Meier 2012). In Western Fennoscandia (Sweden) deer ked have existed since the 18th century (Linnaeus 1758). There, moose densities are currently three times higher than in Finland (Lavsund *et al.* 2003), yet the deer ked's range limit in Sweden is around 60°N (Välimäki *et al.* 2010), suggesting that, additionally, geographical or environmental factors drive the population dynamics of off-host stages. Unfortunately, there is no recent knowledge on Russian deer ked populations.

2.2 Offspring life histories in seasonal environments (I, II, III)

The first part of the thesis examines how the life-history characteristics of the deer ked offspring vary through the main reproductive period extending from autumn to spring. I study the basic biology of the deer ked, but the main focus is on the consequences of high variation in offspring age. I observe maternal and seasonal effects on offspring life-history traits in relation to the different conditions that each pupa will experience outside the host. I test whether there is seasonal variation in offspring life histories, such as offspring size, survival, diapause and development.

2.2.1 Material collection and preparation

All the collected pupae in studies I-III originated from the moose population inhabiting the surroundings of Siikalatva, Finland (64°30′20″N, 25°39′00″E; 60 metres above sea level). The first observations of deer ked in the area were approximately in 2005 (Juho-Antti Junno, pers. comm.). The deer ked density at the studied northern invasion frontier is very high: visual examination of bedding sites during winter (see Kaunisto *et al.* 2009) showed that nearly all moose in the study area are heavily parasitised by the deer ked.

I used pupae collected throughout the main reproductive period from October 2009 to April 2010. Reproductive adult deer keds cannot be reared in the laboratory due to their blood-feeding habits and close dependence on the host environment. All the data were therefore collected using individuals from a wild deer ked population. Black pupae are easy to find in the wild during the snowy winter period but difficult to locate in the soil without any snow cover. Due to the lack of a permanent snow blanket until mid-winter, I collected pupae directly from hunted moose during the first three months of the study period. Afterwards, the pupae were collected from moose bedding sites. I am aware that the pupae on a host may not be of similar quality with those collected from the bedding sites, but this was the only way to acquire material throughout the main reproductive period. For example, the death of the host individual may increase physiological stress for the ectoparasite female and potentially affect offspring provisioning (Clutton-Brock, 1984). The potential effects of the method were taken into account during collection (see below) and analysing the data (I).

From October to December I collected pupae from the pelts of a few recently killed moose (killed by local hunters each month during the moose hunting season). The pupae (on average 300 pupae/moose) were picked from the pelt within 18 hours after the moose was shot. A deer ked female gives birth to a white third instar larva, a pre-pupa. Pupation (incl. hardening and melanisation of the puparium) takes several hours after birth (Bequaert 1953). A complete puparium is dark black in colour, but if pupation fails, the puparium remains partly soft and the colour remains partly white/red/brown. Failed pupation leads to an unviable pupa (L. Härkönen, unpublished observations), and therefore only completely pupated individuals were selected for the following experiments. This selection guaranteed that, at the time of collection, newborn pupae were viable and, with high probability, born before the moose were killed. From January to April I searched for recently used bedding sites (indicating that the pupae had been born during the last few days) by following fresh moose tracks, and I collected completely pupated individuals from the snow cover.

2.2.2 The experiment on life histories (I) and diapause (III)

The experiment described in articles I and III were conducted simultaneously using the same pupae collected monthly between October and April. I used different parts of the data to examine seasonal variation in offspring size, diapause duration, survival through pupal period (I), and ecophysiological diapause determination and timing of adult emergence (III).

I divided the pupae within each collection month into three chilling and two photoperiod treatments. The pupae in the first chilling treatment, i.e. 'no diapause', were placed immediately in either a short (8 h/light: 16 h/dark) or a long photoperiod (16 h light: 8 h/dark) at a constant high temperature (17 ºC). Before exposure to the photoperiodic treatments, the pupae in the second chilling treatment, 'short diapause', were kept in a dark cold room (5 °C) for one month (30d), and the third chilling treatment, 'natural diapause' for the cold period that each pupa would have experienced in nature: chilling time in the latter treatments thus varied from one month (for April pupae) to seven months (for October pupae). Table 2 shows in detail which chilling treatments were used in articles I and III.

Table 2. The chilling treatments used to examine seasonal variation in offspring life-history characteristics. Pupae collected between October and April were exposed to three different lengths of diapause (chilling treatment). Different treatments and parts of this data were used to study seasonal variation offspring life-history characters (I) and timing of the diapause and life cycle (III) based on predictions presented in Table 1.

