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Nectar resource limitation in agricultural landscapes: effects on behaviour and life-history traits in the meadow brown butterfly (*Maniola jurtina*)

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Summary

Diverse animal diets are associated with different constraints, and ultimately different life styles in different environments. In holometabolous insects, which are usually characterised by vastly different diets in the larval and adult stages, growth and development are accomplished at the larval stage, but adult food sources may make significant contributions to the energy and nutrient budget to survive and to reproduce. In nectar-feeding insects like butterflies, the quantity and quality of adult food resources have been shown to impact life-history traits. In productive, intensive agricultural landscapes, floral resource limitation has been suggested to cause strong declines in populations of flower-visiting insects including pollinators. Flower rarefication (both in abundance and diversity) is only one of the modifications induced by agriculture intensification. Other factors include habitat fragmentation, landscape simplification, and general and functional biodiversity loss.

This PhD thesis aims at evaluating the impact of a reduction in nectar quality, quantity and both combined on life-history traits and adult behaviour of a grassland butterfly in intensive, nectar-poor *versus* extensive, nectar-rich agricultural landscapes. The central study species used as a model for hypotheses testing was the Meadow brown, *Maniola jurtina*.

Butterflies are often considered opportunistic nectar consumers that visit a wide variety of flower species. However, butterflies may face changes in floral diversity and abundance. This may, in turn, have consequences for their adult behaviour and the use of nectar. We identified different foraging patterns of *M. jurtina* populations and individuals living in either nectar-rich grasslands and in flower-poor hay meadows. We identified clear preference

for knapweed *Centaurea jacea* and thistles (*Cirsium* sp.) in nectar-rich, extensively managed grasslands. In absence of the preferred flower species (i.e. in nectar-poor sites), red clover *Trifolium pratense* is visited, amongst other present species.

Having identified nectar use by butterflies in meadows under different management intensity, we showed the consequences of floral nectar limitation and use in each meadow type on the performance of *M. jurtina*. In outdoor flight cages, we simulated the typical floral nectar supply of nectar-poor, intensively managed meadows and nectar-rich, extensively managed meadows. A 48-h stay of young adult butterflies under nectar-poor conditions had a strong negative effect on body condition, flight activity and lifetime survival compared to butterflies under nectar-rich conditions. Butterflies could not mitigate this effect by compensatory feeding on diluted honey after the experiment, suggesting a key role of specific nectar components (like amino acids). Agricultural landscapes that provide limited amounts of floral nectar, and no high-quality, preferred nectar sources relative to the needs of the flower-visiting species may create ecological sinks. From an insect performance viewpoint, the simple presence of nectar is not necessarily functionally adequate.

Next, we investigated the effects of variation in nectar quality, quantity, and both combined, on life-history traits, energy metabolism and flight performance of *Maniola jurtina*. Testing individuals from intensive and extensive agricultural landscapes suggested adaptations to altered resource availability relative to agriculture intensification. We simulated the nectar offer of intensive agriculture land and of flower-rich, semi-natural landscape, along with intermediate treatments, and placed wild-caught young butterflies for two days in one of the treatments. Effects on life-history traits depended on the origin of the butterflies: all females performed compensatory feeding after the experiment and had similar

values of longevity and fecundity, except for those from intensive landscapes, which, when both nectar quality and quantity were reduced, lived half as long as the others and laid less than a fifth of the number of eggs laid by the females in other treatments. Females from intensively managed agricultural landscapes had reduced Resting Metabolic Rate (RMR) and stronger flight capacities, which were conserved under all nectar treatments, and at the expense of lifespan and fecundity under food stress. Females from extensively managed landscapes were more plastic in their metabolic rates and flight capacity responses to variation in the adult diet. The results suggest that butterflies in intensively managed landscapes have altered flight capacities, physiological pathways and life-history trade-offs as a response to strong selection regimes in anthropogenic environments such as intensively managed agricultural landscapes. Our results stress the importance of the impact of reduced nectar sources in intensive agricultural landscapes and underline the consequences for population viability and dynamics under dramatic nectar limitation in simplified agricultural landscapes.

Sparing zones from mowing has been proposed to improve local conditions for survival and reproduction of insects in hay meadows. We studied population densities of butterflies before and after mowing in the refuge zone of 15 meadows, and the behavioural consequences for *M. jurtina*. Densities of grassland butterflies in this zone doubled on average after mowing. In line with the idea of increased scramble competition in the refuge zone after mowing, *M. jurtina* increased the time spent on nectar feeding, the preferred nectar source was visited more frequently, and females made more use of non-preferred nectar sources. The resulting concentration effect altered the time allocated to different activities, nectar use and movements. These aspects raise questions about optimal quantities and quality of uncut refuge sites for efficient conservation of grassland arthropods in agricultural landscapes.

This PhD thesis contributes to our understanding of the importance of nectar feeding in adult butterflies, and of the significance of nectar source limitation for flower-visiting insect population viability in changing anthropogenic landscapes.

Résumé

Dans le règne animal, la variété des régimes alimentaires créée des styles de vie différents correspondants à des environnements et des régimes alimentaires différents. Chez les insectes holométaboles, le régime alimentaire change en général du tout au tout entre les stades larvaires et adulte. La croissance et la majorité du développement sont effectuées aux stades larvaires, mais les ressources alimentaires des adultes peuvent contribuer significativement aux besoins énergétiques et en nutriments nécessaires à la reproduction et à la survie. Chez les insectes nectarivores comme les papillons, la quantité et la qualité de la nourriture jouent un rôle majeur pour les traits d'histoire de vie. Dans les paysages agricoles intensifs, la disparition des ressources florales, en termes de diversité et d'abondance, est souvent pointée du doigt comme étant une des causes du déclin des pollinisateurs, mais fait en réalité partie d'un ensemble de modifications engendrées par l'intensification de l'agriculture, avec par exemple la fragmentation des habitats, la simplification du paysage et un déclin de la biodiversité générale et fonctionnelle.

Cette thèse de doctorat vise à évaluer l'impact d'une réduction de la qualité et/ou de la quantité du nectar, de manière similaire à celle rencontrée en milieu agricole, sur les traits d'histoire de vie des papillons, en utilisant le myrtil *Maniola jurtina* comme espèce modèle.

Les papillons sont souvent considérés comme étant des opportunistes en ce qui concerne le butinage. Cependant, un changement dans l'abondance et la diversité des ressources florales disponibles peut engendrer des modifications dans leur comportement de butinage. Nous avons étudié les différences dans le comportement de butinage de *Maniola jurtina* dans des prairies semi-naturelles, riches en fleurs, et dans des prairies de fauche gérées de manière intensive, offrant peu de nectar. Nous avons clairement

mis en évidence une préférence pour la centaurée *Centaure jacea* et les chardons (*Cirsium* sp.) dans les prés fleuris. En l'absence de leur espèce préférée (dans les prés intensifs), les papillons se nourrissent entre autres sur le trèfle *Trifolium pratense*.

Nous avons ensuite utilisé les deux espèces nectarifères identifiées comme constituant la majeure partie du régime de *Maniola jurtina* dans chacun des types de prairies (*C. jacea* et *T. pratense*), pour étudier les conséquences en termes de traits d'histoire de vie d'un régime nectarifère correspondant à chacun des types de prairies. Le régime nectarifère similaire à celui des prés intensifs a fortement réduit la longévité des papillons et leur état physiologique, comparé au régime nectarifère des prés fleuris, et ce après seulement deux jours dans ces conditions. Etant donné que les papillons n'ont pas été capables de compenser après le traitement en se nourrissant d'eau sucrée, nous suggérons que certains nutriments essentiels ont dû être limitants dans le traitement offrant peu de nectar. Cette première expérience nous permet d'affirmer que le nectar est effectivement une ressource essentielle pour les papillons dans les paysages agricoles, et que la qualité et la quantité de nectar doivent être préservées pour favoriser le maintien des populations de pollinisateurs.

Ensuite, nous avons examiné les effets d'une réduction de la qualité et de la quantité de nectar séparément, et de manière conjointe. Nous avons réalisé cette expérience chez des papillons provenant d'un paysage agricole géré de manière extensive, ainsi que chez des papillons provenant d'un milieu agricole intensif pour tester les adaptations des seconds à une disponibilité réduite des ressources. Pour cela, des jeunes papillons capturés sur le terrain ont été placés pendant deux jours dans des conditions simulant soit l'offre en nectar des prés fleuris, soit celle des prés intensifs, soit un des intermédiaires en termes de quantité ou qualité de nectar. La réponse des papillons en termes de traits d'histoire de vie

dépendait de leur origine. Seules les femelles provenant du paysage agricole intensif soumises à une réduction simultanée de la quantité et de la qualité du nectar ont été affaiblies: leur fécondité et leur longévité ont été fortement réduites. Les femelles des paysages agricoles intensifs avaient un taux métabolique plus faible et des capacités de vol plus fortes, qui sont de plus maintenues même dans des conditions de stress alimentaire importantes, alors au détriment de la longévité et de la fécondité. Les papillons provenant du milieu agricole extensif ont répondu avec plus de plasticité aux différents régimes nectarifères. Leur stratégie consistait en un ralentissement du métabolisme et un changement de comportement (pas de volonté de voler) lorsque les conditions n'étaient pas optimales, afin de préserver la longévité et la survie. Nos résultats suggèrent une forte pression de sélection dans les paysages agricoles intensifs sur les papillons, qui y répondent en adaptant leur capacité de vol, leur physiologie et leurs stratégies de compromis d'allocation des ressources entre les différents traits d'histoire de vie. Malgré ces adaptations, les conséquences d'une offre en nectar limitée restent dramatiques et peuvent avoir de sérieux impacts sur la viabilité des populations en milieu agricole intensif.

Pour améliorer localement les conditions environnementales dans les prés de fauche pour la survie et la reproduction des insectes, épargner une zone de la prairie lors de la fauche est une méthode suggérée, et utilisée. Nous avons étudié la densité en papillons dans ces zones non-fauchées, avant et après la fauche, dans un ensemble de 15 prairies, ainsi que les conséquences en termes de comportement pour *M. jurtina*. La densité des papillons des prés y est en moyenne doublée après la fauche. La compétition pour *C. jacea*, l'espèce nectarifère préférée de *M. jurtina*, est plus forte, et les papillons y passent en conséquence plus de temps à se nourrir, visitant même plus souvent qu'avant la fauche d'autres espèces nectarifères de second choix. Ces résultats indiquent que l'abondance et la

diversité des espèces florales doivent être prises en compte dans la planification des mesures de conservation de la nature en paysage agricole.

Globalement, cette thèse de doctorat contribue à la compréhension de l'importance du nectar pour les papillons, et son rôle clé dans la conservation des espèces dans les paysages modifiés par l'homme.

Chapter 1 - General introduction

Food and energy requirements in animals

Food provides the organism with the essential energy and building blocks for growth and development, survival, reproduction and all other activities. It is fundamental for three main animal needs: i) it provides energy for cellular respiration; ii) it brings organic raw materials used to synthetize biomolecules, and iii) it supplies essential nutrients which cannot be synthetized by the animal (Reece et al., 2011). Energy is obtained through oxidation of macromolecules in the cells, mainly carbohydrates, lipids, or proteins when other molecules become rare (Munro, 1951). It is fundamental for all activities, but also for maintaining basic functioning of an organism (resting metabolism, or basic metabolism for vertebrates) (Sinclair et al., 2011). As heterotrophs, animals are not able to synthetize organic molecules from inorganic materials. Molecules needed for growth and development, and for tissue replacement, are manufactured from organic fragments (mainly organic carbon and organic nitrogen) obtained from food. Finally, the essential chemical substances that cannot be bio-synthetized by animals are called essential nutrients. They vary interspecifically with the ability of the species to synthetize molecules. Four main classes of essential nutrients are generally recognized: essential amino acids, essential fatty acids, vitamins and minerals (Reece et al., 2011). But water must also be recognised as a vital component of nectar to its consumers.

Animals vary in their diet from carnivores eating only other animals to herbivores eating only primary producers (i.e. plants), and omnivores that combine both food types. Mechanisms of food acquisition are also diversified. Most animals ingest pieces of food of variable size and use more or less specialised body parts (e.g. specialised mouthparts in insects;

McGavin, 2001) to manipulate and take up the food. Many species or groups of species evolved feeding specialization and appropriate feeding apparatus. Butterfly larvae for examples have mandibles that allow chewing plant materials. Adult butterflies have mouthparts that are transformed into a proboscis allowing suction of fluids, and in several cases they are no longer capable of ingesting solid food (Krenn, 2010). *Heliconius* butterflies do, however, ingest pollen, which is a rich source of amino acids, and allow them to reach extended adult longevity (e.g. up to 6 months); however there is no indication of adapted mouthparts compared to other butterflies (Krenn and Penz, 1998).

Food and energy in butterflies

Holometabolous insects change the way of acquiring food, and hence their diet type, during their life. They go through four developmental stages to accomplish their life-cycle: the egg (or embryo), the larva, the pupa and the adult (or imago). Most of the growth and development are completed at the larval stages, whereas reproduction happens at the adult stage. Food acquisition and ingestion may occur both at the larval and adult stage. Some insect species only feed at the larval stages, they are referred to as strict capital breeders (Jönsson, 1997). Species relying on adult feeding for reproduction are referred to as income breeders, but most species do not rely entirely on adult feeding for reproduction, and are placed along the continuum from strict capital breeders to income breeders (Bonnet et al., 1998).

In this PhD-thesis, we will focus on one butterfly species, the meadow brown butterfly (*Maniola jurtina*) as a study model in this context. In most species of the Lepidoptera, both larvae and adults are herbivores, but the mechanisms of food acquisition change completely throughout the lifecycle, hence resulting in completely different diets. Larvae feed on host plant and ingest plant fragments, whereas adult butterflies feed on nectar,

on nectar and pollen, or on other food sources such as dung, honeydew or rotten fruits (Erhardt and Mevi-Schütz, 2009), which makes them not always strict herbivores.

Larvae possess chewing mouthparts and their food is constituted of protein-rich plant foliage (Geister et al., 2008; Roeder and Behmer, 2014; Telang, 2001). Proteins and carbohydrates are two important macronutrients that influence animal survival, growth and reproduction (Karasov and Martinèz del Rio, 2007). They provide essential amino acids and energy, respectively. For most herbivores, feeding on a specific protein/carbohydrate ratio (often expressed as its reverse: C/N ratio) results in optimal performance (Behmer, 2009; Raubenheimer and Simpson, 2003). Plants materials can be highly variable with respect to the C/N ratio (Behmer et al., 2002; Bernays and Chapman, 1994; Schoonhoven et al., 2005). To get food at their optimal C/N ratio, many species either feed on a range of plants or plant species (generalists) (Chambers et al., 1996; Singer et al., 2002) or alternatively, feed on different parts (or vegetative tissues) of a single plant (expected for specialists) (Thornber et al., 2006). With the ingested food, larvae grow and store energy and essential nutrients. Energy is essentially stored in the form of lipids, and essential amino acids are stored in hexamerins (proteins) in the haemolymph and fat body (Pan and Telfer, 2001). In the wild, host plants may be limited in quantity (e.g. by drought, overgrazing, competition, soil type and soil conditions, pH), in quality (changes in C/N ratio and other micronutrient concentrations in response to fertilizers) (Cahenzli and Erhardt, 2013a; Joern et al., 2012), or access to high-quality food may be limited by high levels of predation (Hawlena and Schmitz, 2010) or parasitism (Choutt et al., 2011).

A reduction in the quantity of larval food at the very end of larval development resulted in reduced adult body mass and forewing length

reduction in *Speyeria mormonia* (Boggs and Freeman, 2005), in lower pupal mass (Bauerfeind et al., 2009), longer development time, reduced growth rate, adult body size and fecundity in *Bicyclus anynana* (Bauerfeind and Fischer, 2005). Effects of changes in larval food quality are various and depend on the species and on the type of quality change (Awmack and Leather, 2002). Nitrogen content of the larval host plant, for example, can vary a lot, especially with high levels of fertilisation and atmospheric-nitrogen input. Generally, larvae fed with fertilized host plants have higher growth rates than conspecifics on unfertilized host plants, and decreased developmental time. However, high levels of nitrogen content may become detrimental, as it reduced pupal survival and adult size in *Lycaeana tityrus* for example (Fischer and Fiedler, 2000). Many butterfly species are, however, able of compensatory feeding in response to reduction in their host plant quality or food quantity. At the larval stage, they may eat more of the host plant (Cahenzli and Erhardt, 2012a; Lavoie and Oberhauser, 2004). Alternatively, individuals may eat more, or differently by changing feeding behaviour (in case of a generalist species) at the adult stage (Mevi-Schütz and Erhardt, 2005).

In Lepidoptera, adult food diet varies considerably among species (Erhardt and Mevi-Schütz, 2009). Food is ingested by suction through their specialised feeding organ, the proboscis (Krenn et al., 2001). In Europe, the majority of species are strict nectar feeders at the adult stage.

Besides water, sugars are the main constituents of nectar (Nicolson and Thornburg, 2007). Fructose, glucose (both hexoses, monosaccharides) and sucrose (disaccharide) are the three main sugars in nectar, and the ratio sucrose / (fructose + glucose), which is relatively constant within a species (Rusterholz and Erhardt, 1998; Torres and Galetto, 2002), is related to the pollinator type (Baker and Baker, 1990, 1983; Freeman et al., 1991). Other sugars can be present in trace amounts (Baker and Baker, 1983). Xylose has

been identified as an important nectar sugar in some species of the *Proteaceae* family, although it is not preferred by the majority of flower visitors and its presence is more likely to be due to plant physiology than to pollinator-plant coevolution, insects are averted from xylose and not able to metabolize it. (Jackson and Nicolson, 2002). Amino acids are universally present in nectar in concentrations of up to 4 mg ml^{-1} (Baker, 1977). The amino acid assemblage and their relative proportions is usually constant at the intraspecific level (Baker and Baker, 1977; Gardener and Gillman, 2002), and is likely to provide a distinct taste for each nectar (Baker, 1977) since some insect chemoreceptors react differently to similar groups of amino acids (Shiraishi and Kuwabara, 1970). Plant species with nectar that is more concentrated in amino acids are pollinated by organisms that do not have alternative sources of proteins or amino acids, such as settling moths, butterflies and many wasps (Baker and Baker, 1986; Baker, 1977). Lipids and antioxidants are present in lower concentrations, in a minority of nectars, but mainly in tropical areas, as well as alkaloids, phenolic substances and glycosides, which are frequently considered as chemical defences, deterring nectar feeders (Kessler and Baldwin, 2007), or potentially toxic substances (Baker, 1977; Elliott et al., 2008). More generally, nectar composition is believed to have coevolved with pollinator groups (Baker and Baker, 1975). Some minor nectar constituents are known to attract only a limited number of flower visitors, which are the pollinators (Kessler and Baldwin, 2007), or to deter flower visitors but the pollinators (Elliott et al., 2008). Nectar quantity per flower and/or inflorescence and nectar quality in terms of sugars are generally higher in perennial plant species than in annual species (Petanidou, 2007). As a consequence, perennial species are more attractive to flower-visiting insects, at least in some ecosystems (Petanidou, 2007).

Water is a required component both at the larval (Tabashnik, 1982) and adult stage (e.g. (Murphy et al., 1983). Plant leaves (and other vegetal

parts), which are ingested by butterfly larvae, vary significantly with respect to their water content (Gardner and Ehlig, 1965). Development on drought stressed host plants generally reduces fitness in butterflies (Gibbs et al., 2012; Vande Velde et al., 2012). Adult butterflies acquire water through nectar ingestion (Nicolson and Thornburg, 2007). Adult butterflies may also puddle at mud or other wet soil to complement their water needs, although it is believed that puddling behaviour is mainly used to supplement nitrogen or sodium needs (Molleman, 2010).

Adult butterflies allocate their resources to reproduction, maintenance, survival or further foraging (Boggs, 1992). When resources are limited, trade-offs are made in the allocation of resources to each activity type and associated life-history traits (Boggs, 1992). For example, in the butterfly *Speyeria mormonia* under dietary restriction, lifespan can be maintained at the expense of fecundity (Boggs and Ross, 1993), or flight capacities may be preserved at the cost of fecundity (Niitepõld et al., 2014). In other cases, all traits may be negatively affected by low nectar quality and/or quantity (e.g. Murphy et al., 1983).

Flight is highly energy demanding (Suarez, 2000) and is involved in many activities (e.g. escape from predator attacks, searching for mates in males and for oviposition sites in females, etc.) and may, hence, related to several other life-history traits.

In several laboratory studies, quantitative and qualitative nectar restrictions were shown to impact life-history traits of adult butterflies. However, physiological aspects such as the impact on the energy metabolism, have rarely been investigated (but see Niitepõld et al., 2014). Some butterfly species are known to lower their Resting Metabolic Rate (used as a measure of maintenance cost) in response to food stress (Niitepõld et al., 2014) and, consequently, to preserve allocation levels to other traits.

Moreover, the physiological cost of flight is often underestimated as, in many studies, butterflies were kept in small cages throughout the experiment (e.g. Boggs and Ross, 1993; J. Mevi-Schütz and Erhardt, 2003). Yet flight is highly energy demanding (Suarez, 2000) and represents a large fraction of butterfly activities (Cormont et al., 2010).

Insects may use different energy sources to fuel flight (Beenakkers et al., 1984), from carbohydrates (Suarez et al., 2005) to lipids (van Marrewijk et al., 1980) or even amino acids (especially proline) (Scaraffia and Wells, 2003; Weeda et al., 1980). In butterflies, carbohydrates are used in the first place to fuel flight, but generally they are quickly consumed and sustained flight is then fueled by lipids (Beenakkers et al., 1984).

Costs of reproduction vary between males and females as females have to manufacture the eggs whereas males produce a spermatophore transmitted to the female during copulation. Butterfly eggs are mainly composed of water (*c.* 80% in mass), proteins and lipids (Karl et al., 2007). Hence, females use, and hence need, all these compounds for successful egg-laying. During copulation, male butterflies transfer a spermatophore to the female, which contains sperm but also nutrients. More particularly, the spermatophore contains lipids (Marshall and McNeil, 1989), proteins (Boggs and Gilbert, 1979) and sugars (Watanabe and Sato, 1993). Hence, the production of spermatophore bears an energetic cost as well (Watanabe and Hirota, 1999).

Nitrogen is often thought to be the limiting element for adult butterflies, in particular for reproduction (O'Brien et al., 2002). This is especially the case for egg manufacturing in females, since nitrogen in contrast to carbon, is not present in high amounts in adult food (Morehouse and Rutowski, 2010). Several nectar-feeding butterfly species are known to hydrolyse their flight muscles located in the thorax (Karlsson, 1994; Stjernholm et al., 2005) in

order to reallocate proteins to reproductive investment (Stjernholm and Karlsson, 2006). Because Lepidoptera acquire most of their proteins at the larval stage, it was long believed that larval nutrition only determined reproductive potential and the level of adult somatic maintenance (Boggs, 1981; Jervis and Boggs, 2005; Mevi-Schutz and Erhardt, 2005; O'Brien et al., 2002). However, it became clear that quantity and quality of adult butterfly diet also affect reproduction and lifespan in many species, independently or not of larval food quality and quantity (e.g. Murphy et al., 1983).

Restriction of nectar quantity reduced fecundity in females of *Speyeria mormonia* and *Colias eurytheme* (Boggs and Ross, 1993; Niitepõld et al., 2014), but not lifespan. Egg energy provisioning decreased with age only in food limited females of *Bicyclus anynana* (Karl et al., 2007). In several butterfly species, females increasingly used carbon from adult diet for egg production (O'Brien et al., 2004). Nectar quality also affects butterfly life-history traits. When fed with nectar of high sugar concentration, males of *Coenonympha pamphilus* (Cahenzli and Erhardt, 2012b) and females of several *Mycalesis* species (Braby and Jones, 1995) lived longer, and in some species both longevity and fecundity increased (Hill, 1989; Murphy et al., 1983). Nectar amino acids also influence butterfly fitness (Jervis and Boggs, 2005). Nectar amino acids increased fecundity in *Arashnia levana* (Mevi-Schütz and Erhardt, 2005), but only for individuals that were raised as larvae on nitrogen-poor host plants. In *C. pamphilus*, addition of amino acids in nectar mimics enhanced reproductive performances in females (Cahenzli and Erhardt, 2012c) and males (Cahenzli and Erhardt, 2013b). Amino acids were used to enhance offspring quality (heavier larvae, increased hatching success) and offspring quantity only by females that were raised on nitrogen-poor host plants (Cahenzli and Erhardt, 2012c). Amino acids used by adult butterflies are thought to represent compensatory feeding mechanisms across developmental stages to respond to larval food stress. In other species, addition of amino acids in

the diet had no effect, but larvae were all grown under the same conditions and tests for compensatory feeding mechanisms were not possible (e.g., *Maniola jurtina*: Grill et al., 2013; *B. anynana:* Molleman et al., 2008). Effects may impact egg size (e.g. *E. editha*; Murphy et al., 1983), but larger eggs are not systematically more advantageous. For example, smaller eggs were shown to have higher energy density in *B. anynana* (Karl et al., 2007)

It has been proposed that some nectar-feeding butterfly species may increase their amino acid intake with diluted pollen in the nectar (Baker and Baker, 1973; Erhardt and Baker, 1990), but this depends on flower morphology. Some species have been observed puddling, i.e. ingesting water together with micro-nutrients from mud or puddles (Molleman, 2010), most probably to complement their nutritional needs.

Foraging behaviour, i.e. the decisions made by an organism as to which resources to exploit will determine resource intake, and life-history patterns depend on reallocation of resources (either stored from the larval stages, or acquired by adult feeding) to fitness-related activities (Boggs, 1992). Although some species have been recognized as nectar specialists (Tudor et al., 2004), several butterflies feed on a variety of flower species (Grundel et al., 2000; Sharp et al., 1974). Some species feed mainly on a single nectar species, but may change nectar source, for example in the absence of their main source. *Boloria eunomia*, for example, mainly feeds on *Polygonum bistorta*, but has been reported to feed on other nectar sources as well (Goffart and Waeyenbergh, 1994).The vast majority of butterfly species feed, however, on several nectar sources, and show some level of preference for colour (Goulson and Wright, 1995; Ômura and Honda, 2005; Pohl et al., 2011), nectar constituents (Erhardt, 1992; Jovanne Mevi-Schütz and Erhardt, 2003) or floral scent (Andersson et al., 2002; Ômura et al., 1999). Depending on the local abundance of floral resources,

they may change their foraging behaviour, for example by visiting other flower species, or a wider variety of species (e.g. Goulson et al., 1997).

Although many studies reported on the effect of modification in the quantity or quality of adult diet in butterflies, few studies have linked variation in foraging behaviour and available nectar sources to fitness consequences (but see Mevi-Schütz and Erhardt, 2003, 2005).

Intensively managed agricultural landscapes and the challenges they represent for flower-visiting insects

Biodiversity currently faces rates of extinction of populations and species that are much faster than expected background rates (De Vos et al., 2014; Pimm et al., 2014). The main reason for this biodiversity crisis is the growing human population, and the associated environmental impact (United Nations, Department of Economics and Social Affairs, 2013). Intensive agriculture is a major driver of biodiversity loss, through the multiple changes imposed on natural ecosystems. In western Europe, agriculture is the dominant land use (Robinson and Sutherland, 2002; Stoate et al., 2009). Agricultural techniques introduced during the last century have brought faster changes to the agricultural landscapes than ever before (Stoate et al., 2001; Tscharntke et al., 2005). Agricultural intensification occurs at two spatial scales: the local, field scale and the landscape scale.

At the local scale, increasing use of inputs, technical progress in agricultural engines, competitive and productive crop selection are the three main causes of intensification. Use of fertilizers has largely increased from the 1960s (Tilman et al., 2001), causing losses of biodiversity and changes in species compositions in terrestrial ecosystems, and soil acidification (Sutton et al., 2014; Vitousek et al., 1997). Pesticides are, by definition, made to kill one or several organisms considered as pests for agriculture. The most direct effect of herbicides for flower-visiting insects is the loss of (key)

resources that make up their functional habitat, through disappearance of nectar and host plant species (McLaughlin and Mineau, 1995). Pesticides are also known to detrimentally affect non-target organisms, depending on their mode of action (review: Sánchez-bayo (2012)), and may for example accumulate in nectar (Barker et al., 1980). Field size has increased over time (Tscharntke et al., 2005), but agricultural management actions also have more impact, perturbing the environment and consequently the co-occurring wild organisms. Tilling, for example, renders soil much more susceptible to erosion and affects the levels of organic carbon and nitrogen in the top layer of the soil (McLaughlin and Mineau, 1995). Semi-natural grasslands were traditionally used for grazing and hay making, but most of them have been transformed into intensively used grasslands and fields (Cizek et al., 2012). Modern hay meadows are usually fertilized, sown with highly productive grasses, and mown several times a year (Humbert et al., 2010). In the short term and at the local spatial scale, mowing may have a strongly perturbing effect on wild organisms like flower-visiting insects since it removes all vegetation, and hence most resources from the local meadows (Dover et al., 2010). Modern pastures are often under high grazing pressure, causing (amongst other factors) reduced plant biodiversity, soil compaction and fertilization (Stoate et al., 2001).

At the landscape scale as well, many changes have occurred (reviewed in Tscharntke et al., 2005). First, conversion of (semi)-natural ecosystems into agricultural land always happens at the cost of (semi-)natural habitat loss (Tilman et al., 2001). Hence, (semi-)natural biotopes either remain as very small scattered fragments in the agricultural landscapes, or they totally disappear in so-called cleared agricultural landscapes. This has already caused several population extinctions of species confined to those biotopes (Robinson and Sutherland, 2002). Increasing field sizes by assembling different plots by suppressing field boundaries or removing hedgerows and by specialization to one or a few arable crops decreases

landscape diversity and has led to strong landscape homogenization and habitat fragmentation (Tscharntke et al., 2005). Indirectly, management practices, landscape homogenization, loss of biotopes, and also pesticide and fertilizer use may change microclimatic conditions (Holland and Luff, 2000; Matlack, 1993). Populations in intensive agricultural landscapes have generally lower densities because of reduced habitat quality (Butler et al., 2007), and increased fragmentation (Krauss et al., 2003). Reduced population density and reduced diversity may lead to cascade effects on similar or higher trophic levels in the local or regional food webs (e.g. May et al., 1981).

The link between intensive agriculture and biodiversity loss has been addressed in many studies, covering several taxa (Benton et al., 2003; Butler et al., 2009, 2007). Plant diversity and abundance are declining in agricultural landscapes (McCollin et al., 2000), for reasons already put forward in the previous part of this introduction. Declines of birds have also been extensively studied. Farmland bird species are declining across the whole of Europe, and there is a significant negative correlation between national trends of all farmland bird species and indices of national agricultural intensity (Donald et al., 2006). Many farmland birds nest on the ground and are strongly affected by modification of sward structure and by intensive exploitation of meadows or fields (Vickery et al., 2001). Obviously, bird species nesting in trees and shrubs are negatively affected by the disappearance of landscape elements providing nesting sites. Food availability is also recognized as a consequence of agriculture intensification that directly impacts farmland bird populations, either carnivorous (Butet et al., 2010), herbivorous (Smart et al., 2000) and seed-eating birds (Robinson and Sutherland, 2002). Invertebrates are also known to widely decline in agricultural landscapes (Potts et al., 2010; Schweiger et al., 2005), and butterflies are no exception (Van Dyck et al., 2009).

Consequently, many studies have focused on biodiversity in agricultural landscapes, exploring links between landscape elements or management practices with abundance and diversity of organisms. Butterflies are often used as model organisms in that framework, because they are easy to work with, there is good to excellent knowledge on their ecology, and field techniques for the study of butterflies are well developed, though often labour-intensive (Thomas, 2005). Moreover, butterflies are declining in agricultural landscapes (van Swaay et al., 2006; Van Swaay et al., 2013), and this decline has been demonstrated even for common and widespread species (Van Dyck et al., 2009). Many studies relate butterfly communities with flower abundance and diversity (e.g. Aviron et al., 2011; Blake et al., 2010; Croxton et al., 2005; Dover, 1996; Feber et al., 1996; Feest et al., 2014; Haaland and Bersier, 2011; Pywell et al., 2004; Saarinen et al., 2005; Sparks and Parish, 1995). This suggests that nectar source rarefication in agricultural landscape is one of the causes leading to butterfly declines (Wallisdevries et al., 2012). Although patterns of butterfly abundance and diversity have often been related to flower diversity and abundance, mechanisms linking the two have rarely been tested explicitly. For nectar rarefication to play a role in butterfly declines (in abundance and diversity), one must link foraging behaviour in intensively managed farmland to negative fitness consequences compared to the situation in extensively managed semi-natural ecosystems, and their ultimate impact on population viability.

But flower source rarefication is only one of the changes due to agriculture intensification. Habitat fragmentation and isolation, with low inter-patch connectivity, reduces inter-population flows and may for example also contribute to population extinctions (Baguette and Van Dyck, 2007).

The degree of ecological specialisation of a species will influence its ability to adapt to rapidly changing environments, with specialists being more

vulnerable than generalists given their limited variety of (potential) resources. In a Japanese study on butterfly communities along a gradient of human disturbance, the number of specialist species decreased with increasing human disturbance (Kitahara et al., 2000). Species mobility (*i.e*. dispersal propensity and flight capacity) is also an important factor relative to understanding the responses of species to changing environments, although mobility (including dispersal) may be subject to selection (Mcpeek and Holt, 1992). In the U.K., over a 30 years period of increased habitat degradation, particularly the more mobile generalist species succeeded in colonizing newly created habitats at their northern distribution area thanks to climate change (Warren et al., 2001). Ecological specialization and dispersal are thought to be part of a trade-off (Jocque et al., 2010) although it is debated (Stevens et al., 2012).

Species able to maintain viable populations are expected to respond to the environmental changes induced by agriculture intensification through phenotypic adjustments (Ghalambor et al., 2007; Reznick and Ghalambor, 2001) in order to avoid local extinction, unless conservation programs can provide complementary resources to buffer negative impacts. Phenotypic adjustments may originate from genetic adaptations or from phenotypic plasticity (Hendry et al., 2008).

Phenotypic plasticity is present when a genotype produces different phenotypes in response to different environmental conditions (West-Eberhard, 2003), and is graphically represented with reaction norms (trait values as a function of the environment). Plasticity can be adaptive or not; adaptive plasticity results in a phenotype that is in the same direction as the optimal value favoured by selection when facing environmental changes whereas non-adaptive plasticity is often due to an environmental stress imposed on organisms and results in phenotypes that have a lower fitness value (Ghalambor et al., 2007). Adaptive plasticity is the quickest way for

phenotypic adjustment to fast environmental change (Ghalambor et al., 2007). The ability to respond plastically can be constrained and costly as well, as environmental cues guiding phenotypic responses might be unreliable and the maintenance of machinery to detect these cues may be costly for the organism (DeWitt et al., 1998). Studies of plasticity in butterflies are numerous (e.g. Merckx and Van Dyck, 2006; Pijpe et al., 2008; Serruys and Van Dyck, 2014; Steigenga and Fischer, 2009). For example, in a study on the speckled wood butterfly *Pararge aegeria*, Sibly et al. (1997) showed major among-population differences in reaction norms for body size and developmental period in responses to developmental temperature. More recently, developmental plasticity found in *Aglais urticae* in response to microclimatic and host plant quality changes was understood as a mechanism to allow this butterfly to be successful in agricultural landscapes (Merckx et al., 2015).

Genetic adaptations are possible if enough genetic variation is available within the population for the traits under selection (Hoffmann and Willi, 2008). Rapid evolution is now widely recognized as a mechanism for organisms to respond to environmental changes on time scales overlapping with ecological time scales (Carroll et al., 2007). A well-known example of rapid evolution refers to pesticides resistance in insects as an adaptation to the strong selection pressure imposed by the pesticides (Mallet, 1989). Aphids, for example, have been shown to develop pesticide resistance on the short term (e.g. Feng and Isman, 1995; Kerns and Gaylor, 1992).

In the host-specialist butterfly *Euphydryas editha*, two populations evolved in different directions with respect to host plant species, in response to human land-use (logging and cattle ranching), and in one case, it was based on genetic changes, indicating rapid evolution (Singer et al., 1993). In *Pararge aegeria*, butterflies from agricultural landscapes have evolved

different aspects of flight behaviour than their woodland conspecifics (Merckx et al., 2003).

Chapter 2 - Thesis objectives and thesis outline

Butterfly and pollinator declines in agricultural landscapes have been partly attributed to the increasing scarcity of nectar resources (Biesmeijer et al., 2006; Wallisdevries et al., 2012). Many studies correlated butterfly abundance and diversity with nectar resources, amongst other factors (Feber et al., 1996; Haaland and Bersier, 2011; Marshall and Moonen, 2002; Sparks and Parish, 1995), but experimental, causal evidence of nectar-related consequences are still rare. In this framework, the main objective of my PhD thesis was to evaluate ultimate consequences of variation in nectar availability on butterfly life-history traits (Figure 2.1) as experienced by butterflies in agricultural landscapes. To reach this objective, we used an approach that combined field observations and experiments on the one hand, and laboratory experiments in semi-controlled conditions on the other.

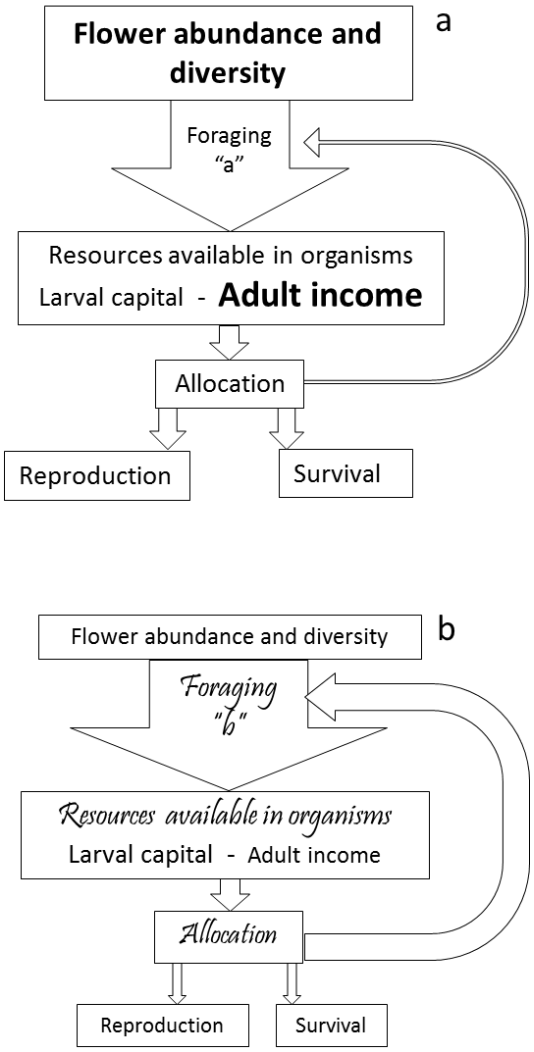

Figure 2.1: Interconnections between foraging and life history for a nectar feeding butterfly in a) extensively managed agricultural landscape, and b) intensively managed agricultural landscapes. Adapted from Boggs (1992). Smaller characters represent smaller quantities or values. Changes in chemical composition or processes are represented by different font characters. Arrow size represents costs of different allocation pathways in terms of resource use. Changes in floral resource availability from one landscape to the other are expected to change foraging patterns and associated costs. Consequently, the total pool of resources (carbohydrates, lipids, proteins, essential nutrients, water) available to, and finally in, the organism will vary and hence affect allocation trade-offs between reproduction, survival or further foraging.

Both approaches were needed as they are strongly complementary. Field observations and experiments were used to identify the typical nectar offer in meadows under different management intensity and to link them to patterns of foraging behaviour. Laboratory experiments were conducted in order to evaluate life-history consequences of the observed foraging behaviours. We chose as a model species the meadow brown butterfly, *Maniola jurtina*, because of the following reasons:

i) It is a widespread grassland species that is able to maintain populations in relatively intensively managed agricultural landscape (Van Swaay et al., 2013).

ii) It is a strict nectar feeder and adults feed on a variety of flower species (Brakefield, 1982a).

iii) Larvae feed on a variety of grasses, hence host plants are not likely to be a limiting resource.

iv) The species is considered as moderately mobile (Schneider et al., 2003). Hence we may assume that distances of several tens of kilometres may affect populations structure, whereas it is clearly able to move between grassland habitat remnants in intensively managed agricultural landscape (Delattre et al., 2013).

