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Impacts of summer drought on plant-herbivore interactions:  
the Glanville fritillary metapopulation as a case study

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ACADEMIC DISSERTATION

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# Impacts of summer drought on plant-herbivore interactions: the Glanville fritillary metapopulation as a case study

Ana Lucía Salgado Maldonado

**The thesis is based on the following articles, which are referred to in the text according to their Roman numbers:**

- I Salgado AL, Kahilainen A, Israel D and Saastamoinen M. Responses to drought and the role of habitat type in a perennial herb at its northern range margin. Manuscript.
- II Salgado AL and Saastamoinen M. (2019). Developmental stage-dependent responses and preference to host plant quality in an insect. *Animal behaviour*, 150, 27-38.
- III Salgado AL, DiLeo MF and Saastamoinen M. Narrow oviposition preferences put herbivore insect at risk under climate change. Manuscript.

## Table of contributions

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Data analyses	ALS	ALS	MFD
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# ABSTRACT

The life-history and performance of insect herbivores can be significantly affected by environmental stress, and the responses may vary across the life cycle of the insect. This variability in the responses depends on the specific demands of different life stages, but also on their potential to adjust behaviourally by seeking better environmental conditions. Drought is one of the most dominant environmental stressors that can affect natural populations. The increased prevalence and severity of droughts during the last decades have generated radical changes in ecosystems with further consequences for biotic interactions. Therefore, it is necessary to study the effects imposed by drought on trophic interactions. Insect herbivores are intimately dependent on their host plants, which means that the effects of drought on host plant may impact life-history traits and behaviour of the insect herbivore. Drought may, for example, alter the nutritional quality of the host plant with subsequent impacts on the herbivores acquiring resources from these plants.

This PhD thesis investigates the responses of the Glanville fritillary butterfly (*Melitaea cinxia*) to variation in host plant quality, by exposing one of its prominent host plants (*Plantago lanceolata*) to drought. In my research, I combined lab experiments with detailed field measurements and existing long-term monitoring data. The lab experiments demonstrated that drought-exposed host plants are nutritionally richer than well-watered host plants, and that the larval responses to host plant quality changes imposed by drought are

developmental stage-dependent. Furthermore, I show that post-diapause larvae and adult females each preferred host plants that enhanced their growth rate or survival of their offspring, respectively. Using long-term monitoring data and detailed information of the habitat conditions from the field, I found that maternal oviposition preference was directed towards drought-exposed microhabitats with high host plant abundance. This preference was correlated with higher overwinter survival of the offspring on normal years. However, as females did not show an ability to adjust their oviposition preferences in a year of severe drought, this led to very few surviving offspring. These data suggest that the metapopulation may be at risk under increasing drought conditions in the future.

The results from this dissertation highlight that both the host plant and the insect herbivore are somewhat adapted to live under variable environmental conditions. The host plants show ability to respond to drought conditions and also the insect herbivore. The insect herbivore is, to some extent, also able to adjust its behaviour in response to variation in host plant quality induced by drought. Additionally, my results show that microhabitat conditions are important in determining insect herbivores' performance. However, if conditions become even drier in the future this may impose risks. In conclusion, to fully understand the potential impacts of anthropogenic climate change, we need to understand their impacts on the interactions of species, such as host plants and insect herbivores.

# TIIVISTELMÄ

Ympäristöstressi voi vaikuttaa merkittävästi kasvinsyöjähyönteisten elinkiertoekologiaan ja suorituskykyyn ja nämä vaikutukset voivat vaihdella hyönteisen elinkaaren ajan. Vaihtelu reaktioissa riippuu eri elämänvaiheiden erityisvaatimuksista, mutta myös niiden mahdollisuudesta mukauttaa käyttäytymistä etsimällä parempia ympäristöolosuhteita. Kuivuus on yksihallitsevimmistaympäristöstressereistä, jotka voivat vaikuttaa luonnossa eläviin populaatioihin. Lisääntynyt kuivuus ja sen vakavuus viime vuosikymmeninä ovat aiheuttaneet radikaaleja muutoksia ekosysteemeissä, joilla on lisävaikutuksia biotottiin vuorovaikutuksiin. Siksi on tarpeen tutkia kuivuuden vaikutuksia troofiin vuorovaikutuksiin. Kasvinsyöjähyönteiset ovat läheisesti riippuvaisia isäntäkasveistaan ja tästä johtuen kuivuuden vaikutukset isäntäkasveihin voivat vaikuttaa hyönteisten elinkiertoekologisiin piirteisiin ja käyttäytymiseen. Kuivuus voi esimerkiksi muuttaa isäntäkasvin ravitsemuksellista laatua ja myöhemmin vaikuttaa niihin kasvissyöjiin, jotka saavat resursseja näistä kasveista.

Tämä väitöskirja tutkii täpläverkkoperhosen (*Melitaea cinxia*) reaktioita isäntäkasvien laadun vaihtelulle altistamalla yksi sen pää-asiallisista isäntäkasveista (*Plantago lanceolata*) kuivuudelle. Tutkimuksessani yhdistin laboratoriokeet yksityiskohtaisiin kenttämittauksiin ja olemassa olevaan pitkän aikavälin seurantatietoon. Laboratoriokeet osoittivat, että kuivuudelle altistuneet isäntäkasvit ovat ravitsemuksellisesti rikkaampia kuin hyvin kastellut isäntäkasvit ja että kuivuuden aiheuttamat muutokset isäntäkasvien laadussa vaikuttavat toukkiin eri tavoin eri kehitysvaiheissa. Lisäksi osoitan, että talvihoroksen

jälkeiset toukat ja aikuiset naarasperhoset valitsivat isäntäkasveja, jotka paransivat niiden kasvunopeutta tai vastaavasti jälkeläisten eloonjäämistä. Käyttäen pitkäaikaista seurantatietoa ja yksityiskohtaista tietoa elinympäristöolosuhteista, huomasin, että perhosnaaraat suosivat muninnassa kuivuudelle altistuneita mikrohabitaatteja, joilla on runsaasti isäntäkasveja. Tämä mieltymys korreloi korkeampaa jälkeläisten selviytymistodennäköisyyttä normaaleina vuosina. Naaraat eivät kuitenkaan osoittaneet kykyä muuttaa munimismieltymyksiään vakavan kuivuuden vuonna, mikä johti hyvin pieneen määrään eloon jääneitä jälkeläisiä. Nämä tiedot viittaavat siihen, että metapopulaatio voi vaarantua tulevaisuudessa kuivuuden lisääntyessä.

Tämän väitöstutkimuksen tulokset korostavat, että sekä isäntäkasvi että kasvinsyöjähyönteinen ovat jossain määrin sopeutuneet elämään vaihtelevissa ympäristöolosuhteissa. Isäntäkasvit ja hyönteiset molemmat osoittavat kykyä reagoida kuivuusolosuhteisiin. Hyönteiset pystyvät myös jossain määrin säättämään käyttäytymistään vasteena kuivuuden aiheuttamille isäntäkasvien laadun vaihteluille. Lisäksi tulokseni osoittavat, että mikrohabitaattiolosuhteet ovat tärkeitä kasvinsyöjähyönteisten suorituskyvyn määrittämisessä. Olosuhteiden muuttuminen tulevaisuudessa vielä kuivemmiksi saattaa aiheuttaa riskejä. Yhteenvetona voidaan todeta, että ihmisen aiheuttaman ilmastomuutoksen mahdollisten vaikutusten ymmärtämiseksi meidän on ymmärrettävä vaikutukset lajien, kuten isäntäkasvien ja kasvinsyöjähyönteisten väliseen vuorovaikutukseen.

# ABSTRAKT

Växtätande insekters livshistoria och framgång kan påverkas avsevärt av miljöbelastning och responsen kan variera över insektens livscykel. Denna variation i respons beror på de specifika kraven i olika livsfaser, men också på deras potential att anpassa sitt beteende genom att söka bättre miljöförhållanden. Torka är en av de mest dominerande miljörelaterade stressfaktorer som kan påverka naturliga populationer. Den ökade förekomsten och svårighetsgraden av torka under de senaste decennierna har åstadkommit radikala förändringar i ekosystem med ytterligare konsekvenser för biotiska interaktioner. Det är därför nödvändigt att studera vilken effekt torka har på trofiska interaktioner. Växtätande insekter är direkt beroende av sina värdväxter, vilket innebär att följderna av den torka som värdplantan genomgår kan påverka livshistoriska egenskaper och beteende hos växtätande insekter. Torkan kan till exempel förändra värdplantans näringskvalitet med efterföljande påverkan på växtätande insekter som får resurser från dessa växter.