Chilling treatment	Study I	Study III
No diapause	Offspring size, size-dependent survival	Offspring age, photoperiod and temperature regulating diapause termination and seasonal variation in diapause intensity
Short diapause		
Natural diapause	Diapause survival	

2.2.3 Offspring size, survival and cold tolerance (I, II)

In the deer ked, maternally derived energy reserves during larval incubation determine the resources for the entire non-feeding, off-host period and offspring resistance to starvation and environmental adversities outside the host.

In article I, I studied seasonal variation in offspring size and its relationship with diapause survival. I measured pupal size through the main reproductive period immediately after collection (i.e. birth size between October and April), and investigated the effects of pupal size and varying diapause length (no diapause or natural diapause) on survival to the point of adult emergence (I; see 2.2.2. and Table 2 for the treatments used here).

In article II, I investigated the effects of pupal size, birth month and cold acclimation on the cold tolerance of the deer ked through its off-host life cycle (III). I collected pupae within intervals of six weeks from autumn to spring, to study the cold tolerance of newborn pupae that drop off the host without acclimation. To avoid lethal freezing, an insect may lower its freezing point, but the time it survives in a supercooled state varies with the duration and severity of frost (Knight *et al.* 1986). I therefore tested cold tolerance by measuring the freezing points (SCP) using DTA analysis (see detailed methods in III) and measured survival after exposure to extreme frosts (-15 °C or -20 °C) for three days. I also measured cold-hardening capacity through all four seasons: in addition to newborn pupae produced between October and April, I measured SCP during pupal development in summer and during the adult stage in autumn.

2.2.4 Timing of diapause termination and adult emergence (III)

A further aspect of the work was to study how seasonal and age-dependent features affect diapause and its duration. Photoperiodic and thermal conditions experienced by a deer ked pupa differ, depending on its birth time: reproduction begins in autumn when the day-length decreases but temperatures may still be favourable for development. Offspring production lasts through the darkest and coldest times of the year and ends a few months after the vernal equinox, when day-length increases and temperatures are high again.

Using all the diapause treatments (see 2.2.2 and Table 2), I tested whether the responses to environmental cues and diapause intensity vary seasonally with pupal birth month and age. It is expected that correct cues must be received during diapause, or else the diapause will continue until the insect dies (Tauber *et* *al.* 1986). For example, if long day-length and/or a period of chilling are obligatory to start post-diapause development, then no adults should emerge in treatments with short day-lengths and/or too short chilling times. By observing emergence success I tested how two environmental cues, day-length and chilling time, regulate diapause termination. I then studied variation in diapause intensity by testing the effects of photoperiod, chilling time and birth time of a pupa on adult emergence time (Kimura & Masaki 1998).

To test the synchronisation of life cycles in pupae of varying age, I compared adult emergence times after each diapause treatment (III) and in relation to pupal size (I). I defined synchrony as a situation when the time required for adult emergence does not vary between birth months, indicating that the diapause is terminated in synchrony.

2.3 Predicting northwards expansion (IV)

In the second part of my thesis, I investigated pupal performance in the more extreme conditions that prevail at the current deer ked range limit. To test the effect of spring and summer temperatures on survival and development time, pupae were collected from moose bedding sites in late winter (March–April). Five study sites were chosen along a geographical gradient reaching from central Finland to northernmost Finnish Lapland (almost 1000 km): from south to north the field sites were Konnevesi 62°N and Oulu 64°N (present range environments), Kuusamo 65°N, Rovaniemi 66°N and Utsjoki 70°N (north of the present range) (see detailed descriptions of the study sites in IV). Each treatment consisted of pupae collected from three different geographical origins (southern, central or northern Finland). The first set of pupae was introduced to each field site at the end of March (representing the circumstances when falling off the host in late winter). In the middle of May, another set of pupae was introduced to Konnevesi, Rovaniemi and Utsjoki, to experience only summer conditions.

3 Results and Discussion

The results showed high seasonal variation in offspring life histories in the viviparous deer ked. A short summary of my main results and their conclusions are presented in Table 3.

The resources a mother allocates to offspring provisioning largely determined the performance of offspring outside the host. In contrast to my predictions, early born offspring with the longest diapause and exposure to low temperatures were the smallest, and offspring size increased during the second half of the reproductive period (I, Fig. 1). Small autumn pupae with the longest diapause had very low survival, 3%, compared to the 61% survival rate of the largest spring pupae with a short diapause (Fig. 2). Surprisingly, photoperiod had no role in diapause regulation, but the deer ked overwinters at a thermally opportunistic diapause. This means that neither is a cold period required to start development, but the diapause is a rapidly broken once exposed to high temperatures. Diapause termination was also affected by seasonal variation in diapause intensity, i.e. adult emergence time varied with pupal age and chilling time. The longest, natural chilling for all pupae resulted in earlier emergence, also enhancing the synchrony of the adult stage. The deer ked tolerated extremely harsh temperatures during diapause, but also through all the off-host stages and four seasons.