More precisely, we pursued the realisation of 5 goals that are developed in the different chapters of this thesis:

1) Describe patterns of nectar use by butterflies in flower-rich, extensively managed hay meadows on the one hand, and in flower-poor, relatively intensively managed hay meadows, on the other. We also confronted the assumption that our studied species is a generalist nectar feeder (Brakefield, 1982a; Dennis, 2004) by testing for flower preference as opposed to opportunistic foraging behaviour (Grundel et al., 2000; Sharp et al., 1974). These questions were addressed by means of field observations and experiments in grasslands submitted to different management intensities (**Chapter 4)**.
2) Assess life-history consequences (**Chapters 5 and 6**) and effects on energy metabolism and flight capacity (**Chapter 7**) of suboptimal nectar foraging, as experienced in intensively managed hay meadows, on butterflies. We expected suboptimal nectar offer to negatively affect butterfly life-history traits, as in other species (Boggs and Ross, 1993; Niitepõld et al., 2014). As other species are known to maintain flight capacity under quantitative dietary restriction, whereas slowing their resting metabolic rate (Niitepõld et al., 2014; Roark and Bjorndal, 2009), we expected the same in *M. jurtina*. This question was addressed by testing wild-caught butterflies in large outdoor flight cages in which we simulated different levels of nectar quality and quantity. We used real flowers that were preferred by *M. jurtina* in field situations as nectar sources to be as close as possible to the nectar offer under field conditions (i.e. combining simultaneous effects of nectar quantity

and quality (Baker and Baker, 1975)). Outdoor flight cages allowed flight behaviours and interindividual interactions.

3) Disentangle the effects (life-history traits, energy metabolism and flight capacities) of nectar quality form those of nectar quantity. This issue is developed in **Chapters 6** and **7**. We addressed this question by using 4 different nectar tretments in outdoor flight cages following the same semi-controlled experimental setup as for the previous objective. We used two flower species often visited in the field to vary nectar quality: *Centaurea jacea* is preferred over *Trifolium pratense*. The first one is the most often visited flower species in extensively managed hay meadows, whereas the second one is one of the most often visited species in intensively managed hay meadows. We varied nectar quantity by using different numbers of flower heads in the flight cages.
4) Test for adaptive differences between butterflies originating from populations of intensively managed agricultural landscape and from populations of extensively managed agricultural landscape in their response to low nectar availability (in quantity, quality or both). This was addressed by comparing life-history trait (**Chapter 6**), energy metabolism and flight capacity (**Chapter 7**) consequences of living under the different nectar treatments already explained above. We expected butterflies from the intensively managed agricultural area to be less impacted assuming they are adapted to low nectar resource levels (**Chapter 6**). Grassy habitat remnants in agricultural landscapes (field boundaries, road verges, hedgerows, meadows) (Dover, 1996) are generally less distant than average mobility of species as *M. jurtina*, we predict populations of our

study species originating from intensive agriculture conditions to be more mobile, and to show better flight capacities (**Chapter 7**).

5) In **Chapter 8**, we tested the effects of a sudden reduction of nectar resources for grassland butterflies, in hay meadows where a refuge zone was left uncut after mowing. We expected increased population density in uncut zone after mowing as it has already been shown for orthopterans (Humbert et al., 2012). Higher population density might increase competition for nectar (Schultz and Dlugosch, 1999), predation rates (e.g. Sang and Teder 2011) and male harassment for females (e.g. Odendaal et al., 1989). We predicted an increased competition for the preferred nectar source in the refuge zone after mowing, and accordingly, changes in foraging behaviour of grassland butterflies.

Chapter 3 - Materials and methods

Study species

The meadow brown, *Maniola jurtina* L., is a butterfly from the Nymphalidae family belonging to the subfamily of the Satyrinae. The species occurs all over Europe, from Siberia to North-West Africa and the Canary Islands, and it can be found up to 2500m in altitude (Tolman and Lewington, 1999).

Maniola jurtina is a medium-sized butterfly. It has a proboscis of approximately 10 mm long and an average wing loading of 0.67 N m^{-2} (SEM: 0.054, on 10 individuals; Corbet, 2000). Female forewing length is approximately 26 mm; males are smaller (forewing length: 24 mm) and much lighter (female mean fresh weight around 100 mg, male fresh weight: around 55 mg). Sexual dimorphism is also present in wing colour patterns (Figure 3.1). On the dorsal surface of the forewing, both sexes possess an orange area (smaller in males) with a black-and-white eyespot that may be doubled in females. The ventral surface of the forewing is mainly orange, and possesses a black eyespot centred in white, which is larger in females.

Figure 3.1: Wing patterns of females (top) and males (bottom) of *M. jurtina*, on the dorsal (left) and ventral (right) surface. Pictures: J. Lebeau.

The ventral surface of the hindwing, light brown in females and grey-brown in males, shows a postdiscal band of variable width, which is more pronounced in females. Variable numbers of small black spots are present in the postdiscal band. These spots and their varying patterns and numbers have been studied extensively (Dowdeswell, 1961; Handford, 1973; Brakefield and van Noordwijk, 1985).

Maniola jurtina lives in open habitats and has a relatively large ecological niche and therefore can be found in a wide variety of open environments such as grasslands, glades, along hedgerows or pathways, or even on road verges and road intersections (Valtonen and Saarinen, 2005). This widespread butterfly is one of the most abundant species found in the agricultural landscapes of western Europe, and certainly in Belgium, both in Wallonia (Fichefet et al., 2008) and in Flanders, although a little less in this

northern part of Belgium (Maes and Van Dyck, 2001). Despite its abundance and large distribution area, recent data in the framework of the European grassland butterfly index suggest a decline at the European scale (Van Swaay et al., 2013). In some intensively cultivated landscapes where agricultural intensification is extreme, the species is even becoming rare (Asher et al., 2001).

The meadow brown is a strictly univoltine species. In Belgium, it is on the wing from late May to the end of August (*pers. obs.*), with continuous but decreasing adult emergence during this period (Brakefield, 1982b). The species exhibit a protandry of about 10 days, hence males only are flying at the very beginning of the flight season, while only females can be found at the very end (Brakefield, 1982b; Grill et al., 2006; Scali, 1971). In Belgium, adult emergence generally starts in June or in late May. June maximum temperature and accumulated temperature in March are key factors in determining the adult emergences date (Brakefield, 1987). Average adult lifespan is between 5 and 12 days (max 22 days observed in the field) (Brakefield, 1982b). In the south of its distribution area, females have been reported to undergo an aestivation period (imaginal diapause during the hottest season) (Scali, 1971), hence their longevity is much higher. In a recent laboratory experiment, Grill et al. (2013) found no increase in longevity or fecundity when females where fed with nectar mimics containing amino acids compared to females fed with plain sucrose solution.

Young adult males are ready for copulation soon after emergence (Scali, 1971), whereas females emerge without eggs in their genital tract; egg maturation happens progressively throughout female lifespan (Bink, 1992) although we think it happens quickly after emergence in our populations. Females mate only once (Dowdeswell, 1981; Svärd and Wiklund, 1989), usually within 24 h of emergence, whereas males have been observed to

mate several times (Brakefield, 1982b). Once mated, females reject males with a specific behaviour consisting of rapidly shaking their wings (Delattre, 2010).

Males spend more time flying than do females (Grill et al., 2006; Merckx and Van Dyck, 2002). Males patrol to locate females, or alternatively, they perch on the vegetation to intercept passing females, but do not show aggressive defence of their perching spot (Dennis & Shreeve, 1988). Females alternate periods of resting with feeding and egg laying and feed more frequently than males (Brakefield, 1982a). The species has often been observed aggregated around flower clumps (Brakefield, 1982a; Clausen, Holbeck, & Reddersen, 2001; Dennis, 2004; Schneider, Dover, & Fry, 2003). Males and females use a wide range of nectar sources (Blake et al., 2010), and preferences for several species have been observed: knapweed (*Centaurea* sp.) and thistles (*Cirsium* sp.) (Brakefield, 1982a), bramble (*Rubus fruticosus*) and ragwort (*Senecio jacobae*) (Dennis, 2004).

Females lay eggs one by one on a support, not specifically on a host plant (Delattre et al., 2010), which reflects a rather unselective egg-laying behaviour (Bink, 1992). In laboratory conditions, females were observed to lay a total of 179 (SEM: 15.2) eggs (with a peak of daily egg production 2 to 5 days after mating, most eggs laid after 2 weeks were infertile) and were estimated to produce 66 eggs in field conditions (Brakefield, 1982b). Grill et al. (2013) observed a total of 200 to 350 eggs laid per female, in laboratory conditions. Eggs were estimated to have a volume of around 0.08 mm^3 when assimilated to a sphere (García-Barros, 2000), although they have effectively a truncated cone shape (*pers. obs*., Figure 3.2).

Figure 3.2: Egg of *Maniola jurtina*. Eggs are estimated to have a volume of around 0.08 mm³ when assimilated to a sphere (García-Barros, 2000) Picture: G. San Martin Y Gomez.

The egg hatches after 2 to 3 weeks (pers. obs.). Young caterpillars are diurnal (Delattre, 2010). Host plants are numerous, common species in the *Poaceae* family (*Holcus lanatus, Poa pratensis, Festuca ovina, Lolium perene, Bromus erectus*, etc.) (Bink, 1992; Blake et al., 2010; Brakefield, 1982b; Tolman and Lewington, 1999). Overwintering takes place as a stage 1 or 2 larva, which spends most of its time deep down among the grass tussocks (Dowdeswell, 1961). From late April onwards, the caterpillars (measuring on average 10 mm) become nocturnal and active again, and feed on grass stems (Dowdeswell, 1961). Pupation occurs between May and mid-July and lasts approximately a month (Delattre, 2010).

The meadow brown has several known parasitoids, *Apanteles tetricus* (Dowdeswell, 1961), *Erigorgus melanops*, *Ichneumon caloscelis*, *Meteorus versicolor*, *Cotesia tetrica* and *Coelopisthia pachycera* (Shaw, Stefanescu, & Van Nouhuys, 2009 and references therein). In the Canary Islands, the main predators of the adults are lizards and birds and males exhibit more wing damage than females (Owen and Smith, 1990). In Belgium, we expect birds and spiders to be the main predators.

Most *M. jurtina* individuals usually stay in a restricted area. The mean movement distance recorded in CMR studies was 100 m (Grill et al., 2006), 322 ± 21 m (Schneider et al., 2003), and the home range was estimated as 0.850 ± 0.065 ha (Brakefield, 1982b). Its perceptual range is estimated to be between 100 and 150 m (Conradt et al., 2000; Conradt, Roper, & Thomas, 2001). However, the species is also capable of long-distance flight: 2100 m (Schneider et al., 2003) and 2116 m (Grill et al., 2006) are the maximum distances recorded in CMR studies. *Maniola jurtina* shows dispersal rates typical of butterfly metapopulations in fragmented habitat; the individuals recognize boundaries between habitat and non-habitat (Conradt et al., 2003); In particular, the species is known for its non-random systematic foray loops, a strategy used in habitat searching (Conradt et al., 2003) and its dispersal mode, a tendency to shift from a search flight to a flight in a straight line when leaving the habitat (Delattre et al., 2010; Dennis, 2004).

Maniola jurtina is an interesting model to study nectar use in agricultural landscapes as it is widespread even in intensively managed agricultural landscape, as it has an average mobility and adults feed on a variety of nectar sources. Moreover, the species is also considered a generalist at the larval stage, feeding on many different grasses, reducing the chances of host plants being limiting. Furthermore, its ecology (Brakefield, 1982a, 1982b, 1987; Dennis, 2004), spot pattern and genetics (Creed et al., 1959; Dowdeswell, 1961; Handford, 1973a, 1973b; Scali & Masetti, 1973; Brakefield & van Noordwijk, 1985; Goulson, 1993) and phylogeography (Dapporto et al., 2009; Habel et al., 2010; Schmitt, Röber, & Seitz, 2005) have been extensively studied. More recently, it has become a model in the study of dispersal (Conradt et al., 2001, 2003; Schneider et al., 2003; Conradt and Roper, 2006; Ouin et al., 2008; Delattre et al., 2010, 2013).

Study areas and collection sites

In order to compare the consequences of nectar limitation for butterflies from extensively managed agricultural landscapes and from intensively managed agricultural landscapes, we collected young adult *M. jurtina* butterflies (**Chapters 5, 6 and 7**) in two landscapes differing in their agricultural management intensity (Figure 3.3).

The land use in the intensively managed landscape (located in Walloon Brabant; center: 50°35'02" N, 4°39'09" E) mainly consisted of agriculture, which mostly included arable land with crop culture. Grassland habitats were confined to roadsides, hedgerows and intensively managed meadows and pastures. In this landscape, butterflies were captured in 9 sites distant of at least 1km of each other. In addition, butterflies occasionally observed on the road between the sites were also collected.

The extensively managed landscape (located in the Famenne region; center: 50°08'22" N, 5°03'01" E) consisted of meadows, pastures and forests. Types and intensity of grassland management varied among sites, but a large part was composed of relatively extensively managed hay meadows and other grasslands favorable to several grassland butterfly species (Figure 3.4).

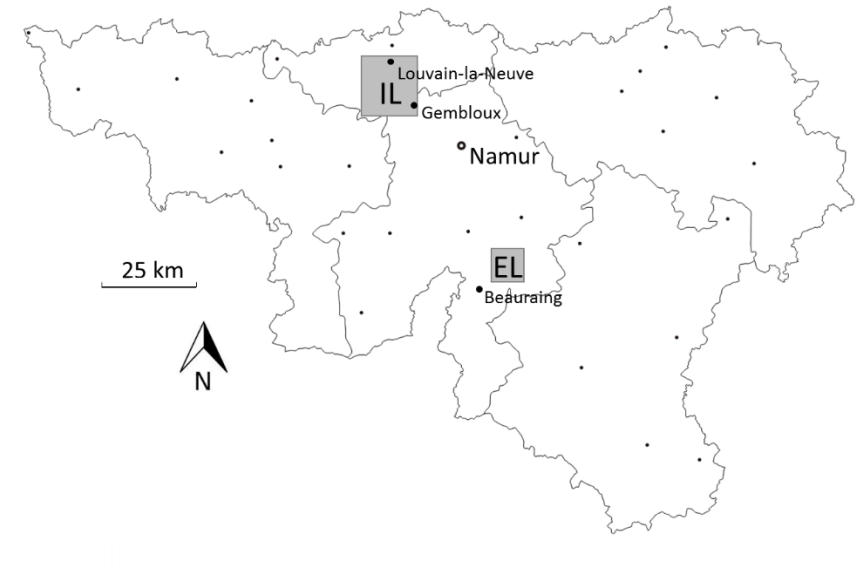

Figure 3.3: Location of the intensively managed agricultural landscape (IL) and of the extensively managed agricultural landscape (EL) in Wallonia (South of Belgium). Both areas were used for butterfly collection of chapters 5 (EL only), 6 and 7 (IL and EL). Field observations and experiments were all performed in the EL (Chapter 4, 5 and 8).

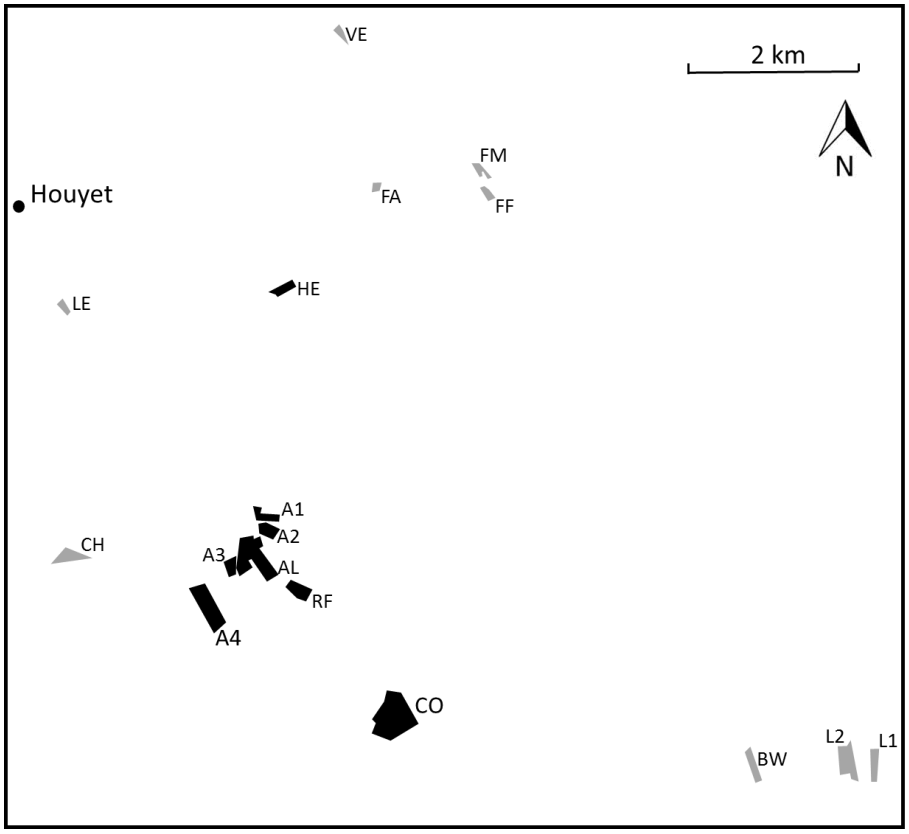

Figure 3.4: Study sites in the extensively managed landscape. Sites that were more intensively managed are represented in grey. Sites that have always been under extensive management are in black.

Field observations and experiments

All field observations and experiments were performed in the EL, in the municipalities of Houyet, Beauraing and Rochefort, astride two geological regions: the "Famenne" (mostly heavy clay soils, flat territory) and the southernmost slope of the "Condroz" (hills with calcareous fertile soil in the valleys and poor sandstone on the tops). The area is mostly occupied by woods and grasslands (pasture and hay meadows), in a relatively extensively managed hedged farmland, since the soil is not appropriate for crop cultivation.

A total of 15 sites were used (Figure 3.4). They were all hay meadows under Belgian (Walloon) agri-environmental schemes (MAE 2 or 8; Demotte & Lutgen, 2008). No intervention is allowed between 1 January and 15 June (MAE2) or a later date in July specific to the site (MAE8). After the date, the site can either be mown or grazed. Our study sites were all mown and never grazed during the study period. No (MAE 8) or low (MAE 2, limited to a single yearly manure spreading) fertilizer addition and no chemicals are allowed. When mown, hay must be collected and a minimum of 5% (MAE 2) or 10% (MAE 8) must be left unmown. The localisation of the unmown zone may vary from year to year. However, sites vary regarding to their historical management. Some sites had always been under extensive management (Figure 3.5a and b), and other were only recently placed under agri-environmental scheme, and therefore looked more like productive hay meadows in terms of plant diversity and flower abundance (Figure 3.5c). Using meadows under these agri-environmental schemes allowed butterfly observation in the field until mowing, and even after but in the refuge zone only, contrary to other more intensive grasslands that are mown several times a year. Sites also varied in size, ranging from 0.7 ha to 15 ha. We classified the sites according to their past management (Table 3.1, Figure 3.4), which reflected their flower diversity and abundance, and grass diversity.

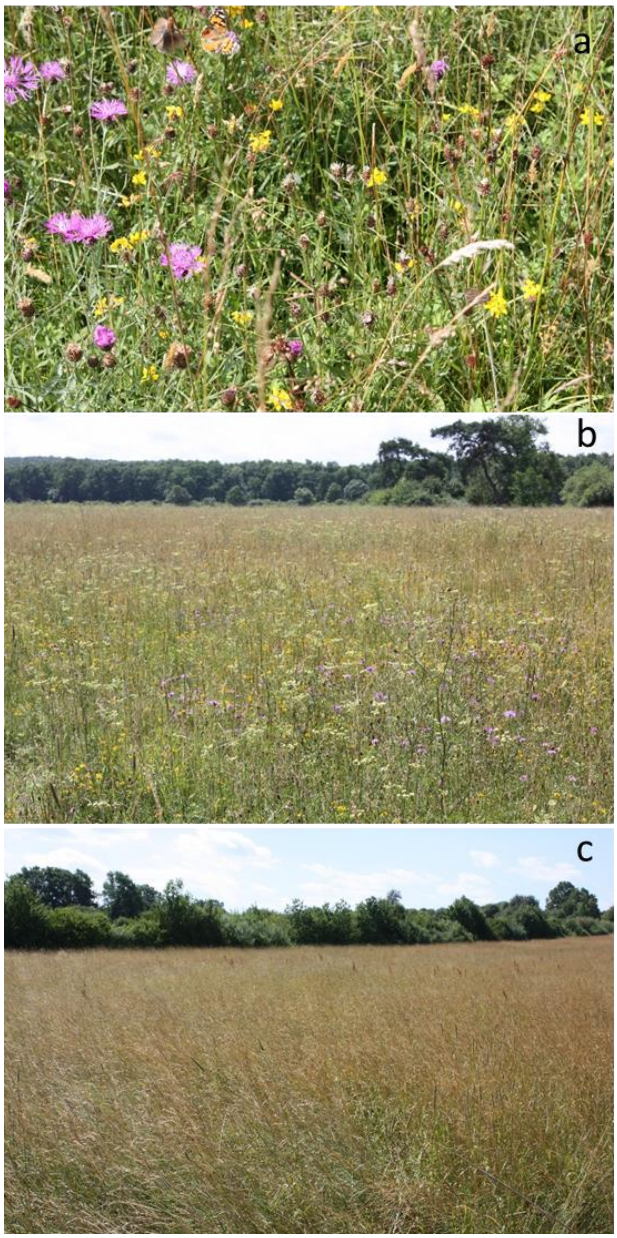

Figure 3.5: Study site extensively managed for a long period (a and b) and only recently placed under agri-environmental scheme but intensively managed until recently (c). Pictures: J. Lebeau

Table 3.1: Studied sites coordinates, mowing date and past management (I: intensive management, E: extensive management) and agri-environmental scheme (2: "semi-natural grassland" or 8: "grassland of high biological interest"). 'No mowing date' indicates that the site was mown after the end of the flying season of *Maniola jurtina*, or that is was not recorded.

site	coordinates	Mowing date			Past management	MAE
		2009	2010	2011		
A1	50°09'11"N - 5°02'53"E		July the 4rd	July the 3rd	E	2
A2	50°09'03"N - 5°03'02"E		July the 4rd	July the 3rd	E	2
A3	50°08'51"N - 5°02'38"E		July the 4rd	July the 3rd	E	2
A4	50°08'33"N - 5°02'26"E		July the 4rd	July the 3rd	E	2
AL	50°08'57"N - 5°02'48"E				E	8
BW	50°07'35"N - 5°07'38"E				I	2
CH	50°08'57"N - 5°01'03"E	June the 29th			I	2
CO	50°07'46"N - 5°04'06"E	July the 28th			E	8
FA	50°11'11"N - 5°04'06"E	July the 13th	July the 1st		I	2
FF	50°11'08"N - 5°05'08"E	June the 29th			E	2
FM	50°11'18"N - 5°03'08"E	June the 29th		July the 3rd	E	2
HE	50°10'34"N - 5°03'10"E			July the 3rd	E	2
L1	50°07'36"N - 5°08'49"E	July the 14th	July the 26th		I	2
L2	50°07'30"N - 5°07'39"E	July the 14th			I	8
LES	50°10'28"N - 5°01'02"E	July the 13th	June the 29th		I	2
RF	50°08'42"N - 5°03'17"E	July the 12th	July the 2nd		E	8
VE	50°12'07"N - 5°03'45"E	June the 29th		July the 3rd	I	2

All sites were characterized in terms of floral units and butterfly abundance and diversity. We set out two 100m² transects (5m x20m) in each site. One was placed randomly in the central area of the site (that would be mown), and the other in the (future) uncut zone. We recorded all floral units in ten 1-m² squares randomly chosen within each transect. One floral unit equals one flower or one inflorescence, depending on the species. Butterflies were recorded in the delimited transects with the Pollard walk technique (Pollard and Yates, 1993). We performed transect counts at least three times before mowing (when possible), from the beginning of the flying season of *M. jurtina*. Whenever possible, transects counts were also performed on three different dates after mowing, in the uncut refuge zone only, because preliminary studies showed that transects in the mown zone were always empty of butterflies (no butterfly observed in the transect placed in the mown zone in a total of 5 sites each observed at 3 different dates after mowing).Comparing densities of butterflies in the refuge zone before and after mowing tested for the refuge effect of these uncut zones (**Chapter 8**). For this question, we used all sites that were mown during the flight period of *M. jurtina* in the summer of 2009 and 2011.

We studied the foraging behaviour of *M. jurtina* and how it varied with nectar resource availability, with behavioural tracking of butterflies in four sites (**Chapter 4**), in 2010 and 2011. Two sites (CO, AL) had been extensively managed for a long time and offered a high diversity and abundance of flowers, whereas the two others (FA, LE), were only recently placed under agri-environmental schemes and were flower-poor. Because of low butterfly abundance in flower-poor sites, we had to group two nearby sites (L1 and L2) and their surroundings, and to consider it as one unique site (further called LE) for the behavioural trackings. We also performed a field experiment to test the strength of flower preference in the two type of site (flower-rich sites: CO, at two extremities; flower-poor sites: L1 and BW). In each site, 4 experimental flower arrays were introduced. Each array

consisted of a mix of 18 wild-picked inflorescences: 9 *C. jacea* and 9 *T. pratense* (Figure 3.6). They were placed as to offer the same number of inflorescences of each species (3 of each), at the same distance relative to the inflorescence placed in the centre of the array. The total number of inflorescence in the array was chosen as to be the maximum that was still feasible to observe by one observer, hence it had a trapezium shape as to have 18 flower heads (9 of each species) disposed with respect to these rules. Inflorescences in the array were placed at 60 cm from each other. We assumed that this distance ensured detectability of the surrounding flower heads by the meadow brown, while avoiding butterflies on adjacent flower heads to disturb each other. All wild inflorescences within the array were removed, as well as those within a 1-m buffer around the array (Figure 3.7).

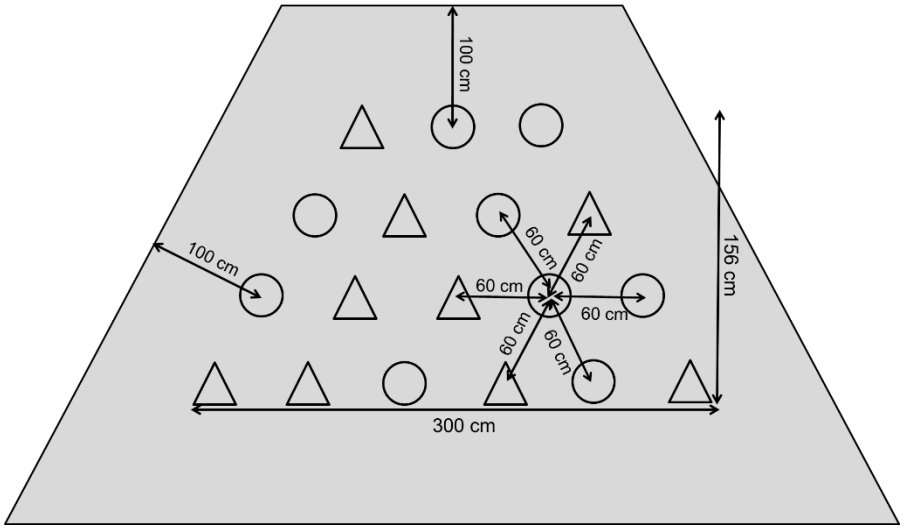

Figure 3.6. Schematic representation of the introduced nectar array. Δ represent *Centaurea jacea* flower heads, ○ represent *Trifolium pratense* inflorescences. All wild flowers present in the grey area were removed before the experiment. The observer stood at a distance of 1,5 m from the array.

Figure 3.7: Experimental nectar array, with individually marked inflorescences of each nectar species. Picture: J. Lebeau.

Inflorescences were placed in the array, in water-filled tubes attached to sticks in order to present the inflorescences at a height of *c.* 35 cm (Figure 3.8). Although nectar secretion in the florets of the flower heads used in this experiment probably stopped as soon as we cut the stem (or a few moments later), we assumed that nectar content of the disposed flower heads was maximized due to visitor prevention with the exclosure bags.

Figure 3.8: Water-filled tube used to present the flower head at approximately 35 cm off the ground. Picture: J. Lebeau.

We also performed behavioural tracking in 2009 in CO, before and after mowing, in order to test for changes in behaviour and flight patterns in response to higher population densities in the refuge zone after mowing (**Chapter 8**). An increase in population density generally creates more competition for resources. We further studied the use of two frequently

visited nectar sources before and after mowing, by *M. jurtina*. For that purpose, we introduced potted nectar plants (*C. jacea* and *T. pratense*) in the site CO (in 2009) and recorded flower head visits by *M. jurtina* during 1 h by video camera (JVC Everio).

Laboratory experiments and measurements

We tested the effect of a short stay (48 h) in outdoor flight cages offering different levels of nectar quality and quantity, on life-history traits (**Chapters 5, 6 and 7**), metabolism and flight-related traits (**Chapter 7**) of *M. jurtina* from populations of extensively managed agricultural landscape (**Chapters 5, 6 and 7**) and intensively managed agricultural landscape (**Chapters 6 and 7)** (Figure 3.3).

We used two identical semi-cylindrical outdoor cages (plastic greenhouses; dimensions l x w x h = 9.0 m x 3.7 m x 1.8 m (at maximal height)) located in Louvain-la-Neuve. In a first experiment, we compared two nectar treatment, and used a whole cage for each treatment (**Chapter 5**). In this experiment, one nectar treatment offered 100 *C. jacea* flower heads, whereas the other had 10 *T. pratense* inflorescences. The quantities of displayed flower heads reflected the natural abundance of each species in the wild, in flower rich sites (*C. jacea*) or flower-poor sites (*T. pratense*). *Centaurea jacea* flower heads came from the site CO, whereas *T. pratense* plants were cultivated in Louvain-la-Neuve, in outdoor conditions.

In a second experiment (**Chapters 6 and 7**), we simulated four levels of nectar availability and divided each cage into two identical halves (4.5 m x 3.7 m x 1.8 m), with a relatively opaque veil carefully outstretched in the middle of the cage (Figure 3.9). In this second experiment, we tested differentially for effect of nectar quality, quantity, or both. The four nectar treatments were 100 *C. jacea*, 10 *C. jacea*, 100 *T. pratense* and 10 T*. pratense*. *Centaurea jacea* flower heads were wild-picked in sites CO and

AL, and *T. pratense* came from cultivated experimental plots in Louvain-la-Neuve.

On the day before their use, flower heads of each species were covered with exclosure bags (Figure 3.10) to preclude any insect visitation and ensure maximum nectar content.

On the day of their use, flower heads were cut and placed in the flight cages in small 10-cm high plastic vials filled with water and half buried in the ground (Figure 3.11). They were placed at regular distances on the floor (Figure 3.9). As discussed previously, we assumed that nectar content at the beginning of the experiment was maximized in our disposed inflorescences, despite their cut stems. It is unlikely that they continued to secrete nectar during the 48 h of the trial. However, we believed that initial nectar quantities in the different treatments was respected. We cannot exclude that nectar was totally depleted in all flower heads from the low nectar quantity treatments.

Figure 3.9: The interior of a flight cage, here separated into two identical parts by a green veil halfway along the cage. The floor is covered in wood chips, and plastic vials with inflorescences are regularly spaced on the ground (treatment with 100 flower heads shown here). Picture: S. Blareau.

Figure 3.10: Exclosure bags placed on *T. pratense* flower heads prior to use in the experimental setup. Picture: S. Blareau.

Figure 3.11: Plastic vial half-buried in the ground and filled with water containing a *C. jacea* flower head in an outdoor flight cage. Picture: S. Blareau.

The first experiment focused on life-history consequences of nectar offer limitation as found in intensively managed agricultural lands. Butterflies were collected in CO in 2011. The second experiment (**Chapters 6 and 7**) we conducted aimed at testing life-history consequences, flight capacities and metabolism after a short stay (48 h) in different combinations of nectar quality and quantity on *M. jurtina* populations from intensively and extensively managed agricultural landscapes. In the summer of 2012, freshly emerged wild butterflies were sampled in two Belgian agricultural landscapes: the extensively managed landscape (EL) and the intensively managed landscape (IL) (Figure 3.3).

After the short stay in the flight cage, surviving butterflies were collected back and were then randomly assigned to measurement groups.

In 2011 (**Chapter 5**), they were either directly frozen (–18°C) for further lipid extractions, or placed in cages for longevity measurements, with

maximum 5 individuals per cage (Figure 3.12), sexes separately, with unlimited access to water-diluted honey on cotton. Lipid extractions were performed by placing individuals in refluxing diethyl ether in a Soxhlet apparatus (Figure 3.13). We compared dry body mass before and after lipid extraction to obtain the total lipid mass remaining in the individual after the short stay (Marden, 1989).

In the second experiment, surviving males were either frozen (–18°C) for further physiological extractions or used for longevity measurements (**Chapter 6**). Females were either frozen, used for longevity and fecundity measurements, or tested for metabolism and flight capacity (**Chapters 6 and 7**).

Butterflies that were used for longevity (and fecundity) measurements were placed in small individual cages (30 x 30 x 30 cm) in controlled conditions (light/dark: 16 h/8 h; 25°C during the day and 14°C at night) with unlimited access to water-diluted honey on cotton (Figure 3.14).

Cages were cleaned every day, their relative location within the climate room was changed, and all eggs laid by each female were collected (Figure 3.15). All eggs were brought back to the site where the female was captured.

Figure 3.12: Cages in outdoor conditions used for longevity measurements in 2011. a) overview of the cages under the shelter; b) one cage with females. Pictures: C. Marchand.

Figure 3.13: Lipid extraction in Soxhlet apparatus. Butterflies were placed in paper filter bags in a cartridge (b); Diethyl ether was warmed up (f) in a balloon (c) and evaporated; it cooled down in the condenser (a)cooled by running water (d) and sank back in the balloon with the overflow system (e). Picture: C. Marchand.

Figure 3.14: Individual cages in controlled conditions in the climate room used for measuring longevity and individual egg production in 2012. Picture: S. Blareau.

Figure 3.15: *M. jurtina* female laying an egg on the wall of its individual cage in the climate room. Picture: S. Blareau.

We obtained metabolic rates of *M. jurtina* females (**Chapter 7**) by measuring CO_2 emission within a flow-through system using a dual-sensor oxygen analyser (Sable Systems International Oxzilla II) connected to a CO_2 analyser (Sable Systems CA-10) (Figure 3.16a). The tested butterfly was placed in a metabolic chamber, which had been flushed of its CO_2. The chamber was then hermetically closed for 10 min, during which the butterfly emitted CO_2. After 10 min, the chamber was flushed again, and the amount of CO_2 measured corresponded to production by the butterfly.

Resting metabolic rate was obtained from CO_2 emission after 10 min in a small chamber (Figure 3.16b) covered by a thick dark cloth to prevent the tested butterfly from being active. Flight capacities and flight metabolic rate were simultaneously measured. The tested butterfly was glued by its thorax to a flight mill placed in a metabolic chamber (Figure 3.16c), and CO_2 emission was measured after a 10-min flight, stimulating the butterfly to be active if necessary by tapping on the chamber. The number of rounds was automatically recorded by a Hall Effect sensor detecting two small magnets attached to the flight mill (Labview v 8.6).

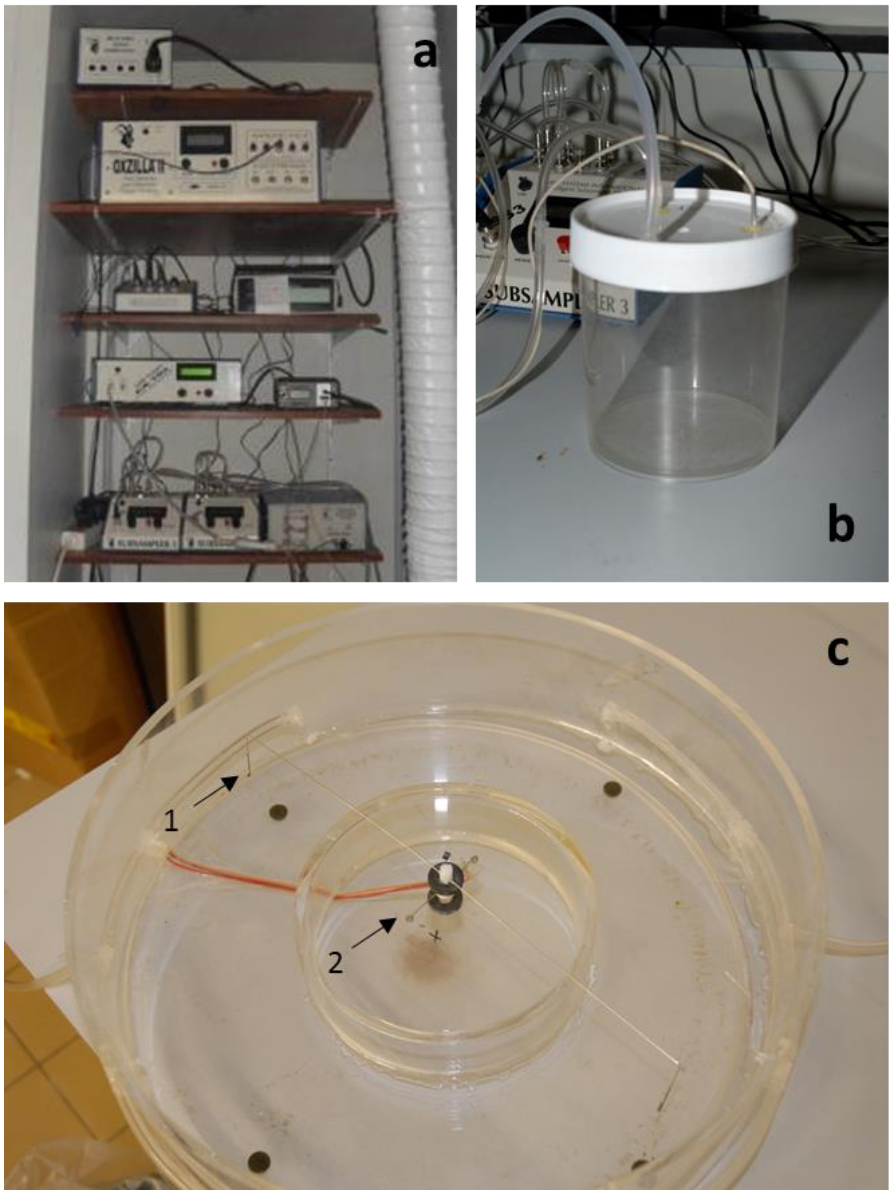

Figure 3.16: Measurements of metabolism and flight capacities. a) engines of the flow-through respirometer; b) chamber for resting metabolic rate measurement; c) flight mill inside the chamber for flight metabolic rates measurement: 1) attachment point of the butterfly; 2) magnets for automatic recording of the number of rounds.

Chapter 4 - Flower choice in the grassland butterfly *Maniola jurtina*: a generalist with a strong preference?

Julie Lebeau, Renate A. Wesselingh and Hans Van Dyck

Author contribution: Study design: J.L.; Field work: J.L.; Data analysis: J.L. (with feedback by R.W. and H.V.D.; Interpretation and writing: J.L., R.W. and H.V.D. Thanks are due to Adeline Louvigny, Céline Marchand, Paul Saunders and Morgan Dalmas for field assistance.

Abstract

Butterflies are often considered as opportunistic nectar consumers that visit a wide variety of flower species. However, the degree of specialisation in foraging behaviour varies considerably at the interspecific level, from highly specialised to generalist species. In generalist species, intraspecific variation in the use of floral resources may arise since different populations may face differences in floral diversity and abundance. By means of behavioural tracking, we identified foraging patterns of the butterfly *Maniola jurtina* in nectar-rich and nectar-poor grasslands. For both males and females, we identified a significant preference for the brown knapweed *Centaurea jacea* and thistles (*Cirsium* sp.) in nectar-rich, extensively managed grasslands. In the absence of these preferred flower species, as is typically the case in nectar-poor, intensively managed sites, *M. jurtina* made use of *Trifolium pratense* or other abundant species. However, the visits were on average shorter on these flower species compared to visits of the preferred nectar sources. These observational results were confirmed by an experimental approach in the field (in a wild flower introduction experiment). In extensively managed grasslands, foraging bouts were generally confined to patches of flowering plants and did not involve long flights between flower visits, whereas in intensively managed meadows, butterflies performed longer flights, and ignored more flowers between two consecutive visits. Despite the variety of flower species visited, this 'generalist' butterfly does show a strong preference for a limited species as nectar sources.

Introduction

Biodiversity conservation involves understanding how organisms deal with changing landscapes, and the traditional dichotomist view of the landscape as habitat and matrix may not be helpful (Dennis et al., 2006). Instead, adopting a resource-based definition of habitat, where the habitat of a species comprises all resources needed for each stage of the life cycle, allows the integration of resource variability and behaviour for a better understanding of resource use (Dennis et al., 2003; Vanreusel and Van Dyck, 2007). Butterflies are popular study systems to address such issues as their ecology is well known and they are easily observed in the field (e.g. Rosin *et al.* 2011; Kalarus, Skorka & Nowicki 2013).