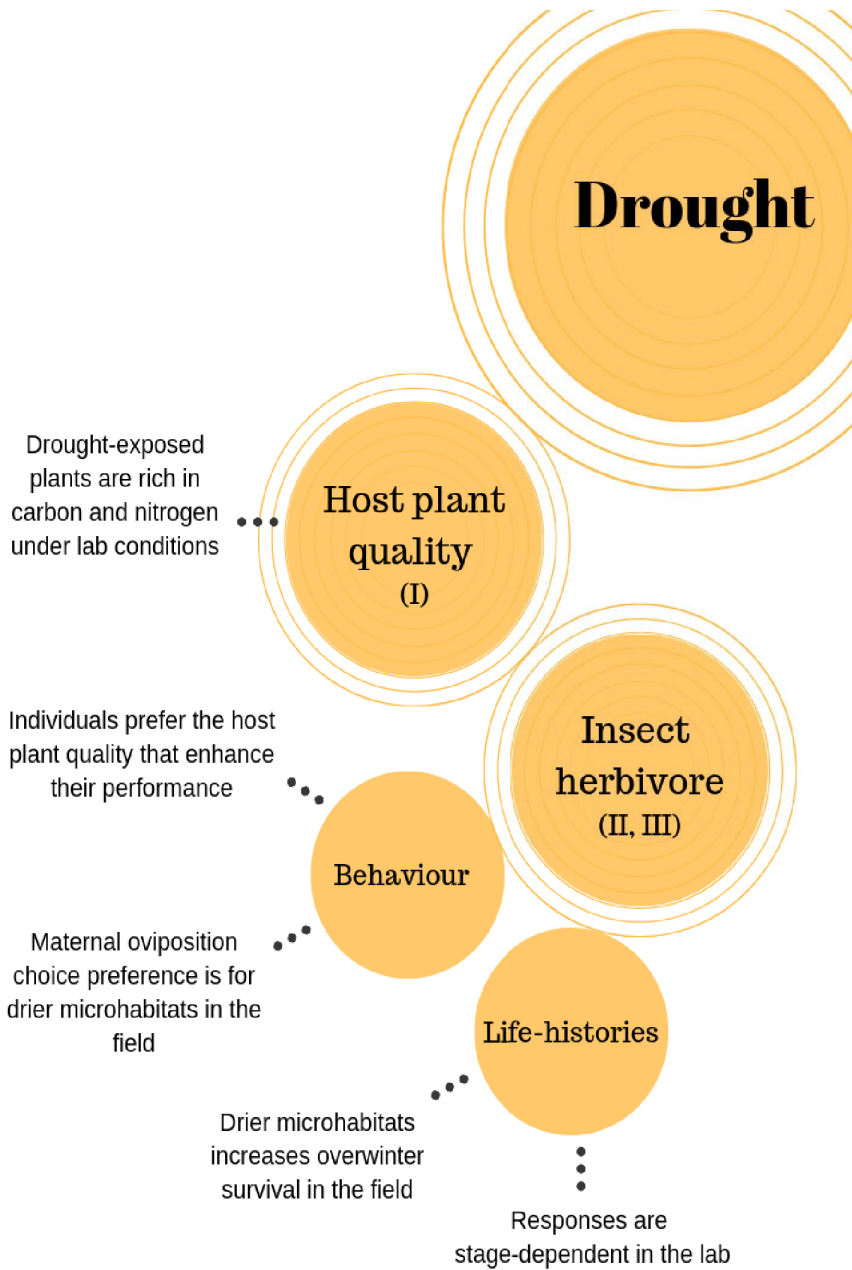
Denna doktorsavhandling undersöker Ängsnätfjärilens (*Melitaea cinxia*) respons till variation i värdplantans kvalitet genom att utsätta en av dess framstående värdväxter (*Plantago lanceolata*) för torka. I min forskning kombinerade jag laboratorieexperiment med detaljerade fältmätningar och befintliga långsiktiga övervakningsdata. Laboratorieexperimenten visade att värdplantor som var exponerade till torka är näringsrikare än välvattnade värdväxter, och att ängsnätsfjällarvrens respons på förändringar i värdväxtens kvalitet, som påverkats av torka, beror på

utvecklingsstadiet. Dessutom visar jag att larver efter diapaus samt vuxna honor föredrog värdväxter som förstärkte deras tillväxthastighet respektive överlevnad hos deras avkommor. Med hjälp av långsiktiga övervakningsdata och detaljerad information om livsmiljöerna i fält, fann jag att maternell äggläggning var riktad mot mikrohabiter som var utsatta för torka och hade ett stort överflöd av värdplantor. Denna preferens korrelerades med högre överlevnad över vintern hos avkommorna under normala år. Eftersom honorna emellertid inte visade förmåga att anpassa sina naturliga cykler för äggläggning under ett år med svår torka, ledde detta till mycket få överlevande avkommor. Dessa data tyder på att artens metapopulation kan vara i riskzonen i framtiden under ökande förhållanden med torka.

Resultaten från denna avhandling framhäver att både värdväxten och den växtätande insekten är något anpassade för att leva under varierande miljöförhållanden. Värdplantorna visar förmåga att svara på torka och även den växtätande insekten. Den växtätande insekten kan till viss del också anpassa sitt beteende som respons till variation i värdplantans kvalitet som har påverkats av torka. Dessutom visar mina resultat att förhållandena i mikrohabitat är viktiga för att bestämma växtätande insekters framgång. Men om förhållandena blir ännu torrare i framtiden kan det innebära risker. Sammanfattningsvis, för att fullt ut förstå de potentiella effekterna av klimatförändringar orsakade av människan, måste vi förstå deras påverkan på interaktion mellan arter, såsom värdväxter och växtätande insekter.



# GRAPHICAL ABSTRACT



# SUMMARY

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## 1 INTRODUCTION

### 1.1 DROUGHT

Human activity has dramatically affected ecosystems in the last century, bringing the study of the effects of anthropogenic climate change to the forefront of biology (Mishra & Singh, 2010; Otto, 2018). The list of ecosystem alterations induced by climate change include the melting of ice caps in the poles, accelerating species extinction, and the accumulation of unprecedented levels of greenhouse gases (Otto, 2018). All environments on Earth have suffered from human pressure, ushering a new era: the Anthropocene (Lewis & Maslin, 2015).

Climatic conditions have been constantly changing along Earth's history. However, human activity has accelerated changes beyond historical rates, and unusual weather events have become more frequent with drastic effects on wild populations (Geber & Dawson, 1993; Mishra & Singh, 2010; Otto, 2018). For example, the intensity and prevalence of drought, which has increased substantially by the end of the 20th century, has had considerable effects on multiple ecosystems (Dai, 2011, 2013). Drought has been defined as a reduction in the precipitation levels from the comparable mean of an ecosystem, and it can be prolonged for a few days, months or years. Any climatic zone on Earth can be affected by drought, and its effects are vast, including alteration in carbon cycling, soil erosion and inducing mortality of vegetation with cascading effects on higher trophic levels (Cook, Mankin, & Anchukaitis, 2018; Lake, 2011; Mishra & Singh, 2010; van der Molen et al., 2011). Predictions for the next decade estimate an increase of drought at large scale all around the world (Mishra & Singh, 2010). Therefore, it is critical to

understand the effects of drought and the potential mechanisms that mitigate these effects on wild populations.

Species with complex and tight biotic interactions are particularly vulnerable to environmental change, both through direct effects by the altered conditions, and through indirect effects of interacting species responding to the novel conditions (Gryj, 1998). Thus, alterations in climatic conditions could generate susceptible populations and erode the synergy between species (Ayles, 1993). Nevertheless, the indirect effects of climate change on species are complex and difficult to predict, and consequently have only recently been incorporated into studies (Clissold & Simpson, 2015).

### 1.2 PLANT-HERBIVORE INTERACTIONS

Plant-herbivore interactions are the most dominant processes occurring in a vast number of ecosystems (Burkepile & Parker, 2017). Plant-herbivore interactions are important because they link primary production and food webs (Burkepile & Parker, 2017). The link occurs mainly because many animals rely on plants for energy to grow and live (Schoonhoven, Jermy, & Loon, 1998). This has driven the co-evolution between plant defence strategies and mechanisms in herbivores to overcome these defences, which in turn has increased the diversity of both plant and animals on Earth (Ehrlich & Raven, 1964; Thompson, 1999). Due to their importance, plant-herbivore interactions have

been extensively studied to address diverse questions in ecology and evolution. They are also useful in addressing questions on how environmental change will modify natural populations and their interactions (Gryj, 1998; Lenhart, Eubanks, & Behmer, 2015).

Many insects depend on plants for development and survival, and also rely on ambient temperature to regulate their metabolism and activity. Hence, insects are great candidates to investigate the effects of environmental stress on plant-herbivore interactions (Bauerfeind & Fischer, 2013; Caillon, Suppo, Casas, Arthur Woods, & Pincebourde, 2014). On one hand, climate change will directly manipulate key traits of insect herbivores, such as the effects of temperature on the performance of insects. On the other hand, climate change can indirectly affect insect herbivores by modifying the quantity and quality of the host plants they rely on (Clissold & Simpson, 2015; Jamieson, Trowbridge, Raffa, & Lindroth, 2012). In this dissertation, I focused on the indirect effects of drought on the performance of an insect herbivore by studying the changes drought induce in the nutritional quality of its host plant and how this influenced life-history traits and behaviour of the insects.

### 1.3 EFFECTS OF DROUGHT ON HOST PLANTS

Plants are especially susceptible to changing environmental conditions due to their sessile lifestyle, and their success (as in other organisms) depends on the ability to respond to environmental change (Box 1; Agarwal, Reddy, & Chikara, 2011). Plant adaptations to drought are extraordinary diverse and complex, involving morphological, physiological, and biochemical changes (Agarwal et al., 2011; Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). At the morphological level, drought stressed plants tend to reduce growth, resulting in smaller individuals with fewer leaves and reduced reproductive traits. However, roots tend to be larger in order to increase water uptake (Farooq et al., 2009; Mattson & Haack, 1987). At the physiological level, plants close their stomata to limit water loss, which generates a reduction in photosynthesis and stomatal conductance. This has cascading effects at the biochemical level, because nutrient uptake is reduced and several enzymes are limited (e.g. Rubisco; Farooq et al., 2009).

Particularly, the accumulation of nutrients such as amino acids, sugars, nitrogen, secondary compounds and volatiles in aboveground tissue are often observed in plants under drought stress (Mattson & Haack, 1987), but the responses vary among plant species. From the perspective of an insect herbivore, all of the drought-induced changes can modify the quantity and quality of the host plants, which in turn can impact insect performance (Albanese, Vickery, & Sievert, 2008; Mattson & Haack, 1987; Sconiers & Eubanks, 2017; Thompson, 1988).