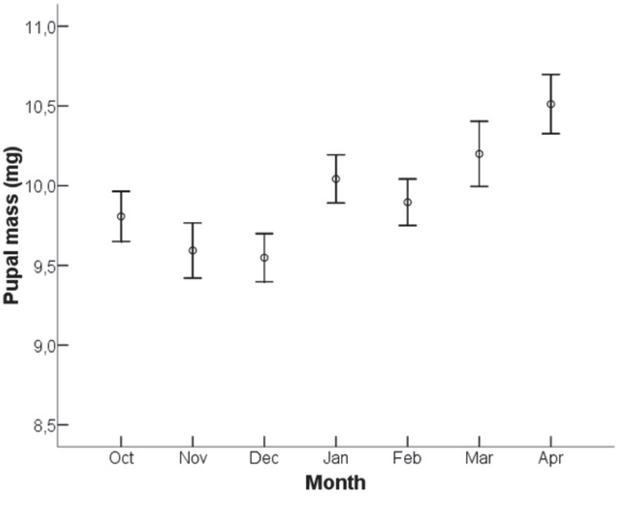

Fig. 1. Seasonal variation in pupal mass (mg ± 95% CI) of the deer ked (I).

Table 3. Summary of empirical results and their conclusions based on predictions.

Study	Predictions	Observations	Conclusions
I	Offspring size and survival decrease with maternal age Offspring size reflects resources for varying diapause duration	**NO**, offspring size and survival increase with maternal age **NO**, diapause consumes energy, decreasing survival, and larger pupae areproduced when diapause will be shorter	Smallest first-born pupae have low survival: offspring provisioning does not reflect diapause requirements. Offspring size determined by selection for long reproductive lifespan or seasonal variation in host condition
II	Most tolerant offspring produced in winter Large size increases cold-hardening capacity Cold-hardening capacity high in diapausing pupae and low in active summer stages	**NO**, the most tolerant offspring are produced in spring **YES**, large size increases tolerance to long frosts, but **NO,** size does not affect supercooling point **NO**, supercooling points remain low through all stages	High cold tolerance through all seasons but during diapause large pupal size increases tolerance to long frosts
IIII	Short day-length always maintains diapause chilling obligatory to start post-diapause development Autumn pupae have deeper diapause and require longer chilling than pupae produced later in winter and spring	**NO**, diapause maintained at low, ended at high temperature **NO,** cold experience not required to start development **YES**, diapause intensity at birth is high in autumn and weak in spring and it decreases as diapause progresses	Opportunistic diapause is thermally regulated and reversible, regardless of season or offspring age. Photoperiod does not have any role. Seasonally varying diapause intensity synchronizes life cycles in spring.
IV	Decreasing temperatures decrease pupal performance along the latitudinal gradient	**YES**, low spring or summer temperatures decrease pupal survival, prolong development and shorten flight time	The deer ked has potential for further invasion, but the invasion rate slows down towards the north

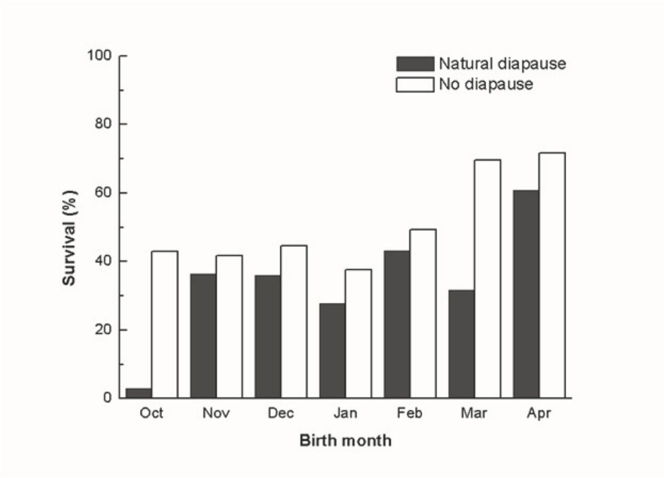

Fig. 2. Survival of pupae from birth until adult emergence after direct development (i.e. no diapause) and natural diapause duration. In the latter, pupae produced in October by young females had the longest diapause (seven months, on the left), and pupae produced by old females in April had the shortest diapause (one month, on the right) (I).