Butterflies are often considered as opportunistic nectar consumers that visit a wide variety of flower species (e.g. Grundel, Pavlovic & Sulzman, 2000; Sharp, Parks & Ehrlich, 1974). However, the degree of specialisation in foraging behaviour may vary considerably at the interspecific level. Some species are highly specialized and feed on a single plant species; they are called true nectar specialists. This can be the result of coevolution between a plant species and its pollinator species (e.g. Yucca moths; Pellmyr & Krenn 2002; Pellmyr 2003). Other nectar specialists are butterfly species that use the same plant species as host for the larval stage and as adult nectar source, although often observation of adult foraging on other species have been reported (e.g. *Boloria eunomia*, Turlure, 2009; *Polyommatus icarus*, Janz, Bergström & Sjögren 2005). Most species feed, however, on a wide range of resources and therefore adopt a generalist strategy. Yet, butterflies do not randomly visit flowers and show nectar preferences (Jennersten, 1984; Murphy, 1984). Butterflies with high wing loading and a long proboscis generally prefer flowers with deep corollas that are aggregated, providing many flowers at a very small spatial scale, whereas butterfly species with low wing loading and short proboscis more often visit

scattered flower species (Corbet, 2000). For example, *Vanessa* species, which have long tongues and high wing loading, are often observed visiting *Buddleja davidii*, and lycaenids, with their short tongues and low wing loading, often visit flowers of the Asteraceae family (Corbet, 2000).

Nectar is mainly composed of water and sugars, but also contains amino acids and micronutrients (Baker and Baker, 1983, 1973). Butterfly species generally prefer nectar that is more concentrated in sucrose than in fructose and glucose, and prefer fructose over glucose (e.g. Rusterholz & Erhardt, 1997; Watt, Hoch, & Mills, 1974). As for nectar amino acid preference, patterns vary among butterfly species, with some species preferring nectar mimics with amino acids (Erhardt and Rusterholz, 1998; Erhardt, 1991; Mevi-Schütz and Erhardt, 2002) and others not, although it remains unclear if they are able to detect nectar amino acid concentrations (Erhardt and Mevi-Schütz, 2009). Nectar quantity per flower (and/or inflorescence) and nectar quality in terms of sugars are generally higher in perennial plant species than in annual species (Petanidou, 2007). Consequently, perennial species are more attractive to flower-visiting insects than annual species at least in some ecosystems (Petanidou, 2007).

Finally, some species have an innate preference for a specific flower colour (Pohl et al., 2011; Weiss, 1997). For example, the butterfly *Vanessa indica* has an innate preference for yellow and blue colours (Ômura and Honda, 2005).

Here we focus on flower preferences at the intraspecific level. Males and females have different nutritional needs and may, as such, prefer different flower species for their nectar properties (Rusterholz and Erhardt, 2000). Females need to manufacture eggs, which are mainly constituted of water, lipids and proteins (Karl et al., 2007), whereas males, apart from spermatophore production, mainly use carbohydrates (and lipids) to fuel

flight. Moreover, intraspecific flower preference can be altered by learning. For example, butterflies of several species were able to associate a sugar reward with a colour (Pohl et al., 2011; Rodrigues and Weiss, 2012; Weiss, 1997). Butterflies locate and select flowers primarily by visual cues, and secondarily by olfaction (Andersson & Dobson, 2003; Ômura, Honda, & Hayashi, 1999; Hisashi Ômura & Honda, 2005). Butterflies have good vision in the human visual spectrum, but also in the UV-spectrum (Briscoe and Chittka, 2001). Many flowers show species- specific visual patterns in the near ultraviolet (e.g. Jones & Buchmann, 1974). Butterfly flower preference may also vary with local flower abundance and diversity (Erhardt, 1995; Goulson et al., 1997a).

Intensification of agriculture has led to biodiversity loss in several taxonomic groups, including flowering plants (Wilson et al., 1999). Hence, butterfly flower preference is expected to target different species in extensively managed, flower-rich agricultural landscapes and in intensively managed, flower-poor and highly productive agricultural lands, because flower abundance and diversity are much lower in the latter.

The terms “preference” and “constancy” are used in many studies, but their definitions differ between authors. Here, we use the term preference when a flower species is visited more often than expected from random choice relative to the relative abundance of nectar resources. The term constancy will be used only when a butterfly feeds consecutively on two flowers of the same species. Otherwise its behaviour will be labelled as ‘switching’.

Here, we studied flower visitation in males and females of a common grassland butterfly in nectar-rich grasslands under extensive management and nectar-poor grasslands under more intensive management. We tested for flower choice preferences under both conditions. We aimed at identifying used nectar sources and expected them to vary according to

grassland management type. In a second step, we experimentally tested in the field the strength of their preference for the preferred nectar source of both meadow types, in each of the two environments. If both nectar species are equally preferred, we expect butterflies to keep their site preference, assuming that short-term history does influence their flower choice. If one nectar source is preferred over the other, then we expect butterflies to show preference for the more profitable species in both environments, provided that they are able to detect their preferred species.

Material and methods

Study species

The meadow brown (*Maniola jurtina* L.) is a widespread satyrine butterfly. The species is strictly univoltine and occurs in open grassy habitats (e.g., meadows, road verges, heathlands, glades and along hedgerows). In Belgium, it is on the wing from late May to the end of August (Fichefet et al. 2008). Males emerge earlier than females, from a few days to up to 10 days (Brakefield 1982). Caterpillars feed on a variety of *Poaceae.*

Males spend more time flying than do females (Merckx & Van Dyck 2002). Males patrol to locate receptive females, or alternatively, they perch on the vegetation to intercept passing females. Females alternate periods of resting with feeding and egg laying and feed more frequently than males (Brakefield, 1982a). The average individual of a population is not very dispersive and may have an action radius up to 300 m (Schneider et al., 2003).

Study sites

Our study sites were all located in the Famenne area in Belgium (municipalities: Houyet, Beauraing and Rochefort). We studied butterfly flower choice in four different hay meadows under agri-environmental scheme imposing a single yearly cut (not before the end of June) and banning the use of pesticides. Sites were selected according to their flower abundance and diversity, and to their previous and current management (Figure 4.1).

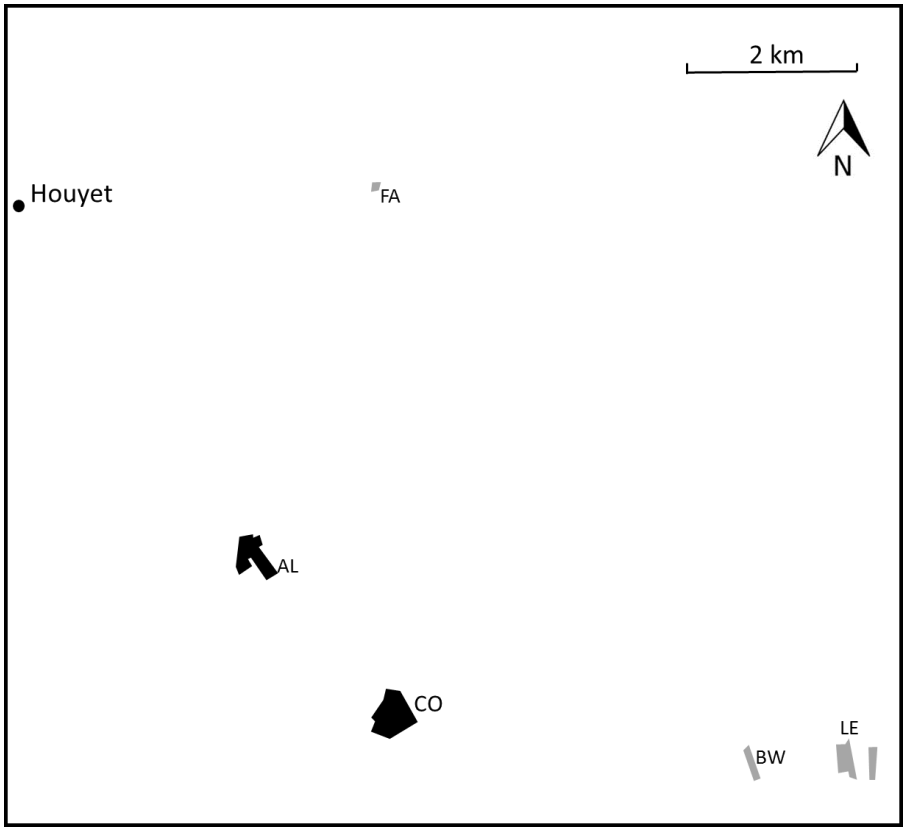

Figure 4.1: Localization of the studied meadows. Black: sites that have always been under extensive management; grey: sites that have only recently been placed under agri-environmental scheme.

Two sites (CO: 50.15°N, 5.04°E; AL: 50.08°N, 5.02°E) were extensively managed for at least 50 years (no input, one single yearly cut, no or very restricted grazing) and were therefore flower-rich (in abundance and diversity), they will be referred to as nectar-rich sites ('NR'). The two other sites (FA: 50.11°N, 5.04°E; LE: 50.07°N, 5.08°E) were placed under agri-environmental scheme for a maximum of 10 years (a single yearly manure application and low grazing allowed from September onwards) and were intensively managed before. Since they had been under more intensive management, they had therefore lower flower abundance and diversity,

and we refer to them as nectar-poor sites ('NP'). We characterized the local nectar supply of each site in 2010 by recording the number of floral units (i.e., 1 flower or 1 inflorescence, see next section) at least twice in each site in 10 squares of 1m² randomly chosen within two transects (5 m x 20 m) located in the centre and at the border of each site, respectively. We summed the number of floral units per species for the 20 squares recorded on each date. We calculated per flower species the mean for all dates. From these means, we calculated the total flower abundance. We also calculated Shannon's equitability (equation 4.1) and Simpson's diversity (equation 4.2) indices because species richness and total flower abundance do not provide information about relative abundance of the flower species.

$$E_{H=} \frac{n \log(n) - \sum_{i=1}^{k} f_i \log(f_i)}{n * \log(k)}$$

Equation 4.1: Shannon's equitability index (Peet, 1975)**. n: total flower abundance; k: number of species; f_i: abundance of species i. Lower values indicate higher diversity, an index value of 1 means that all species have the same frequency.**

$$L = \frac{\sum[n_i(n_i - 1)]}{N\ (N - 1)}$$

Equation 4.2: Formula for calculation of Simpson's diversity index (Peet, 1974)**. n_i: abundance of the i^{th} species. N: total flower abundance.**

Behavioural tracking

Butterflies were tracked from the beginning of the flight season until mowing in the four sites in the summers of 2010 and 2011 on sunny days between 10.00 h and 17.00 h. Butterflies were caught with a hand net and individually marked on the ventral hind wing. After marking, the butterfly was returned to the net and was released after a 1-min recovery period (Cormont et al., 2010). Next, the butterfly was tracked for a maximum of 10

min (unless the butterfly was lost) from a distance of *c*. 1 m to avoid perturbing it (Loos et al., 2015). The trajectory was recorded by a handheld GPS (Pathfinder Data logger SiRf Star III, GPS-DL R8; relative spatial accuracy: ± 0.1 m). The following behavioural categories were recorded using a digital voice recorder (Olympus VN-5500PC): flying, feeding, basking, resting, mating, egg laying (females) and interactions (intra- and interspecific) (Dover, 1997). For flower visits, the nectar plant species was also recorded. All along the track, we dropped numbered white plastic plates, and coloured plastic plates were placed to identify nectar plants species that were visited. After recording the track, we retraced the flight path of the butterfly to record all the encountered flower units in each flight path section between two flower visits (in 50 cm on both sides of the path). We assumed that butterflies could detect flowers in the area delimited in this way. The flower unit was one inflorescence (*Asteraceae*, *Apiaceae*, *Fabaceae* and other species with grouped flowers) or a single flower (e.g. *Convolvulaceae*). Flower units were not recorded when the tracked butterfly did not visit any flower. If the tracked individual visited at least one nectar source during the 10-min track, it was ranked as "did feed", otherwise as "did not feed".

We analysed the proportion of individuals observed feeding during the tracks with a GLMM model with binomial distribution and sex, management type and the interaction effect as fixed factors, and site (nested within management type) and year as random factors. Based on the butterflies that were observed feeding, we analysed the mean number of visits performed during the 10-min period by a GLMM model with a Poisson distribution and sex, management type and the interaction effect as fixed factors and site (nested within management type) and year as random factors.

We analysed the duration of a feeding event (based on the most frequently visited species) using a mixed model with sex, management type, flower species and all interaction effects as fixed factors, and individual, year and site (nested within management type) as random factors. Data were ln transformed to reach normality (Shapiro and Wilk, 1965).

We analysed flower preference by considering each encountered floral unit as a binary event (i.e. visited (1) or not (0)), only on the flower species that received more than 10 visits in total. Since there was no difference between years, we lumped the data of both years. For each sex and management type, we applied a GLMM model with a binomial distribution, with flower species as fixed factor, a fixed factor called "same as previous flower visited" (1 when the encountered species was the same as the last one visited, 0 otherwise) and the interaction, and individual as random factor. Because of very low sample sizes, we could perform the analysis on a subset of flower species and for females only.

We then classified each floral unit that was encountered but not visited along a flight path between two visits into 3 categories: same species as the flower that was visited right before the flight (same as before), same species as the flower visited at the end of the flight (same as the next one) and different species (different) (as in Goulson, Ollerton & Sluman, 1997) (Figure 4.2). This was done for the whole data set (i.e. including species not often visited). We were interested in three aspects. First, we wanted to verify whether butterflies switch visited flower species between two visits in case of a low (relative) abundance of the species that was visited right before the flight bout (Figure 4.2a). Second, we were interested in testing, in case of a switch, whether they visited at the end of the flight bout the species that was more often encountered during the flight (Figure 4.2b). We expected more floral units of another species encountered in case of a switching than a constancy behaviour. Third, we analysed, in case of a

constancy behaviour, the number of encountered floral units of species different than the one visited (in this case, the same before and after the flight bout) compared to the number of the same species, expecting a higher number of the same. We analysed the number of floral units encountered between two visits, for the sexes separately, with a GLMM model with a Poisson distribution with the constancy between the two visits (binary), the species category of the proportion (same as before, same as next, different), the management type and all interactions as fixed factors, and individual as random factor. Year was removed from the analysis because no differences between years were observed. Total number of flower units encountered between two visits was calculated to give an estimation of the distance travelled between two visits (although it also depends on flower density) and we expected it to be overall higher in NR meadows given the higher flower density.

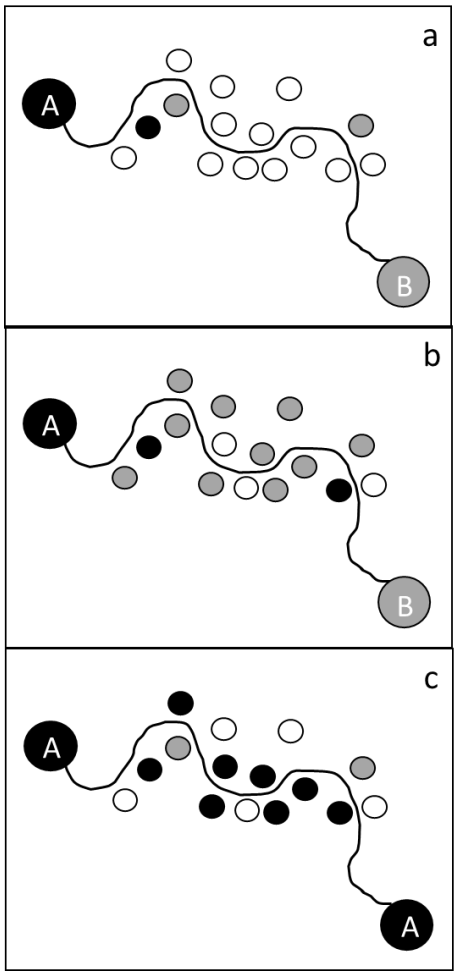

Figure 4.2. : Schematic representation of a flight bout (continuous line) between two visited flower units (A, first species visited, before the flight; and B, second species visited, after the flight bout). Circles represent flower units along the flight paths that were encountered but not visited, and different species are represented by different colours. Encountered flower units could be of the same species as the one visited right before the flight (black circles, species A), of the same species as the one visited at the end of flight bout (grey circles, species B, in case of a switching behaviour; or black circles, species A in case of a constancy behaviour) or of different species (white circles). a) Switching behaviour where the species visited before (here in black) is not abundant. b) Switching behaviour where the next (at the end of the flight bout) visited species (here in grey) is abundant. c) Constancy behaviour with large abundance of encountered flower units that are of the same species as the visited one (here in black).

Nectar array experiment

In the summer of 2010, we also performed a choice experiment in the field to establish butterfly preference for *Centaurea jacea* and *Trifolium pratense* in extensively and intensively managed areas (see also Results), two nectar-rich species known to be important nectar source for butterflies (Clausen et al., 2001; Goulson et al., 1997b; Rusterholz and Erhardt, 1998; Tudor et al., 2004). *Centaurea jacea* is present only in flower-rich, extensively managed sites whereas *Trifolium pratense* is present in both intensively and extensively managed sites.

The experiment was performed in two different landscapes in the same region as for butterfly trackings. In each landscape area, two representative meadows under NR and NP conditions were selected. Meadows within the same landscape were separated by at least 800 m (Figure 4.3).

In each meadow, two experimental flower arrays were introduced (separated by at least 200 m from each other). Each array consisted of a mix of 18 wild-picked inflorescences: 9 *C. jacea* and 9 *T. pratense* (Figure 4.3), which were individually marked. All wild inflorescences within the array were removed, as well as those within a 1 m buffer around the array.

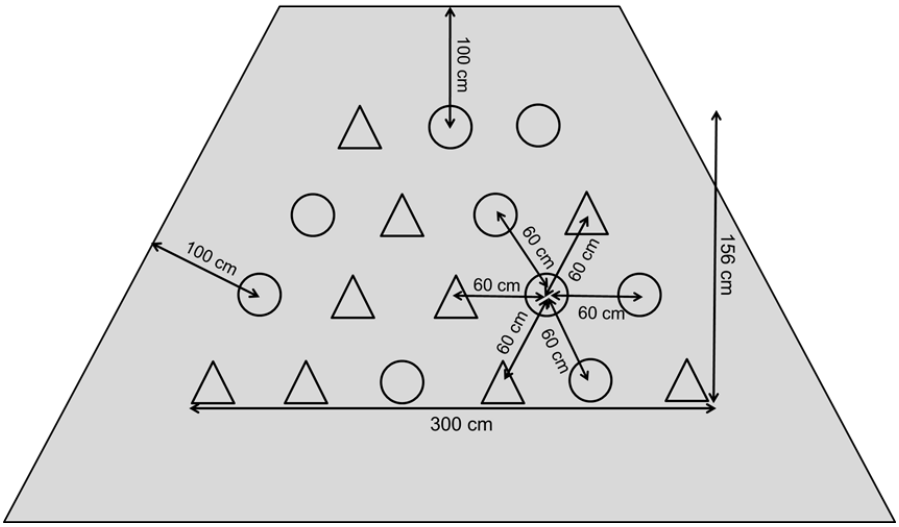

Figure 4.3. Schematic representation of the introduced nectar array. Triangles represent *Centaurea jacea* flower heads, circles represent *Trifolium pratense* flower heads. All wild flowers present within the array and in the 1-m buffer around it (grey zone) were removed before the experiment. The observer stood at a distance of 1.5 m from the array.

The day before the observations, small bags were placed over a number of inflorescences with open florets of *C. jacea* and *T. pratense* in order to exclude access by flower-visiting insects (in both species, mainly the recently opened florets produce nectar). Hence, these flowers were expected to have had sufficient quantities of nectar during the experiment since their overnight production of nectar was not emptied by visitors. The day of the experiment, the bagged inflorescences were cut and placed without the bags in the array, in water-filled tubes in order to present the inflorescences at a height of *c*. 35 cm. Observation sessions lasted for 2 h. When a *M. jurtina* butterfly entered the patch, its behaviour was recorded and timed until it left the array. We marked butterflies once they left the array whenever possible to avoid pseudoreplication. All other visiting insects were chased away from the experimental array by the observer. The two arrays located in one site were simultaneously observed by two

different observers. All observations were done on sunny days, between 10.00 h and 17.00 h on seven days in July 2010. The experiment was repeated on two different days in each site. In total, we worked in 4 sites (2 of each management type), with two arrays in each site, and the experiment was repeated in each site at 2 different dates. At the end of the observation period, exclosure bags were placed back on all inflorescences and 5 florets per inflorescence were sampled for remaining nectar (if possible). Nectar was sampled with 1-µL microcapillaries, a single microcapillary was used for all five florets of an inflorescence because of the low nectar volume collected in individual florets. The amount of nectar in the capillary in µL was determined by measuring the length of the nectar column with digital calipers and dividing this number by the total length of the capillary. We counted the number of open florets per inflorescence to estimate the volume of remaining nectar per inflorescence.

Additionally, we determined the quantity of nectar initially available in our arrays (nectar standing crop of an array) by sampling nectar in 10 additional inflorescences of each species that were collected and treated in the same way as the ones used in the arrays. These inflorescence were not used for the experiment and were kept covered by exclosure bags until sampled. Nectar was sampled with the same protocol as for inflorescences in the arrays, but before beginning the observation session, and nectar sugar concentration was measured with a hand-held pocket refractometer (Eclipse 0-50 BX LV, Bellingham + Stanley) (Baker, 1975). The refractometer was especially designed for very small volumes of nectar (< 1µl), measuring a range of sugar concentration between 0 and 50° Brix. Since we collected even smaller volumes of nectar, samples were diluted to fill the microcapillary and, next, final sugar concentration was measured.

The number of *M. jurtina* entering the array was analysed by two-way nested ANOVA with management type and site as random effect nested

within management type as factors. Prior to analysis, data were ln-transformed (ln (Number of *Mj* +1)) to achieve normality (Shapiro and Wilk, 1965) and homogeneity of variances.

We analyzed the proportion of visits directed towards *C. jacea*, for each butterfly which visited a minimum of 2 inflorescences, with a GLM on the binomial variable "visiting *C. jacea* or not" using management type, sex, and the interaction effect as fixed factors.

The length of a visit on each nectar species was analyzed using a GLMM on log-transformed data considering management, sex, nectar species and all interactions as fixed factors, and site (nested within management type) as random factor.

Simultaneously to the test arrays, two additional arrays for each date at each site (16 supplementary arrays in total) were left unobserved for 2 hours; hence all visitors were allowed to visit the displayed inflorescences and we evaluated flower visits by any insect. We will refer to these as control arrays, while the observed arrays are the test arrays. At the end of each observation period, all inflorescences of the four arrays were bagged again to avoid any additional visits, and the remaining nectar was sampled using the same method as for the test arrays.

The volume of remaining nectar was analyzed by a mixed model with management type, nectar species, type of array (test or control) and all interactions as fixed factors, and site nested within management type, order of nectar sampling, and number of sampled florets as random factors.

Statistics

All analyses were performed in SAS 9.3 (SAS, 2012). We used Analysis of Variance (ANOVA), Mixed Models and Generalized Linear Mixed Models (GLMM), respectively with the GLM, MIXED and GLIMMIX procedures. For the two latter, covariance parameters were estimated by REML (residual maximum likelihood) and the denominator degrees of freedom for the tests of fixed effects were calculated using the Satterthwaite method. Final models were always obtained by backward selection.

Results

Floral diversity and abundance

A total of 24 taxa (species or groups of species in the case of *Apiaceae*) were recorded during our floral unit counts. The two NR sites displayed on average more species than the intensive ones (Figure 4.4). The most abundant species in NR sites were *Centaurea jacea*, *Lotus corniculatus*, with *Cerastium fontanum* and *Stellaria graminea* mainly in the AL site (Table 4.1). In NP sites, *Leucanthemum vulgare* and *Trifolium repens* were dominant in FA, whereas only *Lotus corniculatus* was observed along the transects of the LE site (Table 4.1). In the LE (NP) site, *Cirsium arvense*, *Trifolium pratense* and *T. repens* were present in low densities, but they were not observed on the transects.

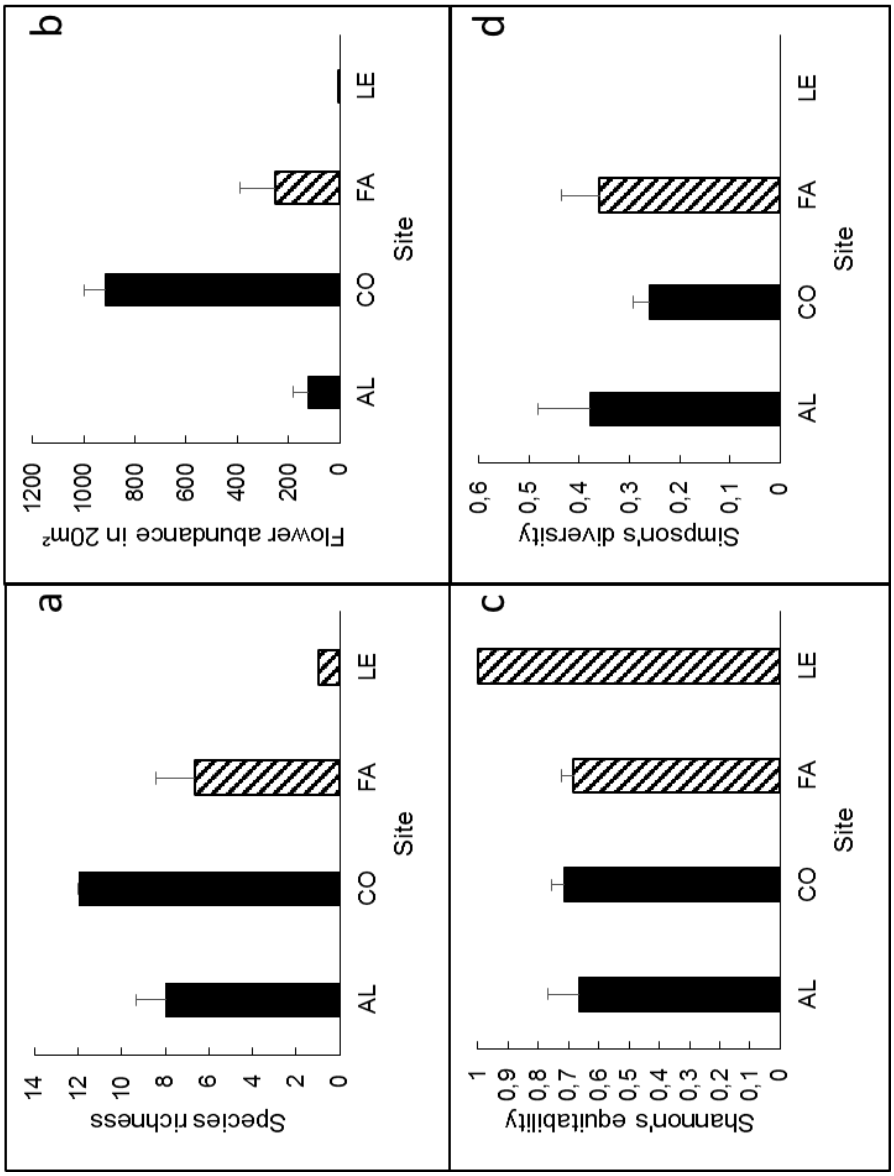

Figure 4.4: Flower diversity and abundance based on floral unit counts within transects: a) species richness, b) floral unit abundance, c) Equitability and d) Simpson diversity index. Filled columns: NR sites (AL, CO); dashed columns: NP sites.

Table 4.1: Identified flower species in the transects of the different sites, with their relative abundance within the transects in the site. Values are in % of total abundance by site. AL and CO: NR sites. FA and LE: NP sites.

	Site			
	NR sites		NP sites	
Flower species	**AL**	**CO**	**FA**	**LE**
Achillea millefolium	1,8			
Apia sp.	0,4	0,3		
Capsella bursa-pastoris			0,3	
Centaurea jacea	12,9	19,9		
Cerastium fontanum	19,0	2,3	2,1	
Cirsium arvense		3,1		
Geranium molle	1,4		0,4	
Hypericum dubium	2,2			
Knautia arvensis	0,8			
Leucanthemum vulgare	1,2	3,5	43,2	
Lotus corniculatus	12,9	43,6		100,0
Malva muschata	4,8			
Myosotis scorpioides		6,3		
Plantago lanceolata	0,8	<0,1		
Ranunculus acre	1,6	4,1	9,6	
Rhinantus minor	6,2	<0,1		
Rubus fruticosus	2,0			
Senecio jacobae			<0,1	
Stellaria graminea	25,0	7,2	8,6	
Trifolium dubium		1,4	12,1	
Trifolium pratense	2,6	2,1	2,0	
Trifolium repens	1,2	4,5	18,3	
Veronicha camaedris	0,2		3,6	
Vicia sp.	3,2	1,7		

Butterfly foraging behaviour

We tracked a total of 181 and 306 butterflies in 2010 and 2011, respectively. In NR sites, proportionally more males were observed feeding than females, but this was not the case in NP sites where the proportion of individuals observed feeding was equal for both sexes; a greater proportion of the observed individuals tended to feed at least once during tracking in the NR sites (GLMM with binary distribution: Management type: $F_{1,2.58}$ = 11.74, P = 0.052; Sex: $F_{1,433}$ = 1.34, P = 0.248; M x S: $F_{1,2.58}$ = 14.32, P = 0.0002; Figure 4.5).

Both sexes visited flowers more frequently in the NR sites (Figure 4.6; GLMM with Poisson distribution: Sex: $F_{1,116}$ = 5.68, P = 0.0187; Management type: : $F_{1,116}$ = 46.11, P < 0.0001).

Females performed 347 visits in total (296 in NR sites and 51 in NP sites). In NR sites, they visited a total of 7 nectar species, but 84.4% of the visits were on *Centaurea jacea*. In NP sites, 6 flower species were visited and visits were more evenly distributed among the different flower species, although *Cirsium* species were frequently visited, if present. Males performed 452 visits (414 in NR sites = 91.6 %). In NR sites, they directed 92 % (381) of their visits to *C. jacea*.

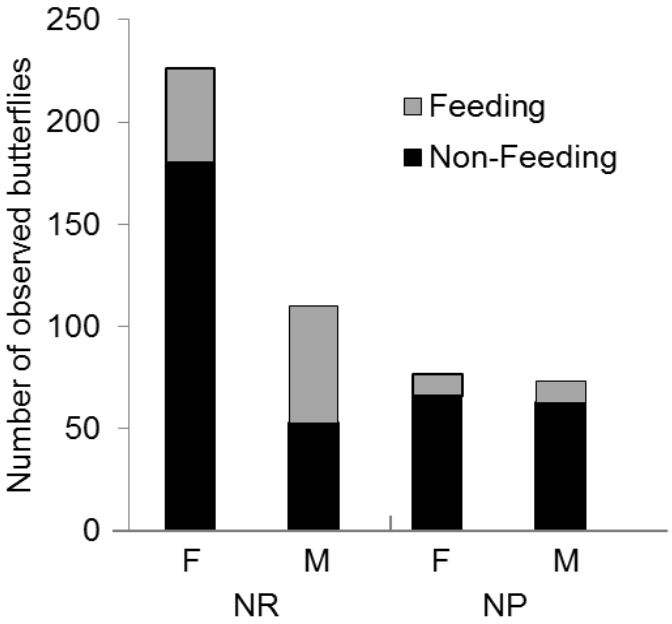

Figure 4.5: Numbers of individuals of *M. jurtina* feeding during behavioural tracking by sex and management type. Grey: butterflies observed feeding; black: individuals that did not feed. NR: nectar-rich sites, NP: nectar-poor sites.

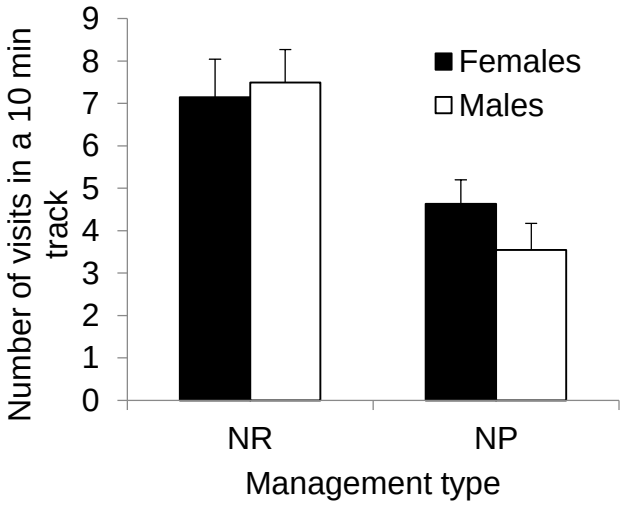

Figure 4.6: Mean (± SEM) number of visits performed by individual *M. jurtina* butterflies during 10-min behavioural track, in nectar-rich sites (NR) and nectar-poor sites (NP). Based on 124 tracked individuals (46 females and 57 males in NR sites, and 11 females and 10 males in NP sites).

During all the tracks, butterflies encountered 39 flower taxa (species or group of species; see above). Several species were encountered but never visited : *Achilea ptarmica, Agrimonia eupatoria, Anthemis arvensis, Apiaceae* sp.*, Campanula rotundifolia, Centaurium erythraea, Cerastium fontanum, Convulvulus arvensis, Crepis biennis, Cruciata laevipes, Filipendula ulmaria, Galium verum, Geranium molle, Malva moschata, Plantago lanceolata, Potentilla anserina, Potentilla erecta, Ranunculus repens, Rhinanthus minor, Jacobaea vulgaris, Stellaria graminea, Trifolium dubium* and *Veronica chamaedrys*. Other species were very rarely visited: *Achillea millefolium* (5 visits), *Crepis capillaris* (1 visit), *Hypericum perforatum* (1 visit), *Heracleum mantegazzianum* (1 visit), *Knautia arvensis* (2 visits), *Lotus corniculatus* (3 visits), *Mentha aquatica* (2 visits), *Myosotis scorpiodes* (1 visit)*, Ranunculus acris* (1 visit) and *Trifolium repens* (9 visits). These species were excluded from the rest of the analyses. Hence, for the analyses of flower choice and feeding duration, we restrained the analyses to the most often visited species: *Cirsium* species (78 visits), *Centaurea jacea* (634 visits)*, Leucanthemum vulgare* (29 visits) and *Trifolium pratense* (28 visits).

Overall females fed for longer periods than males, and the duration of feeding events was on average longer on *C. jacea* and on *Cirsium* species than on *L. vulgare* and *T. pratense* (Figure 4.7; GLMM: Sex: $F_{1,101} = 14.03$, $P = 0.0003$; Management type: $F_{1,270} = 0.41$, $P = 0.52$, Flower species: $F_{3,452} = 7.86$, $P < 0.0001$).

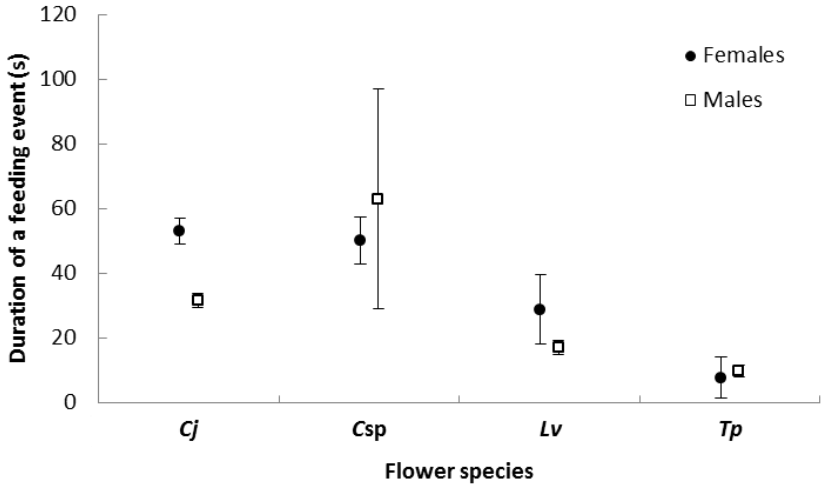

Figure 4.7: Mean (± SEM) duration of a feeding event observed during the behavioural track of *M. jurtina* butterflies on the most often visited species, in both management types combined: *Cj: Centaurea jacea; Csp: Cirsium species; Lv: Leucanthemum vulgare and Tp: Trifolium pratense*.

Out of the total number of encountered flower units, females visited a significantly greater proportion of the encountered *C. jacea* and *Cirsium* species (Table 4.2, Figure 4.8a) than of the other species in NR sites. In NP sites, they clearly visited more *Cirsium* and *T. pratense* flower heads than expected by random choice, and this was not the case for *L. vulgare* (Table 4.2, Figure 4.8b).

Compared to the available (i.e. encountered) offer of nectar species in NR sites, males selected a greater proportion of the available *C. jacea* (Figure 4.8c). In NP sites, they tended to use more species, including *T. pratense* and *Cirsium* species (Figure 4.8d).

Males in NR sites were more likely to visit an encountered *Cirsium* when the previous flower visited was also a *Cirsium*, whereas this trend was reversed for *C. jacea* and *T. pratense* (Table 4.2; mean (± SEM) probability of visiting

an encountered flower head for: *Cirsium*: 0.53 (± 0.4) and 0.02 (± 0.01); *C. jacea* : 0.07 (± 0.009) and 0.15 (± 0.08); and *T. pratense* : 0.01 (± 0.006) and 0.09 (± 0.06); when the last flower visited was of the same species or not, respectively).

Table 4.2: Mixed models with binary distribution on the probability of visiting an encountered flower, for each sex of *M. jurtina* and management type, with species of the encountered flower (Flower species: *Cirsium, C. jacea, L. vulgare* and *T. pratense*), whether it is the same of the last flower visited or not (Constancy with last visited), and their interaction as fixed factors, and individual as random factor. Females from NR sites could only be tested on *C. jacea* and *Cirsium* species, and on *Cirsium*, *L. vulgare* and *T. pratense* for NP sites. Analyses on males flower choice were performed on the four most often visited (group of) species: *Cirsium* species, *C. jacea*, *L. vulgare* and *T. pratense*.

Sex	Management type	Factor	Num. DF	Den. DF	F	*P*
F	**Nectar-rich**	Species	1	2873	0.04	0.84
		Constancy	1	3040	1.15	0.28
	Nectar-poor	Species	2	9.08	15.66	**0.001**
		Constancy	1	490.4	0.78	0.38
M	**Nectar-rich**	Species	3	7504	5.56	**0.0008**
		Constancy	1	7504	6.53	**0.01**
		Species x Constancy	2	3188	5.86	**0.003**
	Nectar-poor	Species	2	1	5.1	0.26
		Constancy	1	1	5.1	0.19

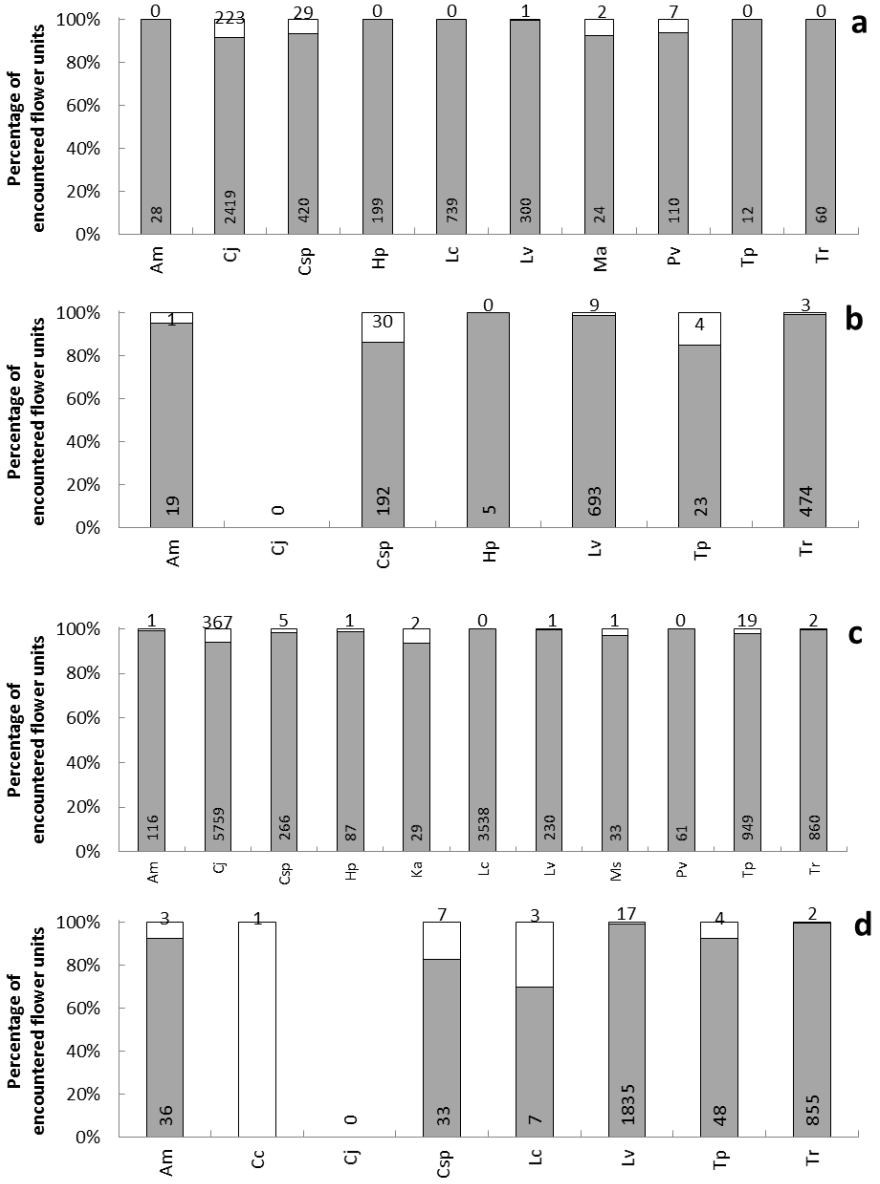

Figure 4.8: Proportion (y axis) and numbers (tags) of encountered floral units that were visited by *M. jurtina* (white, numbers on top) or not (grey, numbers at the bottom) for a) females in nectar-rich sites, b) females in nectar-poor sites, c) males in nectar-rich sites and d) males in nectar-poor sites; by flower species: Am: *Achillea millefolium*; Cc: *Crepis capillaris*; Cj: *Centaurea jacea;* Csp: *Cirsium* species*;* Hp: *Hypericum perforatum*; Ka: *Knautia arvensis*; Lc: *Lotus corniculatus*; Lv: *Leucanthemum vulgare*; Ma: *Mentha aquatica*; Ms *Myosotis scorpioides*; Pv: *Prunella vulgaris*; Tp: *Trifolium pratense* and Tr: *Trifolium repens*. Plant species that were encountered but never visited are not represented.