Furthermore, the severity, duration and timing of drought impact the responses (Farooq et al., 2009; Huberty & Denno, 2004; Sconiers & Eubanks, 2017). When drought is intermittent, insect herbivores are more prone to attack plants (Sconiers & Eubanks, 2017). There is evidence that drought can create pest outbreaks because water stressed plants have increased nutrient content, especially nitrogen, and reduced investment in defence compounds (i.e. secondary compounds). The nutritional changes of drought exposed plants can increase the development and survival of insect herbivores (Ayres, 1993; Huberty & Denno, 2004; Mattson & Haack, 1987). Under severe drought, however, the host plant quality is altered further and can entire desiccate the host plants. Thus, severe drought is more likely to limit both the quantity and quality of resources available for insect herbivores (Jamieson et al., 2012; Lenhart et al., 2015). However, we still know relatively little about how host plant quality altered by drought affects wild populations of insect herbivores.

### 1.4 INDIRECT EFFECTS OF DROUGHT TO HERBIVORES

Studies assessing the responses of insect herbivores to drought have mainly focused on the direct effects. For example, drought is associated with high temperatures, which may affect the development time and growth of insects directly because they are ectotherms that regulate their metabolism according to the ambient temperatures (Caillon et al., 2014; Huey et al., 2012; Mattson & Haack, 1987; Scheffers, Edwards, Diesmos, Williams, & Evans, 2014). Few studies have focused on the indirect effects, whereby drought alters the host plants and the food quality of the insect herbivores (Ayres, 1993; Bauerfeind & Fischer, 2013; Jamieson et

al., 2012; Lenhart et al., 2015). Here, I will assess how drought-induced changes in host plant quality affect the preference and performance of a specialist insect herbivore. I study these effects across different life-stages (pre-diapause, post-diapause and adult stages) of the Glanville fritillary butterfly.

### 1.4.1 HOST PLANT QUALITY AND NUTRITION

Host plants under drought stress are often nutritionally rich because they tend to concentrate nutrients (Clissold & Simpson, 2015; Jamieson et al., 2012; Mattson & Haack, 1987). This may benefit insect herbivores as many of these nutrients (e.g. nitrogen and carbon), are key components for insect development and reproduction (Awmack & Leather, 2002; Lenhart et al., 2015). The optimal concentration of nutrients for insect herbivores are, however, often species specific (Nestel et al., 2016).

If the host plant cannot provide adequate nutrients and the herbivores cannot switch host plants because they have limited mobility or non-suitable host plants are available in the vicinity, detrimental effects may become evident (Janz, 2003). Poor nutritional conditions during development in insects can reduce body size, diminish resistance to stress, and reduce the immune defence (Awmack & Leather, 2002; Boggs & Freeman, 2005; Cotter, Simpson, Raubenheimer, & Wilson, 2011; Lee, Kwon, & Roh, 2012; Nestel et al., 2016). The effects of nutrition shortage at larval stages often persist to adult stage, and can even be transmitted to the next generation through transgenerational effects (Rodrigues et al., 2015; Woestmann & Saastamoinen, 2016).

The effects of the nutritional conditions will also depend on the life-stage of the individuals due to the differential requirements. For example, the amount of lipids needed from the diet can depend on the sex and the age of individuals (Barrett, Hunt, Moore, & Moore, 2009; Simpson et al., 2015). The larval stage is the crucial phase for acquiring resources that will be allocated to growth and storage for future stages (Nestel et al., 2016; Rodrigues et al., 2015). In the adult stage, most of the resources acquired are allocated for reproduction and body maintenance (Awmack & Leather, 2002; Nestel et al., 2016). In

general, nitrogen, carbohydrates and minerals, as well as the water content available in the host plant are critical resources for insect herbivores' performance (Mattson & Haack, 1987; Scriber, 1977).

### 1.4.2 HOST PLANT QUALITY AND INSECT HERBIVORE'S BEHAVIOUR

Individuals may have the ability to modify their behaviour in order to tackle adverse conditions (Awmack & Leather, 2002; Danks, 2002; Jamieson et al., 2012). Due to the importance of accumulation of nutrients at the larval stages, it is expected that individuals will actively modify their behaviour to regulate the intake of a diet that permits them to maximize their development (Nestel et al., 2016; Scriber & Slansky, 1981). However, their capability is likely to depend on their life stage. The microclimatic and nutritional conditions experienced by early larval stages in Lepidoptera and other insects are often linked to the maternal oviposition choice because these stages are small and sessile. Hence, the conditions experienced depend on the location where the larvae hatch (Albanese et al., 2008; Rausher, 1979; Tjørnlov, Kissling, Barnagaud, Bøcher, & Høye, 2015). The older and more mobile larval stages, on the other hand, may have the ability to adjust their location by searching for other host plant individuals (Clissold & Simpson, 2015; Scriber & Slansky, 1981).

For many insect herbivores, maternal oviposition choice is an important determinant of the performance of the offspring. Choosing an appropriate habitat for offspring impacts their development, phenotype and maybe even survival, and hence have direct consequences on the mother's fitness (Jaumann & Snell-Rood, 2017; Refsnider & Janzen, 2010). This premise is known as the preference-performance hypothesis (Gripenberg, Mayhew, Parnell, & Roslin, 2010). Maternal oviposition preference can be based on many factors, such as availability of host plant, host plant quality, predation risk or microhabitat type (Janz, 2013). While choosing the microhabitat for oviposition, females can take into consideration factors such as plant size, colour and odour, but also environmental characteristics such as microclimate, terrain slope and aspect (Albanese et al., 2008; Janz, 2003; Slansky, 1982). Having habitat preference provides and maintains optimal conditions required

## BOX 1. HABITAT VARIATION AND ADAPTATION

Environments have significant spatial and temporal variation in conditions (Sultan 1995), and only limited areas within the landscape can be used by individuals. Habitat is a natural area that contains the ideal abiotic and biotic conditions for the development, reproduction and survival of individuals (Hanski, 2005; Kaweski, 2008). Microhabitats, which are small areas of the habitat, represent units with the ideal conditions and resources for living (Hanski, 2005). In many insects, the larvae are restricted to the microhabitat where they were deposited due to their sessile life style (Albanese et al., 2007). Plants are also sessile organisms and dependent of the surrounding conditions for their development (Linhart & Grant, 1996), hence microhabitats are important for these individuals to persist.

Microhabitats are not exempt from anthropogenic climate change, thus these units will present environmental changes, and organisms living within boundaries that become uninhabitable will need to disperse or adapt or they will perish (Davis et al., 2005). Genetic adaptation and phenotypic plasticity are the main mechanisms that organisms use for adjusting to environmental alterations (Chevin & Hoffman, 2017). Genetic adaptation occurs under selection, where individuals with high fitness persist and adapt to the conditions (Linhart & Grant, 1996). Phenotypic plasticity is the ability of one genotype to express a unique phenotype for each of environments encountered (Chevin & Hoffman, 2017; Sultan, 1995), and it can be adaptive if the produced phenotype can maximize individual's fitness (Sultan, 1995). In harsh environments phenotypic plasticity will help to increase the survival of animals and plants (Chevin & Hoffman, 2017).

In animals, behaviour can be adaptive and affect the fitness of the individuals (Kaweski, 2008). Habitat preference is a behavioural trait that permits individuals to select specific conditions that enhance their development and survival, and also often the performance of their offspring. Such is the case for many insects, where the earlier larval stages are sessile and their performance is dependent on the maternal oviposition site choice (Jaumann & Snell-Rood, 2017). As the larvae become bigger and often more mobile, they are able to search for the conditions that enhance their development (Jaenik & Holt, 1991). Thus, maternal oviposition choice and larval mobility are important behaviours for microhabitat selection. Moreover, microhabitats play a crucial role in the ecophysiology of ectotherms as they may buffer against extreme conditions occurring at the habitat level (Caillon et al., 2014; Scheffers et al., 2014). However, the buffering effects of microhabitats can only be achieved if a population show variation in habitat use (Derhé et al., 2010; Scheffers et al., 2014).

for the development of the offspring but other benefits include avoiding competition and predation (Refsnider & Janzen, 2010). For evolution of maternal oviposition choice to occur, it is necessary to have a genetic basis for the preference, and also a strong correlation between the maternal preference and offspring performance (Thompson, 1988). Indeed in some species of Lepidoptera, host plant preference has been shown to be highly heritable (Singer, Ng, & Thomas, 1988; Thompson & Pellmyr, 1991).

## 2 AIMS OF THE STUDY

The aim of this thesis is to give comprehensive insight into how environmental stress, studied through drought, influences plant-herbivore interactions. The Glanville fritillary (*Melitaea cinxia*) is an ideal system to study these questions as both host plant (*Plantago lanceolata*) and insect herbivore ecology is well understood. As this specialist herbivore is highly dependent on the specific host plant species, the effects of host plant quality are likely to have a

strong impact on their performance. The aim was approached by modifying precipitation regimes of the host plant in laboratory conditions (I, II), and assessing natural conditions by studying wild populations of the herbivore (III). I applied multiple approaches including experimental studies, collecting natural history data and analysing long-term ecological monitoring data. Specifically, I sought to understand how drought modifies host plant quality and how these changes impact the life-history traits and behaviour of the insect herbivore.

## 2.1 SPECIFIC AIMS

In the first chapter, I investigated how drought affects host plants, including their potential quality, by assessing different trait responses to drought. I collected seeds from natural populations and assessed traits of the host plants under two water conditions. I hypothesize that drought stress on host plants may increase concentrations of nutrients that are important for insects (Huberty & Denno, 2004; Mattson & Haack, 1987).

In the second chapter, I assessed how feeding on drought-exposed host plants modifies the life-history and behaviour of the insect herbivore. The goal was to determine if individuals showed preferences for such host plant quality that increased their performance. Specifically, I tested if postdiapause larvae preferred to feed on the host plant quality that enhanced their development and survival, and if females preferred to oviposit on host plants that increased their offspring performance. I hypothesize that larvae feeding on nutritionally rich plants will have a faster development and higher survival, and a preference towards those host plants.