3.1 Winter survival: diapause and cold tolerance (I, II, III)

One of my most surprising findings was that photoperiod had no role in regulating the seasonal shifts in the deer ked, but instead winter dormancy was maintained by low temperature and terminated by high constant temperature. Unexpectedly, diapause was broken by an approximately four-day exposure to constant high temperature (17 ^{o}C): post-diapause development started without a cold period, even in autumn. Insects that have an opportunistic diapause are able to reverse their dormancy promptly once the ambient temperature becomes suitable for further development (see Masaki 1990, 2002, Ando 1993). This contradicts the general view, that before completion of diapause development, diapause is irreversible even under favourable conditions (Tauber *et al.* 1986).

My results support the theory that a thermally regulated pupal diapause is characteristic of hippoboscid louse-flies (Kennedy *et al.* 1975). A reversible, thermally-dominated diapause is not a common form of insect hibernation in the temperate zone, as the annual and seasonal fluctuations in temperature are assumed to offer less predictable signals for an insect than do constant photoperiodic cues (Tauber *et al.* 1982, Tauber *et al.* 1986). However, the majority of deer ked pupae are produced during the unfavourable seasons, when low temperatures will maintain diapause until spring. A similar diapause has been reported in some insects, for instance, in the temperate fly *Agromyza frontella* (Agromyzidae; Tauber *et al.* 1982). Nijhout (1998) reports that diapause in the moth *Manduca sexta* (Sphingidae) and the butterfly *Papilio polyxenes* (Papilionidae) is terminated by high constant temperature and without absolute requirements for cold.

During diapause the pupae lost a significant amount of their weight, which had a negative effect on post-diapause survival (I). If a pupa lost more than 21% of its body mass during its diapause, it did not survive to adulthood. The relative weight loss increased with diapause duration and was relatively higher for small pupae. Small individuals often have higher metabolic rate than large individuals (Peters 1983; Matsuo 2006), so small deer ked pupae may suffer from relatively higher energetic costs during a long dormancy. It is often thought that the negative effects of resource loss on further lifespan following diapause (e.g. additional mortality and fewer reproductive opportunities) are only involved in a diapause that extends to several years (Leather *et al.* 1993, Matsuo 2006). Surprisingly, my results show that diapause may have a crucial role in offspring performance even if it is short. My results suggest that variability among offspring in their size and diapause duration may be an important component of deer ked life history evolution, indicating that it would be useful to examine overwintering effects on further lifespan in future life-history studies.

In article II, I observed low freezing points (-26 °C), even without cold acclimation. Winter cold tolerance in the deer ked thus contradicts the general assumptions that winter cold-hardening would require days or weeks before actual severe winter conditions arise (Tauber *et al.* 1986). Ectoparasites that leave the warm host during winter may face a sudden risk of freezing, since they have had no time to prepare themselves for harsh winter conditions. Some temperate species may respond to low temperatures at short notice and even during the favourable seasons: rapid cold-hardening processes protect the insects from cold injury if they experience a sudden fall in ambient temperatures (e.g. Czajka & Lee 1990, Kelty & Lee 2001). The ability to cold-harden rapidly may increase the survival probability of newborn deer ked pupae when they have just fallen off the host

The mortality of the deer ked pupae increased when the duration of harsh frost was prolonged. For example, at -20 °C they survived relatively well for three days but four days at this temperature killed them all. Large size increased tolerance to long frosts, and therefore cold tolerance was higher in spring than in winter. Frosts below -20 °C occur only during a few months (Dec–Mar) even in northernmost Finland (Kevo/Finnish Meteorological Institute Database, 2010), whereas the deer ked pupae are produced during a period lasting at least nine months. A large proportion of pupae will be covered by snow, which protects the overwintering pupae.

The cold-hardening capacity of the deer ked remained high throughout all four seasons and free-living life-history stages (SCP ≤ 20 °C; II): the developing pupae and adults never experience such temperatures in summer or autumn. The majority of insects enter diapause well in advance in order to withstand decreasing autumnal temperatures. Supercooling points of -30 °C during diapause are not uncommon among hibernating insects in temperate regions. So far, only some Arctic and Antarctic freeze-intolerant species have been reported to retain a high degree of cold-hardiness all year round (Tauber *et al.* 1986, Bale *et al.* 2001).

The high cold tolerance all year round and through free-living stages observed in deer ked may correlate with other factors increasing their off-host survival, such as resistance to starvation or drought (Tauber *et al.* 1986, Dautel & Knülle 1997). In the deer ked, high cold tolerance may be a side effect of offspring provisioning for their long non-feeding period. For example, it has been suggested that high supercooling capacity in the ectoparasitic tick *Argas reflexus* (Argasidae) is a consequence of its ability to survive prolonged periods of starvation and desiccation when outside the host (Dautel & Knülle 1997). Body fat is an important energy source during starvation (Colinet *et al.* 2006), and fatty acid composition has been found to be involved in the cold-hardening of dipterans (Bennett *et al.* 1997, Ohtsu *et al.* 1998). Age also has an important role in cold-hardening capacity (Bowler & Terblanche 2008). Although the cold tolerance of the deer ked remains high after diapause, energy reserves decrease with age (i.e. length of starvation), which is likely to gradually reduce cold tolerance.