Females encountered more flower units between two successive visits in NP sites than in NR sites (GLM with Poisson distribution on total flower units encountered: Management type: $\chi_1 = 3290.7$, $P < 0.0001$; Constancy: $\chi_1 = 1892.3$, $P < 0.0001$; Species group: $\chi_2 = 0$, $P = 1$; Management x Constancy: $\chi_1 = 1014.9$, $P < 0.0001$; Figure 4.9a and b).

Overall, when two consecutive visits were on different flower species, a larger number of flowers of other than the visited species was encountered than between constant visits. Moreover, the species that was eventually visited was less frequently encountered in a switching sequence than in a constant one. In NP sites, females encountered more flowers, and this was especially true when they finally fed on a different species than the previous one (GLMM with Poisson distribution: Constancy: $F_{1,407.9} = 82.29$, $P < 0.0001$; Species group: $F_{2,1} = 90.63$, $P = 0.0741$; Management type: $F_{1,43.28} = 13.12$, $P = 0.0008$; Constancy x Species group : $F_{2,1} = 286.49$, $P = 0.041$; Constancy x Management type: $F_{1,398.8} = 54.85$, $P < 0.0001$; Figure 4.9a and 4.9b).

Males also encountered on average more flowers between two successive visits in NP sites than in NR sites (GLM with Poisson distribution on total flower units encountered: Management type: $\chi_1 = 4264.38$, $P < 0.0001$; Constancy: $\chi_1 = 3920.85$, $P < 0.0001$; Species group: $\chi_2 = 0$, $P = 1$; Management x Constancy : $\chi_1 = 96.79$, $P < 0.0001$; Figure 4.9c and 4.9d).

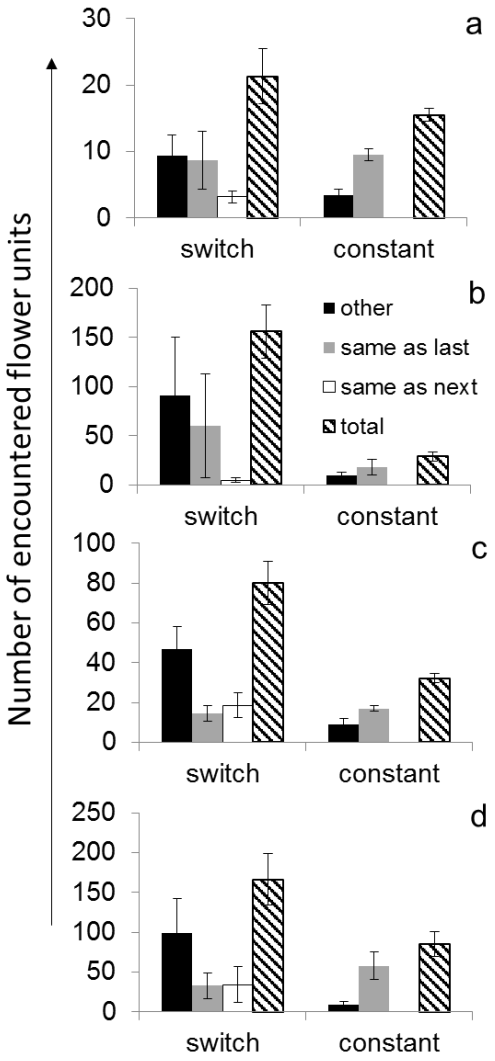

Figure 4.9: Mean (for all individuals, ± SEM) number of floral units encountered between two visits by *M. jurtina* for a) females in nectar-rich sites, b) females in nectar-poor sites, c) males in nectar-rich sites and d) males in nectar-poor sites; for flights between two visits on the same flower species (constant) or on two different species (switch). White represents the number of floral units belonging to the same species as the next one visited; grey the number encountered that were the same of the same species as the last visited flowers; and black represents the number of floral units that were not of the same species as the last visited flowers nor at the next one. Based on 53 flights (11 switch, 42 constant) of females and 74 (20 switch, 54 constant) of males.

Differences in the number of flowers encountered by management type, species group (same as either the previous or the next one visited) and flower constancy between two consecutive visits showed the same results as observed for females (GLMM with Poisson distribution: Constancy: $F_{1,1123}$ = 35.71, $P < 0.0001$; Species group: $F_{2,1}$ = 36.92, P = 0.1156; Management type: $F_{1,55.88}$ = 1.14, P = 0.2898; Constancy x Species group : $F_{2,1}$ = 1159.92, P = 0.021; Constancy x Management type: $F_{1,1123}$ = 17.48, $P < 0.0001$; Figure 4.9c and 4.9d).

Nectar array experiment

A total of 232 *M. jurtina* visited our test arrays, 213 males and 19 females. We recorded many more individuals visiting the introduced arrays with inflorescences in NP than in NR sites: the mean number of adults visiting the nectar patch during 2 h was 27.8 ± 6.1 and 1.5 ± 0.6, respectively (Two-way nested ANOVA: Management type: $F_{1,2}$ = 212.78, P = 0.0047; Study site: $F_{2,12}$ = 0.019, P = 0.82). All together they performed 959 visits. A male butterfly entering the array visited on average (mean ± SEM) 2 ± 0.64 flowers in NR sites and 4.28 ± 0.34 flowers in NP sites, and females 2.33 ± 1.33 and 3.75 ± 1.54 in NR and NP sites, respectively. In NR sites, the first species visited was always *C. jacea*. In NP sites, females oriented 81 % of their first visits to *C. jacea* and males 95%.

Note that only 12 butterflies visited the test arrays in NR sites (9 males and 3 females). Females performed a total of 58 visits, 53 of them in NP sites and 5 in NR sites, of which respectively 5 and 2 on *T. pratense*. In NR sites, males never foraged on *T. pratense*, whereas 13% of the visits performed by the 204 males in the arrays displayed in intensively managed sites were on *T. pratense* (113 out of 874; Figure 4.10).

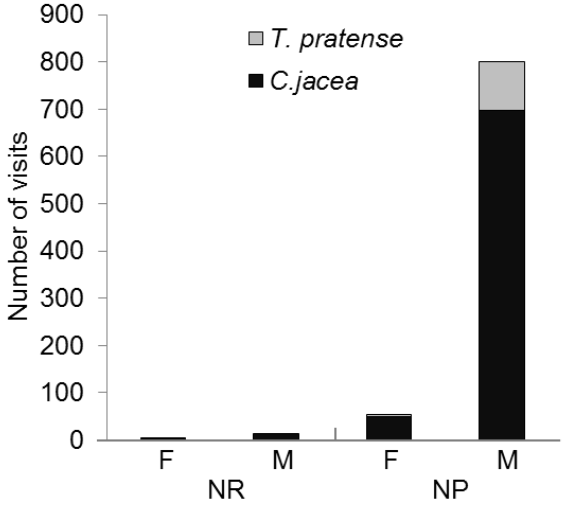

Figure 4.10: Number of visits by *M. jurtina* on *C. jacea* (black) and *Trifolium pratense* (grey) in the nectar arrays, for males (M) and females (F) in both types of site (NR: nectar-rich, flower-rich sites and NP: nectar-poor, flower-poor sites). GLM: Management type: $\chi_1 = 0$, *P* = 1; Sex: $\chi_1 = 3.6$, *P* = 0.057; M x S: $\chi_1 = 5.49$, *P* = 0.019. Based on 32h of arrays observation (16h in each management type).

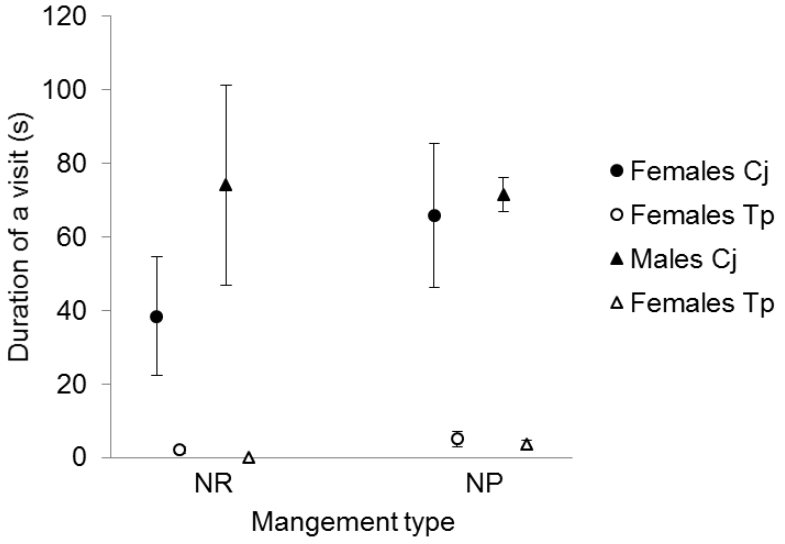

Figure 4.11: Mean (± SEM) duration of a feeding event observed in the test arrays with wild flowers by female *M. jurtina* (circles) and males (triangles) on *Centaurea jacea* (filled symbols, Cj) and *Trifolium pratense* (open symbols, Tp).

Foraging bouts lasted always longer on *C. jacea* than on *T. pratense* (GLMM on ln-transformed data: Management type: $F_{1,954} = 0.84$, $P = 0.36$; Flower species: $F_{1,954} = 230.75$, $P < 0.0001$; Sex: $F_{1,954} = 0.31$, $P = 0.57$; Figure 4.11).

Both flower species had similar numbers of open florets per flower head, quantity of nectar and nectar sugar concentration varied between species, and for each species, nectar properties differed slightly from the literature (Table 4.3).

Table 4.3: Nectar characteristics of *Centaurea jacea* (*Cj*) and *Trifolium pratense* (*Tp*) as observed from flower heads used for nectar standing crop in our arrays and in the literature.

	Nectar species	N	Number of open florets	Volume per floret (µl)	Sugar conc. (%)
This study	***Cj***	45	28.82 ± 1.75	0.059 ± 0.008	60.09 ± 3.13
	Tp	23	22.83 ± 2.67	0.035 ± 0.009	46.96 ± 4.82
(Rusterholz and Erhardt, 2000)	***Cj***	5	-	0.18 ± 0.02	50.95 ± 24.13
	Tp	5	-	0.21 ± 0.02	43.08 ± 13.42

Both flower species had similar remaining nectar volumes in control arrays, whereas in the test arrays, *T. pratense* always had more nectar remaining than *C. jacea*; for *T. pratense*, more nectar remained in the test arrays than in the controls, whereas *C. jacea* were similarly emptied in both array types and in both management types; in NR sites, more nectar remained in the test arrays than in the controls, while in NP sites, there was no difference between array types (GLMM: Flower species: $F_{1,567}$ = 2.1, *P* = 0.148; Array type: $F_{1,566}$ = 1.74, *P* = 0.187; Management type: $F_{1,2}$ = 0, *P* = 0.999; Array type x Flower species: $F_{1,566}$ = 6.15, *P* = 0.013; Array type x Management type: $F_{1,566}$ = 5.37, *P* = 0.021; Figure 4.12).

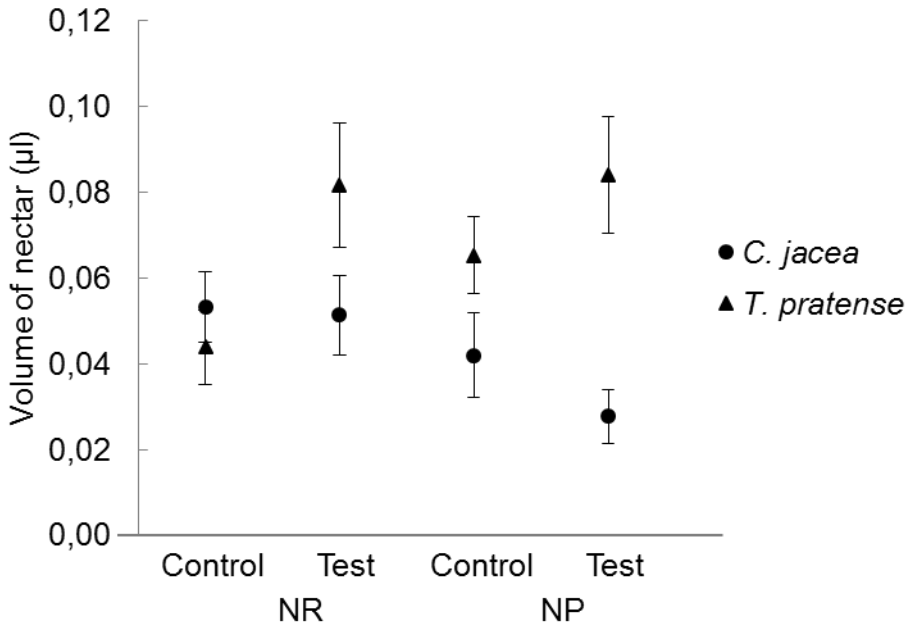

Figure 4.12: Mean (± SEM) volume of remaining nectar in inflorescences of the test and control arrays, for *Centaurea jacea* (circles) and *Trifolium pratense* (triangles) in both management types: NR: nectar-rich sites; NP: nectar-poor sites.

Discussion

Nectar-feeding butterflies obtain energy and other nutrients from nectar; it is now well established that nectar supply enhances fitness in several butterfly species (e.g. Boggs & Ross 1993; Niitepõld, Perez & Boggs 2014). Nectar quantity, sugar composition and concentrations, and nectar amino acids (and other nutrients) composition and concentrations vary between flower species (Baker and Baker, 1983). Hence, foraging behaviour (i.e. the flower species they choose) will determine the quantity and composition of nutrients obtained from nectar feeding. Generalist butterflies, feeding on a variety of nectar sources, have been considered as opportunists for a long time (Grundel et al., 2000; Sharp et al., 1974). Our study demonstrated, with both field observations and a choice experiment, that the generalist butterfly *Maniola jurtina* does not forage randomly on available nectar sources, but rather shows a strong preference for *Centaurea jacea* and *Cirsium* species. Moreover, we showed that flower preference was context-dependent, with a preference for *Trifolium pratense* expressed only in the absence of *C. jacea*.

We conclude from our behavioural observations that *M. jurtina* has a strong preference for a few flower species and does not use nectar sources randomly or relative to their abundance. Although a total of 13 flower species were visited during our observations in NR sites, the preferred flower species of females are the abundant *C. jacea* and less abundant *Cirsium* species. In NP sites, where *C. jacea* was absent, females preferred *T. pratense* along with *Cirsium* species. Preference patterns were similar for males, though less pronounced; they visited a larger panel of flower species but sample sizes were in some cases too small to allow definitive conclusions. If *T. pratense* and *C. jacea* were present in similar proportions, as it was the case in our experimental nectar arrays, *M. jurtina* still preferred *C. jacea* over *T. pratense*.

Nectar offer, as shown in Figure 4.4 and Table 4.1, does not reflect field reality, as samples were taken in only 20 m² spread in two delimited zones of the sites. Diversity and abundance of flowers encountered during behavioural trackings might be more representative of site nectar offer, although it is based on different sample sizes (different number of tracked individuals, over variable distances). Flower species that were never visited were either small (e.g. *Cerastium fontanum, Galium verum, Stellaria graminea, Geranium molle* and *Myosotis scorpioides*), yellow in the human visible spectrum (e.g. *Potentilla* species, *Jacobaea vulgaris*, *Ranunculus acris*) or very rare (*Centaurium erythraea*). Vision has been shown to be the predominant sense for butterflies to detect flowers at a distance (Andersson and Dobson, 2003; Ômura et al., 1999). Tiny flowers were often crossed by our tracked butterflies, but never visited (except for a single visit to *Myosotis scorpioides*), either because *M. jurtina* could not detect them easily or because they were ignored. In butterflies, the visual perception of flower colour extends into the UV-spectrum (Ômura & Honda 2005;Briscoe & Chittka 2001). Based on our results, *M. jurtina* females always used purple flowers; males preferred purple flowers when they were relatively abundant (NR sites), and visited white and yellow flowers when purple flower abundance decreased (NP sites). In a study on flower visitation in North European butterflies, Jennersten (1984) found that the majority of the observed butterfly species were more attracted to blue and red colours, which is consistent with our results. In his study however, yellow flowers (*Ranunculus acris* especially) were also frequently visited by some butterfly species. The same was found in other studies, e.g. *Thymelicus flavus* preferring the yellow *Lotus corniculatus* (Goulson et al., 1997a); *Battus philenor* (Weiss, 1997) and *Danaus plexipus* (Rodrigues and Weiss, 2012) preferring yellow flowers, a strong preference for yellow flowers in *Lysandra bellargus* (Rusterholz and Erhardt, 2000). We observed sex-specific flower preferences, with females always preferring purple flowers

clustered in flower heads. They preferred *T. pratense* only in absence of *C. jacea*. Males preferred *C. jacea* when present (NR sites), and diversified flower species use in terms of colours and shapes in NP sites. In *Lysandra bellargus*, females feed on a wider variety of flower species than males, which is the opposite of what we found (Rusterholz and Erhardt, 2000). The authors attributed these differences to the nectar properties of the visited flower species and to the sex-specific nutritional needs of the butterflies, females being generally more demanding than males because of egg manufacturing.

Several butterfly species prefer nectar with higher amino acid concentrations (Alm et al., 1990; Rusterholz and Erhardt, 2000; Watt et al., 1974). This preference for particular amino acids is interpreted as compensatory feeding mechanisms under poor larval growth conditions in some species (Jovanne Mevi-Schütz and Erhardt, 2003; Rusterholz and Erhardt, 2000). Butterfly preference for nectar with high relative sucrose concentration has also been demonstrated (Erhardt, 1992; Rusterholz and Erhardt, 2000, 1997). We observed *C. jacea* to have slightly more nectar per floret than *T. pratense*. Hence, there should be more nectar per flower head, although reverse trends were found by Rusterholz & Erhardt (2000). The nectar of *C. jacea* is slightly more sugar-rich, and its nectar sugar composition corresponds more to butterfly preferences. It is also richer in amino acids than *T. pratense* nectar (Rusterholz and Erhardt, 2000). Hence, *M. jurtina* flower use and preference correspond to previous findings based on nectar properties.

Flower visits were always longer on *C. jacea* (and *Cirsium* species) than on *T. pratense* (and *L. vulgare)* (cf. results of behavioural tracking and experimental nectar arrays). This could be due to three different potential mechanisms. First, the butterflies could have difficulties handling *T. pratense*. *Maniola jurtina* has a proboscis of *c.* 10.45 mm long (mean of 10

individuals; Corbet 2000). The corolla tube of *T. pratense* is *c*. 8.65 mm long (Comba et al., 1999), while the tube of *C. jacea* is varies considerably between *c*. 6mm (J. Lebeau, pers. obs.) to more than 30 mm (Lázaro et al., 2011). Hence, corolla depth is not making nectar inaccessible in *T. pratense*. However, the corolla shape (recess of corolla tube around the top, as to close the floret) and width (narrower than *C. jacea*) of *T. pratense* might create handling difficulties. However, we know from field observation and other laboratory experiments that feeding on *T. pratense* does not pose problems for *M. jurtina*. Secondly, it could be that *M. jurtina* only detects non-preferred nectar once the butterfly makes contact with the flower and its nectar. In *Pieris rapae* for example, it was shown that tarsal contact with a host plant (cabbage) induced more frequent landings on plants of gravid females whereas tarsal contact with a non-host plants reduced this tendency (Traynier, 1979). Hence, tarsal contact might be involved in flower recognition. However, other mechanisms of flower (visual) or nectar (scent) recognition must be involved, otherwise we would not have observed a strong preference for *C. jacea* for the first visit in our test arrays. Finally, nectar acquisition might be more rapid on *T. pratense* than on *C. jacea*, allowing butterflies to fly away after a shorter feeding period, as feeding rate is known to vary with nectar sugar concentration (Boggs, 1988; Pivnick and Mcneil, 1985) and *T. pratense* has a lower sugar concentration. However, given the magnitude of the differences in feeding time, the most probable explanation is likely to be a combination of the first two hypotheses. Besides the detection of non-preferred nectar when probing it, *M. jurtina* appears to be able to remotely detect differences – either visually or olfactory – between flower species to orient its choice (Plepys et al., 2002). In NP sites, butterflies were supposedly naïve with *C. jacea*. Hence, without an innate preference for *C. jacea*, half of the first visits in the nectar arrays would have been to *T. pratense*. As this was not the case, either the butterflies were able to remotely detect differences between

flower species, or they were not naïve to *C. jacea* and had a learned preference. Although the second explanation is possible for some individuals dispersing from NR sites, it is unlikely that it is the case for all butterflies.

Optimal foraging theory predicts that flower visiting insects should optimize their net energy gain per time unit while foraging (Oster and Heinrich, 1976). However, our butterflies flew over a large number of suitable and preferred flower units without visiting them; they visited only *c*. 10% of the encountered preferred nectar sources. Similar patterns were observed in the butterflies *Pieris rapae* (Lewis, 1989) and *Thymelicus flavus* (Goulson et al., 1997a). However, foraging is not the only activity that should be considered in this context (e.g. thermoregulation, oviposition, and mate searching; Waser 1986). Moreover, most of these conceptual applications have been developed for central-place foragers like bees and may be less applicable to insects like butterflies since they rarely focus on foraging only, but rather mix different activities

As discussed by Goulson, Ollerton & Sluman (1997), we consider butterflies capable of perceiving the relative abundance of flower species as they fly. This information is then used for further flower choice. Switching occurred more often when a large number of flower units were encountered, whereas fewer floral units were in average encountered between two consecutive visits on the same species. A high number of encountered flowers is either due to a high flower density, or to a long flight between the two successive visits. Most likely, the majority of constant flights were due to consecutive visits on flowers in patches in NR sites, and switching was the result of long flights in NP sites. Flight between two visits might lengthen for two reasons. First the individual also carried out other activities. Secondly, it had to fly over a long distance to find a suitable nectar species. This is likely to happen when preferred flower species are

rare, as in NP sites where butterflies are then forced to search for a long time, eventually ending up by feeding on an alternative abundant nectar source, such as *L. vulgare* and *T. repens*.

Trifolium pratense was not frequently visited by butterflies entering our experimental nectar arrays. However, the species was readily visited by other flower-visiting insects in the control arrays compared to the test arrays (only *M. jurtina* allowed). Flower heads of *T. pratense* had even less nectar in control arrays in NR sites than in NP sites, suggesting that even more visits occurred in NR sites, most probably due to higher insect abundance on these sites. *Centaurea jacea* flower heads on the other hand were similarly emptied in control or test arrays, and in both types of sites.

Very hot and dry conditions during our experiment may have caused the nectar to be more viscous, and this might be more accentuated in *C. jacea* than in *T. pratense*, given different flower morphology. Too viscous nectar might not have been correctly sampled by the way of a microcapillary, inducing measurement errors. A very small number of females were observed entering our experimental arrays. This could be explained by two non-exclusive hypotheses. First, because of particularly dry and hot weather conditions, *M. jurtina* females may have sheltered in more fresh and humid microclimates (bushes). Secondly, females could simply not have been attracted by our flower arrays, for instance because of the great concentration of males therein, and hence the risk of being harassed.

Generalist species are often assumed to select the most abundant resources (Grundel et al., 2000; Sharp et al., 1974), but they often do not make such a simple use of resources (Goulson et al., 1997a; Rusterholz and Erhardt, 2000). We showed strong flower preference in *M. jurtina*, and we show variation in the preferred species and in the strength of preference depending on resource availability. Other studies showed similar patterns

for butterflies foraging on nectar sources (Goulson et al., 1997a; Lewis, 1989; Rusterholz and Erhardt, 2000). Our study focused on nectar sources, but choice of oviposition sites in non-specialist species might also be more complex than suggested by the term generalism, as discussed by Janz, Nylin & Wedell (1994). We suggest that the term generalist, as opposed to specialist, must be used with care, and in the sense of the absence of a strict specialisation, meaning the opportunity to use a wider range of resources, without actually being opportunistic.

In conclusion, *M. jurtina* showed strong flower preference for *C. jacea* and *Cirsium* species. In absence of the preferred *C. jacea*, males visited a variety of other flower species, whereas females preferred *T. pratense*. This butterfly thus seem to prefer purple/pink coloured flower species. *C. jacea* was strongly preferred over *T. pratense*, which is either due to flower morphology, nectar properties or a combination of both. When preferred species were rare, *M. jurtina* switched to feed on non-preferred nectar sources. In NP sites, lower flower abundance may drive butterflies to search for longer for suitable nectar sources, and they eagerly fed on experimentally introduced preferred flower species, conveying their need for nectar. Further work is needed to test the effects on fitness and consequences for local populations in differently managed grasslands and landscapes.

Chapter 5 - Floral resource limitation severely reduces butterfly survival, condition and flight activity in simplified agricultural landscapes

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Author contribution: Study design: J.L., R.W. and H.V.D.; Field/Lab work: J.L.; Data analysis: J.L. (with feedback by R.W. and H.V.D.); Interpretation and writing: J.L., R.W. and H.V.D. Thanks are due to Adeline Louvigny, Céline Marchand and Paul Saunders for field assistance.

Abstract

Agricultural intensification has a strong negative impact on farmland biodiversity, but understanding the mechanisms requires experimental work. Among the causes of pollinators collapse in agricultural lands, floral resources disappearance is often pointed out. We test the level of hunger of butterflies in agricultural landscapes with different management intensities and show the consequences of nectar limitation on the performance of a flower-visiting insect, the meadow brown butterfly *Maniola jurtina*. We introduced nectar resources in intensive, nectar poor meadows and in flower rich grasslands and counted visitors. In outdoor flight cages, we simulated the typical floral nectar supply of each agricultural landscape and placed young butterflies for 48 h therein. Despite higher butterfly abundance in semi-natural grasslands, our introduced nectar sources were more visited in intensively managed meadows, conveying the lack of floral resources. The 48 h stay under nectar-poor conditions had a strong negative effect on body condition, flight activity and lifetime survival compared to butterflies under nectar-rich conditions. Female lifespan was reduced by 22% and male lifespan by 43%, signifying important consequences for population dynamics. Agricultural landscapes that provide limited amounts of floral nectar, and no high-quality, preferred nectar sources relative to the needs of the flower-visiting species may create ecological sinks. From an insect's performance viewpoint, the simple presence of nectar is not necessarily functionally adequate. The effectiveness of agri-environmental schemes for flower-visiting insects (e.g. flower strips) could be improved based on ecological and evolutionary insights on the effects of specific nectar quantities and qualities.

Introduction

Increased food production and productivity by improved technical equipment, selected or modified crops, and the use of agro-chemicals have resulted in high levels of loss and fragmentation of semi-natural habitats and, consequently, in landscape homogenization and simplification (Butler et al., 2007; Potts et al., 2010; Tscharntke et al., 2005). Although patterns of the loss of agro-biodiversity are well documented (Benton et al., 2003; Biesmeijer et al., 2006; Butler et al., 2009), we need better insights into the ecological and evolutionary mechanisms causing these patterns as they will provide key information about ways to mitigate the negative situation for biodiversity in agricultural landscapes (Scherr and Mcneely, 2008). Declines in wild flower diversity and abundance are assumed to be major contributors of regional population collapses of flower-visiting insects in landscapes under intense agricultural use (Biesmeijer et al., 2006; Wallisdevries et al., 2012). This is of particular relevance since flower-visiting insects provide essential ecosystem functions including pollination of crops and wild plants (Issacs et al., 2009; Kennedy et al., 2013) and food provisioning for higher trophic levels (Smart et al., 2000). In cleared agricultural landscapes (*sensu* Tscharntke et al. 2005) non-crop nectar sources are typically rare, but nectar supply can vary considerably in non-cleared, but simplified agricultural landscapes. Several agri-environmental schemes favour measures that increase nectar availability assuming opportunistic nectar use amongst flower-visiting insects (e.g. in syrphid flies; Haenke et al. 2009). Butterflies have also been considered nectar opportunists, but experimental work over the last decade has shown evidence for a relationship between nectar preferences and butterfly fitness (Jervis and Boggs, 2005; Mevi-Schütz and Erhardt, 2005). Observational field data also suggested higher degrees of nectar specialization than had been appreciated before (Tudor et al., 2004).

Here we examined the effect of nectar supply on butterfly performance by comparing typical nectar supply between landscapes that differ in agricultural intensification. Recently, the European grassland butterfly index showed that butterfly numbers in grasslands have decreased by almost 50% between 1990 and 2011 (Van Swaay et al., 2013). We used the meadow brown (*Maniola jurtina* L.) as a model species to address the issue of different nectar supply; it is a widely spread, but moderately declining grassland butterfly that occurs in extensively managed and more intensively managed agricultural landscapes (Bergerot et al., 2011; Ouin et al., 2004). Butterflies are popular study systems in ecology, evolution and conservation (Watt and Boggs 2003) and floral nectar is a well-recognised key adult resource (Dennis et al., 2003). Although several field studies have focused on correlations between flower and butterfly abundance (e.g. Haaland and Bersier 2011) and a number of laboratory studies have analysed the impact of nectar consumption on life-history traits (e.g. O'Brien et al. 2002), there are few studies that combined both approaches. Correlative field studies typically suffer from confounding, co-varying explanatory factors (e.g. low nectar and high pesticide use). Therefore, we adopted an experimental approach that allowed testing specific effects of nectar supply. Our experimental setup and the use of semi-natural conditions in outdoor flight cages in particular, provide an interesting opportunity to carefully test the often assumed impact of floral nectar limitation on adult butterfly performance. The nectar supply treatments in the cages simulated nectar-rich and nectar-poor conditions, corresponding to extensively and intensively managed grasslands as observed in the field. Our study aim is to test for differences in adult survival, physiological condition and flight activity.

Material and methods

Meadow brown: study species

The meadow brown (*Maniola jurtina* L.) is a satyrine butterfly occurring in t grassland habitats. Females lay one egg at a time and use several species of Poaceae as host plants. Both males and females can make use of a range of floral nectar sources, but they have a preference for certain species, including brown knapweed (*Centaurea jacea* L.) (**Chapter 4**). In more intensively managed hay meadows these nectar species are typically absent and then they feed on non-preferred nectar sources, such as red clover (*Trifolium pratense* L.). For long, *M. jurtina* has been used as a study species in evolutionary genetics (e.g. Dowdeswell et al. 1949), movement ecology (e.g. Delattre et al. 2010), and habitat use (e.g. Brakefield 1982; Merckx and Van Dyck 2002). Therefore, and because it remains relatively abundant in agricultural landscapes, it represents an ideal model species for our study.

Nectar supply field experiment

A first experiment was conducted in four sites in Belgium: two intensively managed, Nectar Poor hay meadows (further labelled as 'NP') and two extensively managed, Nectar-Rich hay meadows (labelled as 'NR'). Two experimental arrays with real flowers were introduced in each site on two different days in the summer of 2010 (in total 16 arrays in 4 sites). Within a meadow, the arrays were separated by 200 m. Each array consisted of a mix of 18 wild-picked inflorescences: 9 *C. jacea* and 9 *T. pratense*, which were individually marked (Figure 5.1). All wild inflorescences within the array were removed, as well as those within a 1-m buffer around the array. Brown knapweed (*C. jacea* L.) is abundant in NR meadows, but typically absent in NP meadows; it is preferred by pollinators including *M. jurtina*. In NP meadows, nectar source diversity is reduced to a few species at a lower total abundance. Under such conditions, *M. jurtina* makes use of *T. pratense*.

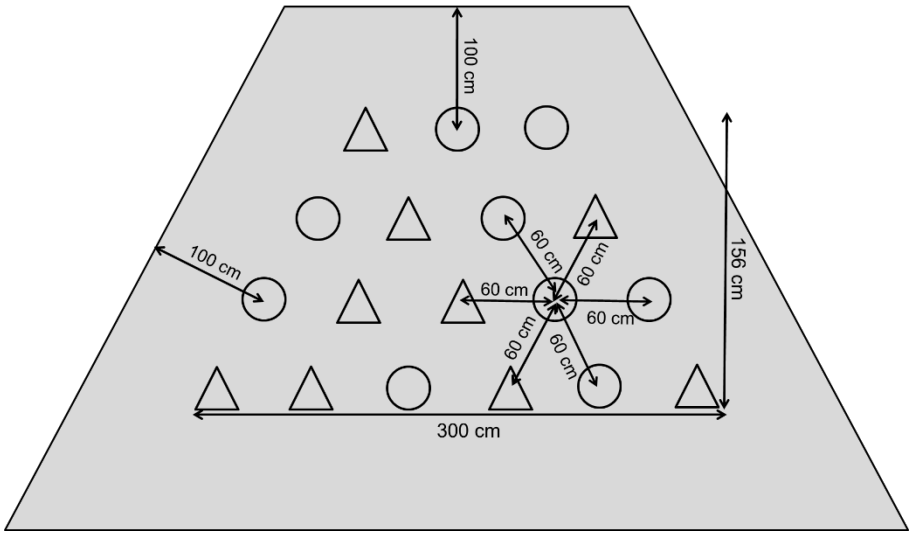

Figure 5.1. Schematic representation of the introduced nectar array. Δ represent *Centaurea jacea* flower heads, ○ represent *Trifolium pratense* flower heads. Flower heads were at similar distances from each other. All wild flowers present within the array and in the one meter buffer around it (grey zone) were removed before the experiment. The observer was at a distance of 150 cm from the array.

The day before the observations, small bags were placed over the inflorescences to exclude access to flower-visiting insects. The day of the experiment, the bagged inflorescences were cut and placed without the bags in the array, in water-filled tubes (at a height of *c.* 35 cm). All visits to each array by *M. jurtina* butterflies were recorded by simultaneous observations during 2 h, as well as the time they spent in the array. Observations were done on sunny days between 10.00 h and 17.00 h on seven days in July 2010.

Relative butterfly numbers

We estimated relative densities of *M. jurtina* in each study site by standard transect counts ('Pollard walks': Pollard 1977) in two 5 m x 20 m transects at each site on four days in July 2010. One transect was placed in the central zone of the site and the other one near a border. We assumed constant detectability of butterflies (Pellet, 2008).

Experiment in outdoor flight cages

We compared fitness indicators of young adult butterflies that lived for 48 h under either NP or NR conditions in outdoor flight cages. As it is the case in the field (**Chapter 4**), both quantity and quality of nectar differed between the NP and NR treatment groups. The NR-treatment simulated extensively managed grassland by placing 100 inflorescences of *C. jacea* plants in the cage, while in the NP treatment, we used 10 inflorescences of *T. pratense*. For each of the eight repetitions, equal numbers of males and females (10 each) were kept in each flight cage for 48 h and they were free to fly and feed on the disposed inflorescences. Outdoor butterfly cages are useful tools to address ecological questions under semi-natural conditions, including questions of conservation relevance (Norberg and Enfja, 2002). Young adults of *M. jurtina* (i.e. no or limited wing wear) used in this experiment came from a large population in South Belgium (50.13°N, 5.06°E). Butterflies (20 males and 20 females for each repetition) were collected between 13.00 h and 15.00 h and individually marked on their ventral wings using a fine, non-toxic permanent marker. They were transported to the laboratory to be weighed to the nearest µg (Mettler Toledo MT5 Balance). They were randomly assigned to one of two treatments. Average body mass of the butterflies of each treatment was not different ($F_{1,287}$ = 0.39 , P = 0.53). Flight cages were two identical plastic tunnel greenhouses (i.e. a semi-cylindrical outdoor cage: 9.0 m long, 3.7 m wide and 1.8 m at maximal height). The bottom of each cage was covered with wood chips.

For the NP treatment, 10 potted *T. pratense* were placed at regular intervals on the floor. Each had a single inflorescence previously protected by an exclosure bag to avoid nectar consumption. They were watered once, just before the butterflies were introduced into the cage. For the NR treatment, *C. jacea* inflorescences were collected at the same site as the

butterflies. They were protected from flower visitors and were cut with a long stem and immediately placed in water. In the flight cage, they were placed individually in small pots at regular intervals on the floor. During the day, to avoid too high temperatures, the flight cages were cooled by sprinkling the roof with water on the outside. Two thermoprobes (HOBO Pro v2 Temp/RH U23-001) per cage recorded ambient temperature every 15 min. Diurnal temperature in the cages was on average (± St. Dev.) 23.8 ± 6.6 °C. In the afternoon of each day of the experiment, a video camera was placed at one end of each cage to record butterfly movements for 1 h simultaneously in the two cages. At the end of the second day, the butterflies were caught and randomly assigned to be either killed immediately by freezing (-18°C) to study their physiological condition (i.e. lipid content), or were placed in small outdoor cages protected from rain to study adult survival. Sexes were kept separately and at a maximum 5 individuals per cage, with unlimited access to water-diluted honey on cotton. The small cages were checked every day to remove dead individuals and renew the food source. Dead butterflies were individually identified and the day of death was recorded to determine longevity.

Lipid reserves

Frozen butterflies were dried for 24 h in an incubator at 70 °C. Their wings were then carefully removed by cutting them at their articulation point on the thorax. Body (head + thorax + abdomen) mass was measured for each individual to the nearest µg (Mettler Toledo MT5 Balance). Body lipids were extracted with chloroform in a Soxhlet apparatus for 8 h (Marden, 1989; Vande Velde and Van Dyck, 2013). After extraction, we dried butterflies in the same way as before lipid extraction, then we reweighed the bodies and calculated total lipid mass as the difference in mass before and after extraction.

Statistical analyses

The number of *M. jurtina* entering the array was analysed by two-way nested ANOVA with management type and site as random effect nested within the management type as factors. Prior to analysis, data were transformed by logarithm (ln (Number of *Mj* +1)) to achieve normality (Shapiro and Wilk, 1965) and homogeneity of variances. Difference in relative density of *M. jurtina* on the transects was analysed by two-way nested ANOVA with the same factors of interest.

For the outdoor cage experiment, we analysed initial body mass, dry body mass, lipid content and longevity using generalized linear mixed models. Covariance parameters were estimated by REML (residual maximum likelihood) and the denominator degrees of freedom for the tests of fixed effects were calculated using the Satterthwaite method. Nectar treatment and management type were considered as fixed effects; flight cage (factor) and capture date (continuous variable, transformed to Julian date) were random effects in the models. Dry body mass after the treatment and lipid content were normally distributed; longevity was logarithmically transformed to achieve normality.

Results

Nectar deprivation in simplified agricultural landscapes

A first field experiment conducted both in NR and NP grasslands showed that butterflies were indeed deprived of abundant, high-quality nectar sources in the latter type of landscape. Although densities of *M. jurtina* were significantly lower on NP grasslands than on NR grasslands (mean number ± S.E.M. of *M. jurtina* observed along 5 x 20 m transects: 2.87 ± 0.70 and 4.81 ± 0.78, respectively; two-way nested ANOVA: Management type: $F_{1,2}$ = 192.2, P = 0.0052, Study site: $F_{2,28}$ = 0.02, P = 0.98), we recorded many more individuals visiting the introduced arrays with inflorescences in NP than in NR grasslands: mean number of adults visiting the nectar patch during 2 h was 27.8 ± 6.1 and 1.5 ± 0.6, respectively (two-way nested ANOVA: Management type: $F_{1,2}$ = 212.78, P = 0.0047; Study site: $F_{2,12}$ = 0.019, P = 0.82). Moreover, butterflies stayed on average three times longer within the nectar patch under NP than under NR conditions (mean residence time: 381 ± 116 s and 127 ± 64 s, respectively). Although nectar was available on the NP sites (*T. pratense*, but not the preferred *C. jacea*), the results indicate constrained nectar acquisition in the NP agricultural landscape.

Adult survival under nectar-poor conditions

Female lifespan after the NP treatment was reduced by 22.8% compared to females of the NR treatment (18.3 ± 3.1 days and 23.7 ± 3.6 days, respectively; Figure 5.2a). The reduction in lifespan was even more dramatic in males: 43.2% (NP: 7.5 ± 2.3 days and NR: 13.2 ± 2.4 days; Figure 5.2C).