Finally, the aim of the third chapter was to determine how fine-scale variation in the natural environment correlates with females' oviposition preference. I investigated the spatiotemporal variation of habitat conditions, focusing especially on the variation in abundance and drought sensitivity of the host plant over four summers (2015-2018). I used survey data (2009-2018) and further related the maternal oviposition site choice to over winter survival of the offspring. I hypothesize that maternal oviposition choice is apparent, it varies across years,

and consequently matches well with the survival of prediapause larvae.

## 3 METHODS

### 3.1 THE GLANVILLE FRITILLARY

The Glanville fritillary (*Melitaea cinxia*) reaches its northern distribution in the Åland islands in the southwest of Finland. In this islands it persists as a classical metapopulation i.e., local populations are dynamic, frequently going extinct and are recolonized through dispersal (Hanski, 1999; Nieminen, Siljander, & Hanski, 2004). The species in the islands inhabits c.a 4500 dry meadows or pastures and uses two host plants, *Plantago lanceolata* and *Veronica spicata* (Ojanen, Nieminen, Meyke, Pöyry, & Hanski, 2013). In Åland, the species is univoltine, with one generation per year (Fig. 1). Adult females emerge at the beginning of the summer and mate in July, and within the habitat boundaries, the mated females search for suitable host plants to oviposit the egg clutches. The eggs are deposited in batches of 100-200 eggs on the ventral side of the host plant leaves very close to the ground. The small and sessile larvae hatch during late summer, and these prediapause larvae develop and feed together on the host plant where their mother oviposited (Saastamoinen, van Nouhuys, Nieminen, O'Hara, & Suomi, 2007; Saastamoinen, Hirai, & van Nouhuys, 2013). The larvae move to a new host plant as a group only when they have defoliated the previous one. At the beginning of the autumn, the fifth instar larvae (sometimes as fourth instar) spin a dense silk web and overwinter gregariously the next months until next spring, when they break diapause and start searching for the fresh host plants in order to feed and develop two more instars before pupation (Kuussaari & Singer, 2017).

### 3.2 HABITAT CONDITIONS

The habitats of the butterfly in Åland are highly fragmented and vary in size and quality. The majority of the habitats are small (average = 1500 m<sup>2</sup>) and host plant abundance within the habitats is high but variable from year to year (Nieminen et al., 2004; Ojanen et al., 2013). The distribution of the two

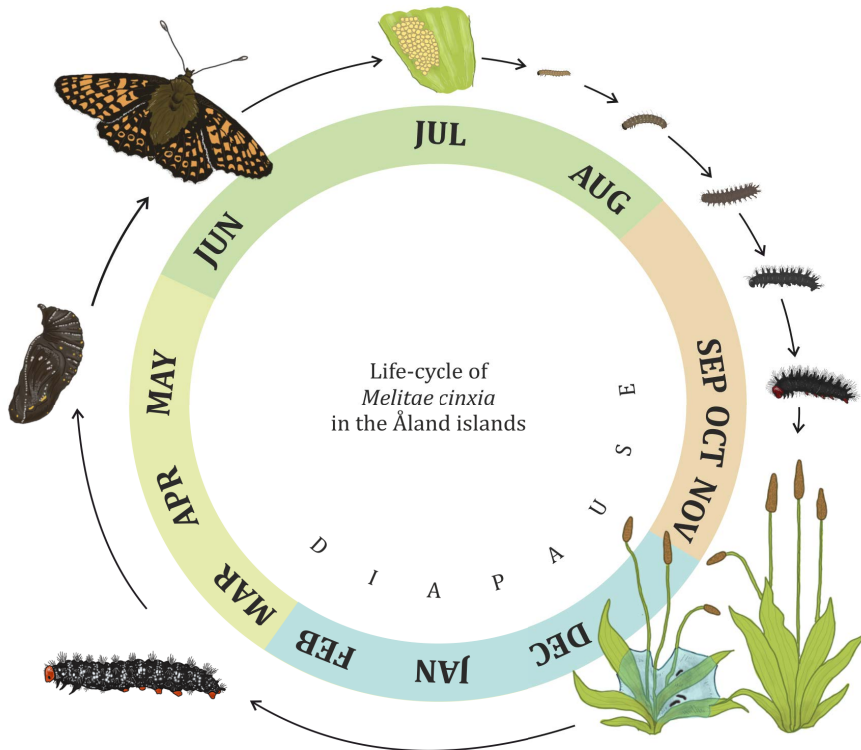


Figure 1. Life cycle of the Glanville fritillary (*Melitaea cinxia*) in the Åland Islands.  
Design: Meri Lähteenaro.

host plant species across Åland is uneven, with *P. lanceolata* being found in most of the meadows, but *V. spicata* being present only in the north-west part of the archipelago (Nieminen et al., 2004). The climatic conditions are important for the long-term population dynamics of the butterfly, as population size has been linked to the precipitation regimes experienced on the islands. The precipitation in Åland is temporal and spatially variable (Hanski et al., 2017; Kahilainen, van Nouhuys, Schulz, & Saastamoinen, 2018; Laine, 2004; Tack, Mononen, & Hanski, 2015), and together with human alterations, affects the quality of the habitats. Drought has become more frequent and severe in the region, especially during summer (Hanski & Meyke, 2005). These prolonged and intense dry summers can affect the prediapause larvae, because the small and sessile individuals are dependent on the host plant where they were oviposited by their mothers (Saastamoinen et al., 2013). The deterioration of the

host plants in drier years can put prediapause larvae at risk of starvation, which is likely to reduce their overwinter survival as smaller individuals at diapause are more likely to die (Saastamoinen et al. 2013). The entire desiccation of the host plants is likely to cause high rates of mortality of larval families, and possibly even drive local populations to extinction (Kuussari, Van Nouhuys, Hellmann, & Singer, 2004; Nieminen et al., 2004).

### 3.3 DATA COLLECTION

The data utilised for this thesis is a collection of field measurements and laboratory experiments (fig. 2). To study the direct effects of drought on the host plants in chapter I, *P. lanceolata* seeds were collected from different habitats in the field and grown under common garden conditions. Once plant shoots were

approximately 10 cm long, the plants were set to experience either drought or well-watered conditions for ten weeks ( $N = 277$ ; fig. 2A). The plant height, number of leaves, leaf length and width of the longest leaf were measured to represent investment in growth. The number of flowers and the length of inflorescences were measured as investment in reproductive traits. Additionally, the net photosynthetic rate ( $A_{net}$ ), stomatal conductance ( $g_s$ ) and instantaneous water use efficiency (IWUE) were measured as physiological traits. Carbon, nitrogen, protein, sugar and starch content were measured as nutritional traits.

The responses of the insect herbivores to drought-exposed host plants was assessed in chapter II by exposing postdiapause larvae, adults and a second generation of prediapause larvae to different host plant treatments. Following a full-factorial design the seventh instar postdiapause larvae from different lab-generated families ( $N = 13$ ) were assigned randomly



Figure 2. Laboratory and field experiments were used in this thesis. (A) *Plantago lanceolata* plants were exposed to different water treatments in the green house (I). (B) The females of the Glanville fritillary performed oviposition choice tests (II). (C) Habitat of the butterfly under an extreme drought that occurred in 2018 (III). (D) The prediapause larvae build a silk nest to overwinter and this characteristic allows the field assistants to locate and mark the coordinates of the nests (III).

to feed on drought-exposed or well-watered host plants. The larvae also underwent a food choice tests. The development time and survival of individuals until adulthood were recorded. Once adults, females were mated and set on enclosures for oviposition trials where the two types of host plants were offered (fig. 2B). The choice for oviposition was recorded and the eggs were collected and reared. The number of eggs deposited, the hatching success of the eggs, and the lifespan of the females were recorded under laboratory conditions. Once the prediapause larvae hatched, 20 larvae per clutch ( $N = 49$ ) were assigned to feed on the plants from the two host plant treatments until they reached diapause. The developmental time, weight and survival until diapause were assessed for this second generation.

The third chapter is based on intensive field surveys over 12 habitat patches from the main Åland Island collected over the summers 2015-2018 (fig. 3A). These habitats were selected using 21 years of data from the butterfly metapopulation autumn surveys (1991-2012; Ojanen et al., 2013). The habitats were filtered and selected to ensure stable local populations of the butterfly (i.e. relatively high abundance of both larval groups and host plants across years) in order to avoid the risk of extinction during the forthcoming years. Additionally, the habitats were selected according to their variability in precipitation regimes. The estimate for host plant abundance is a categorical variable from one to three obtained during the autumn surveys, where two reflects a dense group of plants that can be used by at least one larval group (Ojanen et al., 2013). The average host plant abundance of the selected habitats was 2.67. For more details in the habitat selection, please see material and methods of chapter I.