3.2 Seasonal variation in offspring size and performance (I, II)

In the deer ked, the mean offspring size increased as the mothers became older (Fig. 1). The effects of maternal age on offspring size thus contradicted the general trend reported in invertebrates, i.e. that size often decreases with maternal age. The opposite trend has mainly been found in species that continue growth after maturation (Marshall *et al.* 2010) and in vertebrates (Clutton-Brock 1991, but see Kindsvater *et al.* 2010). This pattern may result from the fact that old mothers with low residual reproductive value are able to transfer an increasing proportion of their remaining resources to offspring provisioning (referred to as terminal investment; e.g. Clutton-Brock, 1991). However, care should be taken when considering only the effects of maternal age on offspring size in the deer ked, as also other factors, such as maternal body size, are likely to affect offspring size (Marshall *et al.* 2010).

Mothers may modify offspring size in order to increase their own fitness rather than to increase survival of an individual offspring (Smith & Fretwell 1974, Begon & Parker 1986, Marshall & Uller 2007). Each propagule that a viviparous deer ked female produces is extremely large among invertebrates: a pupa weighs approximately the same as the blood-consuming female herself (see Paakkonen *et al.* 2010). A high reproductive effort early in life may shorten adult lifespan and reduce their lifetime fecundity (Stearns, 1992). If also the survival probability of offspring is low, females may be expected to postpone their reproduction to an older age (Stearns 1992, Marshall and Uller, 2007). In viviparous species a relatively small increase in the size of already large offspring rarely exceeds the benefits of increasing the offspring number (Schrader and Travis, 2008). Due to large propagule size and low fecundity, I assume that the deer keds may be selected rather to guarantee a long reproductive lifespan and to increase the offspring number than to increase offspring size or postpone reproduction. This phenomenon may have been useful in the history of the species' evolution, and is seemingly not very detrimental in the current distribution area of the species.

I found that large pupal size increases off-host survival in the deer ked, but seasonal variation in offspring size did not reflect seasonal variation in the expected offspring diapause duration or cold tolerance. It thus seems that temporal variation in offspring size does not result from seasonally cued plasticity in offspring size: the first-born offspring, which had a long diapause ahead, were provisioned with the lowest energy reserves. Old females have been reported to produce larger offspring if they have sufficient resources to do that (Fox 1993). In ectoparasites, the variation may rather result from the direct effects of host condition and from the effects of seasonal changes in the quality of host blood on females (Langley & Clutton-Brock 1998). The body condition of moose declines as the winter progresses, due to decreased food availability (Sæther & Gravem 1988). When the host condition declines, it is less able to develop and maintain costly immunological or physiological defence mechanisms, and their lowered resistance increases the quality of resources for blood-feeding ectoparasites (Roulin *et al.* 2003; Tschirren *et al.* 2007). Accordingly, I found that offspring size in the deer ked increased from mid-winter towards spring, which may indicate seasonal changes in the host effect on the ectoparasite's resource quality and thus on its reproductive performance. However, further work on blood-feeding ectoparasites is still needed to distinguish between the effects of maternal trade-offs and host effects on offspring size and performance in seasonal environments.

3.3 Timing of the life cycle and adult emergence (III)

Although I found that deer ked diapause is terminated by constant high temperatures and without absolute requirements for cold, post-diapause development starts more quickly after chilling. This pattern has also been reported in other hippoboscids, and it indicates that diapause development is completed already during the cold period (Kennedy *et al.* 1975). In the deer ked, the effect of chilling time varied with pupal birth season. I found that early-born pupae (until January) required longer chilling to complete their diapause development in cold conditions than did the late-born pupae (from February on). This indicates that diapause intensity varies seasonally, being higher in autumn than in spring, and also with age, as it decreases with the increasing number of days at low temperature (Masaki 2002). A deep diapause is expected in first-born offspring that will receive diapause terminating signals, such as long day-length and high temperature before winter (Tauber *et al.* 1986, Ando 1993, Masaki 2002). Because of high diapause intensity in autumn, the deer ked pupae require longer exposure to high temperatures than in spring to start their development. Our results thus support Ando (1993), who reports that high diapause intensity may prevent development before winter, even when diapause is thermally reversed.