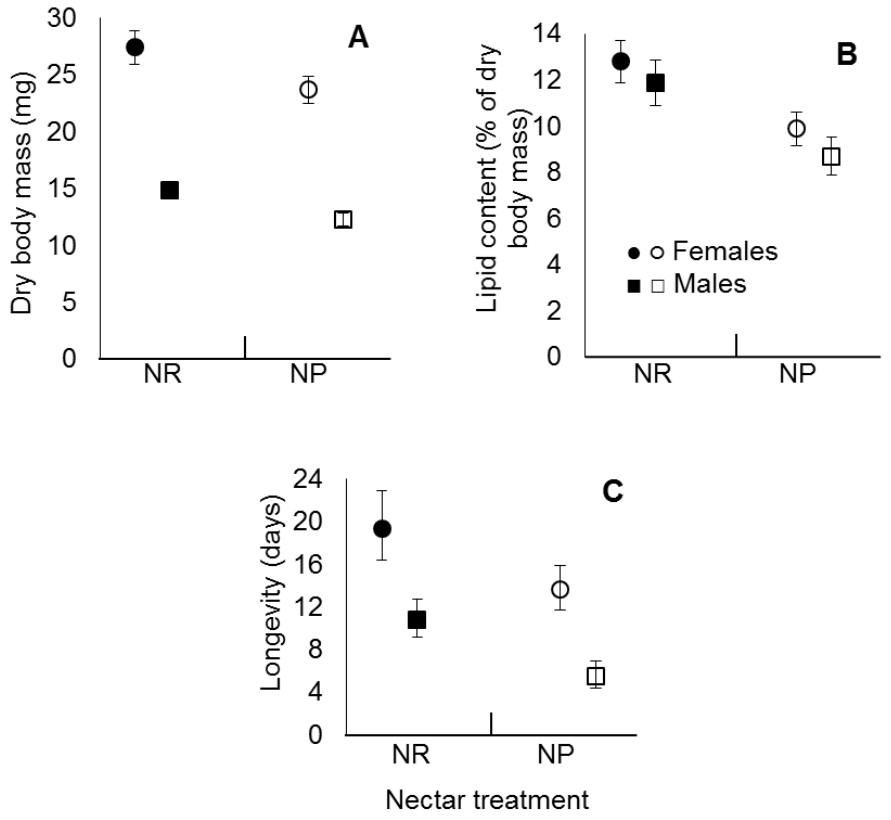

Figure 5.2. Effects of poor versus high-quality nectar availability during 48 h on the physiological state and longevity of *M. jurtina* butterflies (tested in outdoor flight cages): A) Mean dry body mass ± SEM. Butterflies (males and females) from the nectar-rich (NR) treatment were heavier after the treatment than those from the nectar-poor (NP) treatment (Nectar treatment: $F_{1, 62.1} = 7.65$, $P = 0.0075$; Sex: $F_{1, 62} = 82.72$, $P < 0.0001$; Nectar treatment x Sex: $F_{1, 62} = 0.07$, $P = 0.794$); N=67; B) Mean lipid content (% of dry body mass ± SEM). The lipid content after the treatment was significantly lower in butterflies of the NP-treatment compared to the ones of the NR-treatment in both sexes (Nectar treatment: $F_{1, 61.3} = 13.08$, $P = 0.0006$, Sex: $F_{1, 61.2} = 1.17$, $P = 0.283$; Nectar treatment x Sex: $F_{1, 62.2} = 0.1$, $P = 0.753$); N=67; C) Inverse transformation of ln(longevity) ± SEM. Butterflies of the NR-treatment lived significantly longer after the treatment than those of the NP-treatment, when kept under the same, post-treatment conditions (Treatment: $F_{1,62.4} = 8.87$, $P = 0.0041$; Sex: $F_{1,62.3} = 20.09$, $P < 0.0001$; Nectar treatment x Sex: $F_{1,62.1} = 1.03$, $P = 0.314$). N= 68.

Body mass and lipid content under nectar-poor conditions

Two days in the flight cages were sufficient to weaken the physiological condition of the butterflies of the NP-treatment significantly as they had lower body mass compared to conspecifics of the NR-treatment (mean dry body mass after treatment: NP females 23.6 ± 1.2 mg, NR females 27.4 ± 1.4 mg; NP males 12.2 ± 0.5 mg, NR males 14.8 ± 0.4 mg) (Figure 5.2a). The physiological state of the butterflies in the NP-treatment group was poorer after the treatment as they had lower lipid content than conspecifics of the NR-treatment group (% of dry body mass: NP females 9.8 ± 0.7 %, NR females 12.7 ± 0.9 %; NP males 8.7 ± 0.8 %, NR 11.8 ± 1.0 %) (Figure 5.2b), representing 2.9 ± 0.3 mg and 4.4 ± 0.4 mg for NP and NR females, and 1.5 ± 0.1 mg and 2.3 ± 0.2 mg for NP and NR males, respectively.

Decreased flight under nectar-poor conditions

Flight activity levels in the cages were lower in the NP-treatment (number of flights through window of camera per h: NP 32.0 ± 6.4; NR 61.9 ± 17.3; Mixed Model: Treatment: $F_{1,26}$ = 4.26, P = 0.049, Day: $F_{1,26.2}$ = 2.05, P = 0.16, Treatment x Day: $F_{1,26}$ = 1.68, P = 0.20), suggesting that these butterflies switched to a less energy-demanding mode of life compared to the butterflies in the NR-treatment.

Discussion

These results show that the cost of life is significantly higher when butterflies are obliged to feed on limited quantities of non-preferred nectar sources as typically is the case in NP agricultural landscapes. The butterflies in the NP-treatment faced double jeopardy as they had lower nectar intake (including water, sugars and (essential) amino acids; Boggs 2009) and they had to rely more on lipid reserves for all functions than did butterflies in the NR-treatment. Consequently, flight activity levels in the cages were lower in the NP-treatment, suggesting that these butterflies switched to a less energy-demanding mode of life compared to the butterflies in the NR-treatment. This could have, in turn, consequences for mobility and dispersal at the landscape level; local populations would actually benefit from increased mobility to track scattered resources (Baguette and Van Dyck, 2007). But as we showed, the butterflies of the NP-treatment could not maintain similar lipid reserve levels as the NR-treatment group by reducing flight activity only. Butterflies use and reallocate both their capital (lipid reserves) and income (nectar) to reproduction, survival and movement (including foraging and dispersal) (Geister et al., 2008). Nectar contributes to egg production by supplying carbohydrates and amino acids (O'Brien et al., 2004). Nectar carbon is also used for egg amino acid manufacturing with nitrogen coming from endogenous reserves (Cahenzli and Erhardt, 2012c). Not only nectar carbon plays a significant role for fecundity, but nectar amino acids also increase fecundity (Jervis and Boggs, 2005; Mevi-Schütz and Erhardt, 2005). Hence, butterflies under the NP treatment are expected to have reduced reproductive potential. We cannot exclude that observed differences in dry body mass and lipid content of the females was due to differentl egg-laying behaviours according to nectar treatment. However, should the females have oviposited more in the NP treatment, body mass and lipid content differences between the nectar treatments would then be less pronounced in males, which obviously do not lay eggs. This was not the

case in our experiment. Furthermore, ovipositions were never observed, neither by camera recording nor researcher live observations. For these reasons, it is unlikely that different oviposition behaviour according to nectar treatment would have caused the observed differences in the measured traits.

Water is the main nectar constituent (Baker and Baker, 1983). Hence, butterflies in the NP-treatment may have suffered from water stress on top of nutrient stress. However, in the wild, nectar resources rarefication also reduces water acquisition from nectar intake.

Because weather is a significant modifier of activity in flying heliotherms such as butterflies, and because mating and host plant selection depend on flight (Berger et al., 2012), lifespan is of high significance to reproductive success under variable weather conditions like in temperate zone regions. Female lifespan after the NP treatment was reduced by >20% compared to females of the NR treatment and the reduction in lifespan was even double as high in males. Females have more lipid reserves than males, which offers better scope for reallocation from reproduction to maintenance and survival. Butterflies were not able to compensate the negative effects of a stay in a NP environment by additional feeding on artificial nectar; this probably emphasizes the role of components other than water and sugars (e.g. amino acids) (Kennedy et al., 2013).

Our experiments showed evidence of significant ecological effects of poor nectar availability and acquisition on butterfly performance. Poor nectar conditions in simplified landscapes could also induce evolutionary change. As butterflies under NP conditions cannot rely on high income for breeding and dispersal, they instead have to rely more heavily on their (energetic) capital that was built up during larval development. Natural selection could favour higher levels of capital breeding and dispersal in NP environments,

which in turn could result in larger body size. The idea of evolutionary change to living under adult resource limitation warrants careful testing. Through their different quantitative and qualitative nectar supply, intensively and extensively managed agricultural landscapes could impose different selection regimes on life-history traits for species like *M. jurtina*. In mixed landscapes and given our observed relation between nectar supply and activity level or mobility, there could be consequences for dispersal between NP and NR areas. Hence, our insights may provide a mechanistic basis to better understand dispersal asymmetries at the metapopulation and landscape level, which have actually been documented in *M. jurtina* in Swedish grassland systems (Öckinger and Smith, 2007a).

Based on our results, a strong population impact of low nectar quantities and qualities of modern, simplified agricultural landscapes on widespread flower-visiting insects like *M. jurtina* and probably other grassland butterflies seems likely. Agri-environmental schemes for such flower-visiting species would benefit from going beyond the simple presence of nectar by incorporating explicitly both quantities and qualities of nectar sources (Blake et al., 2010). Flower strips in agricultural landscapes will attract local insect species, but if nectar acquisition remains poor in quantity and quality, then they represent suboptimal habitats to pollinators and their ecosystem functions. For mitigation programs, it is now warranted to consider the contributions of nectar quality and quantity independently of each other. If an NP scenario negatively affects mobility, local populations will become more dependent on immigrants from populations with access to adequate nectar sources stressing the importance of nectar-rich, (semi)natural habitats in agricultural landscapes. Our work contributes to the important efforts, and further needs, to restore networks of plant-insect mutualisms across our anthropogenic landscapes (Menz et al., 2011).

Chapter 6 - Landscape of origin affects the impact of quantitative and qualitative nectar limitation on life-history traits in a grassland butterfly

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Author contribution: Study design: J.L., R.W. and H.V.D.; Field/Lab work: J.L.; Data analysis: J.L. (with feedback by R.W. and H.V.D.); Interpretation and writing: J.L., R.W. and H.V.D. Thanks are due to Sophie Blareau for field assistance.

Abstract

Since wild flower diversity and abundance are strongly reduced in intensive agricultural landscapes, flower-visiting insects may experience limited nectar quantities and qualities. Adult insects that rely on energy-rich nectar income for flight, survival and reproduction are expected to be much more affected than insects that rely on their larval reserves. We dealt with this issue at the intraspecific level by comparing the responses of several life-history traits to different nectar regimes between butterflies originating from intensive and extensive agricultural landscapes (IL and EL). We studied the grassland butterfly *Maniola jurtina* in outdoor flight cages in which we simulated four treatments of low/high nectar quality and low/high quantity. IL individuals were heavier than EL individuals. IL butterflies survived better than EL butterflies and survival was highest in the high nectar quantity and quality treatment. EL females lost body mass in all treatments, but less so in the high nectar quantity and quality treatment. IL females were able to buffer, or even increase, their body mass in the high nectar quantity treatments, but differences with EL females disappeared under low nectar quantities (independent of nectar quality). In males, body mass losses were always larger in EL compared to IL individuals. 40% of the females showed complete reproductive failure in the low quantity/low quality treatment compared to *c*. 7% in the other treatments. In the latter treatment, realized fecundity decreased strongly in IL females, but not in EL females. Egg size was not affected in the high quality nectar treatments, but showed very different responses relative to landscape of origin in the treatments with low quality nectar. Our results showed strong effects of reduced nectar supply on fitness-related traits, but responses were different between origins with contrasted nectar regimes. We discuss the consequences for population viability and dynamics in nectar-poor landscapes.

Introduction

The intensification of agriculture has, worldwide, resulted in simplified landscapes characterized by a strong decline of ecological resources (i.e. consumables and utilities) that are essential to wild organisms, including those species that adapted previously to living in agricultural landscapes of low management intensity (Benton et al., 2002; Stoate et al., 2001). Wild flowers providing pollen and nectar are such essential resources that are much scarcer, to locally almost absent, in intensively managed agricultural landscapes (Potts et al., 2010; Tscharntke et al., 2005). Remnant fragments of flower-rich vegetation have been shown to act as source populations of insects to the surrounding landscape (Öckinger and Smith 2007, Kohler et al. 2008). Besides reduced quantities of floral resources, a different spectrum of nectar plant species is found in intensively managed compared to extensively managed landscapes that are richer in semi-natural vegetation (Marini et al., 2007; Wilson et al., 1999).

Pollinating insects are likely to face adult food limitation and altered food quality in intensively managed farmland compared to extensively managed, flower-rich grasslands. For short-living, strict nectar feeders, like many butterflies, scarcity of nectar may have a strong impact on life-history traits (e.g., longevity and fecundity; Geister et al. 2008).

Butterfly species vary in the amount and the type of resources acquired for reproduction and survival at the adult stage. Some species rely entirely on resources gathered during the larval stage (i.e. capital breeding), whereas other species depend on resources to be acquired by active adult feeding (i.e. income breeding) (Stephens et al., 2009). In typical nectar-feeding butterfly species, most nitrogen is acquired at the larval stage, and carbohydrates used for energy demanding activities such as flight principally come from the adult diet (Boggs and Freeman 2005).

Butterflies may experience spatial and temporal variation in nectar supply. Climatic factors, but also vegetation management, affect the presence, abundance and phenology of flowering species (Erhardt and Mevi-Schütz 2009). Anthropogenic systems like agricultural regimes may impose different selection pressures on wild organisms, leading to rapid evolution (Sih et al. 2011). Although many Lepidoptera rely on nectar or other food in the adult stage, evolution has also produced species that do not feed at all as adults (Tammaru and Haukioja 1996). However, at the intraspecific level, nectar-poor, intensively managed agricultural landscapes could select for life styles that depend much less on adult income feeding compared to populations in nectar-rich, extensively managed agricultural landscapes.

Direct ecological responses of butterflies to variation in nectar regime are well documented. Quantitative restriction in nectar intake reduced female fecundity, but not lifespan (Boggs and Ross, 1993; Niitepõld et al., 2014). Egg provisioning decreased with age in food limited females (Karl et al., 2007). In several butterfly species, females used carbon from adult diet for egg production (O'Brien et al., 2004). Nectar quality may also affect life-history traits (Geister et al., 2008). Together with water, sugars are the main constituents of nectar; their types and concentrations vary among flower species (Baker and Baker, 1983). When fed with nectar of high sugar concentration, butterfly species lived for longer (Braby and Jones 1995, Cahenzli and Erhardt 2012b) and both longevity and fecundity may increase as well (Hill 1989, Murphy et al. 1983). Nectar amino acids also play a significant role for female butterfly fitness (Jervis and Boggs, 2005). Nectar amino acids increased fecundity in some species (Mevi-Schütz and Erhardt 2005, Cahenzli and Erhardt 2012a, 2013), but had no effect in others (Molleman et al. 2008, Grill et al. 2013). Nectar intake may also constrain egg size (Murphy et al. 1983).

Butterflies facing temporary reduced nectar supply are expected to respond with altered fitness. Selection pressure often results from a combination of stressing environmental factors; hence isolating the adaptive response to a single cause is often complicated in natural populations. Under restricted adult diet selection regime, butterflies could shift to a higher degree of capital breeding (Tammaru and Haukioja 1996) and hence be heavier at emergence. Other possible adaptations may involve changes in resources allocation patterns, to allow better resistance to food stress.

We recently demonstrated experimentally the direct negative effects of poor nectar supply for young adults of the common grassland butterfly *Maniola jurtina* (**Chapter 5**). The poor nectar supply corresponded to the quantity and quality of nectar as typically observed in meadows of intensively managed agricultural landscapes. The experiment included butterflies of nectar-rich grassland origin only, which were heavily impacted in terms of survival, activity and physiological condition. Here, we tested the effect of nectar quantity and quality on *M. jurtina* adult males and females from extensively managed and intensively managed agricultural landscapes. We simulated the four combinations of low/high nectar quantity and quality in outdoor flight cages to quantify the impact in terms of life-history consequences in butterflies of different landscape origin. Nectar quantity restriction is expected to reduce longevity and/or fecundity depending on the allocation trade-offs. Butterflies from the intensive, flower-poor agricultural landscape are expected to be less affected by nectar limitation. Nectar quality restriction is expected to impact females more than males, because of their nutrient investment in egg manufacturing. Because nutrient pools could vary between the landscapes of origin, we expect butterflies from extensively managed agricultural landscapes (i.e., flower-rich grasslands) to be more strongly impacted by nectar quality reduction assuming they rely much more on an income breeding strategy than conspecifics from the intensively managed

landscape. When nectar quality and quantity are simultaneously reduced, we expect negative consequences for both longevity and fecundity in all butterflies.

Materials and methods

Model species

The meadow brown (*Maniola jurtina*) is a widespread European butterfly. It is univoltine and occurs in grassland habitats and caterpillars feed on a variety of grasses. Adults visit a range of floral nectar species, but show a preference for knapweed *Centaurea jacea* in our study sites (J. Lebeau, unpubl. data). In the absence of the preferred species, they feed on alternatives, including red clover (*Trifolium pratense*) or thistle species (*Carduus, Cirsium*). One to a few days after emergence, females start to lay eggs (Grill et al. 2013). Egg maturation happens progressively; there are no mature eggs at adult emergence (Scali, 1971). Females lay eggs one by one (Scali 1971). Eggs have the shape of a truncated ribbed cone.

Butterfly sampling

In the summer of 2012, freshly emerged wild butterflies were collected from two Belgian agricultural landscapes that differed in management intensification (Figure 3.3): an extensively managed landscape (EL) (located in the Famenne region; center: 50°08'22" N, 5°03'01" E; area: ± 20 km²) and an intensively managed landscape (IL) (located in Walloon Brabant; center: 50°35'02" N, 4°39'09" E; area: ± 40 km²). Both areas were separated by 70 km. In each landscape, butterflies were sampled in several sites distant of each other from 500m to 10 km. The first landscape (i.e. extensively managed) mainly consisted of grassland and woodland. Types and intensity of management varied among sites, but a large part is composed of relatively extensively managed hay meadows and other grasslands favorable to several grassland butterfly species. The intensively managed landscape mainly consists of agricultural fields and a small proportion of grasslands in the form of roadsides, intensively grazed pastures or productive meadows that are mown several times a year. The sampling area was larger in the IL landscape compared to the EL landscape because

of the lower butterfly abundance. We performed repeated trials with young field-caught EL butterflies and IL butterflies in July and early August 2012 (5 and 6 trials (among which two with only some of the nectar treatments) in total respectively). On the day of launching a trial, we collected 48 males and 48 females of *M. jurtina* in one of the two landscape types and assigned them to the four treatments (i.e. 12 butterflies of each sex for each treatment). We captured only young individuals with a hand net; age was estimated based on wing wear (Watt et al., 1977). Before releasing them in the cages, individuals were weighed (Ohaus Explorer balance; accuracy ± 0.1 mg) and marked on their ventral wings using a fine, non-toxic permanent marker. A total of 960 butterflies were used in our experiment, 480 from IL and 480 from EL.

Experimental setup

We used two identical semi-cylindrical outdoor cages (plastic greenhouses; dimensions l x w x h = 9.0 m x 3.7 m x 1.8 m (at maximal height) that were separated each in two. Hence, we had four equally-sized compartments. We applied four nectar treatments: i) high abundance of preferred nectar (i.e. 100 inflorescences of *C. jacea*, C100), ii) low abundance of preferred nectar (i.e. 10 inflorescences of *C. jacea*, c10), iii) high abundance of non-preferred nectar (i.e. 100 inflorescences of *T. pratense*, T100) and iv) low abundance of non-preferred nectar (i.e. 10 inflorescences of *T. pratense*, t10). Treatments were assigned randomly to the four compartments at each new session. Flowers of *T. pratense* contain slightly more nectar than those of *C. jacea* (mean nectar volume per single flower ± SEM: 0.21 ± 0.02 µl and 0.18 ± 0.02 µl; Rusterholz and Erhardt 2000), but they have similar numbers of simultaneously available flowers per flower head (23.2 ± 1.1 and 21.1 ± 0.8; unpubl. data). Nectar of *C. jacea* is known to be more suitable for butterflies; sugar concentration, sucrose:(glucose+ fructose) ratio and amino acid concentration are higher and flowers of *C. jacea* have

a wider corolla, which is usually preferred by butterflies (Rusterholz and Erhardt, 2000). Flower heads used in the nectar treatments were collected in the field. Two days before their use, we precluded any insect visitor by covering the flowers with exclosure bags to ensure maximum nectar content. On the day of their use, stems were cut, placed in water filled recipients. Within 2 hours of the launch of the trial, we cut every stem as to leave a 10-cm stem under the flower head, removed the exclosure bags and placed them in the flight cages, in small 10-cm high plastic tubes filled with water and half buried in the ground. They were placed at regular intervals on the floor. Prior to each repetition, the bottom of each flight cage was abundantly sprinkled with water to ensure a minimum level of relative humidity during the experiment. Butterflies were released in the cage compartments in the evening (20:00h local time) and stayed there for 48 h. Ambient temperature and relative humidity were measured every 30 min in each cage compartment (Thermoprobes HOBO Pro v2 Temp/RH U23-001). Mean diurnal temperature during observations was 22°C and mean relative humidity 65%.

Body mass, longevity and fecundity

After 48 h, we recaptured the surviving individuals, collected the dead ones and noted eventually missing individuals (e.g. due to occasional spider predation). Survivors were weighed again. Half of the surviving males (max 6 per treatment and trial) and a third of the surviving females (max 4 per treatment and trial) were used to assess longevity, and potential and realized fecundity were recorded in females. The individuals were placed in small individual cages (25 x 25 x 25 cm) in a climate room (light/dark: 16 h/8 h; 25°C during the day and 14°C at night) and had access to water-diluted honey on cotton. Positions of the cages in the climate room were changed daily and the cotton renewed. Dead individuals were removed and stored in the freezer (-20°C). For females, eggs were counted and removed. Five eggs

per female per day were randomly chosen and photographed with a Panasonic Super Dynamic WV-CP450 camera through an optical microscope Leica MZ6. Viability of the eggs was scored after visual examination of the picture. The exact position of the egg when it was photographed varied somewhat. Therefore, we took this into account (3 categories according to position). Egg size was measured from size-calibrated digital pictures (ImageJ, freely available on http://rsb.info.nih.gov/ij/). Finally, the abdomens of dead females were dissected in order to count the remaining eggs. The eggs laid during the experiment were brought back to the field sites.

Statistical analyses

We analyzed the factors of interest (Origin, Treatment and the interaction effect) relative to initial body mass, mass loss during experiment, survival, post-experiment longevity and fecundity variables. We used General Linear Models (GLM), Generalized Linear Models (GzLM) or Generalized Linear Mixed Models (GLMM). Final models were obtained by backward selection. We removed interaction term when non-significant to obtain the final model. We considered as fixed factors in the initial model: origin (intensive or extensive), treatment (C100, c10, T100 and t10) and the interaction effect. Analyses were performed separately for males and females. Whenever needed, data were log transformed to reach normality of residuals. We tested for differences in fresh mass before the experiment between the two origins using a GLMM on log-transformed data; with date of capture as a random factor and we verified that no biases were introduced between nectar treatments.

Survival during treatment was analyzed using a GzLM with binomial distribution and taking into account only the individuals that were effectively recollected at the end of the treatment. We subtracted initial mass from the mass after the treatment to obtain the change in mass

during nectar treatment. We used residuals of change in mass by initial mass to remove the effect of initial mass prior to the analysis and worked with GLMM, adding temperature in the flight cage during the experiment as a random factor. Longevity after nectar treatment was analyzed by fitting residuals of log (longevity+1) by initial mass with a GLM. Fecundity was analyzed in females only. We first analyzed the proportion of egg-laying females with a GzLM with binomial distribution. Realized fecundity (i.e., total number of eggs laid) and potential fecundity (calculated as the sum of the number of eggs laid and the number of oocytes remaining in the ovaries at death) were studied in two different ways. First, they were analyzed with a GLM applied to log-transformed data (log(Y+1); in a second analysis, we used the residuals of log-transformed data by log(longevity+1) to remove the effect of longevity, and we applied the same GLM. For the calculation of the residuals, we used log-transformed data for Y and X since the relation had a linear shape but fecundity parameters were not distributed normally. The proportion of potential fecundity that was actually transformed into real eggs (realized fecundity) was analyzed with a GLMM with binary distribution error (laid or remaining in the abdomen), adding “individual” as random factor.

The proportion of viable eggs was analyzed by a GLMM with a binary distribution, incorporating “individual” and “day after treatment” as random factors. Egg size of viable eggs was analyzed with two GLMM. The first modeled egg size differences relative to origin and nectar treatment, adding to the initial model “egg position” and “individual” as random factors. It was then separated into two analyses, per origin, and we analyzed the evolution of egg size with female age by adding to the previous model the continuous factor ‘number of days after treatment’, used as a proxy for female age.

All statistical analyses were performed with SAS Enterprise Guide 5.1 (SAS Institute Inc., 2012), except for the quasi GLM that was performed in R (R Development Core Team, 2013). Covariance parameters were estimated by REML (residual maximum likelihood) and RSPL (Random Solution Pseudo-Likelihood; SAS Insitute Inc. 2012) for mixed models and mixed binomial regressions, respectively. Denominator degrees of freedom for the tests of fixed effects in GLMM were estimated by the Satterthwaite method. Means are given ± S.E.M.

Results

Initial fresh mass

IL butterflies were heavier at capture than EL butterflies (IL females: 102.1 ± 1.0 mg; EL females: 97.7 ± 0.9 mg; IL males: 55.3 ± 0.5 mg; EL males: 53.1 ± 0.6 mg) (GLMM on mass at capture date: Females: Origin: $F_{1,471}$ = 15.52, P < 0.0001; Treatment: $F_{3,474}$ = 1.65, P = 0.18 – Males: Origin: $F_{1,475}$ = 31.8, P < 0.0001; Treatment: $F_{3,474}$ = 1.38, P = 0.25).

Adult survival

Overall, females survived the treatments better than males (N total = 733 individuals). Female survival ranged from 70.5% (EL c10) to 100% (IL C100). A greater proportion of EL females died during the experiment compared to IL females (13.6% vs. 4.5%), and for both landscapes of origin, survival was higher in the C100 treatment (% survival: EL C100: 96.4; EL c10: 70.5; EL T100: 89.8; EL t10: 86.; IL C100: 100; IL c10: 93; IL T100: 93; IL t10: 96.2) (GzLM with binomial distribution; Origin: χ^2_1 = 12.74, P = 0.0004; Treatment: χ^2_3 = 18.2, P = 0.0004). EL males survived less well than IL males (44.2% vs. 59.5%), and both origins survived better in the high nectar quantity treatments (% survival: EL C100: 64.3; EL c10: 34.4; EL T100: 63.6; EL t10: 13.6.; IL C100: 76; IL c10: 37.5; IL T100: 71.8; IL t10: 44.8) (GzLM with binomial distribution; Origin: χ^2_1 = 4.41, P = 0.036; Treatment: χ^2_3 = 34.39, P < 0.0001).

Change in body mass

In total, 355 females and 142 males survived the nectar experiments and were used to analyze change in body mass; 124 females and 89 males were used for longevity and fecundity measurements. Heavier individuals lost relatively more of their body mass than did lighter individuals of both sexes (Linear regression of change in mass by mass at capture (parameter estimate ± S.E.); Females: -0.18 ± 0.04, $F_{1,353}$ = 21.68, P < 0.0001 – Males: -0.41 ± 0.07, $F_{1,140}$ = 36.22, P < 0.0001). EL females lost body mass in all

treatments, but this loss was six times lower in the C100 treatment compared to the other treatments. IL females responded differently. They were able to buffer, or even increase, their body mass in the high quantity treatments (C100 and T100), but not in the two low quantity treatments for which they showed body mass losses similar to EL females (Figure 6.1a; GzLM on residuals of change in mass by mass at capture: Origin: $F_{1,376}$ = 3.95, P = 0.047, Treatment: $F_{3,375}$ = 18.8, P < 0.0001, P x T: $F_{3,375}$ = 7.01, P = 0.0001). Contrary to females, male body mass was not affected by the treatment, but IL males were systematically better to buffer mass loss than were EL males (Figure 6.1b; GzLM on residuals of change in mass by mass at capture: Origin: $F_{1.157}$ = 10.63, P = 0.001, Treatment: $F_{3,156}$ = 1.1, P = 0.353).

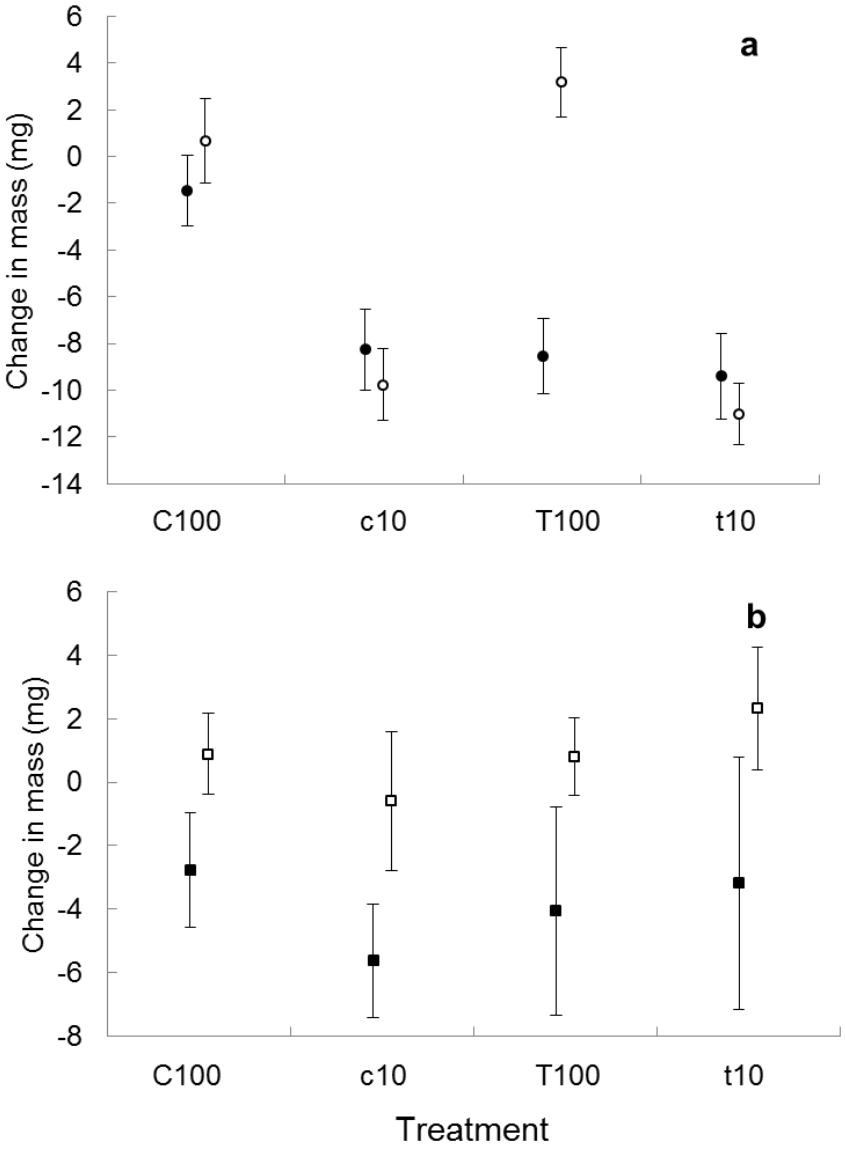

Figure 6.1. Change in total body mass after treatment (means ± S.E.M) in females (a) and males (b). Open symbol: IL origin, Filled symbol: EL origin. Positive values indicate a gain in body mass, negative ones a loss.

Post-treatment longevity

Female longevity was not influenced by initial body mass (Linear regression of log (longevity+1) by mass at capture; P = 0.15). After the experiment, females survived up to 79 days (EL female in T100 treatment), but the average female longevity was much lower (13.9 ± 1.4 days). IL females in the t10 treatment had the shortest longevity (4.8 ± 0.6 days), but there was no overall origin effect across the treatments (Figure 6.2a, GLM on residuals of log (longevity) by initial mass: Origin: $\chi^2_1 = 1.38$, $P = 0.24$; Treatment: $\chi^2_3 = 11.66$, $P = 0.009$). Heavier males lived longer after the experiment (Linear regression of log (longevity+1) by mass at capture; parameter estimate ± S.E.: 0.012 ± 0.005, $P = 0.03$). EL males lived longer after the treatment than IL males, but post-treatment longevity varied with nectar treatment in a different manner for each origin (Figure 6.2b, GLM on residuals of log (longevity) by initial mass: Origin: $\chi^2_1 = 7.44$, $P = 0.006$; Treatment: $\chi^2_3 = 20.72$, $P = 0.0001$; O x T: $\chi^2_3 = 7.9$, $P = 0.048$). There were no differences between IL and EL males in both the C100 and t10 treatments, but EL males lived longer than IL males in the two intermediate treatments.

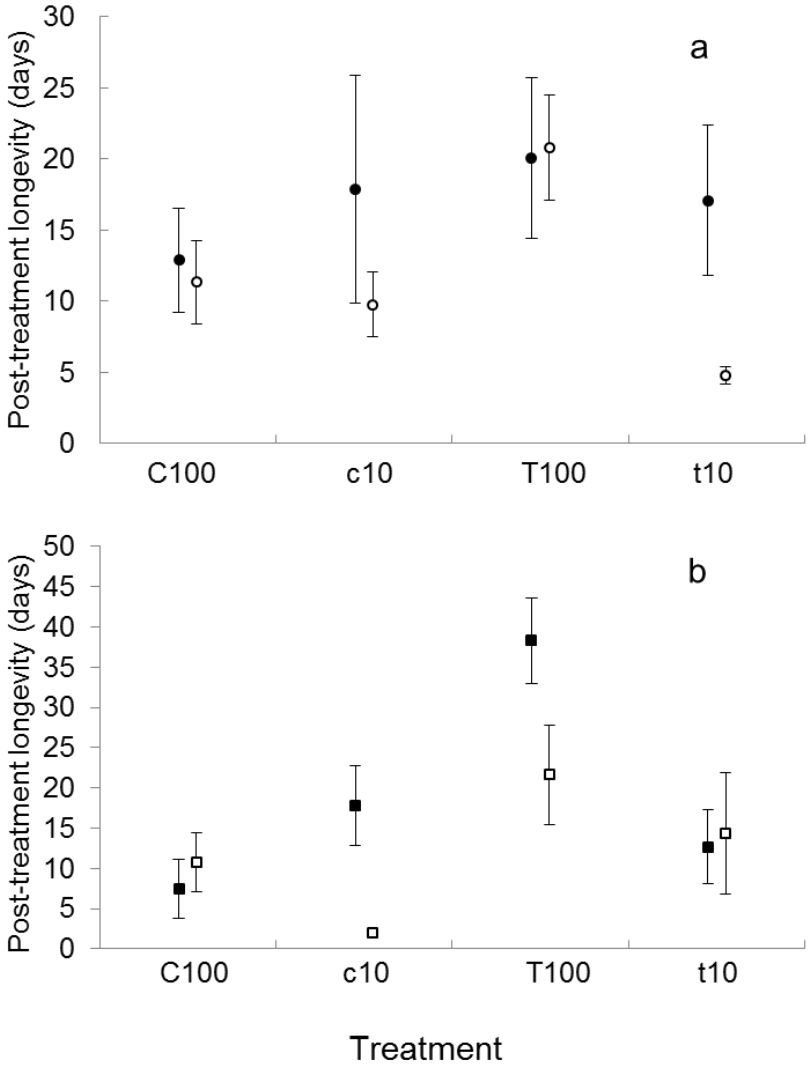

Figure 6.2. Longevity after the treatment (means ± SEM) of females (a) and males (b). Filled symbols: EL origin; open symbols: IL origin.

Female fecundity

The proportion of females that did not lay any eggs at all differed significantly among treatments, but it was independent of origin (Logistic regression: Origin: $\chi^2_1 = 0.02$, $P = 0.9$; Treatment: $\chi^2_3 = 16.26$, $P = 0.001$). Reproductive failure was quite high in the t10 treatment (40%), but much lower in the others (7%). After the nectar treatment, a total of 9832 eggs were laid by the 124 females that were studied for longevity and fecundity. The overall lifetime number of eggs laid was 79.3 ± 9.9 eggs (max: 513 in an EL female after C100 treatment).

Realized fecundity was not affected by origin and treatment (Table 6.1; Figure 6.3a). However, whereas EL females laid similar numbers of eggs in all treatments (ANOVA on log (realized fecundity +1) EL females only: Treatment: $F_{3,50} = 0.63$, $P = 0.59$), realized fecundity decreased dramatically in IL females after the t10 treatment (ANOVA IL females: $F_{3,66} = 8.67$, $P < 0.0001$). Realized fecundity increased strongly with longevity (Linear regression of log (realized fecundity +1) by log (longevity): parameter estimate ± S.E.: 3.00 ± 0.29, $F_{1,122} = 102.32$, $P < 0.0001$). For the same longevity, both origins had the same pattern of realized fecundity under the different treatments, i.e. they laid more eggs in the C100 nectar treatment (and even more than expected by their longevity), intermediate numbers in the c10 and T100 nectar treatments, and low numbers in the t10 nectar treatment (even less than expected by their longevity) (Table 6.1; Figure 6.3b).

Potential fecundity was the same for both origins and all treatments (Table 6.1), whether longevity was taken into account or not (Figure 6.3c and d). Similar to realized fecundity, potential fecundity of IL females tended to decrease in the t10 treatment, whereas this was not the case in EL females (ANOVA on log (potential fecundity): IL females: $F_{3,61} = 2.47$, $P = 0.07$; EL females: $F_{3,50} = 0.29$, $P = 0.83$). EL and IL females laid on average similar

proportions of their potential egg load (overall mean: 57.3%); this proportion tended to be smaller in the t10 treatment (GLMM with binary distribution: Origin: $F_{1, 98.14}$ = 0.14, *P* = 0.71; Treatment: $F_{3, 99.3}$ = 2.31, *P* = 0.08). IL females in the t10 treatment laid on average 17.2 % of their eggs, whereas EL females in the C100 treatment and IL females in the T100 treatment laid 71.9 % and 73.2 % of their eggs, respectively.

Table 6.1. Fecundity-related traits relative to landscape of origin and nectar treatment. Fecundity did not differ between origins, but treatment influenced realized fecundity (with or without taking into account longevity). GLMs applied to realized and potential fecundity.

Variable	Effect	DF	χ^2	*P*
Realized fecundity in absolute values	Origin	1	74	0.39
	Treatment	3	17.8	0.0005
Realized fecundity as residuals against longevity	Origin	1	0.11	0.74
	Treatment	3	15.37	0.0015
Potential fecundity in absolute values	Origin	1	0.96	0.33
	Treatment	3	4.16	0.24
Potential fecundity as residuals by longevity	Origin	1	0.53	0.47
	Treatment	3	0.35	0.95

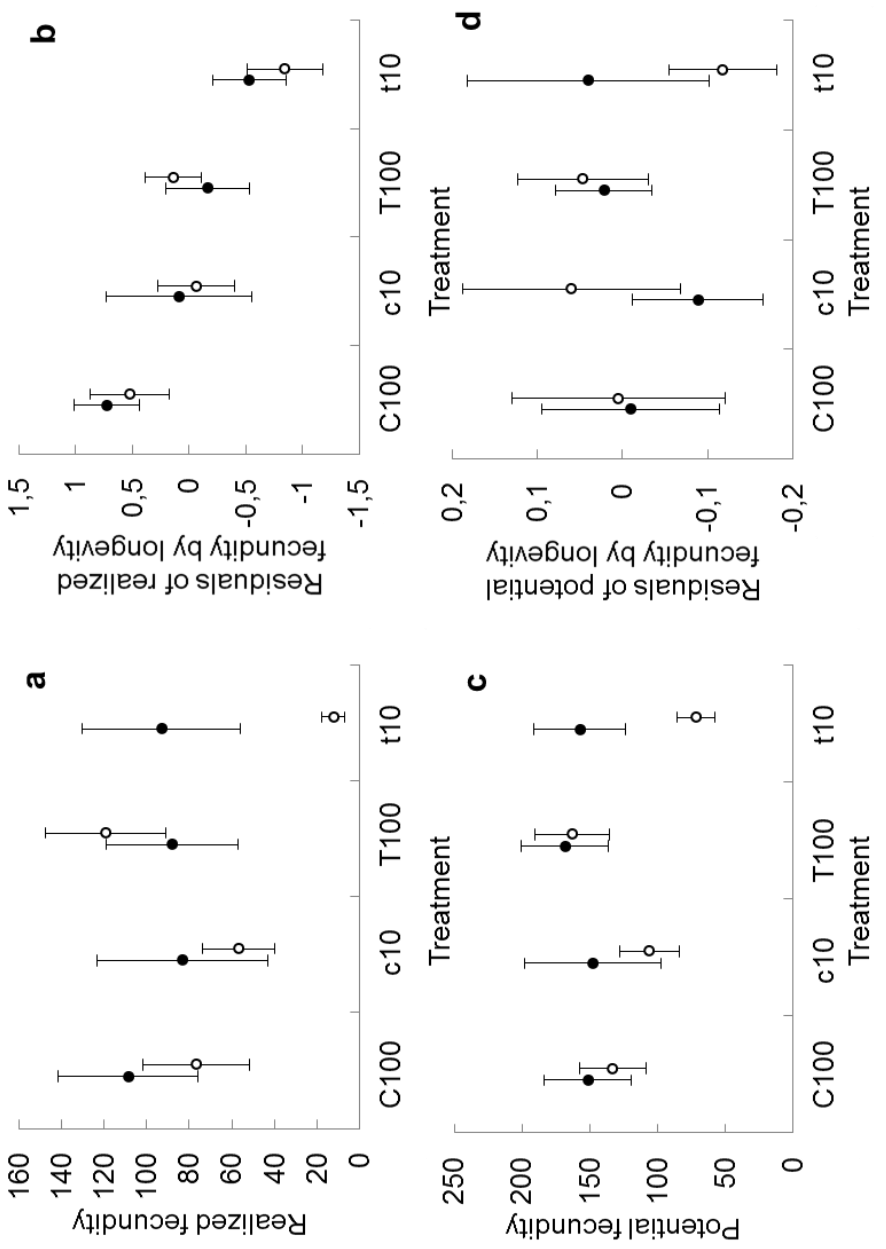

Figure 6.3: Female fecundity of EL individuals (filled symbols) and IL individuals (open symbols) relative to treatment: a) Realized fecundity (total lifetime number of eggs laid); b) Residuals of log (total number of eggs laid+1) by log (longevity); c) Potential fecundity (realized fecundity + number of oocytes remaining in the ovaries after dead) and d) Residuals of log (potential fecundity) by log longevity. Nectar treatments: C100: 100 *C. jacea*; c10: 10 *C. jacea*; T100: 100 *T. pratense*; t10: 10 *T. pratense*.