Each of the selected habitats were divided using a grid of approximately 20x20 m (fig. 3B). Furthermore, each cell within the boundaries of the habitat was characterised in detail with information about the host plant abundance (proportion of the area occupied), host plant drought sensitivity (proportion of plants presenting drought symptoms), vegetation structure and topography. This information was used to define ecological variation within each habitat and to determine the habitat preferred for oviposition (III). Additionally, ten-year data (2009-2018) of the presence of the larval nests in each cell and weather



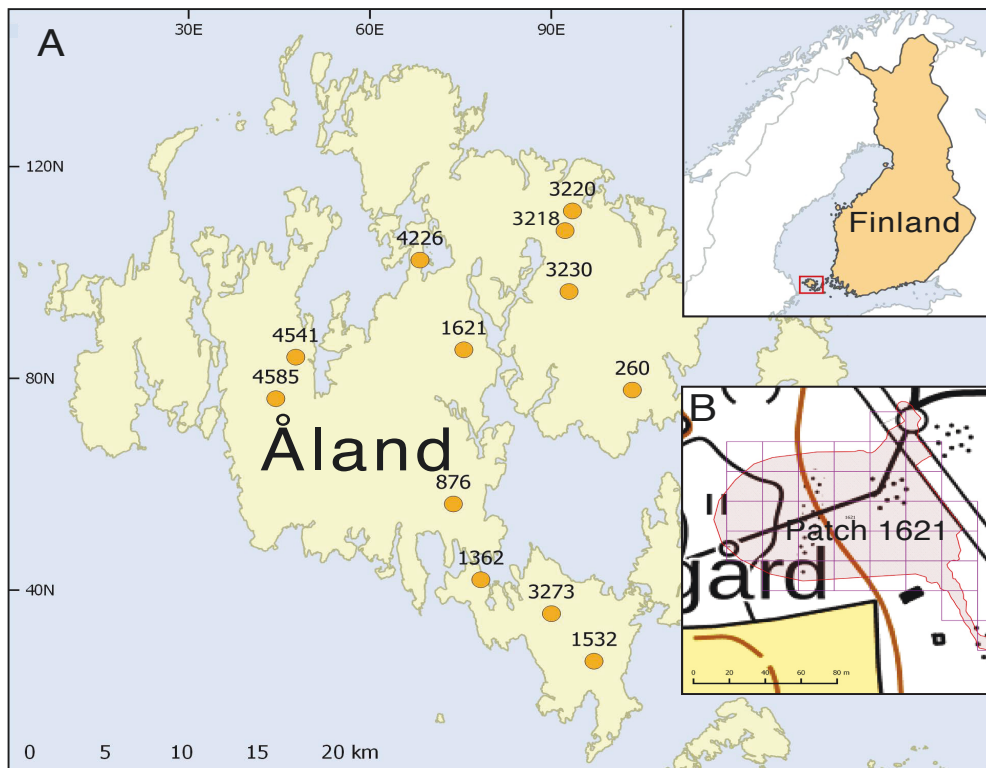


Figure 3. Map of habitat patches selected for the detailed surveys over the three summers used in chapter III (A). Each habitat patch is labelled with a patch number ID. Furthermore, each habitat patch was divided into cells with a 20x20 m grid (purple lines) to collect detailed information about the habitat conditions (B). The red line marks the habitat boundary.

conditions data (obtained from the climatology dataset from the Finnish Meteorological Institute) during early spring (i.e.: May precipitation) were used to determine whether the survival of the larval groups over winter was linked to maternal habitat oviposition preference (III).

### 3.4 DATA ANALYSES

The statistical analyses included Principal Component Analysis (PCA) for the reduction of variables, and Eigenvalues higher than one were retained for further analyses (II, III). Furthermore, generalized linear mixed models (GLMM) were constructed in each of the chapters to determine statistical significance. Dependencies in the data were accounted for by adding

random effects to the models: maternal genotype of the plants were included within habitat ID in chapter I. Family alone or the female ID within family were included in the analyses of chapter II. Cell ID nested within a habitat and within a year was used to estimate variance components (ICC values) in chapter III. Due to the non-independence of the data, a spatiotemporal random effect was added using Integrated Nested Laplace Approximation with Stochastic Partial Differential Equations (INLA-SPDE) to the models in chapter III. Model selection via Watanabe-Akaike information criterion (wAIC) was used to determine the importance of the random effects by comparing models that included the spatiotemporal, spatial and non-spatial effects (III). In a similar way, the Akaike Information Criterion (AIC) was used for model selection (II). To determine food preference, I

identified the effects of the fixed factors with statistical comparisons and a pairwise contrast to determine means differences (II). All the statistical analyses were run using R software (R Core Team, 2018).

## 4 RESULTS AND DISCUSSION

### 4.1 LIFE-HISTORY RESPONSES TO DROUGHT (I, II)

I subjected *P. lanceolata* plants to drought-exposed and well-watered conditions to examine their response to the treatment (I). The common garden experiment showed several modifications in response to drought, which were mainly related to reduced investment on morphological and physiological traits. At the morphological level, drought-exposed plants were of smaller size, had fewer leaves and invested less in reproductive traits than plants under the well-watered treatment. Furthermore, the nutrient content varied between the treatments: the drought-exposed plants had higher content of carbon (fig. 4A) and nitrogen (fig. 4B), but reduced starch (fig. 4C). Such modifications in the nutrient content are connected to the responses at the physiological level, as host plants exposed to drought reduced their net photosynthetic rate by 30% and stomatal conductance by 64%. The observed modifications (I) corresponded with those

of other studies, where the aboveground biomass of *P. lanceolata* was reduced in drought conditions (Ravenscroft, Fridley, & Grime, 2014; van Tienderen & van der Toorn, 1991). The host plants were exposed to a ten-week treatment of continuous drought stress (I), and they were able to deal with this environmental stress without dying. The severity, duration, and timing of drought stress can lead to differential responses in plants (Farooq et al., 2009). Variation in responses may also depend on the degree of drought to which the plants in the wild populations are experiencing, as responses to drought often depend on the genetic background of the plants (Farooq et al., 2009).

From the insect herbivore's perspective, the nutritional quality of the host plants was enhanced by drought as the concentration of both carbon and nitrogen was increased (I). Furthermore, plants under drought stress contained equal proportion of carbon and nitrogen (i.e. carbon-nitrogen ratio; I; fig. 5A). These results suggests that feeding on drought-exposed host plants could increase the performance of the insect herbivore (Gutbrodt, Mody, & Dorn, 2011; Nestel et al., 2016). The proportion of carbohydrates and proteins (i.e. carbon-nitrogen ratio) can strongly influence herbivores' lifetime performance (Clissold & Simpson, 2015), and host plants that have disproportionate amount of nutrients are generally considered as low quality food for insect herbivores. Other important compounds known to impact the development of the *M. cinxia* larvae are the

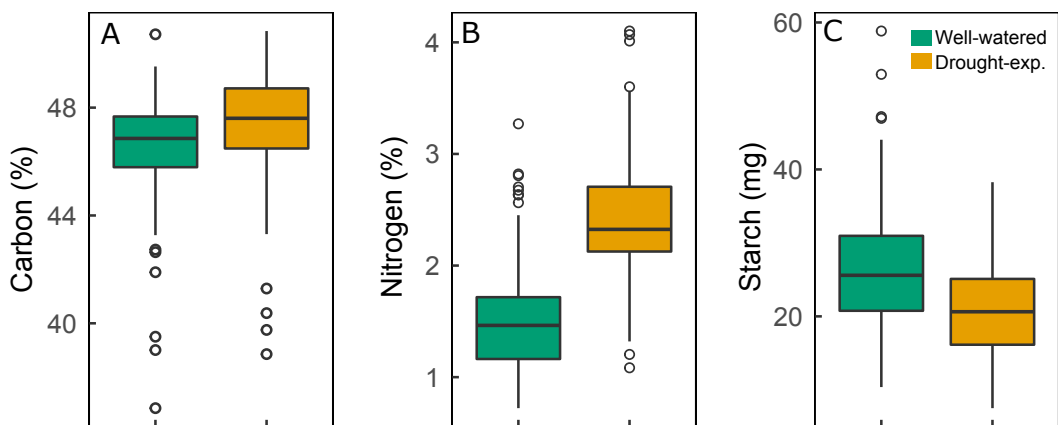


Figure 4. Carbon ( $X^2 = 14.471$ ,  $P < 0.001$ ; A) and nitrogen ( $X^2 = 245.340$ ,  $P < 0.001$ ; B) were significantly increased in drought-exposed host plants, while starch was reduced ( $X^2 = 35.986$ ,  $P < 0.001$ ; C). Box plots represent the median, 25th and 75th percentiles. The whiskers indicate the values within 1.5x inter-quartile range and the open circles represent outliers.

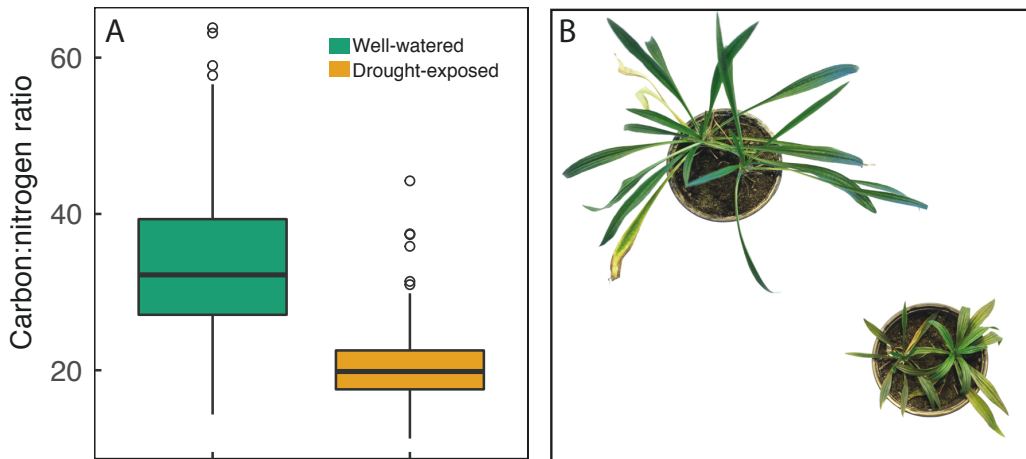


Figure 5. Phenotypes of host-plants under two water treatments. Carbon-nitrogen ratio was significantly lower in drought-exposed host plants ( $\chi^2 = 207.15$ ;  $P < 0.001$ ; A). Box plots represent the median, 25th and 75th percentiles. The whiskers indicate the values within 1.5x inter-quartile range and the open circles represent outliers. B shows the appearance of a well-watered plant on the top left and a drought-exposed host plant in the bottom right corner. Pictures: Aapo Kahilainen.

secondary compounds. Iridoid glycosides, specifically aucubin and catapol, are sequestered from the host plant and used as defence against predators in *M. cinxia* (Saastamoinen et al., 2007). These secondary compounds were not measured in any of the chapters in this thesis. However, Kahilainen et al. (in prep.) analysed the metabolite content of plants under the two water conditions, and found that aucubin and catapol are reduced in drought-exposed plants. In general, drought stress of host plants is predicted to be beneficial for the insect herbivore due to increased host plant quality (Huberty & Denno, 2004; Mattson & Haack, 1987). However, the responses may also depend on the stage of the individuals, their feeding guild and the responses of their host plant (Huberty & Denno, 2004). Consistently to this idea, I found that in *M. cinxia* the responses to drought-exposed host plants were stage-dependent (II; discussed below).