In species that enter diapause at different times of the year, diapause intensity often varies as a response to seasonal cues (Kimura & Masaki 1998, Masaki 2002). The mechanisms for seasonal variation in diapause intensity in the deer ked as well as for diapause induction are currently unknown. In species with a long reproductive lifespan, maternal age correlates predictably with seasonal changes (Mousseau & Fox 1998). If offspring enter diapause at birth, maternal age or environment may determine whether the offspring will enter diapause or not, and how long the diapause is once induced (Denlinger 1972, Mousseau & Dingle 1991). Because the deer ked pupae drop off the warm host after birth, they have no time to prepare for diapause. It may be, for instance, that the mother prepares her offspring for different seasonal conditions: young deer ked females in autumn produce offspring that enter deep diapause compared to the weak diapause of pupae produced by old females in spring. Kennedy *et al.* (1975) suggest that in bird louse-flies, the seasonal condition of host blood may induce variation in offspring diapause rather than the photoperiodic cues experienced by the mother.

Variability in diapause intensity has been found to play an important role in the evolution of seasonal life cycles (Masaki 2002). This has been demonstrated with a geometrid moth *Abraxas miranda,* in which entering pupal diapause is highly asynchronous between autumn and spring. Corresponding seasonal variation in diapause intensity, i.e. higher diapause intensity in autumn than in spring, has been observed to result in more synchronised emergence of adults than would otherwise be expected (reviewed by Masaki 2002). In the deer ked, diapause intensity decreased as a result of chilling. The spring pupae, which will be exposed to cold only for a short period, have lower diapause intensity, so that they will catch up in diapause development with those already born in autumn and experiencing a long cold period. As a result, post-diapause development in all-aged deer ked pupae may start synchronously once exposed to temperature above the developmental threshold in the spring.

In deer ked pupal development, the post-diapause stage lasts exceptionally long, for ca. 90 days (III). An increase in offspring size unexpectedly prolonged pupal development in the deer ked, but Kaunisto *et al.* (2011) reported that large deer ked adults emerge from large puparia. In most invertebrates, offspring from large eggs develop faster and emerge as larger adults than those from small eggs (Roff 1992, Fox 1994). Early emergence and large adult size often increase reproductive success in terms of more mating opportunities, higher mating success and ability to produce larger offspring (Wiklund & Forsberg, 1991, Roff 1992). The opposite pattern I found in the deer ked has previously been associated with their relatively higher rate of energy consumption (Tobin *et al.* 2002). I assume that small deer ked pupae may emerge earlier if their energy reserves are exhausted sooner, whereas large pupae may consume more resources on longer development in order to attain larger body size. However, there seems to be a trade-off between emergence time and adult size in the deer ked (see Zonneveld 1996): is it better to produce small offspring that emerge early or large ones that probably have better resistance to starvation and become bigger adults? The deer ked mainly ambush their hosts, which means that the time available may partly determine the success of host location. However, sedentary waiting for a suitable host may even take several weeks after emergence (Kaitala *et al.* 2009). Early emergence may therefore be of less importance than large size when determining offspring fitness.

Across the northern boreal zone, deer ked adults emerge locally synchronously, in mid-August (Haarløv 1964, Kaitala *et al.* 2009). My results showed that diapause and seasonal variation in its intensity may enhance the synchronisation of adult emergence. Emergence synchrony in parasites is considered essential when their hosts are not continuously available (e.g. Kennedy *et al.* 1975). Deer ked is an ambushing parasite which attacks a by-passing host. Moose are highly mobile, especially in autumn, when in search of mates, and thus the deer ked may have limited opportunities of encountering a suitable host at close quarters. Adults drop their wings immediately after landing on the host and start breeding later in autumn (Ivanov 1981). Local synchrony of adult emergence may increase the possibility that acceptance of any by-passing host will also offer potential mates for later reproduction.

3.4 Does the deer ked have potential for further spread? (IV)

The deer ked was able to survive and complete its development even in arctic environments but its performance deteriorated steadily northwards (Fig. 3. IV). Towards the north, the decrease in survival after experiencing late winter conditions may have been caused by frost kills during late winter. The negative effects of lower summer temperatures on survival increased towards the north. They may not have directly killed the pupae but delayed development, reducing the number of emerged adults as oncoming winter interrupted the emergence period. Since adult emergence in the north was delayed for several weeks, the potential host search time was as much as a month shorter than in the current range.

The results suggest that climate may not fully prevent further spread, and that the time may have been too short for the deer ked to reach the northern parts of Finland. The Finnish deer ked population originates from Central Eurasia (Russia) and it has expanded its range over five latitudes and two climatic zones in less than 50 generations (i.e. 50 years). The native areas in Russia are characterised by high annual variation in temperature (i.e. a continental climate with cold winters and warm summers). My results suggest that the thermal conditions in the past invasion area in Finland may have been within the physiological threshold limits corresponding to the conditions in their original range. Hence no lag period, i.e. time for local adaptations, has been needed to spread further.