Egg size and viability

92.6% of eggs were considered viable (N = 2930 eggs). Egg viability did not differ between origins and among treatments (GLMM with binary distribution: Origin: $F_{1, 86.78} = 0.4$, $P = 0.53$; Treatment: $F_{3, 82.06} = 0.36$, $P = 0.78$). Mean egg size was influenced by treatment, but in an origin-specific manner. Egg sizes were identical for both origins in the treatments providing good quality nectar (C100 and c10), but IL females produced much smaller eggs than EL females in the t10 treatment. However, the reverse pattern was found in the T100 treatment. (GLMM: Origin: $F_{1, 93.2} = 0$, $P = 0.99$; Treatment: $F_{3, 92.7} = 1.67$, $P = 0.18$; O x T: $F_{3, 92.7} = 4.09$, $P = 0.009$; Figure 6.4). Egg size was independent of age in EL females (GLMM: Treatment: $F_{3, 35.3} = 1.11$, $P = 0.36$; Days since treatment: $F_{1, 1341} = 0.16$, $P = 0.69$), although they laid larger eggs when older in both *Trifolium* treatments (T100 and t10). However in IL females, egg size increased over time for all treatments but C100, and the amplitude of this increase was six times stronger in the t10 treatment (GLMM: Treatment: $F_{3, 69.5} = 5.11$, $P = 0.003$; Days since treatment : $F_{1, 164} = 6.15$, $P = 0.01$; T x D: $F_{3, 348} : 2.75$, $P = 0.04$).

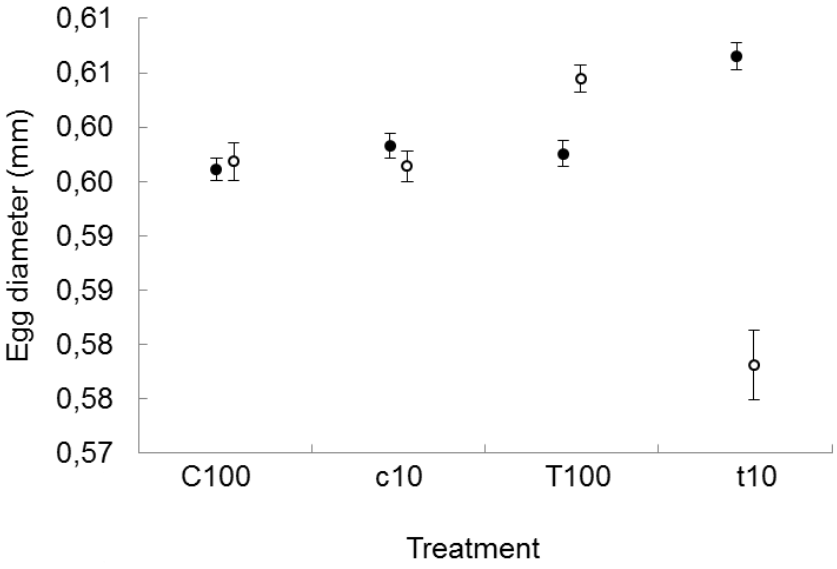

Figure 6.4. Mean egg diameter (± SEM) in EL females (filled symbols) and IL females (open symbols) relative to nectar treatment. Mixed model: Origin: $F_{1, 3690}$ = 0.31, *P* = 0.58; Treatment: $F_{3, 3690}$ = 1.53, *P* = 0.2; P x T: $F_{3, 3690}$ = 5.04, *P* = 0.0017. Assuming a sphere, a diminution of 15 µm (from 595 µm to 580 µm) in diameter corresponds to a diminution of 7.3% in volume.

Discussion

Our experimental study showed clear effects of both nectar quantity limitation and nectar quality limitation on life-history traits in *Maniola jurtina*. These effects were sex-specific and differed relative to the landscape of origin. IL butterflies had better capacities to buffer the effect of a reduction in nectar quality or quantity. Both IL and EL butterflies performed compensatory feeding after the treatment, but when both nectar quantity and quality were simultaneously reduced, IL females were no longer able to compensate and were severely affected in terms of longevity and fecundity. The direct effects of adult food limitation on body mass by nectar quality and quantity treatments were less pronounced in IL butterflies compared to EL butterflies. However, patterns were different for longevity and fecundity. IL females were more negatively affected when both nectar quality and quantity were simultaneously reduced, whereas EL females maintained similar levels of longevity and fecundity in all treatments. Males showed yet another pattern for longevity with no differences between IL and EL in the most and the least favorable treatment, but EL males performed better than IL males in the two intermediate treatments.

Effect of nectar treatments

Any deviation from the preferred nectar choice (i.e., reduction of quantity, change of quality or both), except for IL females in the T100 treatment, resulted as predicted in negative effects on females. These results confirm that meadow browns in the wild direct their flower preference towards the food source that maximizes fitness (Pyke, 1984), here evaluated by survival and mass loss. More individuals survived when nectar supply was favorable. EL females were affected by reductions in nectar quantity and quality, whereas IL females only lost weight when nectar quantity was limited. These results could be explained by different individual life histories and

experiences with flower species. EL females might have been reluctant to feed on a non-preferred nectar source, having already experienced *C. jacea* in the field before being caught. Hence, the quantity of ingested nectar might have been smaller in *T. pratense* treatments than in *C. jacea* treatments. On the other hand, IL females probably foraged in all nectar treatments, those in the *T. pratense* treatments facing a familiar nectar source and those in the *C. jacea* treatments discovering a 'new', but probably innately preferred one. Other generalist flower-visiting insects are known to adjust their flower preference depending on the local ecological context (Gegear et al. 2007). IL females may have fed much more than EL females when nectar became suddenly available with our experiment, whereas EL females probably continued to feed at the same rate as before capture, at least in the *C. jacea* treatments. On the other hand, IL butterflies could show a different level of activity. A lower activity level would imply reduced flight activity, hence less energy expenditure and lower mass loss during the treatment. Other flight-related traits have been demonstrated to vary with habitat fragmentation (Hanski et al., 2004; Mennechez et al., 2004; Schtickzelle et al., 2006). Butterfly habitat is usually more fragmented in intensively managed agricultural landscapes. Hence, IL butterflies could be generally less active (but not less mobile, e.g. Fischer et al. 1999) than EL butterflies, or more economic in their energy expenditure. Differences in body mass loss among females relative to nectar treatment could also be related to different oviposition rates during the stay in the flight cage, but this is unlikely. We have actually never observed oviposition in the flight cages during these 48 h, although it may have happened. Moreover, in that case, we should have observed lower realized and potential fecundity in the groups that supposedly laid more eggs (hence lost more weight) in the flight cages, which was not the case. Water is the main constituent of nectar (Baker and Baker 1983) and water availability could also have caused differences in life-history traits of the butterflies. In that case however, we

would expect nectar quantity, and hence water quantity, to be more important than nectar quality, which was not the case.

Independently of nectar treatment, EL females had similar longevity and fecundity. Only the proportion of ovipositing females was smaller when both quality and quantity of nectar were reduced. As butterflies were kept in individual cages after the 48 h nectar treatment, they had the opportunity of compensatory feeding through unlimited access to water-diluted honey. Absence of differences in measured carry-over life-history traits on longevity and fecundity for the different nectar treatments indicate that females were able to compensate for the stress caused by the applied initial limitation in nectar quality and/or quantity. In a previous experiment using the C100 and t10 treatments only, EL females did not compensate for the stress caused by the low nectar quality and quantity (Lebeau et al., submitted). IL females were not tested in our previous study and butterflies were kept in groups of 10 individuals per cage, under outdoor conditions, while in the current experiment they were in individual cages in a climate room. The immediate consequences for survival and body mass loss of 48 h in the t10 treatment were similar in both our previous and current study. Hence, differences in the ability to compensate can only be explained by the differences in post-treatment conditions. First, competition for access to food may have happened in the collective cages and therefore impaired the butterflies of the t10 treatment even more. Second, outdoor conditions may have created greater difficulty to compensate by active foraging behavior in females of the t10 treatment. Compensatory feeding in response to food stress has been reported in several insects (Lee et al. 2008, Maklakov et al. 2008, Morehouse and Rutowski 2010). Adults of the butterfly Arashnia levana were able to compensate for larval food stress (Mevi-Schütz and Erhardt 2005).

IL females had similar longevity and fecundity across treatments, except in the t10 treatment in which both nectar quantity and quality were reduced. In the latter case, longevity was reduced by more than half, and the butterflies laid less than a quarter of the number of eggs in the other treatments. So, whereas they could – at least partly – compensate in the other treatments, it was no longer possible in this treatment. Although their egg size increased more rapidly over time than for females from the other treatments, they were nevertheless not able to produce eggs as large as in the other treatments. The observation that EL females were able to compensate whereas IL were not in the worst nectar treatment, can be explained by two non-mutually exclusive hypotheses. First, EL females were better able to buffer against adult food stress as they already foraged on *C. jacea* prior to the treatment. We worked with young wild-caught individuals, and such highly valuable nectar sources were hardly (or not) available to IL females on their field sites, although they were heavier at capture. Secondly, because considerable lipid reserves still remained in their abdomen at death (Lebeau, unpubl. data), IL females in t10 treatment may have faced a shortage of essential components (e.g. amino acids) that could not be compensated by the artificial nectar. Since life-history traits were not affected when only nectar quantity was reduced, we conclude that nectar differences between the preferred flower species (i.e., *C. jacea*) and other non-preferred flower species (i.e., *T. pratense*) are reflecting nutritional needs of *M. jurtina* females.

The dramatic declines of longevity and fecundity in IL females under low quantity and low quality treatment raise questions about population viability in intensive agricultural landscapes. Obviously, nectar feeding insects in the wild will be rarely restricted to a single alternative nectar source, but still this result appears alarming. IL butterflies were not immune to the effects of low nectar supply. If wild populations in intensively managed agricultural landscape have to deal with such a reduction in

nectar supply, their low survival and fecundity would have to be compensated by better larval survival or better hatching success for example. In the absence of compensation at any stage of the life cycle, local populations may become sink populations that depend for their survival upon emigration from populations in (nearby) extensively managed sites (Pulliam, 1988), as was shown for butterflies and bumblebees (Öckinger and Smith, 2007b).

Landscape of origin

IL butterflies were heavier at capture than EL butterflies. This is congruent with the idea of selection for capital breeding, since IL individuals can count much less on adult income than EL individuals. This result now warrants further testing by common garden breeding experiments. At the proximate level, several mechanisms may be involved. Developmental conditions for larvae in intensive areas could drive individuals to achieve higher body mass, through different microclimatic conditions, different host plant quality (e.g., due to fertilizer use) or other environmental factors (Goverde and Erhardt, 2003). In intensive agricultural landscapes, regular fertilizer application also causes eutrophication in nearby semi-natural habitat remnants. Hence, larvae in intensively managed landscapes are more susceptible to develop under conditions with fertilized host plants. Climatic conditions in highly fragmented intensive agricultural land are generally warmer (Merckx et al., 2008) and dryer than semi-natural habitats at the same latitude, but with more extremes (Suggitt et al., 2011). Although mean temperature is slightly warmer on intensively managed land, which could drive higher mass in butterflies, clear predictions on the effects of climatic conditions in intensively managed areas during development are currently difficult to establish because extreme temperatures are often detrimental for larval development (Steigenga and Fischer, 2009).

Despite the higher mass at capture, IL individuals did not have higher fecundity than EL butterflies. This further reinforces the hypothesis about their ability of compensatory feeding, because EL females were able to achieve similar longevity and realized fecundity than IL ones, in spite of their lighter weight at capture.

Males: nectar quantity matters for direct survival, whereas nectar quality for longevity

Two days of nectar quantity restriction were sufficient to reduce male survival rate to a mere third. As for females, IL males survived better and even gained weight during the experiment, whereas EL males lost weight in all treatments, probably for the same reasons as discussed above for females. On the other hand, among survivors, EL males lived longer than IL ones, but only in the intermediate treatments (c10 and T100). EL males might be favored by their individual life history prior to our experiment, having probably had more occasions to acquire nectar, and in a less energetically costly way, i.e. without flying for long distances to find nectar sources, as IL males probably did. From another angle, different activity levels in the flight cages during the treatments could explain differential post treatment longevity, although we would then expect similar patterns in mass loss, which was not the case.

Interestingly, the patterns of male post-treatment longevity surprised somehow with both origins living longer when fed with *T. pratense* during the treatment. Males of *M. jurtina* often occur in zones where females are abundant (Brakefield, 1982a) and may therefore forage opportunistically on the nearby flowers that are particularly preferred by females, but this may not reflect male optimal nectar choice independent of female presence. The relevance of the extent to which we observed increased lifespans (e.g. up to more than 20 days) in captivity for butterflies in the wild remains questionable.

Favorable (or even optimal) floral nectar species may differ between males and females in *M. jurtina* and other butterfly species. Male adult behavior is strongly focused on mate location and most of their energy is therefore spent in flight to intercept receptive females. Females, on the other hand, have different functional time schedules as they need to locate suitable oviposition sites, manufacture and lay eggs and therefore need additional nutrients and have different energy allocation patterns. Different foraging patterns have, for example, been shown in *Lysandra bellargus* (Rusterholz and Erhardt, 2000).

Conclusion

We showed evidence for significant consequences of a two-day nectar treatment for the remainder of the butterfly's life. Given the strong effects in individuals from flower-poor landscapes, this raises concerns about the viability of local populations in intensively managed areas without compensatory agri-environmental schemes.

As butterflies achieve their growth and development throughout the larval stages, host plant quality and accessibility are essential and play key roles in determining adult fitness (Boggs and Freeman 2005). For a long time, adult food sources have been thought to be the 'easy' or opportunistic part of butterfly ecology, and also for several other insects. However, adult food source quantity (Boggs and Ross, 1993; Niitepõld et al., 2014) and quality (Mevi-Schütz and Erhardt 2005) have been demonstrated to significantly affect life-history traits. Our present study stresses the importance of adult food resources for nectar feeding butterflies, and also the importance of flower diversity and abundance in agricultural landscapes. Future studies need to address the question of critical thresholds for nectar quantity and quality, and perform further tests on selection away from income breeding towards improved capital breeding in landscapes under intense human use.

Chapter 7 - Flight and metabolic ecology of butterflies in anthropogenic landscapes under strong resource limitation

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Author contribution: Study design: J.L., R.W. and H.V.D.; Field/Lab work: J.L.; Data analysis: J.L. (with feedback by R.W. and H.V.D.); Interpretation and writing: J.L., R.W. and H.V.D. Thanks are due to Sophie Blareau and Augustin Joiris for field and lab assistance.

Abstract:

Flight is an essential ability in many insects, including butterflies. In fragmented habitats, inter-patch movements depend on flight willingness and capacity of adult individuals. But flight is energy demanding and highly costly in environments with few and scattered energetic resources. In such environments, organisms may adopt strategies to save energy for flight at the cost of other functions. Here we tested resting metabolic rate (RMR) and flight capacities in female butterflies of two contrasted landscapes of the butterfly *Maniola jurtina*. One landscape consisted in flower-rich, extensively managed agricultural land and the other in an intensively managed, flower-poor, agricultural land. Nectar acquisition was not limited in the first landscape, but was much more of a challenge in the second landscape. We submitted butterflies to different experimental combinations of nectar quality and quantity for a brief period in their adult life (48h), and measured resting metabolic rate, flight performance and flight metabolic rate. Females from the intensive agricultural landscape had better flight capacities and used less energy for maintenance. Under intense food stress (i.e. when energetic resources were strongly limited), investment in flight was maintained at the expense of longevity and fecundity. Butterflies from the flower-rich landscape were more plastic in their response to variations in nectar diet. They adjusted their flight capacities to the quantity of nectar available, and slowed down their RMR when submitted to a strong food stress. In resource-poor, fragmented landscapes, flight capacities are essential for resources acquisition. Butterflies evolving in an intensively managed agricultural landscapes were not plastic (but canalized) in their flight capacities. Hence, strategies for dealing with resource limitation differed between the origins. Whereas butterflies from a flower-rich environment slowed down all activities, butterflies from resource-poor landscapes maintained higher levels of mobility. We discuss the significance of these findings in an eco-evolutionary framework of the different landscape types for *M. jurtina* butterflies.

Introduction

Much of the success of insects as a large taxonomic group relates to their ability to move around their environment by flight, which originated at least 340 million years ago as a major innovation (Gullan and Cranston, 2005). Flying insects like butterflies use flight for almost all adult activities, including mate location, host plant searching, foraging, escape from predators and dispersal. The power for flight comes from muscles operating against the rigid cuticular exoskeleton and is highly energy demanding. During flight, insects reach some of the highest mass-specific rates of aerobic metabolism known across the animal kingdom (Suarez, 2000). Energy acquisition and adequate metabolic pathways for efficient energy expenditure are of primary importance to fuel the flight machinery of the insect.

Nectar feeding butterflies use up energy acquired during the larval stage, stored in the abdomen in the form of lipids, and acquire carbohydrates from adult foraging (Boggs, 1992). Females may also get some energy and nutrients from the male's spermatophore after mating (Vahed, 1998). The extent to which butterflies rely on resources acquired at the adult stage places them along the income *versus* capital breeding continuum (Tammaru and Haukioja, 1996). Strict capital breeders do not feed at all at the adult stage, and may consequently lack functional mouthparts (Wheeler, 1996) whereas income breeders rely on adult feeding for survival and reproduction. Most butterfly species rely partially on adult resources, and nectar intake positively influences life-history traits in several species (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Niitepõld et al., 2014). Nectar is composed of water and sugars (mainly sucrose, fructose and glucose), but also amino acids (Baker and Baker, 1983, 1973). Nectar quantity, nectar sugar composition and concentration vary between flower species (Baker and Baker, 1983; Rusterholz and Erhardt, 2000; Watt et al.,

1974). Foraging behaviour will affect the quantity and quality of carbohydrate budget available for fuelling flight amongst other activities. The allocation of the energy from larval reserves and adult feeding may lead to trade-offs between somatic maintenance, reproduction, other activities like foraging and further energy storage (Boggs, 1992). Adult butterflies are able to synthetize lipids from nectar, but most typically they will lose body mass and lipids during their adult life (May, 1992; Vande Velde and Van Dyck, 2013). Resting metabolic rate (RMR), measured on quiescent individuals at a standard temperature and corrected for body mass, estimates the compulsory energy cost of self-maintenance that is central to life-history theory (Burton et al., 2011). In butterflies, the measurement of flight metabolic rate (FMR) and flight performances have attracted much attention the last few years (e.g. Mattila and Hanski, 2014; Niitepõld et al., 2009). This is particularly so, as butterflies are popular study systems in a context of dispersal, habitat fragmentation and meta-population dynamics (e.g., Hanski and Saccheri, 2006; Van Dyck and Baguette, 2012).

Anthropogenic environments strongly affect the availability of ecological resources to wild organisms, including energy resources. Intensively managed agricultural landscapes offer, for example, only limited and highly fragmented grassy, semi-natural habitats (Schweiger et al., 2005). Finding resources (both utilities and consumables , *sensu* Dennis et al., 2003) in such an environment may become challenging as these resources are rare and typically scattered across the landscape. Due to the use of fertilizers and agro-chemicals (Robinson and Sutherland, 2002) the quality of the scarcely present resources may alter as well, for instance changes in the assemblage of flowering plants in intensively managed compared to extensively managed landscapes. Butterflies in such fragmented agricultural landscapes may be forced to cover longer distances to complement their resource needs (Ouin et al., 2004) if essential resources are not all co-

occurring in sufficient quantities at the level of local grassland patches or because of high competition levels on remnant resources. Hence, such environmental settings may either become problematic for the regional survival of a butterfly species, or alternatively, the environment may select for higher mobility and/or differences in the energy metabolism. Several studies have shown morphological differences related to flight abilities (Hill et al., 1998; Thomas et al., 1998), in populations from environments differing in degree of habitat fragmentation, or examined butterfly dispersal by the mean of mark-recapture studies (e.g. Hanski et al., 2002) or population genetics (see Stevens et al., 2010 for a review). Responses of populations to habitat fragmentation were either towards higher mobility (Hanski et al., 2002; Hill et al., 1998; Thomas et al., 1998) or lower mobility (Merckx et al., 2003; Schtickzelle et al., 2006). Direction of responses varied according to population age (Hanski et al., 2004), to the studied species (Loos et al., 2015), or to sex (Hanski et al., 2002).

Higher flight performance implies more energy allocated to muscle power output and endurance, and hence, less energy available for other activities, unless the total energy budget is increased under such fragmented conditions. However, low flower abundance, and hence nectar supply, in intensively managed agricultural landscapes may jeopardize butterfly life strategies that rely on higher energy consumption for flight. Food stress reduced RMR in the fruit fly *Drosophila melanogaster* (Djawdan et al., 1997) as well as in two butterfly species (Niitepõld et al., 2014). Under strong food stress, carbohydrate resources will be depleted and the insect has to switch from carbohydrates to reserve lipids as metabolic fuel (Sinclair et al., 2011). Selection for stress resistance may lead to lower RMR (Reinhold, 1999), or alternatively, to higher accumulation of lipids and carbohydrate (energy) rather than a reduction in RMR (e.g., *Drosophila melanogaster* ; Djawdan et al., 1997, and references therein). Flight metabolic rate and flight capacities did not vary with food accessibility at the adult stage in two butterfly

species (Niitepõld et al., 2014), but temperature affected FMR in another butterfly species; reaction norms depended on genotype (Niitepõld, 2010) showing variation in the degree of plasticity for FMR.

Here we used the butterfly *Maniola jurtina*, to test the effect of a relatively short exposure during their young adult life to different combinations of nectar quality and quantity during their young adult life on flight performance, FMR and RMR, for two origins. One landscape of origin originated from an intensively managed agricultural landscape, whilst the other originated from a flower-rich, extensively managed agricultural landscape. Exposure to reduced nectar quantity is expected to decrease RMR, and to have no effect on FMR and flight capacities. We expect butterflies from intensively managed agricultural landscape to have stronger flight capacities and higher FMR, but slower RMR. In other words, we expect them to invest more in traits related to flight performance, and to be more able to increase their energy expense for flight (FMR), independently of the energy needed to maintain basic functioning (RMR). As metabolic rate is generally highly plastic (Niitepõld, 2010), reactions of both origins to adult diet might be different. If nectar quantity reduction is strong, it may provoke depletion of carbohydrates in the tested butterflies, which will have to switch from carbohydrates to lipid reserves in order to fuel flight. Effects of nectar quality are harder to predict, as well as the combined effect of modification in nectar quality and quantity. Adaptations to food stress in butterflies from intensively managed landscape could result in higher levels of lipid storage, hence higher body mass, or slower metabolism in order to save energy for other functions. We further expected butterflies from intensively managed agricultural landscapes to be generally less affected by the imposed food stress, if they are adapted. However, if butterflies from intensively managed landscape do not possess adaptive strategies for dealing with food stress, they might be even more affected by nectar quantity reduction and/or quality change.

Material and Methods

Model species

The meadow brown, *Maniola jurtina*, is a common, widespread European butterfly of grassland habitats. It is univoltine and overwinters as first or second instar larva (Bink, 1992). Caterpillars feed on a variety of grasses. Adults use nectar and they feed on a variety of flower species, but show preference for species like *Centaurea jacea* (**Chapter 4**). In the absence of the preferred nectar source, they feed on alternative flowering species such as *Trifolium pratense* (**Chapter 4**; Brakefield, 1982).

Adults are generally found near nectar sources (Brakefield, 1982a; Dennis, 2004). Within a zone of suitable habitat, they typically show resource-searching flights which are sinuous and relatively slow (Dennis, 2004), but the species is also capable of making less frequent, longer dispersal movements like up to 1 km or more (Schneider et al., 2003) and is known to exhibit a direct linear flight as soon as the habitat is left (Delattre et al., 2010; Dennis, 2004). When leaving habitat or when released in the matrix, the species may also exhibit a systematic non-random search flight (Conradt et al., 2000).

Experimental setup

We used the experimental setup as described in **Chapter 6**, i.e. the same outdoor flight cages with the same 4 nectar treatments: 100 flower head units of the preferred *C. jacea*, 10 of *C. jacea*, 100 of the non-preferred *T. pratense* and 10 of *T. pratense*; one in each flight cage compartment. Individual flowers of *T. pratense* contain slightly more nectar than flowers of *C. jacea* (mean quantity of nectar in a single flower ± SEM: 0.21 ± 0.02 µl and 0.18 ± 0.02 µl, respectively) (Rusterholz and Erhardt, 2000), whereas they have similar number of simultaneously open flower on a flower head (23.2 ± 1.1 and 21.1 ± 0.8 respectively) ($N_{flower\ head}$ = 288 for each species). The sugar composition (i.e. sucrose over glucose and fructose ratio), amino

acid concentration and larger corolla make *C. jacea* a preferred nectar source (Rusterholz and Erhardt, 2000).

Wild freshly emerged butterflies from intensive (IL) and extensive (EL) agricultural landscapes were collected and used for the experiment as explained in **Chapter 6**. Adult butterflies were randomly placed in one of the 4 flight cage compartments before 20.00 h. There were 24 individuals (12 males and 12 females) in each flight cage (half cylinder measuring 4.5 m long, 3.7 m wide and 1.8 m at maximal height) at the beginning of the treatment. A total of 960 butterflies were used for the whole experiment (5 replicates with EL butterflies, and 6 replicates with IL butterflies, among which 2 with only a subset of nectar treatments as to have 5 replicates of each nectar treatment for each origin in total).

After 48h in the flight cage, surviving butterflies were collected and individually weighed to the nearest 0.1 mg (Ohaus Explorer balance). Three of the surviving females of each nectar treatment were placed in individual cages (30 x 30 x 30 cm) with access to water (water imbibed cotton) in a climate room under standardised environmental conditions (light/dark—16 h/8 h; 25°C during the day and 14°C at night), to spend the night.

The remaining females were either placed in individual cages with unlimited access to food to measure longevity and fecundity (**Chapter 6**) or directly frozen for further physiological measurements (Lebeau et al., unpublished). Males were either frozen or placed in individual cages for longevity measurement (**Chapter 6**).

Metabolism and flight capacities

The day after each replicate of the treatment session, we tested the 12 females (3 females from each nectar treatment) for Resting Metabolic Rate (RMR), flight capacities and Flight Metabolic Rate (FMR). We randomized the order in which we tested the females from the different treatment

groups. A total of 118 females were tested for RMR, FMR and flight performance (58 from extensive origin and 60 from intensive origin).

The individual was first weighed to the nearest 0.1 mg (Ohaus Explorer balance; accuracy ±0.1 mg), then placed in a 1.5 L plastic cylindrical metabolic chamber. The chamber was connected to a flow-through respirometer (Sable Systems International) and placed in the dark (i.e. screened from the light source) to avoid butterfly movement under ambient temperatures close to optimal body temperatures for butterflies flight (30.0 ± 0.1 °C). We used a flow-through system with a dual-sensor oxygen analyzer (Sable Systems International Oxzilla II) connected to a CO_2 analyzer (Sable Systems CA-10a) and internal thermoprobe (Physitemp bimetal probe type MT-29/1B connected to Physitemp Model BAT-12 digital thermometer) to measure the CO_2. Data from the CO_2 analyzer and from the thermoprobe were processed by a UI-2 analogue/digital converter (Sable System) and handled and stored on a computer (Sable System ExpeData software).

After 10 min, the chamber was emptied of water and CO_2 by flushing with dry CO_2-free air that had previously gone through three successive columns containing silicagel, drierite and ascarite, respectively. We closed the chamber, waited for another 10 min and then flushed it again at a flow rate of 988 ml/min (Sable Systems International subsample TR-SS3 pump) and the CO_2 gas accumulated (produced by the butterfly) during this period in the chamber was measured, added to the quantity produced during measurement time. RMR was expressed in ml CO_2 mg^{-1} hr^{-1}, based on the volume of CO_2 emitted for 12 min (10 min of measurement and 2 min of flushing).

Next the individual was placed in a flight mill within the metabolic chamber for flight performance and FMR measurements. The flight mill consisted of

a light-weight carbon rod, threaded through a stainless pivot (magnets) to provide a near-frictionless rotation movement. The butterfly was glued by the thorax at one extremity of the rod, while an individually adapted counterweight was placed at the other extremity. Once in the mill, butterflies could only perform tethered flight in a circle (rod diameter, i.e. 29 cm). Movement of the rod could be prevented by placing a magnet on top the mill axis. We fixed a small piece of folded paper in the bottom of the flight chamber at the height of the butterfly legs to stimulated butterflies into flight by tarsal reflex. In order to fix a butterfly to the flight mill, it was first anaesthetized with CO_2. Then, we carefully removed thoracic hairs and cuticular lipids on a dorsal zone of the thorax, with a fine cotton swab soaked with 90° ethanol. The butterfly was then carefully glued (glue: Cyanocrylate ZAP PT09) by the pterothoracic segment to a bent tip of a needle hanging to the flight rod.

The mill was placed in a metabolic chamber consisting of a 7.5 cm high plastic cylinder of 33 cm in diameter. We connected the chamber to the respirometer, screened from the light. During acclimation (butterfly calming down and temperature reaching 30°C in the chamber), we flushed the air in the chamber as described above (*c*. 20 min). Once the chamber was CO_2 free, we closed it, removed the black fabric and the magnet, and allowed the individual to fly for 10 min and the CO_2 to accumulate. The chamber was kept under a light source (Philips HPL-R 400W). The number of tours was automatically recorded by a Hall Effect sensor detecting two small magnets attached to the flight mill (Labview v 8.6). Some individuals started to fly right away. If this was not the case, we tapped gently on the chamber with a pen to stimulate flight. We recorded the intensity of tapping needed to motivate the individual to fly on a scale from 0 (no tapping) to 3 (constant tapping). Propensity to fly was calculated as 3 – intensity of tapping, and scaled from 3 (no tapping, butterflies flying during the whole measurement period) to 0 (continuous tapping, weak flight). After 10 min,

we stopped the experiment and measured the CO_2 by the same method as for RMR. The butterfly was detached from the needle, weighed and frozen (-20°C).

FMR was expressed in ml CO_2 mg^{-1} hr^{-1} and flight distance (m) was calculated based on the number of rounds. We expressed the efficiency of fuel combustion for flight by calculating the distance covered while emitting 1 ml of CO^2 mg^{-1}.

Statistical analyses

Means are always represented with their standard error. All variables except propensity to fly were analyzed using Generalized Linear Mixed Models (GLMM) with MIXED procedure in SAS 9.3 (SAS, 2012), with landscape of origin, nectar treatment and the interaction effect as fixed factors. Temperature in the respirometer and time of day were introduced as random covariates. Covariances were estimated by REML (residual maximum likelihood); denominator degrees of freedom for the tests of fixed effects were estimated by the Satterthwaite method. Model selection was done by backward selection from the initial model, removing non-significant interaction terms. We reported main effects and significant interaction terms.

For each butterfly, we obtained the total volume of CO_2 produced during 12 minutes. We corrected for individual mass and calculated it by time unit, to obtain ml CO_2 hr^{-1} mg^{-1}, and the values we will discuss as RMR are expressed in those units. We worked with $\log_{10}$ (RMR) to obtain normal residuals.

For FMR calculation, we first expressed the values obtained from FMR measurement (total volume of CO_2 produced during 20 min) in ml CO_2 hr^{-1} mg^{-1} (correction for mass and time). Then we subtracted from that value each individual's RMR, to correct for initial variation in RMR. For this

measure, we only analysed the individuals that flew during the measurement (N = 102). We first analysed the absolute values of FMR with the initial model to compare between origins. Because FMR is not independent of the flight distance, we then chose to work separately for each origin, replacing origin in the initial model by flight distance, and performing a second analysis. We also analysed efficiency of fuel consumption with the original model.

Flight distance was analysed with the original model, and considering a flight distance of 0 m for those individuals that did not fly at all (N = 16). For the flying individuals, mean flight speed, as the ratio between flight distance and time spent in flight (m/s), was analysed following the same procedure. We analysed individuals propensity to fly with a GLM using a Poisson distribution on (3 - intensity of tapping) considering origin, nectar treatment, their interaction time of day and individuals' mass as factors, applying backward selection; this analysis was performed in R (R Development Core Team, 2013).

Results

Right before flight and metabolism measurement, butterfly mass did not vary significantly with origin or nectar treatment (Table7.1; Linear Model: Origin: $F_{1,114} = 2.38$, $P = 0.13$; Treatment: $F_{3,114} = 2.49$, $P = 0.06$).

Table 7.1: Female butterflies mass right before flight and metabolism measurements; means ± SEM. EL: extensively managed landscape of origin. IL: intensively managed landscape of origin. C100: 100 *C. jacea*; c10: 10 *C. jacea*; T100: 100 *T. pratense*; t10: 10 *T. pratense*.

Origin	Nectar treatment	N	Mean body mass ± SEM (mg)
EL	C100	15	96.31 ± 4.85
	c10	15	87.41 ± 4.60
	T100	15	87.90 ± 4.52
	t10	15	87.51 ± 2.53
IL	C100	15	99.14 ± 5.43
	c10	12	91,32 ± 3.52
	T100	12	103.69 ± 4.13
	t10	15	88.20 ± 5.36

Flight distance and speed

IL females flew longer distances in the flight mill than did EL females (Figure 7.1). Flight distance increased with butterfly mass in both origins (linear regression of flight distance against body mass: EL females: Distance= -224.6 + 4.05*body mass, $F_{1,56}$ = 12.36, *P*= 0.0009; IL females: Distance = -50 +2.7*body mass, $F_{1,57}$ = 4.84; *P*=0.03). Note that the effect was much more significant in the EL group. In the t10 treatment, EL females tended to fly over slightly shorter distances compared to the other treatments (Contrast: $F_{1, 53.2}$ = 3.89, P = 0.054); the differences between the populations of origin were the clearest in this treatment. Flight speed did not differ significantly with landscape of origin and nectar treatment (GLMM: Origin: $F_{1,95}$ = 0.06, P = 0.81; Nectar treatment: $F_{3,95}$ = 2.16, P = 0.22) (mean flight speed (m/s) ± S.E.M. : 0.58 ± 0.03).

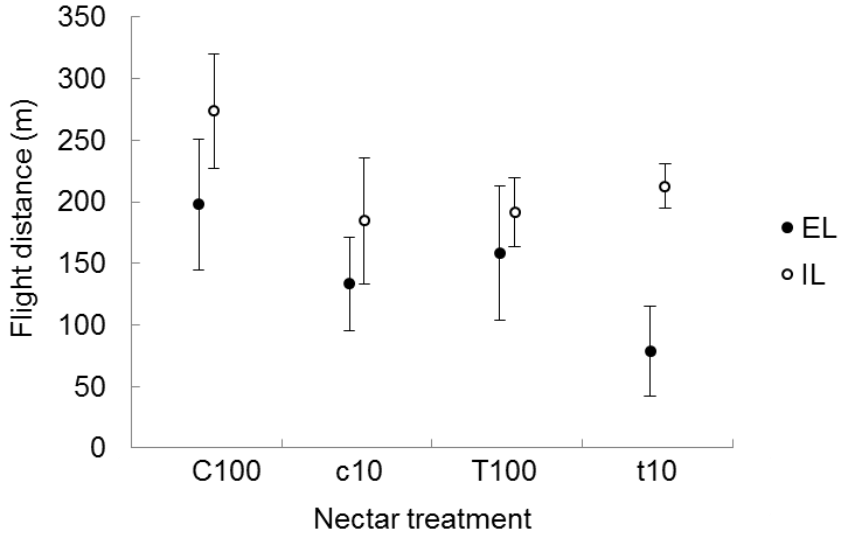

Figure 7.1: Mean flight distance (± SEM) for females by origin (filled symbols: extensively managed landscape of origin (EL); open symbols: intensively managed landscape of origin (IL)) (GLMM: Origin: $F_{1,112}$ = 5.78, P = 0.018; Nectar treatment: $F_{3,112}$ = 1.75, P = 0.16).

Willingness to fly

As predicted, IL females were more willing to fly than EL females; their willingness was not reduced under adult food stress. EL females showed lower willingness to fly when nectar quantity was reduced (i.e. treatment c10 and t10; Figure 7.2).

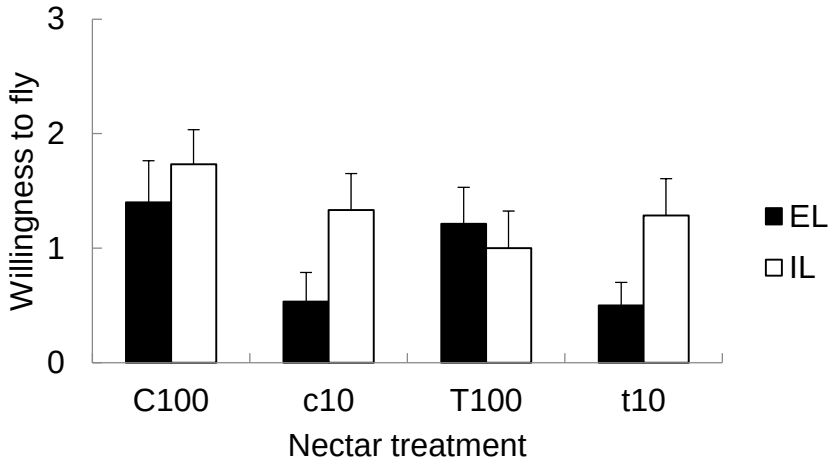

Figure 7.2: Willingness to fly by nectar treatment for both origins (black, EL females; white: IL females). GLM with Poisson distribution: Origin: χ^2_1 =4.72, *P* = 0.029; Nectar treatment: χ^2_3 = 7.14, *P* = 0.068;O x Nt: χ^2_3 = 7.62, *P* = 0.055; Body mass: χ^2_1 = 4.34, *P* = 0.037.

Efficiency of fuel consumption

Butterflies did not differ in their energy consumption in flight (GLMM: Origin: $F_{1,82.1} = 2.31$, $P = 0.132$; Nectar treatment: $F_{3,89.4} = 0.21$, $P = 0.887$; Figure 7.3). Results were the same when data were not corrected by butterfly mass.

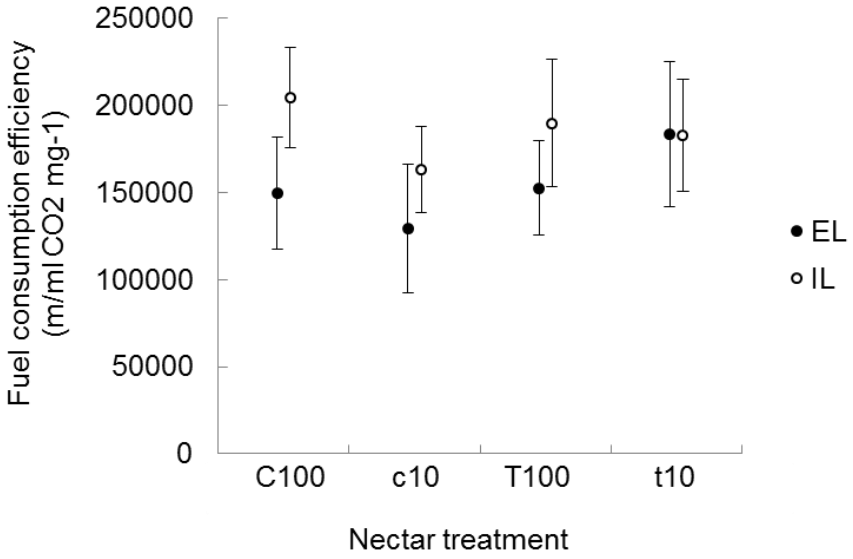

Figure 7.3: Fuel consumption efficiency (distance covered by ml of CO_2 emitted) corrected for body mass, by origin (EL: extensively managed landscape of origin, filled circles; IL: intensively managed landscape of origin, open circles) and nectar treatment.