To assess the potential indirect impacts of drought on the insect herbivore, I fed pre-diapause and post-diapause larvae with drought-exposed and well-watered host plants (II). I determined the life-history responses of larvae feeding on the two types of plants, and found that the responses varied depending on the individual's life-stage (II). While drought-exposed plant diet decreased the weight and survival in pre-diapause larval stage (fig. 6A), it increased

growth rate in post-diapause larvae (fig. 6B; II). One explanation for the stage-specific responses could be that the energetic demands and nutrients required at each larval stage are different (Hahn & Denlinger, 2007; Rodrigues et al., 2015). A previous study in the Glanville fritillary has similarly showed differential stage-dependent responses but to concentrations of iridoid glycosides. In Saastamoinen et al. (2007) high levels of the secondary metabolites at first larval stage slowed the development time whereas at later larval stages increased it. It is noteworthy that the larval development is generally increased by higher levels of these secondary compounds (Saastamoinen et al., 2007). The results by Kahilainen et al. (in prep; details stated above), further indicate that the potential changes in the iridoid glycoside concentrations are not driving the observed differences in larval performance in response to drought-exposed plants (II). Another possible reason for the stage-dependent responses could be changes in the physical traits of the host plant that impact insect herbivores. Drought-exposed plants often have harder and thicker walls (Chaves, Maroco, & Pereira, 2003), which will impose difficulties to the pre-diapause larvae feeding on the nutritious drought-exposed host plants, thus reducing the performance of individuals (Massey & Hartley, 2009). This idea is supported by the results from chapter I, where I observed that host plants under drought

treatment were phenotypically different than plants under well-watered conditions (fig. 5B). However, as the leaf toughness was not assessed and a formal experimentation measuring the physical defences of the host plant are needed to confirm this idea.

The responses of the insect herbivore also depend on the strength of the drought stress imposed to the host plants, and this seems to be the case in the Glanville fritillary. Rosa et al. (2019) fed prediapause larvae on moderately drought stressed plants (compared to a more severe drought that was used in my experiments [I, II]), and found that under milder conditions the pre-diapause larvae exhibited higher growth rate when feeding on drought-exposed plants. In the experiment of Rosa et al. (2019) the nutrition content of the host plant was not assessed, however, the authors speculated that these drought-exposed plants were nutritionally rich and accessible for the larvae (Rosa, Minard, Lindholm, & Saastamoinen, 2019). This conclusion is in accordance with my results in chapter I, where the drought-exposed host plants had higher levels of compounds generally needed for a faster development in Lepidoptera (I). Thus, it is likely that the physical defence of the host plants may be a more reasonable explanation for the observed differential responses between experiments. Potential differences in the genetic background of *M. cinxia* families may also explain some of the contrary results. Kahilainen et al. (in prep.) measured development and the transcriptomics of prediapause larvae (first generation born in the lab) fed with well-watered and drought-exposed host plants, and found considerable variation in the responses among families with some families responding more positively to the drought-exposed treatment than others. The transcriptome analysis also showed that the regulation of gene expression depended mainly on the family (Kahilainen et al., in prep.). Notably, however, in my study, family ( $N = 13$ ) explained only little variation (II).

Additionally, I found that the effects of host plant quality translated to the adult stage, where females that were fed with drought-exposed host plants at postdiapause stage laid eggs with higher hatching success and had longer lifespan than females fed with well-watered host plants during their development (II). Higher hatching success can be explained by higher nutritional food at larval stages. When females are reared in poor diets they tend to

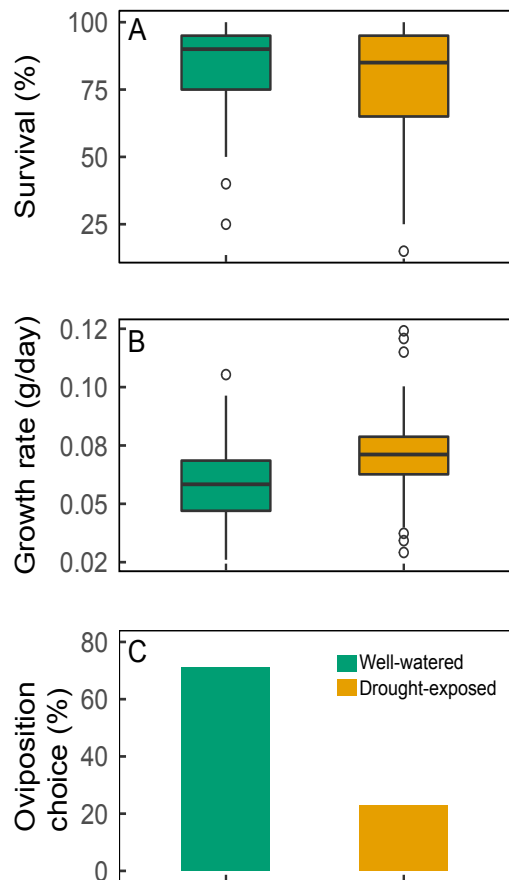


Figure 6. The survival of the prediapause larvae was significantly higher in well-watered than in drought-exposed treatment ( $\chi^2 = 4.480$ ,  $P = 0.034$ ; A). The growth rate of postdiapause larvae rate was higher when feeding drought-exposed host plants ( $\chi^2 = 29.540$ ,  $P < 0.001$ ; B). The mother's showed oviposition preference for well-watered host plants ( $\chi^2 = 24.511$ ,  $P < 0.001$ ; C). Box plots represent the median, 25th and 75th percentiles. The whiskers indicate the values within 1.5x inter-quartile range and the open circles represent outliers.

reduce their fecundity by limiting the number of eggs or by adjusting the amount of nutrients available for egg production (Awmack & Leather, 2002; Mattson, 1980). It has been shown that females can modify the amount of nutrients allocated to eggs. For example, the protein vitellogenin can be allocated according to the availability of nitrogen (Rossiter, Cox-Foster, & Briggs, 1993), which may further impact egg hatching success. The findings of longer adult life span when feeding on drought-exposed host plants during development

(II) could similarly be explained by the importance of larval nutrition in the accumulation of reserves that are used for adult stages. In similar cases it has been demonstrated that food deprivation (i.e. lack of nutrients) at larval stages has negative effects on the survival of adults (Boggs & Freeman, 2005; Swanson et al., 2016), and this has been observed also in the Glanville fritillary (Saastamoinen et al., 2013).

## 4.2 HERBIVORES' BEHAVIOURAL RESPONSES (II, III)

Individuals may also have the ability to respond to environmental change by adapting their behavioural traits, for example, by moving to sunny areas to maximize radiation for thermoregulation (Fey et al., 2019; Huey et al., 2012; Williams, Shoo, Isaac, Hoffmann, & Langham, 2008). In species with complex life cycles, such as insects, the conditions required at different life stages can differ, as well as the individual's ability to adjust. Because the quality of host plants depends on the microhabitat, insects may respond to the available quality by shifting microhabitats (Leimu,

Riipi, & Stærk, 2005). However, such behavioural adjustment are highly dependent on how mobile the individuals are in each life stage (Williams et al., 2008). I assessed whether the insect herbivores showed preferences for the host plants that enhanced their performance (II). First, I used food choice tests in postdiapause larvae to identify their food preference. I found that only the postdiapause larvae that were fed continuously with well-watered host plants (i.e. lower host plant quality) showed a behavioural preference and their preference was for drought-exposed host plants (i.e. better host plant quality). This preference increased with the increase number of trials (fig. 7; II) and further analysis revealed that the preference was significant in the last trials only (II). In any case, this preference indicates the ability of the postdiapause larvae to behaviourally adjust to the host plant quality that enhances performance (i.e. higher growth rate; II). Individuals, in general, can regulate their nutritional intake by choosing the food that provides a better nutrition (Behmer, 2009; Jones & Agrawal, 2017). The data suggests that the mobile postdiapause larvae have the capacity to search for better quality nutritional conditions but they seem to do so only when they have

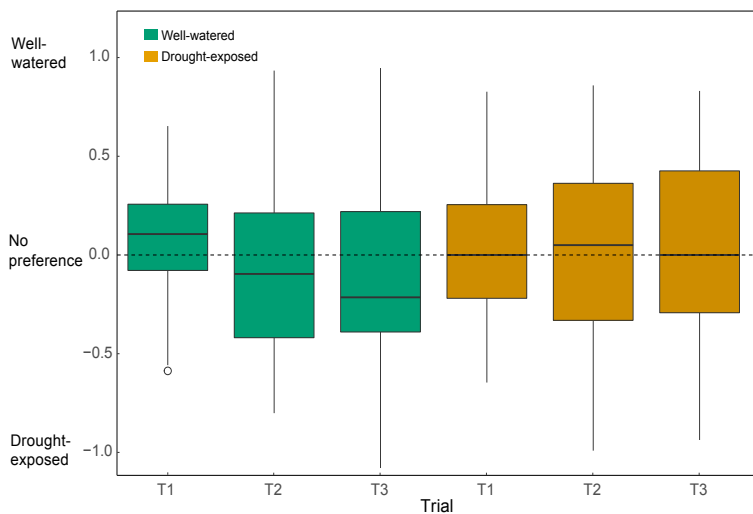


Figure 7. Food preference of postdiapause larvae fed with drought-exposed and well-watered host plants in three consecutive days (trial). The significant interaction between the host plant treatment and the trial ( $\chi^2 = 6.834$ ,  $P = 0.033$ ) indicated that only postdiapause fed-with well-watered host plants induced a preference for drought-exposed host plants as days passed. The post hoc analysis further showed that food preference was significant in the last trial only ( $t = 2.46$ ;  $P = 0.01$ ). Box plots represent the median, 25th and 75th percentiles. The whiskers indicate the values within 1.5x inter-quartile range and the outlier is represented with the open circle.