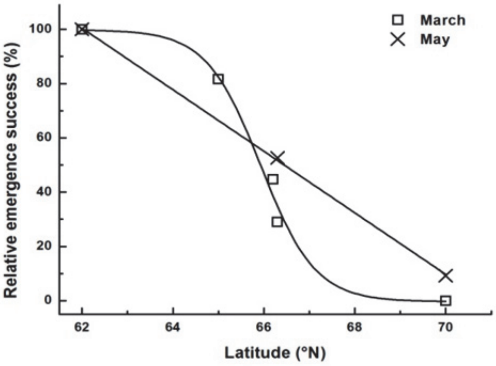

Fig. 3. Relative emergence success of the deer ked pupae along the latitudinal gradient and in relation to the season when the experiment started. Towards north, increased summer was caused mainly by developmental delays whereas in winter and spring, higher mortality may likely be caused by late winter and spring frosts killing larger proportion of the deer ked pupae before development period (IV).

My results on diapause costs indicate that e.g. geographical variation in diapause duration and the factors affecting offspring size variation may be an important determinant for invasion success in this species. If the deer ked reaches more northerly areas in the future, the longer winters may increase the energetic costs (I) and have negative effects on population growth rates. Incorrect timing of seasonal shifts between inactive and active phases may also increase mortality in spring far from the original sites (Bale & Hayward 2010). However, the shift is often timed incorrectly because the predictability of the photoperiod is coupled with suitable thermal conditions in the native range (see Bale & Hayward 2010). Since the diapause of the deer ked is simply regulated by experienced temperature, the timing of seasonal shifts will occur according to the thermal suitability of a given environment.

The ability to cope with a shorter season length and lower sum of heat units are important for establishment in northern environments. Deer ked pupae of northern origin emerged slightly earlier north of the current range than those that originated from more southerly latitudes (IV). Many high-latitude populations are able to compensate for the shorter growing season with higher development rates at low temperatures (Yamahira & Conover 2002). Insects often need to reach a certain number of degree-days in order to emerge (Angilletta 2009). Surprisingly, I found that this is not the case in the deer ked: the thermal time (or thermal sum) needed for adult emergence varies with experienced temperature. In the laboratory, a 1 °C increase at constant temperature prolongs development by approximately 50 degree-days (unpublished data). In the transplant experiment, the first adult in Utsjoki (70° N) emerged almost 200 degree-days earlier than the first adult in Konnevesi (62° N). This indicates that, in the deer ked, the utilisation of the available heat units for development is more efficient at low temperatures. Thus the ability to emerge earlier in terms of shorter thermal time may be an important factor during establishment in higher latitudes.

Emergence synchrony may also increase the invasion success of the deer ked. A newly established population often suffers from the Allee effect (i.e. positive correlation between population size or density and mean individual fitness) and/or inbreeding depression (Davis 2009). When parasite density is still low, synchronised deer ked emergence may result in a decrease in the dilution of individuals in the host population.

The ability to remain active under unfavourable thermal conditions may have an important role facilitating survival of the deer ked in the north. The cold tolerance of the deer ked is exceptionally high through all seasons and life stages outside the host (II). In northern environments, nocturnal frosts may occur even in summer. During autumn, subzero temperatures may occur nightly, whereas day temperatures still remain favourable for flying. Range expansion potential may increase if the shorter season length at high latitudes or altitudes is compensated by the ability to tolerate autumnal frosts, and therefore by extended flight time (Bale & Hayward 2010, Nieminen *et al.* 2012).

In the future, the performance of the deer ked may improve if ongoing global warming raises seasonal temperatures, shortening the harsh winter period and prolonging the growing season in northern ecosystems (IPCC 2007). However, the most important component of range shifts in parasitic species is host availability. Only a part of the deer ked life cycle is spent outside the host, and its further spread will additionally depend on access to a cervid host. Northwards from the current range, high numbers of cervids are available for the deer ked. The current distribution area of the Finnish moose population (ca. 100,000 individuals, post-harvest number) covers the whole country, but their density is significantly lower in Lapland than in the current deer ked range (Pusenius *et al.* 2010). Although the semi-domesticated reindeer population in the northern half of Finland is relatively large (ca. 200,000 individuals in winter stock; Reindeer Herders' Association 2009), the reproductive success of the deer ked on reindeer has been found to be very poor in terms of the short female reproductive lifepan (a few months only) and thus a low number of offspring (Kynkäänniemi *et al.* 2010). As a result, the low number of high-quality hosts may keep population sizes low and thus slow down the invasion towards the northernmost latitudes.