FMR

FMR was not affected by landscape of origin and butterflies reached on average the fastest FMR under the C100 treatment compared to the other treatments (GLMM: Origin: $F_{1,90.7}$ = 0.01, *P* = 0.9; Nectar treatment: $F_{3,90.1}$ = 2.16, *P* = 0.09; Figure 7.4). Results were the same when analysis was performed on data not corrected for body mass. FMR (uncorrected for mass, ml CO_2 hr^{-1}) was positively related to body mass (Linear regression of FMR against body mass:

EL females: FMR= -0.15+0.009*mass, $F_{1,48}$ = 4.94, *P*= 0.031;

IL females: FMR = -0.21+0.006*mass, $F_{1,53}$ = 3.11; *P*=0.083).

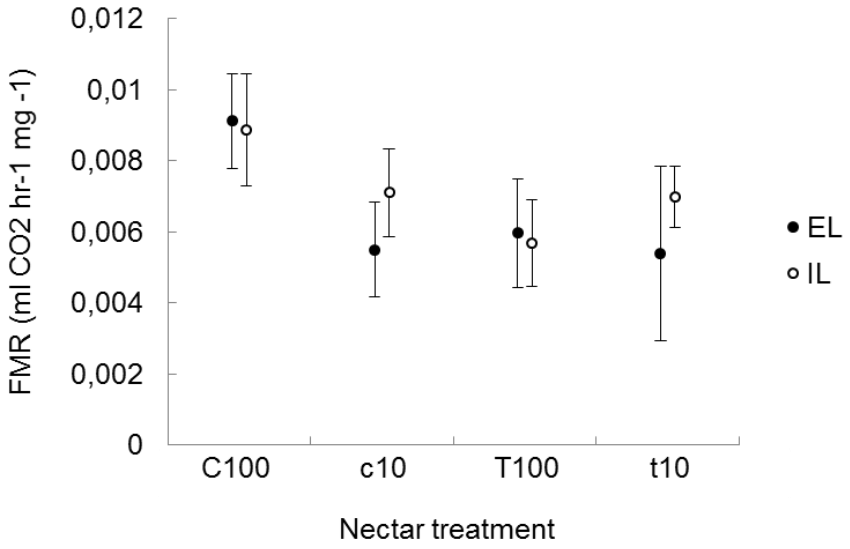

Figure 7.4: FMR (ml CO_2 hr^{-1} mg^{-1}) as a function of treatment and origin (filled symbols, EL: extensively managed landscape of origin; open symbols, IL: intensively managed landscape of origin).

However, the relationship between FMR and distance flown differed between the origins. In IL females, longer distances were associated with higher FMR, whereas this relationship was weaker among EL females (Table 7.2; Parameter estimates (± SE) of the linear regression of FMR by flight distance: EL: $9.23e^{-6} \pm 4.97e^{-6}$, $P = 0.07$; IL: $1.33e^{-5} \pm 3.29e^{-6}$, $P = 0.0002$).

Table 7.2: GLMM on FMR (ml CO_2 hr^{-1} mg^{-1}) during tethered flight in a flight mill analysed separately by population.

Origin	Effect	N. *D.F.*	D. *D.F.*	*F*	*P*
EL	Flight distance	1	38.1	1.05	0.31
	Nectar treatment	3	37.4	0.97	0.41
IL	Flight distance	1	45.9	16.5	0.0002
	Nectar treatment	3	45.1	0.24	0.87

RMR

EL females had higher RMR than IL females, but this pattern was reversed in the most stressful, t10 treatment (GLMM: Origin: $F_{1,108}$ = 0.13, *P* = 0.72; Nectar treatment: $F_{3,108}$ = 0.27, *P* = 0.85; O x Nt: $F_{3,109}$ = 3.51, *P* = 0.018) (Figure 7.5). Hence, IL females reached their fastest pace of life under the high food stress treatment, whereas EL females showed the slowest pace of life in this treatment. RMR did not vary with butterfly mass (all P values > 0.05 for linear regression of RMR against body mass).

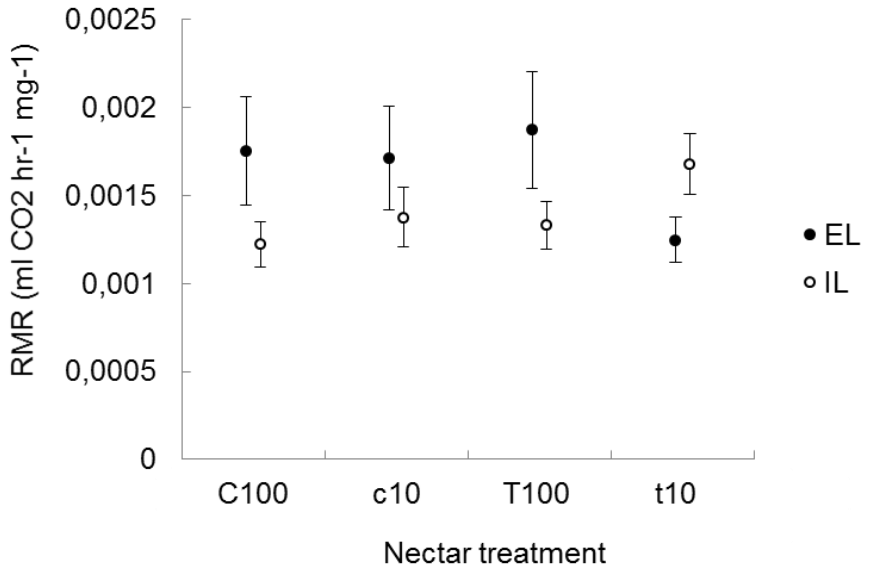

Figure 7.5: Mean RMR (± SEM) for both origins (EL: extensively managed landscape of origin, filled circles; IL: intensively managed landscape of origin, open circles) and nectar treatment.

Discussion

Meadow brown butterflies from landscapes with very different nectar supply showed several differences in flight and activity-related metabolism and performance. As predicted, females from the intensively managed agricultural landscape showed generally stronger flight performance than conspecifics of semi-natural, extensively managed grasslands. Our study also showed significant differences in RMR. Hence, several of the effects of our nectar treatments in outdoor flight cages manipulating nectar quantity and quality had a clear signature of the landscape of origin on individual performance. From a life-history theory viewpoint, our results suggest fundamental differences in the life style, and the underlying metabolic mechanisms, in *M. jurtina* butterflies that have to deal with different nectar quantities and qualities relative to their landscape of origin. First, we will discuss the differences in RMR between the origins, together with the different responses of each origin to variations in the nectar diet. Next, we will discuss flight capacities and differences in plasticity of flight-related traits of each landscape of origin.

RMR is assumed to reflect the cost of self-maintenance for an organism (Burton et al., 2011), and therefore it has often been suggested that a low RMR provides fitness advantages because more energy can be invested in reproduction ('compensation' hypothesis) (Boratyński and Koteja, 2010). On the other hand, higher RMR may sustain higher assimilation of energy for reproduction ('increased intake' hypothesis) (Boratyński and Koteja, 2010). The 'context dependence' hypothesis (Burton et al., 2011) combines the two hypotheses and predicts that faster RMR is advantageous in a favorable environment while lower RMR will drive higher fitness in unfavorable environments, for example when resources are scarce (e.g. Lighton and Bartholomew, 1988; McNab and Morrison, 1963). Accordingly, IL butterflies had slower RMR than EL butterflies, except in the t10

treatment. In our system, one could argue that resource scarcity in intensively managed agricultural landscape would be associated with slower RMR, as individuals would spare energy for other life-history traits. Intraspecific variation in RMR has also been linked to the level of activity of the immune system (Burton et al., 2011 and references therein). Lower butterfly population density in the IL landscape could result in lower density of pathogens. Consequently, the immune system might be less solicited. For EL females from a resource-rich environment, higher RMR could effectively bring fitness advantages under conditions of unlimited food supply. Origins did not react in the same way to the simultaneous reduction of nectar quality and quantity (i.e. t10 treatment). EL butterflies reduced their RMR under food stress, which would save energy that could be subsequently used for reproduction. Food stress reduced RMR in *Drosophila* flies (Djawdan et al., 1997) and in two butterfly species (Niitepõld et al., 2014). Butterflies used in our experiment were wild-caught, young adults, but they all had an individual experience (and of course their evolutionary history) with the feeding conditions in their landscape of origin. Hence, EL butterflies might have foraged on a preferred nectar source in the field prior to capture, and accumulated energy and nutrients from nectar that allowed them to better resist two days of food stress. For IL females, on the other hand, which increased their RMR in the t10 treatment, food stress may have acted as an extension of the stressful conditions experienced in the field. Hence, they may have reached a threshold in expenditure of stored resources so as to reach a point of no return in their physiological state, causing higher RMR. A higher RMR could be due to a shift in the principal fuel source for energy metabolism, from carbohydrates to lipids, or to enzymatic activities cranked up in response to a bad physiological state (Melvin et al., 2007).

We showed differences between the origins that go in the sense of selection for more mobile individuals in fragmented landscape (Hanski et

al., 2002). Our results showed evidence of better flight capacity for IL butterflies than for EL butterflies; they flew longer distances in all nectar treatments. Moreover, whereas IL females were not affected by nectar treatment in terms of flight distances, EL females suffered much more from the simultaneous reduction of nectar quality and quantity. Butterfly mobility may depend on the scale of habitat fragmentation and on the intrinsic willingness and capacity to fly (Van Dyck and Matthysen, 1999). Several behavioural responses can be mediated by boundaries and barriers in the landscape and the perceptual distance to detect resources across the landscape (Merckx et al., 2003; Schultz et al., 2012). For *M. jurtina*, as for other grassland butterfly species, (small) habitat patches may be scattered but numerous in the intensively managed agricultural landscape (field boundaries, road verges, etc.) (Dover and Settele, 2008). Hence, inter-patch distance should be smaller than the maximal exploration range of our focus species (Van Dyck and Matthysen, 1999), which has been reported to be approximately 2 km (Grill et al., 2006; Schneider et al., 2003). Our results show that female butterflies originating from a fragmented landscape with resource scarcity were able to fly for longer distances than conspecifics from a continuous, resource- rich landscape, when tested in a flight mill with tethered flight. Tethered flight does not perfectly imitate free flight in the wild or in experimental arenas (Schultz et al., 2012) or in flight tunnels (Plepys et al., 2002). However, using a flight mill constructed within a metabolic chamber allows the simultaneous measurement of flight and metabolic rate (FMR). In our experimental set-up, butterflies were placed in unnatural conditions to test flight willingness and performance. There is little known about personality-related traits relative to dispersal ability in insects in general, and butterflies in particular. But in principle bold individuals could fly more, whereas shy individuals may be more reluctant to do so (Sih et al., 2004). We showed that IL females were more willing to fly than EL females, which could relate to behavioural differences between

landscapes of origin. Of course our study was not designed for testing personality-related differences, but further work within this context could be interesting (Kralj-Fišer and Schuett, 2014). With EL butterflies being less prone to fly, we could consider the possibility for EL females to have similar physical flight capacities as IL females, but different behavioural propensity. Willingness to fly was similar between the origins in the diet treatments offering high nectar quantity (i.e. C100 and T100, Figure 7.2), and under these conditions, flight distances were indeed not significantly longer for IL butterflies (Figure 7.1). Hence, IL butterflies could be better dispersers than EL conspecifics, which would contribute to their survival in intensively managed agricultural landscapes.

EL females showed a certain level of plasticity in their flight performance in response to nectar diet, whereas IL females did not. This suggests that, whatever the environment, IL females are prone to fly and they fly over longer distances. We may thus think that IL *M. jurtina* butterfly have higher mobility, i.e. greater emigration propensity and dispersal ability, compared to EL butterflies. Emigration propensity and flight capacity appear to be correlated in our study system in response to habitat fragmentation, but this is not necessarily so in all species and landscape settings (Van Dyck and Baguette, 2005).

IL females were much more canalized or less plastic in terms of mobility, but other traits were, however, affected by food stress (i.e. t10 nectar treatment). In **Chapter 6**, we showed negative consequences on fecundity and longevity of IL females under the t10 treatment only. After 48h under all the other nectar treatments, both IL females and EL females were able to perform compensatory feeding, since they were provided with unlimited access to water-diluted honey. Trade-offs in the (re)allocation pathways of resources between the different traits can be identified in limited resources conditions (Burton et al., 2011). It is often suggested that investment in

higher mobility may be traded-off against other life-history traits like fecundity (Hughes et al., 2003) or longevity (Hanski et al., 2006). In **Chapter 6**, we showed both reduction in longevity and fecundity, and fecundity was even lower than expected from the observed longevity. Hence, under limited energy acquisition, the flight performance of IL butterflies, was maintained at the cost of reduced fecundity and longevity.

In conclusion, we showed that butterflies from intensively managed agricultural landscapes had better flight abilities, were more willing to fly, and had lower RMR. Flight related traits were canalized for this landscape of origin, and investment in flight happened at the cost of lower fecundity and longevity under food stress. Butterflies from extensively managed landscapes, on the other hand, were more plastic in their flight-related traits. When submitted to intense food stress, they adopted a strategy consisting in reducing down their RMR probably to spare energy.

Chapter 8 - Butterfly density and behaviour in uncut hay meadow strips: behavioural ecological consequences of an agri-environmental scheme

Julie Lebeau, Renate A. Wesselingh and Hans Van Dyck

Author contribution: Study design: J.L.; Field work: J.L.; Data analysis: J.L. (with feedback by R.W. and H.V.D.); Interpretation and writing: J.L., R.W. and H.V.D. Thanks are due to Murielle Chane for field assistance.

Abstract

Sparing zones from mowing has been proposed, and applied, to improve local conditions for survival and reproduction of insects in hay meadows. However, little is known about the efficiency of refuge zones and the consequences for local populations. We studied population densities of butterflies before and after mowing in the refuge zone of 15 meadows in 2009 and 2011. We also studied the behaviour of the meadow brown (*Maniola jurtina*) comparing nectar use, interactions and flights in the refuge zone before and after mowing. Densities of grassland butterflies in this zone doubled on average after mowing. The density of females of *M. jurtina* increased on average fourfold, while males showed a more modest increase. In line with the idea of increased scramble competition in the refuge zone after mowing, *M. jurtina* increased the time spent on nectar feeding, the preferred nectar source was visited more frequently, and females made more use of non-preferred nectar sources. *Maniola jurtina* did not interact more with conspecifics after mowing, but interactions lasted longer. Flight tracks did not change in linearity, but were faster and shorter after mowing. After mowing, only a part of the local grassland butterflies moved to the uncut refuge zone. The resulting concentration effect alters the time allocated to different activities, nectar use and movements. These aspects have been largely ignored for agri-environmental schemes and grassland management in nature reserves and raise questions about optimal quantities and quality of uncut refuge sites for efficient conservation of grassland arthropods in agricultural landscapes.

Introduction

Agriculture has strongly intensified since the 1950s with well-documented negative impacts on biodiversity (Butler et al., 2007; Robinson and Sutherland, 2002). Semi-natural grasslands under extensive management typically have species-rich communities (Littlewood et al., 2012), but their significance for agriculture has declined considerably; most permanent grasslands have been turned into intensively managed grasslands (with several cuts per year and selected species) or crop fields (Tscharntke et al., 2005). The role of remnants of semi-natural grassland is, however, also recognized in intensive, agricultural landscapes, as these habitats function as population sources of pollinators and other agro-biodiversity (Kohler et al., 2008; Öckinger and Smith, 2007b). In hay meadows, agricultural intensification results in homogenization of the vegetation structure and decline in plant diversity (Tallowin et al., 2005). This in turn affects animals that rely (in)directly upon plant resources and associated environmental conditions.

Extensive grassland management has become a conservation practice in protected areas to control ecological succession of open habitats. Although disturbance, like mowing, is essential to keep semi-natural grasslands from successional development towards shrubland, mowing regimes may also result in increased direct and indirect mortality of grassland fauna, especially invertebrates (Humbert et al., 2010; Johst et al., 2006). Direct mortality caused by the physical impact of mowing can be significant for relatively sedentary invertebrates(Humbert et al., 2010), juvenile insect stages (Dover et al., 2010) and ground nesting birds (Vickery et al., 2001). For flying insects (e.g. butterflies), increased direct mortality may only occur when mowing is done at a time of day when these ectotherms show reduced activity (Cizek et al., 2012; Dover et al., 2010). However, the most significant impact of mowing is likely to be an indirect effect on resource

availability through the sudden disappearance of consumables (especially floral nectar) and altered local microclimatic conditions (Humbert et al., 2009; Johst et al., 2006). Depending on the spatial extent and the timing of mowing, indirect effects may alter individual survival and population viability.

Mowing regimes that leave particular zones uncut have been recommended (Braschler et al., 2009; Cizek et al., 2012; Dover et al., 2010). This body of work provides the rationale for particular agri-environmental schemes and also for rotational mowing management of grasslands in nature reserves (WallisDeVries et al., 2002). To the best of our knowledge, there are surprisingly few studies that show the quantitative effects of uncut refuges in grasslands on specific organisms (but see(Humbert et al., 2012)).

Here we address the practice of leaving refuge strips unmown in hay meadows under agri-environmental schemes focusing on butterflies. We tested the hypothesis of increased use of the uncut refuge zones after mowing. This is often assumed, but rarely tested. After mowing, the majority of the surface of a meadow is cleared from floral nectar resources. This may induce dispersal away from the meadow, but also a concentration effect in the refuge zone. The latter case may in turn alter the living conditions for butterflies (and other insects) in the refuge. Depending on the surface of the refuge and its resources relative to the consumer populations, this may provide scope for further indirect, (sub)lethal effects of mowing regimes. Potential mechanisms include increased competition for nectar (Schultz and Dlugosch, 1999), increased predation rates (e.g. [18]) and increased male harassment for females (e.g. (Odendaal et al., 1989)).

Butterflies are popular study species in the context of agri-environmental schemes (AES), but most studies on these schemes focus on agricultural fields rather than on grasslands (Aviron et al., 2011; Blake et al., 2010; Carvell et al., 2006; Delattre et al., 2013; Field et al., 2006; Meek et al., 2002; Pywell et al., 2004). AES-related studies typically monitor species abundance and diversity between sites submitted to AES and conventionally managed sites (e.g. (Aviron et al., 2011, 2007; Meek et al., 2002)). In this study, we tested (1) the hypothesis of a concentration effect of grassland butterfly species (van Swaay et al., 2006) in the refuge after mowing contrary to other habitat generalist butterfly species; (2) more specifically for such a concentration effect in the grassland species, the meadow brown *Maniola jurtina* L.; and (3) to what extent butterfly life changes in refuge zones by comparing behaviour and resource use before and after mowing in *M. jurtina*. For the latter, we tested differences in nectar use from the supposed increase in competition in the refuge zone after mowing, frequencies of intraspecific interactions, changes in time budget for different activities, and differences in foraging flight paths in the focal zones before and after mowing. In line with the after-mowing concentration effect hypothesis, we predict higher densities in the refuge zone after mowing for *M. jurtina,* and for the assemblage of grassland specialists, but not for the habitat generalists. Assuming increased scramble competition, we predict that *M. jurtina* butterflies spend more time feeding. Rewards per flower visit are likely to be smaller, among-butterfly interactions more frequent and flower visits shorter on average. Under this scenario of increased scramble competition for the preferred nectar, *M. jurtina* is predicted to use less-preferred nectar plants more frequently in the refuge after mowing. Finally, we test the prediction of altered flight pathways with shorter flight bouts in the refuge after mowing.

Material and methods

Study sites and agri-environmental scheme

Study sites were located in a 10 x 10 km area in Belgium (municipalities: Houyet, Beauraing and Rochefort). The landscape mainly consists of forest and grassland, both pastures and hay meadows. We collected data in 15 different hay meadows of various size (range: 0.7-15 ha). All sites were under agri-environmental scheme agreement, which means that meadows cannot be mown before June 15. The sites were located on private lands, and owners gave permission to conduct the study on their lands. The agreement includes limited or no use of manure and bans the use of pesticides. Hay must be removed after mowing and at least 5-10% of the meadow should be left uncut. The location of the unmown refuge zone is allowed to vary between years. Generally, the unmown zone is located on the edge of the meadow and is long and narrow (typically 3 to 5 m large). During our study, all sites were mown, but not grazed.

Study species

We recorded several species of specialist grassland butterflies and generalist butterflies, which may be found in meadows but are not confined to this habitat (as defined in van Swaay et al., 2006). The former group would find most of its resources (e.g. host plants) in the meadows. For all specific behavioural measures we focused on *Maniola jurtina*. This satyrine is a widespread grassland species, but recent data in the framework of the European grassland butterfly index suggest a decline at the European scale (Van Swaay et al., 2013). It is strictly univoltine and males appear up to 10 days before females (Brakefield, 1982a). In Belgium, adults are present from late May to the end of August. Adults live on average 5 to 12 days in the wild (Brakefield, 1982a). Caterpillars feed on a variety of *Poaceae*; adults visit several flower species (Blake et al., 2010; Brakefield, 1982a).

Males spend more time flying than do females (Merckx and Van Dyck, 2002). Males patrol to locate receptive females, or alternatively, perch on the vegetation to intercept passing females. Unlike other butterfly species, *M. jurtina* is not territorial, i.e. males do not defend a specific perching spot as in *Pararge aegeria* for instance (Davies, 1978). Females alternate periods of resting with feeding and egg laying and feed more frequently than males (Brakefield, 1982a). The average individual of a population is not very dispersive and the typical action radius is 300 m or less, however, estimates depend on the scale of the study and movements have observed up to 1 km (Schneider et al., 2003) and more (4700 m, Lebeau et al., unpublished data). Hence, the species is not considered as a sedentary species, but it is clearly not as mobile as other butterfly species that typically fly over several kilometres every day (Cowley et al., 2001; Pollard and Yates, 1993). Our field study did not involve endangered or protected species.

Butterfly density and activity

Butterfly numbers were counted in ten sites in 2009 (June 18–August 4) and in seven in 2011 (June 9–August 2), two of which were also studied in 2009, between 10.00 h and 17.00 h on sunny days (temperature > 18°C, no strong wind) on 5 m x 20 m transects (Pollard walk method; Pollard and Yates 1993). Each site was visited three times before and three times after mowing. The mean number of days (± SE) between the date of the transect count of the three visits and the date of mowing was 7.6 ± 0.6 days before mowing and 4.7 ± 0.7 days after.

Sites were chosen to reflect variation in mowing date relative to butterfly phenology. In 2009, the studied meadows were mown 2 weeks (end of June), 4 weeks (mid July) and 6 weeks (end of July) after emergence of *M. jurtina*. In 2011, meteorological conditions forced farmers to mow at the beginning of July, one month after the beginning of the flight period. Hence, the combined data set provides a reasonable level of overlap between

mowing dates and the butterfly flight season without any particular phenological bias. Additionally, we performed independent transect counts throughout the study period in 2011 in one site that was never mown, to provide reference phenology curves without mowing. This reference site, located within the study area, was visited on 7 days and three transects were done at each date. Butterflies were recorded in the same categories (i.e. grassland specialist species or other habitat generalist species) as for mown sites (van Swaay et al., 2006).

Future refuge zones were delineated before the flight period and we did one transect count per visit per site in this zone before and after mowing. The abundance of all butterfly species was recorded and we distinguished between grassland and generalist species. For *M. jurtina* we also recorded sex and activity (flying, resting/basking, mating, egg-laying or feeding).

Floral nectar supply was quantified in each meadow every 10 days. Along each transect, all flower units were counted by plant species in ten randomly placed 1-m² plots. One inflorescence or one solitary flower equalled one flower unit. The sum of all flower units in all plots of each transect was used as an estimate of the total nectar supply. We also calculated the ratio of the abundance of *Centaurea jacea* L. relative to the abundance of other flower species. In our sites, *C. jacea* is the preferred nectar source of *M. jurtina* and of several other butterflies.

Competition for nectar

In 2009, we introduced a number of potted nectar plants to one of our sites (Site CO; 50°07'54"N – 5°04'12"E) on sunny days between 10.00 h and 17.00 h and recorded flower head visits by *M. jurtina*. We used two nectar plant species: the preferred *C. jacea* and *Trifolium pratense* L. The latter is typically used in the absence of *C. jacea*. The individuals of both nectar species were grown in pots under outdoor conditions. Plants that were

introduced in the field were clipped back to a single inflorescence, protected by an exclosure bag to avoid insect visits prior to the experiment. We placed one flowering plant of each species next to each other in the refuge zone. The exclosure bags were removed and flower visits were recorded during 1 h by video camera (JVC Everio). As we could not distinguish between males and females in every sequence, we used total visit rate by *M. jurtina*. We repeated the experiment on 5 days before mowing (between June 25 and July 16) and on 4 days after mowing (between August 1 and August 12).

Behavioural tracking

In the same year and site, we tracked the behaviour of 41 males (28 before and 13 after mowing) and 68 females (39 before and 29 after mowing) on sunny days between 10.00 h and 17.00 h. Butterflies were caught with a hand net and individually marked on the ventral hind wing. After marking, the butterfly was returned to the net and was released after a 1-min recovery period. Next, the butterfly was tracked for 10 min from a distance of 1 m. The trajectory was recorded by a handheld GPS (Pathfinder Data logger SiRf Star III, GPS-DL R8; relative spatial accuracy: ± 0.1 m). The following behavioural categories were recorded using a digital voice recorder (Olympus VN-5500PC): flying, feeding, basking, resting, mating, egg laying (females) and intraspecific interactions (male-male and male-female), which were used as a measure of the intensity of scramble competition. For flower visits, the nectar plant species was also recorded. Before mowing, tracking was performed in the whole site, since no butterfly stayed within the future refuge zones, which did not differ from the rest of the meadow at that time and were smaller than an individual butterfly's home range. After mowing, tracking was always performed in the unmown refuge zones, since butterflies stayed in these zones. If a

tracked individual left the uncut zone, we continued to follow it up to 10 min.

Statistical analyses

To describe the relative butterfly abundance in the unmown site, we fitted second-order polynomials to the densities observed in the transects at the different dates by least square differences for grassland butterflies, habitat generalist species and for *M. jurtina* males and females.

Mowing and relative abundance

Butterfly densities in the refuge zone before and after mowing were analysed by mixed models on ln (1 + number of individuals observed on a transect). Date (number of days since the beginning of the flight period of *M. jurtina*) and date² were used as continuous, fixed factors. Butterfly group (grassland species vs. habitat generalist species), mowing stage (before or after mowing) and the interaction term were used as fixed factors. Site, year and the site x mowing interaction were introduced as random factors. For the analysis of *M. jurtina* densities, we applied the same model, with sex as a factor instead of butterfly group.

These analyses were performed with SAS 9.3 (mixed procedure) (SAS, 2012). Covariance parameters were estimated by REML (residual maximum likelihood). Denominator degrees of freedom for the tests of fixed effects were estimated by the Satterthwaite method.

Behavioural changes observed during transect counts

A mixed binomial logistic model with a logit link (SAS glimmix procedure) was used to analyse the frequency of individuals observed feeding. Covariance parameters were estimated by RSPL (Random Solution Pseudo-Likelihood, corresponding to maximizing the residual log pseudo-likelihood with an expansion about the current solutions of the best linear unbiased predictors of the random effects; (SAS, 2012)). Denominator degrees of

freedom for the tests of fixed effects were estimated by the Satterthwaite method. The binomial variable for each individual was "1": feeding or "0": not feeding (other activities pooled). Flower abundance was introduced as a covariate. The model contained mowing treatment, sex and the interaction term as fixed factors, while site, year and transect ID (nested within site) were included as random factors.

Flower head visitation rates

For the analysis of visitation rates on introduced flower heads, we performed a zero-inflated Poisson regression with mowing treatment, nectar plant species (*C. jacea* or *T. pratense*) and the interaction term as fixed factors, in *R* 2.15.1 (R Development Core Team, 2013) with packages pscl (Zeileis et al., 2008) and MASS (Venables and Ripley, 2002).

Behavioural tracking: feeding and interacting

The proportion of time spent feeding vs. all other activities was analysed with a mixed logistic binomial model with sex, mowing treatment and the interaction effect as fixed factors, with SAS 9.3 software (SAS, 2012), using the same technique as for the behavioural changes observed during transect counts. Individual was included as a random factor. Next, we applied the same logistic binomial model to analyse proportion of nectaring events on *C. jacea*.

We counted the number of changes in activity per 10-min track and analysed it by negative binomial regression, with mowing, sex and their interaction effect as fixed factors. This was performed in *R* 2.15.1 (R Development Core Team, 2013) with packages pscl (Zeileis et al., 2008) and MASS (Venables and Ripley, 2002).

Tracked females never interacted with other individuals. Hence, we analysed the number of interactions using a quasi GLM with Poisson distribution for males only, in *R* 2.15.1 (R Development Core Team, 2013)

with packages pscl (Zeileis et al., 2008) and MASS (Venables and Ripley, 2002). Mowing was a fixed factor in the model. We analysed the duration of interactions using a mixed model for ln(duration) and with mowing as fixed factor and individual as random factor in SAS 9.3 (SAS, 2012).

Behavioural tracking: flight path analysis

A flight bout was defined as a whole flying event between two stops (either resting, basking or nectaring). We included only those flights that fulfilled at least one of three criteria: i) the flight lasted longer than 3 s; ii) it covered more than 30 cm; or iii) the activity before the flight differed from the activity after the flight. After mowing, we selected only the flight bouts that remained within the unmown zone; the mown meadow is a very different environment from the refuge and alters flight paths from typical foraging to straight dispersal flight (Delattre et al., 2010). For flight bouts, we analysed the i) flight distance (including the sinuosity of the flight track), and ii) net displacement (Euclidean distance between take-off and landing). We also analysed the duration, speed and linearity (i.e., the ratio between net displacement and real distance). Single flight distance, speed and flight duration were analysed using mixed models in SAS 9.3 (SAS, 2012), on log10-transformed data with sex, mowing and the interaction term as fixed effects and individual as a random effect. We used the same model for flight bout linearity after log10 (1-linearity) transformation to reach normality.

Results

Grassland butterfly abundance increased from early June until the end of June, when it reached its maximum value, and then decreased until mid-July, whereas generalist butterflies remained relatively rare throughout the observation period (Figure 8.1a). Females of *M. jurtina* reached their peak abundance in 2011 at the end of June and decreased in abundance afterwards, while males were observed at lower densities throughout the observation period (Figure 8.1b).

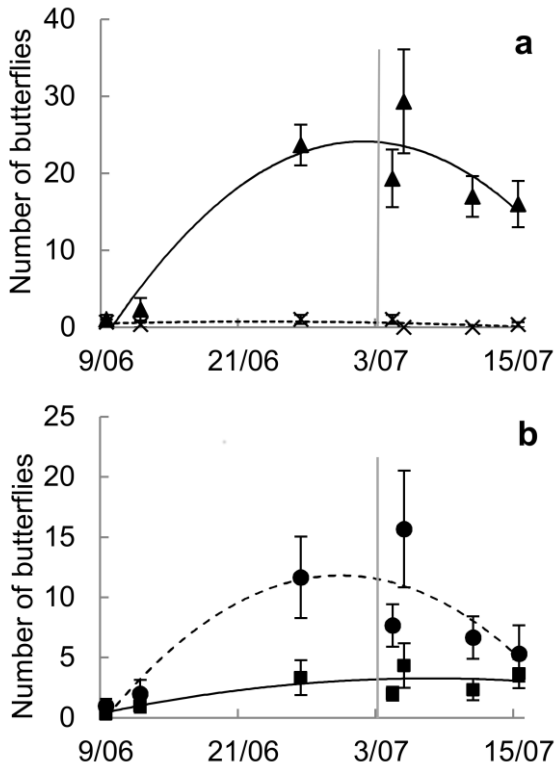

Figure 8.1. Butterfly phenology and mowing date in 2011. Mean number of butterflies (± SEM) recorded during transect counts in a site that was not mown, throughout the observation period in 2011. a) Grassland species (triangles, plain curve) and generalist species (crosses, dotted curve). b) *M. jurtina* females (circles, dotted curve) and males (squares, plain curve). Curves are second-order polynomial regressions fitted through least squares differences. Other studied sites in 2011 were mown on the 3rd of July, indicated by the vertical line.

Mowing and butterfly abundance

After mowing, the abundance of grassland butterflies increased significantly in the refuge zone; it doubled on average, whereas there was no effect on generalist butterfly species (Figure 8.2). An increase in relative abundance was also observed in *M. jurtina*; the effect was modest in males, but female relative abundance increased on average fourfold after mowing (Figure 8.3). For the remainder of the results, we focus exclusively on *M. jurtina*.

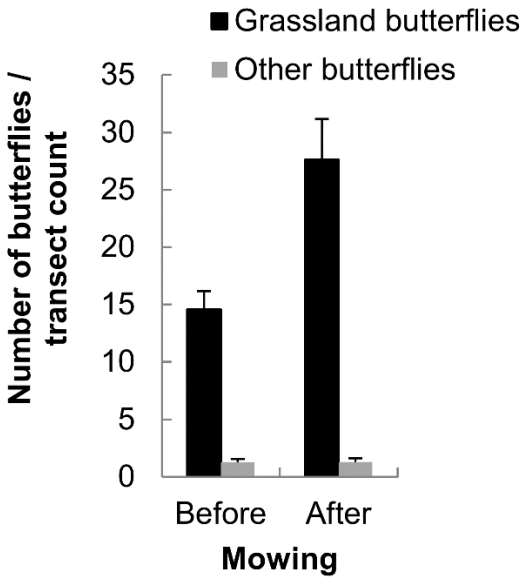

Figure 8.2. Increase in grassland butterflies abundance after mowing. Mean number of butterflies recorded during transect counts before and after mowing (± SE). Mixed model: Date: $F_{1,152} = 6.82$, $P = 0.0099$, Date²: $F_{1,187} = 7.17$, $P = 0.0081$, Mowing: $F_{1,26.5} = 2.25$, $P = 0.145$, Species group: $F_{1,169} = 672.5$, $P < 0.0001$, Mowing x Species group: $F_{1,169} = 11.54$, $P = 0.0009$. Ntotal = 2285 butterflies (809 before and 1476 after). Grassland species: *Maniola jurtina*, *Aphantopus hyperantus*, *Melanargia galathea*, *Coenonympha pamphilus*, *Pyronia tithonus*, *Papilio machaon*, *Thymelicus lineola*, *T. sylvestris* and lycaenid butterflies (*Thecla betulae*, *Heodes tityrus*, *Polyommatus icarus*, and *Lycaena phlaeas*). Other species: *Aglais urticae*, *A. io*, *Arashnia levana*, *Vanessa cardui*, *V. atalanta*, *Aporia crataegi* and pierid butterflies.

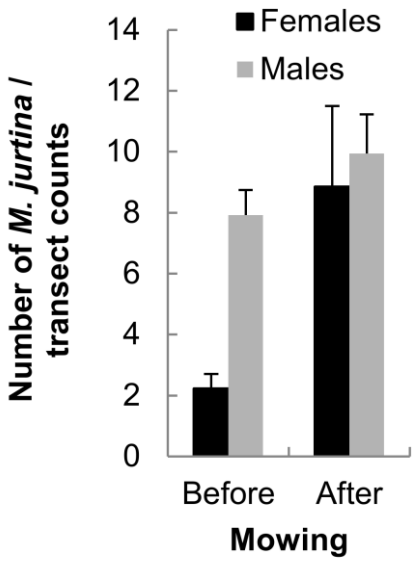

Figure 8.3. Increase in *M. jurtina* after mowing. Mean number (± SE) of *M. jurtina* recorded during transect counts in the refuge zone before and after mowing. Mixed model: Date: $F_{1, 180} = 13.58$, $P = 0.0003$, Date²: $F_{1, 196} = 24.38$, $P < 0.0001$, Mowing: $F_{1, 26.2} = 6.04$, $P = 0.0209$, Sex: $F_{1, 169} = 65.7$, $P < 0.0001$, Mowing x Sex: $F_{1, 169} = 11.04$, $P = 0.0011$. $N_{total} = 1474$ (562 females and 912 males).

Feeding behaviour and nectar use

Flower visiting frequency was significantly influenced by flower abundance, butterfly sex and mowing. Higher frequencies of nectaring were observed in flower-rich sites and females showed higher frequencies than males (54.3% and 3.7%, respectively). The effect of mowing had a sex-specific effect, explaining the highly significant interaction effect (Table 8.1). Before mowing, 29.7% of the females were feeding, whereas this frequency increased to 60.3% after mowing. However, males did not feed more frequently after mowing (< 5% in both periods).

Table 8.1. Mixed binomial regression of feeding frequency of *M. jurtina* during transect counts relative to nectar supply, sex and mowing stage (before or after mowing).

Effect	DF	F	*P*
Nectar offer	24.7	4.83	0.0375
Sex	1469	124.05	<0.0001
Mowing	65.07	0.07	0.7911
Sex x Mowing	1469	6.95	0.0085

Camera observations on potted nectar plants in the field before and after mowing also showed an increase in feeding rate in the uncut strips after mowing (Table 8.2). Interestingly, the effect differed between nectar plant species (Table 8.2): it was very strong for the preferred *C. jacea* (mean number of visits h^{-1} ± SE: before 0.4 ± 0.4; after 5.0 ± 2.1), but absent for *T. pratense* (before 0.6 ± 0.4; after 0.25 ± 0.25).

Table 8.2. Zero-inflated Poisson regression of the number of visits by *M. jurtina* in 1-h video-recordings of visits to inflorescences of *Centaurea jacea* and *Trifolium pratense*

Effect	Estimate	SE	z	*P*
Intercept	18.961	0.224	8.452	<0.0001
Nectar plant species	-29.450	11.086	-2.657	0.0079
Mowing	-23.686	0.913	-2.593	0.0095
Nectar plant x Mowing	32.699	15.313	2.135	0.0327

We tracked 109 individuals of *M. jurtina*, 68 females and 41 males, together they performed 400 nectaring visits: 162 (90 before mowing and 72 after) for females and 238 (118 before and 120 after mowing) for males, respectively. Behavioural data indicated that after mowing a larger part of their time was spent feeding (logistic binomial regression; Mowing: $F_{1, 88.03} = 3.53$, $P = 0.064$, Sex: $F_{1, 88.03} = 2.59$, $P = 0.111$, Mowing x Sex: $F_{1, 88.03} = 1.05$, $P = 0.308$); females spent 24.3 % of their time to nectaring before mowing and 36.8% after, while males allocated respectively 23.3% and 44.8% of their time (Table 8.3).

Table 8.3. Mean duration in seconds (± SEM) of each activity as observed during 10-min behavioural trackings of *M. jurtina* females and males.

Activity	*Females*		*Males*	
	Before	**After**	**Before**	**After**
Flying	46 (8)	55 (10)	175 (27)	189 (25)
***Feeding on* C. jacea**	161 (33)	186 (47)	238 (42)	257 (26)
Feeding on other species	2 (2)	37 (20)	106 (99)	26 (8)
Resting	368 (37)	293 (45)	183 (27)	64 (17)
Basking	10 (8)	17 (9)	77 (21)	47 (10)
Mating	0	0	0	0
Egg laying	8 (5)	9 (3)	0	0
Interacting with conspecifics	3 (2)	2 (2)	21 (3)	32 (6)

After mowing, females visited other species than the preferred *C. jacea* more frequently (before: 1.1%, after: 29.5 %; Table 8.3), while this was not the case for males (before: 13.5 %, after: 7.5 %) (logistic binomial regression: Mowing: $F_{1,115.4} = 3.55$, $P = 0.062$, Sex: $F_{1,115.4} = 0.24$, $P = 0.626$, Mowing x Sex: $F_{1,115.4} = 5.5$, $P = 0.021$). The ratio between the abundance of *C. jacea* and other flower species did not change between the two periods (22.6 % before mowing and 23.5 % after).

Egg-laying behaviour

Before mowing, ovipositing females flew down into the grass to reach ground level, laid a single egg before flying away and repeating the sequence. Five females out of the 39 that were tracked were observed ovipositing and they laid a total of 16 eggs. After mowing, females almost always left the refuge zone and flew over variable distances in the mown zone before landing and ovipositing on a support or on bare ground in the mown zone. Then they flew further away to repeat the sequence before ultimately flying back to the refuge zone. Egg laying was the only activity observed in the mown zone after mowing. A total of 25 ovipositions

performed by eight females (out of 29 tracked females) were observed, 21 of which were outside of the refuge zone.