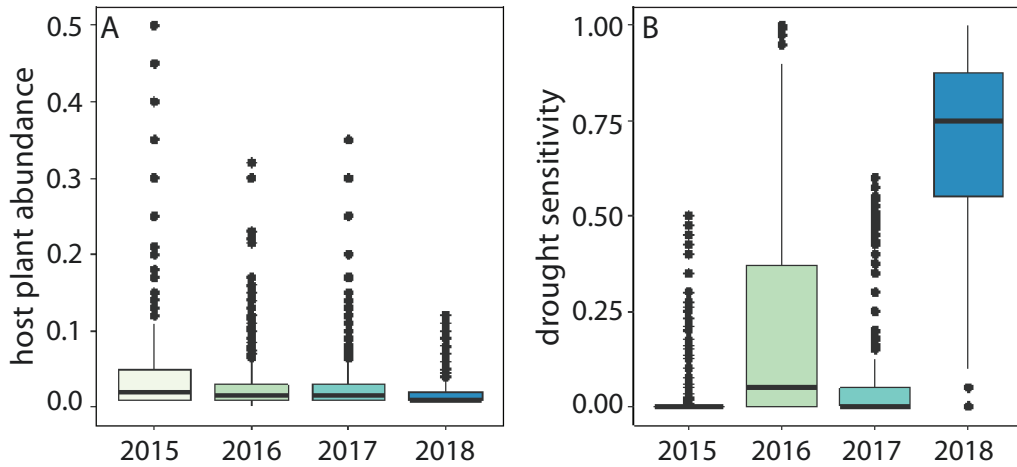


Figure 8. Temporal variation of host plant abundance and drought sensitivity over four summers (2015-2018; III). The host plant abundance was calculated as the proportion of the area covered with the host plant (A) and the drought sensitivity was estimated as the proportion of plants that presented symptoms of drought (i.e.: leaves were yellow or brown, crispy and curled; B). Larger values represent higher abundance and drier habitats, respectively for host plant abundance and drought sensitivity.

been exposed to a poorer diet. As the performance of the more sessile prediapause larvae depends on the maternal oviposition choice (Gripenberg et al., 2010), I tested the maternal oviposition choice and the performance of the pre-diapause larvae in the lab (II). The results showed that the females tended to oviposit on the host plant type (i.e. well-watered plants) that enhanced the performance of their prediapause larvae (fig. 6C). This suggests that maternal oviposition preferences is correlated with the performance of the young offspring (Gripenberg et al., 2010).

In addition, I wanted to investigate if the observed correlation between host plant preference and larval performance occurred under more natural circumstances (III). To test this, I identified the habitat conditions used for oviposition in the field and investigated the influence of maternal oviposition choice on the survival of prediapause larvae (III). I first quantified the spatial and temporal microhabitat variation in the metapopulation of the Glanville fritillary (III). The results showed that there was little temporal variation in host plant abundance (proportion of the area occupied) among years ( $ICC = 0.01$ ; fig. 8A), and that most of the variation occurred within patches ( $ICC = 0.33$ ). Additionally, the drought sensitivity (proportion of plants presenting drought symptoms)

of host plants showed greater variation between years ( $ICC = 0.64$ ; fig. 8B), drawing that the habitats are mainly variable in space on host plant quantity within habitat patches, and highly spatiotemporal variable in the host plant quality. Microhabitats can alleviate the effects of extreme conditions as they create optimal conditions for the species to respond, for example, ectotherms need this environmental variation for thermoregulation (Caillon et al., 2014; Latimer & Zuckerberg, 2019; Scheffers et al., 2014).

I then identified the habitat conditions linked to habitat oviposition preferences using the long-term data and found that nest presences within habitat patches were positively correlated with the proportion of host plant abundance and drought sensitivity (III). Meanwhile, nest probability was negatively correlated with both PC1 (transformed field aspect and the slope corrected transformed aspect) and PC2 (soil depth and canopy coverage; fig. 9; III). This means that females preferred to oviposit in microhabitats with higher proportions of host plants and that were more likely to be dry, and preferred south-facing slopes and open microhabitats (III). The results are in hand with other studies showing the larval groups are distributed in warmer and more open areas with high quality microhabitats and abundant host plants, which tend

increase growth rate of the offspring faster (Curtis & Isaac, 2015; Janz, 2003).

Additionally, the model showed an interaction between drought sensitivity and May precipitation, with a stronger positive correlation between nest presence and drought sensitivity in the driest summers (fig. 10; III). The observed response can likely be attributed to low variability in the drought sensitivity of the host plants in wetter years (fig. 8B; III), and not to a behavioural adjustment in the maternal habitat oviposition preference. May precipitation can alter the host plant quantity and quality available for the prediapause during the next months of summer. Previous research on the metapopulation dynamic of the Glanville fritillary in Åland, found that population growth rates were positively correlated with May precipitation (Kahilainen et al., 2018). The authors further found that an increase in synchrony of climatic conditions in the islands has escalated the risk of extinction by increasing synchrony of colonization/ extinction events across habitat patches (Kahilainen et al., 2018). During my study, the islands experienced extreme high temperatures and low precipitation in May 2018, which had significant consequence for the metapopulation as only 91 larval nests were found in over 3000 meadows surveyed during the autumn (van Bergen et al., in prep.). Based on the long term data

the average number of nests found per year is 2750 (van Bergen et al., in prep). Of these 91 nests, only two larval nests were found within the 12 habitat patches used for this study, and those nests were located in the microhabitat with the lowest average drought sensitivity (III). Moreover, precipitation for 2018 was the lowest ever recorded for the region, leading to a reduction in vegetation productivity, which included a reduction in the host plant quantity and quality available for the prediapause larvae (van Bergen et al., in prep.). Even though behaviour has the potential to buffer the effects of weather conditions, this is only possible if the females have the ability to vary their maternal oviposition preference (Derhé, Moss, Edwards, Carmenta, & Hassall, 2010; Fey et al., 2019; Scheffers et al., 2014). However, we observed no shift in female oviposition site choice preference in 2018 when the environmental conditions were extremely dry.

Finally, I found that in average years the probability of overwinter survival was positively correlated to the drought sensitivity of the microhabitat, but unrelated to host plant abundance and other microclimatic variables (III). The appearance only of drought sensitivity in the model reaffirms the importance of host plant quality, induced by drought, for the survival of the insect herbivore. This variable

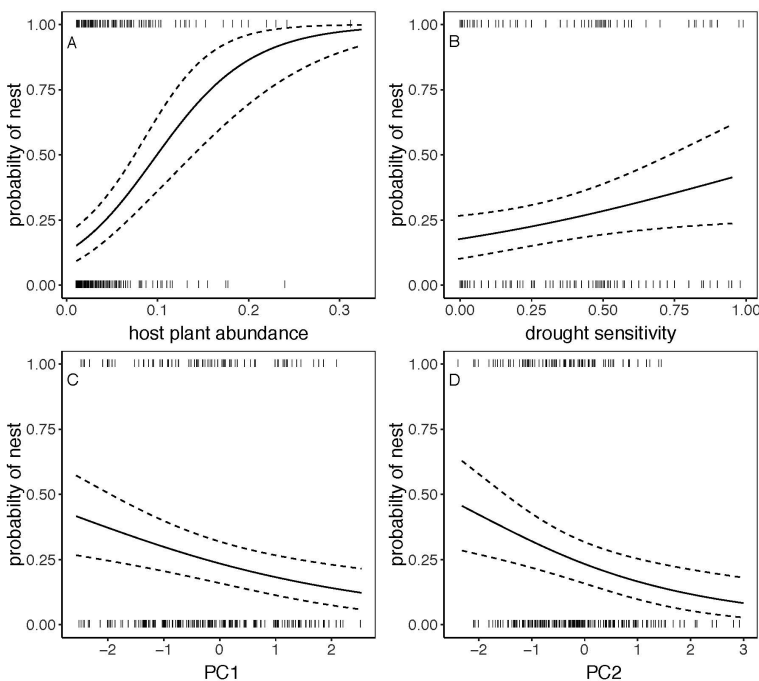


Figure 9. Probability of nest presence in relation to host plant abundance, drought sensitivity, (transformed field aspect and the slope corrected transformed aspect) and PC2 (soil depth and canopy coverage). Predictions are represented in solid lines and the 95% confidence interval in dashed lines. Vertical lines in zero and one represent present or absent observations, respectively.