3.5 Invasion potential of ectoparasites

Blood-sucking insects often have a negative influence on a wide range of vertebrates. Ectoparasites are harmful as parasites and pathogen vectors, but they also have direct fitness costs for their hosts (e.g. Fitze *et al.* 2004, Samuel 2007). Studies on their basic biology are therefore important in order to evaluate their ability to invade northern ecosystems, e.g. due to climate warming. The life-history characteristics of invasive deer ked differ significantly from those of free-living invasive species. Invasion of the free-living insects is often facilitated by high active dispersal capacity and a high reproduction potential. In addition they often produce a large number of small propagules: due to their small body size, they have fast growth and development rates, which result in a short generation time (Roff 1992). In contrast to deer ked, they are also often generalists in their ecological requirements (Mack *et al.* 2001, Sax & Brown 2001, Davis 2009).

An important component of rapid expansion is high propagule pressure, i.e. the number and frequency of recruits arriving in the new areas (Lockwood *et al.* 2005, Davis 2009, Sexton *et al.* 2009). One moose carrying thousands of deer keds often has long seasonal migrations, when the continuous recruitment of new deer ked pupae may compensate for some of the mortality and allow the deer ked to persist in novel areas. The moose also disperses the deer ked pupae over a wide range of habitats. Deer ked performance outside the host presumably requires plasticity and high physiological tolerance already in its current range, and the same characteristics may also facilitate spread of the deer ked (but see Brotons *et al.* 2004). On the other hand, since the offspring of each female are dispersed over different environments, adaptation to local conditions may be slow.

Physiological tolerance to environmental stress and body size are among the best predictors of invasion success (Davis 2009). Diapause increases resistance to the environmental stress which is often encountered during transportation and introduction to novel environments. The introduction of dormant stages is an efficient strategy for dispersal in several aquatic invertebrates (reviewed by Panov & Caceres 2007). The production of large diapausing propagules may thus also be an important determinant of survival in the deer ked in heterogeneous off-host environments, as well as in other ectoparasites that use large vertebrate as vectors for introduction to novel areas. Furthermore, large late-born deer keds may have a selective advantage at high latitude environments because of their short exposure to winter conditions and higher survival rate (I, II). However, my findings also suggest that seasonal or geographical variation in host effects on offspring size and performance should be considered when evaluating the invasion potential of blood-feeding ectoparasites.

4 Conclusions

This study serves to enlighten seasonal variation in the offspring life histories of viviparous ectoparasites, which have been surprisingly poorly studied among invertebrates. I found that in the viviparous deer ked, both the variation in offspring life histories and their seasonal adaptations contradicted several general predictions concerning those reported in other temperate invertebrates. My results therefore provide several new aspects for future life-history studies on ectoparasites.

I found that offspring life histories, especially offspring size, of the deer ked vary seasonally, and the resources provided by the deer ked females determine offspring performance through its off-host period. First, in contrast to most invertebrates with a long reproductive period, young females produced the smallest offspring with the lowest survival. The offspring size then increased towards the spring, and offspring survival and cold tolerance increased accordingly. The diapause period consumed energy reserves, and due to the significant weight loss, survival to adult emergence decreased, especially if the period of diapause (and starvation) were long. Thus seasonal variation in offspring size did not reflect the resources that would guarantee offspring survival during the longest diapause or highest cold tolerance during the harshest winter frosts. Nevertheless, the deer ked was highly tolerant to winter frosts, even without cold acclimation. Unexpectedly, high cold tolerance was not associated only with diapause; also the active life stages tolerated such harsh frosts that they never encounter in nature. The pupae overwintered at an opportunistic diapause, which may be terminated rapidly by exposure to high temperature: photoperiod had no role in regulating the seasonal shifts. Nevertheless, I found that diapause intensity varied with birth time according to the expected winter ahead and enhanced synchronisation of adult emergence.

Seasonal variation in offspring life histories in the viviparous deer ked differs from those variation patterns reported in many invertebrates. This may be due to the extremely large offspring size, and the fact that the maternally-derived resources determine offspring performance through the entire off-host period. Variation in offspring performance is dependent on maternal resources and seasonal variation in moose condition. However, further studies on the life-history evolution of viviparous ectoparasites should include distinguishing between the maternal trade-offs associated with offspring size and the host effect on seasonal variation in reproductive performance.

I also evaluated factors that may have facilitated the rapid northward invasion of the deer ked, as well as the effects of life history variation on further invasion potential. Studies I-III showed that the life history characteristics of invasive deer ked differ significantly from those of free-living invasive species. Based on the common garden experiment, suitable environments for offspring development exist 500 kilometres to the north of the current range. The results suggest that the colder climate may not totally prevent further spread. However, it should be kept in mind that the most important factor driving the rapid invasion of ectoparasites is the accessibility of suitable hosts beyond the range limits.

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