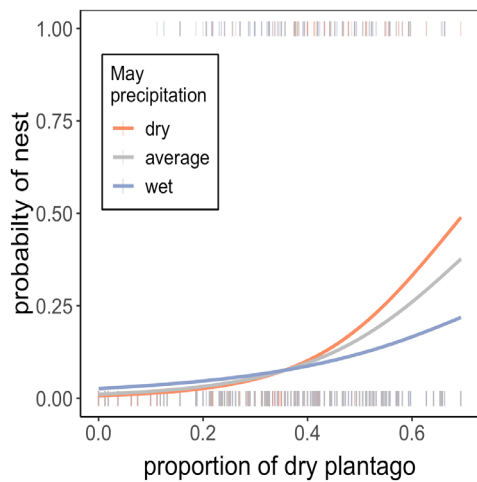


Figure 10. Nest presence according to the interaction between drought sensitivity and May precipitation. The solid lines are the predictions for the interaction between drought sensitivity and May precipitation. May precipitation is represented for dry (precipitation values below one standard deviation of the mean), average (values within one standard deviation of the mean) and wet (values above one standard deviation from the mean) years. The vertical bars at one and zero represent presence and absence of nests, respectively.

reflects that the microhabitats of the prediapause larvae have the adequate conditions for a greater performance, and may contribute to the survival of the larvae during the low temperatures. However, other related life-history traits are also likely influencing overwinter survival. For example, silk nest density have been demonstrated to correlated positively with overwinter survival, and smaller group size can increase mortality during diapause (Duploux, Minard, Lähteenaro, Rytteri, & Saastamoinen, 2018; Kuussaari & Singer, 2017). Lastly, there are other microhabitat conditions that could impact the overwinter survival that were not measured in the present study, such as the amount of snow (Bale & Hayward, 2010; Marshall & Sinclair, 2012).

Both lab (I, II) and natural experiments (III) suggested that drought influences the plant-herbivore interaction. However, several new questions also arose, as some of the results were not consistent. The first one is why do females show an opposite oviposition preference under lab and natural conditions? It is

possible that the cues females use between these studies differ. Under controlled (i.e.: lab) conditions the females are using only host plant phenotype as a cue for oviposition, whereas in the field there are several conditions that affect the oviposition preference of the females. Studies in other Lepidoptera have shown that habitat conditions are taken into account before selecting the actual host plant individual for oviposition (Friberg, Olofsson, Berger, Karlsson, & Wiklund, 2008; Friberg & Wiklund, 2019). In these studies, host plant preference found under laboratory conditions played a secondary role under field conditions (Friberg et al., 2008; Friberg & Wiklund, 2019). The priority of habitat choice over host plant is common in ovipositing insect herbivores (Friberg et al., 2008). For the Glanville fritillary, drier microhabitats in the field allow faster development of the prediapause larvae (Curtis & Isaac, 2015), and faster development might drive the decision for oviposition due to the short time frame of summer in this region. It is also possible of course that the females in the two studies showed variability in oviposition preference due to variation in their condition or genetic background (Davis & Stamps, 2004; Kemp, 2019; Thompson, 1988; Via 1986; Singer & Moore 1991). Furthermore, the females used for the oviposition choice test were lab generated, and the previous generations were reared under controlled conditions. Thus, the females in this experiment (II) could have a preference for well-watered host plants. Meanwhile, the females from the field (III) were naturally selected for the conditions such that females prefer to oviposit in drier microhabitats that enhance a faster development of the prediapause larvae.

The second question that arose from my work is, why did prediapause larvae survive better on well-watered host plants in the lab, but had higher survival on microhabitats with higher drought sensitivity in the field? Some of the potential reasons have already been discussed above. The discrepancy may also be explained by the lack of other multitrophic interactions and other abiotic conditions (e.g. ambient moisture) in the lab that can impact prediapause survival in the wild. Competition, predators, parasitoids, and other biotic factors play an essential role in the decision-making of females; hence, their preference will be to lay eggs in conditions that enhance total fitness (i.e., the sum of different life-history traits; Janz, 2003). Moreover, it is important to keep in mind that in the field the



quality of each individual host plant was not assessed and instead the drought sensitivity was assessed across several host plant individuals at the microhabitat level. Furthermore, survival was measured differently in the two experiments. In the lab, the survival until diapause was assessed at individual level (II), whereas in the field I assessed the overwinter survival for the entire larval nest (III). Both reflect performance but at a different level and at different time point.

Finally, why were females not able to adjust their oviposition preference in a year with extreme drought, especially as in the lab they clearly avoid laying eggs on drought exposed plants? This lack of ability to shift oviposition preference in 2018 resulted in a huge population decline, as only two larval nests were alive in the 12 studied habitat patches in autumn (III). The constraint hypothesis may help to explain this result, which states that rapid environmental change or physiological constraints do not always permit an optimal oviposition choice (Janz, 2003). Microhabitat conditions in 2018 may have changed too rapidly and unexpectedly, preventing females to adjust to these extreme conditions. The question about the limited ability of females to adjust their oviposition preference goes in hand with the question of whether females were able to predict the environmental conditions their offspring would be exposed to. It has been suggested that if environments are too variable and unpredictable, the best strategy for females is to spread their offspring across the available microhabitats (i.e.: bet hedging; Hopper, 1999; Wiklund & Friberg, 2009). Such strategy did not seem apparent in this study, at least not within the habitat patches assessed (III), and these further studies are required.

## 5 CONCLUSION AND PROSPECTS

The goal of my thesis was to understand how plant-herbivore interactions may be potentially altered under future changing climates. Specifically, I investigated how individuals cope with direct and indirect effects of drought by studying responses of both the host plant and insect herbivore feeding on the host plants in both the lab and field conditions.

Plants under drought stress tend to accumulate certain nutritional compounds known to be important

for insect herbivore performance (Mattson & Haack, 1987), as was indicated with the results in the lab experiments where the amount of carbon and nitrogen were enriched by drought (I). Thus, I conclude that the drought-exposed plants have a greater nutritional quality for the insect herbivores than plants under well-watered conditions. More generally, the responses in the plants to drought-stress involved various changes at the physiological, morphological or nutritional level (I). When feeding different life stages of the butterfly on drought-exposed host plants, I found the responses to be stage-dependent (II): drought-exposed host plants enhanced the survival of the postdiapause larvae but reduced it at prediapause stage. These results emphasize the importance of assessing the effects of environmental conditions in different life stages. The Glanville fritillary, both in postdiapause and adult stage, can adjust their behaviour under lab conditions and choose the host plant that benefits performance (II). The postdiapause larvae do it by preferring to feed on drought-exposed plants, while females enhance the survival of their prediapause larvae by preferring to oviposit on well-watered host plants (II). When observing the conditions in natural habitats, host plant quantity and quality explained the maternal oviposition site preference (i.e. the presence of nests within a habitat; III). This observation confirms the relevance of host plant quality, or at least drier microhabitats, to insect herbivore performance. Additionally, my results show the influence of drought to host plant quality (III), highlighting the importance of abiotic factors for habitat selection. Ultimately, insect population persistence depends of the ability of individuals to track and adjust to the environmental changes (Clissold & Simpson, 2015; Williams et al., 2008).

One of the main contributions of this thesis is the incorporation of the indirect effects of weather events in understanding how biotic interactions will be modified in the future - a topic that requires increased attention as the severity and intensity of unusual weather events are becoming more frequent. The results here emphasize that the northern butterfly metapopulation is relatively well adapted to cope with variation in drought conditions. The insect herbivore often benefits and even chooses to feed on drought exposed hosts plants (II, III), and that the drought-exposed host plants provide better nutrients for larval development that even translate to adult performance

(II). However, the results also show that the insect herbivore may face difficulties to deal with potential climatic changes that make conditions even drier, and that this may first impact the more sensitive and sessile pre-diapause stages. Under extremely dry conditions, the host plants desiccate and cannot support the developing larvae, yet females still continued choosing these highly drought sensitive environments (III). In the driest and hottest year studied, the females were not able to adjust their oviposition preference which resulted in high mortality in the larval nests (III). Here, I highlight the importance of behavioural adjustment to habitat preference for the long-term persistence of the species. The butterfly populations' resilience may depend on the females' ability to adjust the habitat choice to fit the changes in the future climatic conditions (Derhé et al., 2010).

Furthermore, studying the impacts of drought under both lab and field conditions, highlight the importance to integrate different approaches in ecological research to help to disentangle the effects of correlated variables. Assessing responses under laboratory conditions allowed me to focus on the main abiotic factor of interest, drought, while controlling for the rest of the conditions (I, II). The field conditions, on the other hand, allowed me to integrate more variable environments and add more ecological relevance to the study (III). However, in my thesis I did not implement field information about the post-diapause larvae and its microhabitat, as these data are not available. In addition, my focus was solely on *P. lanceolata* and not the other host plant, *V. spicata*. This alternative host plant can be crucial for understanding impacts of drought on the herbivore performance in nature, as the host plants are likely to have very different strategies to resist drought. *Plantago lanceolata* seems more vulnerable to desiccation but recovers fast after rain, whereas *V. spicata* is probably more resistant to drought but recovers slowly (personal observation). Additionally, it would have been interesting to study whether drought produces similar or different responses in *V. spicata* as in *P. lanceolata*, and how feeding on drought-exposed *V. spicata* plants influences larval performance.

This thesis brings new insight in the area of ecology and behaviour, and identifies new questions to investigate. My suggested directions of research include identification of the nutritional quality of host

plants in natural conditions. Studying the variability in the nutritional quality available for the insect herbivore in the landscape may help to make more accurate predictions of the effects of future climate change on the long term persistence of the butterfly metapopulation. My thesis shows that such studies should take into account the different requirements of different life stages of the insect herbivore (II) and the genetic variability in the responses of both host plant (I) and herbivore (Kahilainen et al., in prep.) to environmental conditions. An additional direction could be incorporating molecular techniques to find specific genes that regulate the observed behavioural traits. For example, there is potential to determine if the oviposition preference of the Glanville fritillary is genetically variable as a full genome has been sequenced (Ahola et al., 2014). However, molecular techniques are difficult to include in the domain of behavioural ecology, because behavioural traits are mainly linked with transcriptions, epigenetic modifications, ontogeny and other physiological processes, and are modified according to the environment (Bengston et al., 2018). Nonetheless, progress in this area is continuously being made (e.g. Breed & Moore, 2015; Sokolowski, 2001). Fluctuating environments and the difficulty in predicting climatic conditions impose a challenge in science to address questions in natural populations as well. Plant-herbivore interactions bring the opportunity to understand life-history responses and identify the risks ecosystems are facing under anthropogenic climate change.

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