Flight characterization and interactions

We observed 235 interactions between *M. jurtina* males and other butterflies (148 before and 87 after mowing). Males did not interact more frequently with other butterflies after mowing (mean number of interactions during an observation session ± SE: 2.23 ± 0.42) than before (1.89 ± 0.31) (Quasi-GLM with Poisson distribution: Mowing: $\chi_{1,115} = 399.23$, $P > 0.53$). However, intraspecific interactions lasted on average longer after mowing (5.2 ± 0.4 s and 3.9 ± 0.2 s, respectively; Mixed model: Mowing: $F_{1, 33.1} = 5.69$, $P = 0.029$).

Both sexes tended to switch between activities more frequently after mowing (mean number of switches ± SE: females: before: 12.2 ± 1.7, after: 18.8 ± 3.6; males: before: 25.7 ± 2.7, after: 41.4 ± 2.1) (Table 8.4).

Table 8.4. Negative binomial regression analysis of the number of activity changes by *M. jurtina* during behavioural tracks before and after mowing. Parameters estimates for sex give the value for males relative to females, and for mowing the values after mowing were used as reference level.

Variables	β	SE	*P*
Sex	0.789	0.271	0.0035
Mowing	-0.428	0.207	0.0386
Sex x Mowing	-0.047	0.341	0.889

After mowing, single flight bouts of both males and females were significantly shorter; this was true for both total and net displacement. Flight bouts were also faster, but did not differ in linearity (Table 8.5, Figure 8.4). Males always tended to fly over shorter distances than females, but their flights took longer. Hence, males flew slower than females.

Table 8.5. The results of mixed models of flight behaviour variables of *M. jurtina* relative to the effect of mowing and sex.

Variable	Effect	df	F	*P*
Flight duration	Mowing	87.6	7.94	0.0060
	Sex	87.6	47.61	<0.0001
	Mowing x Sex	87.6	1.13	0.2901
Flight distance	Mowing	69.4	6.26	0.0147
	Sex	69.4	1.21	0.2749
	Mowing x Sex	69.4	0.23	0.6310
Net displacement	Mowing	67.8	7.89	0.0065
	Sex	67.8	3.19	0.0786
	Mowing x Sex	67.8	0.15	0.7028
Linearity	Mowing	75.8	1.94	0.1673
	Sex	75.8	0.45	0.5035
	Mowing x Sex	75.8	0.23	0.6294
Speed	Mowing	76.3	5.98	0.0168
	Sex	76.3	13.85	0.0004
	Mowing x Sex	76.3	0.81	0.3723

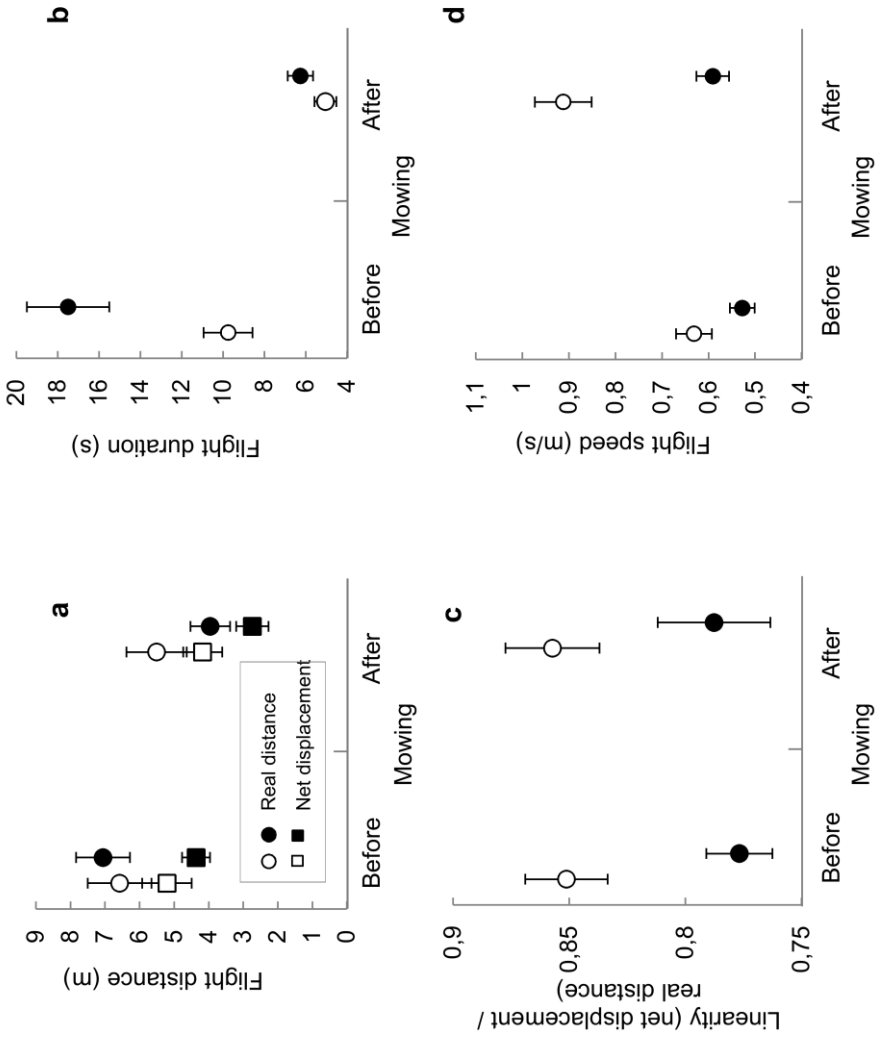

Figure 8.4. Flight traits of tracked *M. jurtina* before and after mowing. Open circles: females (n = 92 flight bouts before, 89 after); filled circles: males (n = 239 before, 111 after); N_{total} = 531. (a) mean flight distance, (b) mean flight duration, (c) mean linearity and (d) mean flight speed. Bars: ± 1 SE

Discussion

The basic challenge of mowing management from an animal conservation viewpoint is dealing with the compromise between short-term negative effects and long-term beneficial effects of keeping the vegetation in favorable condition, although other organisms ay benefit from later successional stages of the vegetation (Balmer and Erhardt, 2000). The issue is highly relevant for species-rich meadows in nature reserves, but also for agri-environmental schemes in hay meadows and other grassy habitats placed on the edges of crop fields such as green lanes or conservation headlands. In hay meadows, sparing a zone from mowing in one year has often been recommended as a solution to control vegetation succession and avoid negative impacts on local animal populations(e.g. [10]). Although benefits are typically assumed (Braschler et al., 2009; Cizek et al., 2012), they are rarely demonstrated (but see (Buri et al., 2013; Humbert et al., 2012)). Here, we provide quantitative evidence for a concentration effect after mowing in the refuge zone for flower-dependent insects, grassland butterflies. Butterfly abundance and diversity were also high in linear grassy habitats on field edges implemented in agricultural landscape to promote biodiversity (e.g. [42]), and are especially associated with nectar sources (Dover, 1997, 1996; Feber et al., 1996). To avoid ecological succession, these linear grassy habitats are frequently managed by mowing, but timing of mowing relative to the butterfly's flight season is known to play a major role for butterfly abundance (Feber et al., 1996; Kulfan et al., 2012). For the meadow brown (*M. jurtina*), several of our results from behavioural observations point at higher levels of competition for resources in the refuge zone after mowing. These aspects have not been fully appreciated by conservation biologists and practitioners so far.

Increased density of grassland butterflies and of M. jurtina in the refuge zone after mowing

The density of grassland butterflies in the refuge zone was almost doubled after mowing, whereas the density of habitat generalist butterflies remained stable. In 2011, mowing occurred after the butterfly abundance peak, hence the increased density in the refuge zone cannot be attributed to phenology. An increase in density in the refuge zone after mowing has recently been shown for grass-feeding insects (Orthoptera; (Humbert et al., 2012))and there is some evidence for displacements from mown to refuge zones in a few other arthropods (Hossain et al., 2002; Thorbek and Bilde, 2004). The absence of a mowing effect on generalist butterfly species at the level of the local meadow most probably relates to their mobile life style and opportunistic use of nectar resources at a wider spatial scale across the landscape, compared to typical grassland butterflies. Hence, the probability of encountering an individual of a generalist species in the refuge remained constant per unit area, resulting in the same densities before and after mowing.

For grassland butterflies like *M. jurtina*, higher densities after mowing are in line with the predicted concentration effect in the uncut refuge zone. At least in 2011, since peak abundance had already been reached before mowing, seasonal effects could be excluded as a cause for this increase. Some of the individuals could be immigrants from nearby meadows, but such an effect is likely to be more important if several meadows are mown simultaneously in a resource-poor landscape. However, Dover et al. (Dover et al., 2010) did not find evidence for increased dispersal of butterflies to nectar resources in adjacent meadows and tracks after mowing.

If we assume i) homogeneous habitat quality and butterfly abundance within a meadow and ii) that all grassland butterflies of a particular meadow would move to the uncut zone after mowing, then the increase in

density should be inversely proportional to the part of the meadow left uncut. In our study, on average 10% of the meadows was left uncut, which would lead to the prediction of a tenfold increase in grassland butterfly density for the refuge zone. However, we observed on average only a doubling of the densities; for *M. jurtina* females we observed on average a fourfold increase. The discrepancy between the predicted and observed densities suggests significant losses for local populations, although with the current data we cannot discriminate between mortality and emigration. Direct killing due to hay cutting has been shown for several organisms (Humbert et al., 2009), but rarely for butterflies at the adult stage, although its potential impact has been suggested by Dover et al. (Dover et al., 2010). Moreover, butterflies from the surroundings could also immigrate to an unmown zone at any moment. In any case, meadows that are mown only once a year should be mown as late as possible to guarantee floral resources to butterflies and other insects (Dahlström et al., 2008; Valtonen et al., 2006).

As our results suggest, unmown refuge zones offer opportunities for part of the local butterfly population after mowing of a hay meadow, but it appears to be far from a full compensation for the habitat loss induced by cutting. Long-term efficiency of a refuge zone in hay meadows has been shown for orthopterans, but they do not rely on nectar sources (Buri et al., 2013).

Behavioural consequences of increased density for Maniola jurtina

Our study took the issue of unmown strips in meadows a step further by also addressing behavioural changes that are significant to butterfly survival and reproduction. Butterflies performed shorter and faster flight bouts after mowing and switched more frequently between different types of activities. This suggests a higher activity level in the refuge zone compared to the conditions before mowing. However, the frequency of intraspecific

interactions did not increase and overall flight paths were similarly tortuous in the unmown zone before and after mowing. We confirmed sexual differences in several flight traits (*c.f.* Brakefield, 1982a), but they did not interact with the effect of mowing (Table 8.5). Increased male harassment could be a bigger issue if mowing occurred very early in the season, since *M. jurtina* is protandrous, but in our sample there was much variation in the timing of mowing (*see* Material and methods, Butterfly density and activity).

In our study areas, refuge zones were relatively narrow, linear elements (width: on average only 3 to 5 m) along the meadow border. It is known from studies on corridors that butterflies fly faster in narrow habitat elements (Pryke and Samways, 2001). As population density did not affect flight speed in another satyrine butterfly (Wickman, 1988), we argue that the observed higher flight speed in the refuge zone after mowing is a result of a 'canalising' side effect of the geometry of the refuge. Little is known about the sensory ecology of flying butterflies under natural conditions, but visual cues are assumed to play an important role. There is an interesting scope for future work on sensory and cognitive issues of refuge use at the landscape scale.

The significantly higher frequency of switches between activities most likely relates to lower nectar rewards per flower visit, which in turn demanded higher frequencies of altering between foraging and flying. This likely implies an increase in the physiological cost, as insect flight is energetically costly and particularly so for take-offs (Berwaerts and Van Dyck, 2004). Frequent switching between foraging and flight include frequent take-offs. Hence, the profitability of flower visits will be lower in the refuge after mowing.

Only a low proportion of the tracked females were observed ovipositing. However, our behavioural tracking was not specifically designed to study oviposition in any detail (unlike for example (Van Dyck and Regniers, 2010)). We argue that the 10-min track did not provide a sufficient time window as to reflect the proportion of egg-laying females in the population. Moreover, the majority of egg-laying occurs between 14.00 and 16.00 h and our behavioural trackings took place between 10.00 h to 17.00 h. That females left the refuge zone after mowing to lay eggs reveals that butterfly may find resources (oviposition sites) within the mown zone. *M. jurtina* is known to emigrate from recently cut or grazed areas (Brakefield, 1982a), and our observation are in agreement with former studies in that sense, since butterflies were absent from the mown zone of the meadows after mowing. However, the high proportion of oviposition episodes observed in the mown zone after mowing, and the fact that the mown zone is used only for egg laying, indicates a preference of females for this zone for oviposition, as previously observed (Kulfan et al., 2012). Actually, the mown part of a meadow is different from the refuge in several aspects, including, microclimate, level of predation and plant quality. Eggs laid in the mown zone have higher probabilities to successfully become adult butterflies than eggs laid in the refuge zone (Lebeau et al., unpublished data). Although the majority of our results showed negative effects of mowing for butterfly communities, we argue, based on these oviposition patterns, that it creates nevertheless new conditions that may be beneficial for part of the community.

Our results showed an effect of mowing and of the consequent density increase for behaviour related to resource use. Butterflies – females in particular – allocated proportionally more time to foraging, shown from our transect and individual tracking data. *Centaurea jacea* is a favourable and preferred nectar source in our sites and this nectar source was visited more often after mowing (based on camera data). This flower species is also

frequently visited and highly appreciated by several bee species (honey bees, bumblebees, wild bees) (Hirsch et al., 2003), that will also be forced to forage within the remaining unmown zones after mowing or to move to other landscape elements. Nectar production in the *Centaurea* genus is fairly continuous throughout the day (Lack, 1982a), so even in the case of total nectar depletion by previous visits, small amounts of nectar are likely to be available on inflorescences, although nectar secretion is relatively slow. However, wild *Centaurea* flowers in the field are always nearly empty, and nectar quantities within the florets were too small to be sampled with a microcapillary (Lebeau et al., unpublished data; (Lack, 1982b)). Within the time spent feeding, a larger proportion of time was used to forage on less-preferred nectar species after mowing than before (based on individual track data). These results are best explained by higher levels of scramble competition for nectar at both the intra- and interspecific level after mowing. Quantity and quality of nectar affect butterfly fecundity, especially in females (Boggs and Ross, 1993). Experiments have established strong relationships between nectar use and butterfly fitness (Jervis and Boggs, 2005; Mevi-Schütz and Erhardt, 2005). Female butterflies are typically more demanding for nectar (including carbohydrates and amino acids), because they have to manufacture eggs (O'Brien et al., 2004).

Management practices favouring grassland butterfly community

Our results raise several questions about the optimal quantity and quality of uncut refuge zones in hay meadows for efficient conservation of grassland butterflies and other insects. The location, surface and nectar supply of a refuge zone are key factors in maximizing local resource availability and local survival after mowing. Agri-environmental schemes and management plans often focus on percentages, but the absolute area and shape of the refuge zone should also be considered. Although management techniques to stimulate nectar supply in the refuge zone

deserve further attention, a local optimization of the nectar resources within a 5-10% strip of a meadow may not be sufficient for harbouring local populations of nectar-feeding insects. We did not study very small or large refuge zones, but based on our results one could still question the '10%-rule' that is often applied for management regimes with a refuge zone. Our observations of oviposition in the mown zone after mowing suggest that the insect community would benefit from applying several cuts a year, on a fraction of the meadow only, as to finish the season with each fraction having been cut at least once, with uncut zones remaining throughout the year and varying in localization within the site. There is also scope for further work on the effects of local movements induced by mowing. Butterflies move to surrounding landscape elements to find more of the limiting resources (complementation), or other resources absent from the refuge (supplementation;(Ouin et al., 2004)). Beside the quantity and spatial configuration of nectar resources, the qualitative aspects appear to be largely neglected. Butterflies have often been considered nectar-opportunistic, but different plant species may provide different nectar quantities and qualities with significant fitness consequences (Erhardt and Rusterholz, 1998; Jervis and Boggs, 2005). Further experiments on the area, configuration and quality of refuge zones are now warranted to provide evidence-based guidelines for hay meadow management.

Conclusion

Sparing zones in hay meadows from cutting provides essential resources to grassland arthropods, including flower-visiting grassland butterflies. The principle is well-known and widely applied, but the consequences for local populations in the refuge zones have been largely ignored. We observed a significant increase in butterfly density in the refuge zone after mowing, but not enough to account for total population densities before mowing, indicating a local decline in butterfly abundance. We also showed evidence

of changes in behaviour and flower visitation after mowing in line with increased scramble competition for (nectar) resources. Our study raises novel issues about the quantity, configuration and quality of refuge zones in grasslands for animal conservation.

Chapter 9 - General discussion

In this PhD thesis, the consequences of foraging behaviour and nectar limitation on adult fitness components have been addressed, using the butterfly *Maniola jurtina* as a model organism. This species is a widespread European grassland butterfly submitted to a wide variation in nectar supply across different landscapes that differ in management intensity.

First, we clearly characterised the foraging behaviour of *M. jurtina* under natural conditions, in flower-rich, extensively managed grasslands and in intensively managed, flower-poor hay meadows (**Chapter 4**). We identified *Centaurea jacea* to be the most frequently used flower species by males and females in extensively managed sites. In intensively managed sites, both sexes often visited other species that were largely ignored in the presence of the preferred *C. jacea.* The principal nectar source under such conditions was *Trifolium pratense*. Preference for *C. jacea* was independent of the landscape context; when introduced in intensively managed sites in equal numbers as *T. pratense*, it was significantly visited more frequently. Female preference for *C. jacea* was found to be stronger than male preference.

Next, we evaluated several life-history consequences of the nectar availability differences between the two levels of management intensity (**Chapter 5**). For *M. jurtina* from extensively managed, flower-rich grasslands, living for two days under nectar limited conditions, was sufficient to have a strong negative effect on the butterfly's condition for the rest of its life. Compared to those exposed to good nectar conditions, butterflies from the nectar limited treatment lived shorter, were less active, and had lower potential fecundity.

We then analysed the effects of nectar quality (flower species) and quantity (number of available flowers) on life-history traits, flight performance and the physiological pace of life of *M. jurtina*. In this experiment, we compared the responses of butterflies from extensively (EL) and intensively (IL) managed agricultural landscapes (**Chapter 6 and 7**). We showed that nectar quality was more important for females than for males. Simultaneous reduction of nectar quality and quantity had a negative effect on flight performance of EL females, whereas flight performance of IL females was not affected (**Chapter 7**), although the imposed nectar regime represented a stress that caused an irreversible decrease in lifespan and fecundity (**Chapter 6**). Under higher food stress, EL females decreased their resting metabolic rate (RMR), whereas IL females increased it.

In **chapters 6 and 7**, we analysed the different responses of the populations to reductions in nectar resources. IL butterflies were better able to buffer the effects of nectar limitation, in quality or in quantity. They also had a slower pace of life (measured by resting metabolic rate) and flight capacities that correspond to higher mobility. However, when both nectar quality and quantity were simultaneously reduced, IL females were no longer able to compensate for the stress caused by the nectar limitation and died shortly after the treatment; they laid less than a fifth of the number of eggs laid by the females in the other treatments.

Finally, we showed the behavioural consequences of a sudden increase in the level of competition for nectar resources (**Chapter 8**). In hay meadows where a refuge zone was left uncut after mowing, we showed an increase in grassland butterfly density in that zone after hay cutting. This increased butterfly density caused the preferred nectar source of *Maniola jurtina,* i.e. *C. jacea,* to be more frequently visited after mowing, and for longer bouts as well. Female butterflies allocated a greater proportion of their time to foraging activities, and visited more often other, non-preferred nectar

sources in the higher population density conditions in the refuge zone after mowing.

This general discussion is divided into three main parts; we will discuss our main results across the different chapters and compare them with the literature, and develop several perspectives for future work. We will first discuss variation in foraging behaviour, identifying the causes (i.e., variations in the balance between costs and benefits) and life-history consequences. We will then review environmental changes due to agricultural intensification and the most common impacts they have on organisms that occur in such agricultural environments. Finally, conservation actions and measures in favour of biodiversity in agricultural landscapes are reviewed and discussed in the light of our results.

Foraging behaviour: causes of variation and life-history consequences

Foraging determines resource intake by an organism and life-history patterns depend on allocation and reallocation of resources to fitness-related activities (Boggs, 1992): growth, reproduction, maintenance and further foraging. In order to fully understand life-history strategies in variable environments from a life-history theory viewpoint, one needs to combine foraging patterns and efficiency of resource-use with trade-offs between life-history traits. My PhD-thesis fits into this eco-evolutionary framework as we tried to link nectar use by the butterfly *M. jurtina* as observed in the field in different environments to the life-history consequences of different nectar diets.

Cost and benefits of foraging and influence on foraging strategy

Foraging behaviour, or the decisions made by an organism as to which resources to exploit and the strategy used to exploit the resources will partly determine the balance between costs and benefits of foraging (Lima et al., 1985; Sih, 1980). Costs of foraging can be attributed to exposure to predation and to the time and energy expenses of foraging (Fewell, 1988). Time spent searching for a resource will reduce the time budget available for other activities. However, many organisms do not focus on foraging only when searching for food. They may also engage in other activities such as territorial defence, reproduction, thermoregulation and mate finding (Brown, 1988) while foraging at the same time. This is also the case for butterflies (e.g. Goulson et al., 1997a). Hence, time and energy expenses and exposure to predation between two feeding events are not necessarily entirely attributed to foraging alone. Furthermore, for flower visiting insects, the time cost of foraging can be increased by switching between visited flower species, or alternatively, by being constant. Many flower-visiting insects that are considered to be generalists actually show variable

levels of preference and may visit one, or a few flower species more frequently than expected by the relative abundance of the plant species (e.g. Feinsinger, 1976; Goulson and Wright, 1995; Lewis, 1989). We showed in **Chapter 4** that this was the case for *M. jurtina*. We concluded from our behavioural tracking that *M. jurtina* butterflies visited *C. jacea* and thistles (*Cirsium pratense* and *Cirsium arvense*) more frequently than expected assuming a random foraging behaviour. In adopting such a preference, they ignored other rewarding flower species on their path. In fact, *T. pratense* , which is known to produce nectar and to be visited by butterflies (Gardener and Gillman, 2002; Rusterholz and Erhardt, 2000, 1998) was ignored in the presence of *C. jacea*, although it was visited otherwise. *Lotus corniculatus* is another example of an abundant flower species that was ignored, although it is preferred by other generalist butterfly species (Goulson et al., 1997a). Switching between flower species may cost time if learning to handle the new species is required (Darwin's interference hypothesis; Darwin, 1876), which depends on flower structure complexity (Gegear and Laverty, 1995; Goulson and Wright, 1995; Goulson et al., 1997b). Alternatively, restraining visits to one or few species may increase time and energy expenditure and predation risks by increasing travelling time between two visits (Gegear and Thomson, 2004; Waser, 1986; Wiegmann et al., 2003), assuming a scattered distribution of the visited flower species. However, in our behavioural observations (**Chapter 4**), we did not find an increased travel time between two consecutive visits on *C. jacea* compared to visits on other species.

Foraging benefits actually depend on the quality and quantity of food acquired (Hainsworth and Hamill, 1993). The quantity of available resources, which directly influences food quantity, obviously depends on resource distribution and abundance. Hence, many species are known to adjust their foraging decisions based on the abundance of resources or on food quantity, and on the physiological needs. During reproduction, wandering albatrosses (*Diomedea exulans*) either travel up to 3600 km in

search of scarce prey during pelagic foraging trips, which provides constant food quantity, or alternatively, they may forage on patches of aggregated squids and fish inshore, which leads to uncertain amounts of food (Weimerskirch et al., 1994). Fur seals (*Arctocephalus gazella*) perform longer foraging trips and allocated more time to foraging when prey abundance is reduced (Boyd et al., 1994). The nectarivorous bat (*Glossophaga soricina*) adopts territorial defence in the case of clumped flower resources but foraged along regularly used feeding routes in the case of a scattered distribution of flowers (Lemke, 1984). The small skipper butterfly *Thymelicus flavus* foraged on another flower species than previously visited if the latter became scarce (Goulson et al., 1997a). Similarly in **Chapter 4**, we showed that *M. jurtina* preferred to forage on *C. jacea* and *Cirsium* species, when present. These flower resources were present in flower rich environments, offering high abundance and diversity of nectar sources (i.e. flower-rich extensively managed grasslands). In this fairly continuous habitat, flowers are common and, consequently, flights between two consecutive flower visits were relatively short. We also observed that reduced flower diversity and abundance forced *M. jurtina* to fly over longer distances between two consecutive flower visits, and also to make use of flower species that were otherwise ignored. In intensively managed hay meadows, offering low flower diversity and abundance, *M. jurtina* fed preferentially on *Cirsium*, *Trifolium* species or *L. vulgare*, that were typically ignored in the presence of *C. jacea*. Among those species, thistles (*Cirsium* spp.) and clovers (*Trifolium* spp.) are known to be nectar rich and are important nectar sources for several butterflies species (Clausen et al., 2001; Goulson et al., 1997b; Rusterholz and Erhardt, 1998; Tudor et al., 2004). However, high resource abundance is often associated with high population density, and consequently, high competition and might therefore not provide high per capita resource availability. Competition may ultimately reduce resource availability. For flower visiting

organisms, it will reduce the amount of food acquired per flower visit, or in extreme cases, totally deplete flowers of their nectar and pollen rewards. As a result, foraging strategies of flower visiting organisms may vary in response to levels of competition and of rewards per flower. Some species forage early or later in the day to take advantage of full nectar refill in flowers during the night, before other visitors (hummingbirds: Feinsinger, 1976; solitary bees: Gottlieb et al., 2005; honey bees and bumblebees: Schaffer et al., 1979). However, this flexibility may be limited by the way body temperature is regulated (e.g. endothermic bumblebees start visiting flowers much earlier in the day than heliotherms like butterflies). Another strategy consists of changing flower species as rewards from the presently visited species start to decrease. Bumblebees, for example, were shown to adjust their constancy level to the quantity of nectar intake per flower, as to maximize their net energy intake rate (Gegear and Thomson, 2004; Wiegmann et al., 2003). Similarly, in **Chapter 8**, our behavioural observations showed that females of *M. jurtina* visited more frequently a non-preferred flower species following an increase in competition on the preferred flower species. Visitation rate of the preferred *C. jacea* was higher under high population density. Moreover, visits were always longer when population density was higher, and females allocated a greater proportion of their active time to foraging. Hence, we assumed that the increase in visitation rate on *C. jacea* depleted its nectar supply more rapidly, and butterflies consequently adjusted their foraging behaviour.

Resource selection by the forager may also depend on resource quality (e.g. Erhardt, 1992; Hainsworth and Hamill, 1993; Watts, 1998). Bees preferred to visit spatially distant artificial flowers if the quality of the reward in nearby flowers decreased, and their behaviour depended on flower colour (Hill et al., 2001). In butterflies, flower preference has been suggested to be influenced by nectar properties (Hainsworth and Hamill, 1993; Rusterholz and Erhardt, 2000) and to influence their fitness. In butterflies, sugar nectar

preference varies among species; *Battus philenor* (Erhardt, 1991) and *Inachis io* (Rusterholz and Erhardt, 1997) prefer, in decreasing order, sucrose, fructose and glucose, whereas *Lysandra bellargus* prefers sucrose (males) or glucose (females) (Rusterholz and Erhardt, 2000). Some butterfly species also prefer nectar that contains specific amino acids (*Battus philenor*, Erhardt (1991); *Pieris rapae*, Alm et al. (1990); *Arashnia levana*, Mevi-Schütz and Erhardt (2003) and *Pieris napi*, Mevi-Schutz and Erhardt (2004)). Although we did not test for preference between nectar constituents, we tested for preference between flower species that vary in their nectar composition, amongst other differences, with experimental flower arrays introduced in the field (**Chapter 4**). Because we used real flowers of two species, we cannot disentangle the roles of flower morphology and colour from those of nectar properties on the preference of butterflies. As discussed in **Chapter 4**, *M. jurtina* preference for *C. jacea* compared to *T. pratense* is probably due to both aspects. The nectar of the preferred flower species, *C. jacea*, should be preferred by butterflies as far as amino acid concentration (Rusterholz and Erhardt, 2000), sugar concentration (Rusterholz and Erhardt, 1998; pers. obs. in **Chapter 4**), and ratios of the different main sugars (Glucose/ Fructose and Sucrose/(Glucose + Fructose); Rusterholz and Erhardt, (1998)) are concerned. Hence, our study confirmed the general tendency in nectar property preferences in butterflies.

The physiological state of the individual may also influence foraging behaviour. For example, lactating mammals have different food preference than non-lactating conspecifics of the same sex (Gibb et al., 1999; Parsons et al., 1994). In butterflies, age influences the quantity of nectar intake (e.g. Boggs, 1988), but also the sex, and this is often attributed to the different life histories of males and females (e.g. Rusterholz and Erhardt, 2000). In **Chapter 4**, we also found sex-specific differences in the foraging behaviour of *M. jurtina*; males were feeding on a wider variety of flower species than

females, suggesting males to be less demanding than females in terms of nectar composition.

In summary, individual foraging behaviour is balanced between the costs (time and energy expenses and risks of predation) and benefits (quantity and quality of food intake). It is largely influenced by resources distribution, diversity and abundance, and it will ultimately determine individual fitness in a specific environmental context.

Life-history consequences of foraging behaviour

Any variation in foraging strategy will have repercussions on allocation trade-offs, and ultimately on life-history traits (Boggs, 1992). Resources acquired through foraging and stored from previous feeding are reallocated to further foraging, growth, maintenance, resources storage and reproduction (Boggs, 1992). Holometabolous insects at the adult stage have completed their growth, and dispose of a defined amount of stored resources from the larval stages, and further forage to acquire more energy, nutrients and water (Boggs, 1997). Many studies describe foraging behaviour, or tested variation in foraging strategies relative to environmental conditions, as described in the previous section of this general discussion. Most of them, however, did not test life-history consequences of the observed behaviours, and assumed increased fitness with higher net energy gain, for instance. Other studies tested the effects of different diets on individual life-history traits, independently of specific foraging behaviour. For butterflies, these are summarized in Table 9.1

Finally, some studies integrated the observed foraging behaviours with their life-history consequences. In a laboratory test, mayflies *Baetis tricaudatus* were shown to balance mortality risk due to predation and food acquisition as to maximize fitness (Scrimgeour and Culp, 1994). Dogwhelks *Nucella lapillus* maximized their net energy intake rate, which in turn

maximized growth (used as a surrogate for fitness) (Burrows and Hughes, 1990). To the best of our knowledge, studies combining foraging behaviour and individual life-history traits in butterflies are rare. Butterflies generally prefer nectar with higher concentration of amino acids (Alm et al., 1990; Erhardt, 1991; Jovanne Mevi-Schütz and Erhardt, 2003; Mevi-Schütz and Erhardt, 2004). In *Arashnia levana*, preference for nectar containing amino acids (Jovanne Mevi-Schütz and Erhardt, 2003) was linked with increased fecundity under larval food stress, which was interpreted as a compensatory feeding mechanism across developmental stages (Mevi-Schütz and Erhardt, 2005). Other studies tested for effects of amino acids in the diet, compared to control diets without amino acids, or for different sugar concentration, on butterfly life-history traits (e.g. Grill et al., 2013; Hill, 1989; Table 9.1). However, this was most often done without linking diets to observed food selection in the wild. Studies of dietary restriction on butterfly life-history traits (Boggs and Ross, 1993; Karl et al., 2007; Niitepõld et al., 2014) are more realistic as to represent variation of food availability in the wild, even if they use nectar mimics and do not make quality variation.

This PhD-thesis provided an integrated approach to link foraging behaviour and life-history consequences for nectar-feeding butterflies. We began with observations of foraging behaviour under natural conditions, with varying levels of resource abundance and diversity (**Chapters 4** and **8**).

Table 9.1: Summary consequences of different adult diets on life-history traits of adult butterflies. AAs: Amino acids

Aspect of nectar modification		Source	Species	Treatment	Consequences
Quantity		Boggs and Ross, 1993	*S. mormonia*	Dietary restriction	Reduced fecundity (potential and realized)
		Niitepõld et al., 2014	*S. mormonia C. eurytheme*	Dietary restriction	Reduced body mass, fecundity and RMR. Conserved flight capacities
		Karl et al., 2007	*B. anynana*	Dietary restriction	Decreased egg provisionning with increasing age
Quality	**Sugars**	Murphy et al., 1983	*E. editha*	Nothing, water, 20% sugar solution, water and AAs, nectar mimic with low or high AAs concentration	Sugar intake increased longevity, body weight maintenance and fecundity
		Hill, 1989	*E. core*	25% sugar solution, with or without AAs 1% sugar solution, with or without AAs	Sugar intake extended longevity and fecundity, 1% group performed compensatory feeding
		Cahenzli and Erhardt, 2012b	*C. pamphilus* (M)	Different sugar concentration	Longer life-span with higher sugar conc.
	AAs	Murphy et al., 1983	*E. editha*	See above	AAs increased egg weighth
		Hill, 1989	*E. core*	Nectar mimics (see above) with or without AAs	No effect
		Mevi-Schutz and Erhardt, 2003	*L. megera*	Nectar mimic with or without AAs	No effect
		Mevi-Schutz and Erhardt, 2005	*A. levana*	Nectar mimic with or without AAs	AAs increased fecundity (if growth on unfertilized hostplants)
		Cahenzli and Erhardt, 2012a	*C. pamphilus* (F)	Nectar mimic with or without AAs	AAs increased hatching success and larval mass
		Cahenzli and Erhardt, 2013	*C. pamphilus* (M)	Nectar mimic with or without AAs	AAs increased progeny's larval hatching mass
		Grill et al., 2013	*M. jurtina*	Nectar mimic with or without AAs	No effect
Quality and quantity		This PhD Thesis	*M. jurtina*	100 or 10 *C. jacea*, 100 or 10 *T. pratense*	Depending of origin of butterfly; various effects on survival, longevity, fecundity, RMR and flight capacities, presented in more details in Table 9.2
		This PhD Thesis	*M. jurtina*	100 *C. jacea* or 10 *T. pratense*	48h with 10 *T. pratense* reduced longevity and lipid reserves

Then, we tested life-history consequences of the observed behaviours in the different environments (**Chapters 5, 6 and 7**) with an experimental design intended to mimic food availability related to each environmental condition. Our experimental approach with flight cages used real flowers as food sources for the tested butterflies. By using two flower species – i.e., the preferred *C. jacea* and the non-preferred *T. pratense* – we varied the diet with respect to nectar quality. Quantity of food was manipulated through the numbers of flowers displayed in the flight cage. In flower-rich environment, *M. jurtina* fed preferentially on *C. jacea*, whereas in flower-poor environments, in absence of the preferred nectar-source, a variety of other species were visited, among which the nectar-rich *T. pratense* (**Chapter 4**). Two nectar treatments represented field conditions in respect to flower availability of the tested flower species (C100 and t10, see **chapters 5 and 6**), and two other intermediate conditions allowed to test separately for the effect of the nectar source (i.e., quality) and nectar quantity (i.e., T100 and c10) (tested in **chapters 6 and 7**). Effects of the different diets on life-history and flight-related traits in *M. jurtina* are summarized in Table 9.2.

Any deviation from the high quality and quantity treatment lowered survival rates during and after the treatments. Combining nectar quality and quantity reduction had dramatic effects on females from intensively managed agricultural landscapes, which were only able to maintain flight performance under those conditions, but showed lower values for all other traits compared to female butterflies in all other treatments. Post-treatment longevity was higher in the T100 group than in the other treatments, but in females, such an extended longevity did not lead to an increase in the total number of eggs laid. Consequently, it did not seem to provide clear fitness advantages. Overall, we conclude that the preference for *C. jacea* as observed in flower-rich sites corresponds to the nutritional needs of *M. jurtina*. Of course, situations in the wild where a *M. jurtina*

butterfly would forage on a single flower species throughout its life are somewhat extreme. We showed that *T. pratense* was not the only visited flower species in flower-poor sites (**Chapter 4**), suggesting that *M. jurtina* supplements its nutritional needs by visiting a variety of different nectar sources (Ouin et al., 2004). Moreover, in **chapter 7**, we argue that butterflies in intensively managed croplands probably move in search of new resources when facing resources scarcity. Nevertheless, our results point at the dramatic consequences of a nectar shortage for *M. jurtina* butterflies.

In a laboratory experiment comparing effects of different diets on young wild-caught *Maniola* butterflies, Grill et al. (2013) showed no effects of additional amino acids in the diet on lifespan, fecundity and egg-laying strategy.

Table 9.2: Summary of the effects of nectar quality and quantity on *M. jurtina* butterflies originating from intensively managed agricultural landscape (IL) and extensively managed agricultural landscape (EL) based on chapters 5, 6 and 7 of this PhD-thesis. Effects are expressed as compared to the value of the considered trait in the C100 treatment: change in nectar source (quantity), reduction in number of available flower heads (quantity) or both combined. Methods and results are explained in the respective Chapters.

		IL			EL			
Sex	**Trait**	**Quantity**	**Quality**	**Both combined**	**Quantity**	**Quality**	**Both combined**	
							Ch. 6/7	**Ch. 5**
Female	Survival to trial	↘	↘	↘	↘	↘	↘	=
	Mass loss during trial	↘	=	↘	↘	↘	↘	
	Longevity	=	↗	↘	=	=	=	↘
	Lipid content after trial							↘
	Proportion of egg-laying individuals	=	=	↘	=	=	↘	
	Realized fecundity	=	=	↘	=	=	=	
	Realized fecundity as expected by longevity	↘	↘	↘↘↘	↘	↘	↘↘↘	
	Egg size	=	↗	↘	=	=	↗	
	RMR	=	=	↗	=	=	↘	
	FMR	=	=	=	=	=	=	
	Flight distance	=	=	=	=	=	=	
	Willingness to fly	=	=	=	↘	=	↘	
Male	Survival to trial	↘	=	↘	↘	=	↘	↘
	Mass loss during trial	=	=	=	=	=	=	
	Longevity	↘	↗	=	=	↗	=	↘
	Lipid content after trial							↘

In their experiment, butterflies were fed with the tested diet throughout their adult life. Hence, they had no opportunity for compensatory feeding. They argued that for univoltine species as *Maniola jurtina*, diet quality at the adult stage is probably less important than endogenous factors, or environmental factors in an earlier developmental stage. We did not test directly for effects of amino acids in the diet, and in both our experiments butterflies were offered the possibility of compensatory feeding after the initial nectar treatment. However, the differences we found (Table 9.2) still suggest that nectar feeding is an important aspect to consider when evaluating *M. jurtina* fitness. We believe that prolonged duration of our treatment would result in stronger effects on the measured traits.

Note that treatment and post-treatment conditions were not exactly the same between the two experiments. In the experiment presented in **Chapter 5**, we placed 10 female and 10 male *M. jurtina* in a flight cage for 48 h, in the C100 or t10 treatments. To measure longevity, butterflies were kept under outdoor conditions (but sheltered from the rain) at a maximum of 5 butterflies per cage and sexes were kept separately with unlimited access to water diluted honey on cotton. In the second experiment, butterflies were placed in one of the four treatments (C100, c10, T100 or t10, see **Chapter 6**) for 48 h in flight cages that were half the size of the previous ones, and with a total number of 24 butterflies (12 females and 12 males). So for the same amount of resources, there were more butterflies in a smaller space. To measure longevity, they were kept in small individual cages under controlled conditions in a climate room, with unlimited access to water diluted honey on cotton. Where differences in the measured traits are observed for the C100 and t10 categories between the two sets of trials, we should not ignore the differences in the experimental conditions. We expected the treatment in itself to be less favourable for the butterflies in the second experiment (**Chapter 6 and 7**) because of the slightly higher level of competition for resources and because of the higher butterfly

density, leading to more interactions between individuals, which are very energy demanding. On the other hand, the post-treatment conditions of the second experiment (**Chapter 6 and 7**) were expected to be more favourable compared to the conditions in the first experiment (**Chapter 5**), thanks to the absence of competition for access to food and to controlled climatic conditions, compared to butterflies of **Chapter 5** which experienced stronger daily variation in temperature and humidity.

In females, mean survival to the treatments did not change between years, whereas it was lower in the second set of trials (**Chapter 6**) for males (Figure 9.1). In females, survival did not differ between treatments in the trials presented in **Chapter 5**, but were lower in the t10 treatment in the trails of the second experiment. Male survival was dramatically low in the t10 treatment in the second set of trials. The survival analysis was based on the number of butterflies that were effectively recollected at the end of a trial, but a non-negligible number of individuals were lost (either they managed to escape from the flight cage, or died due to spider predation) and could not be incorporated in the analyses. On this basis, we suggest that the slight differences observed in female survival do not allow any strong conclusion. In males, however, higher densities in the flight cage combined with low resource quality and quantity, led to increased mortality. The different responses of survival to nectar treatment and density between females and males is probably due to the sex-specific behavioural differences in life style; males spend, for example, more time flying (in search of a mate) than females (Brakefield, 1982a). Moreover, the lower activity level observed in the t10 treatment (**Chapter 5**) also suggests lower energy available for individuals, and hence, lower flight activity. In a denser environment, the number of interactions increased, as observed in **Chapter 8** under natural conditions. So, individuals may be forced to fly even though their energy resources are at a low level.

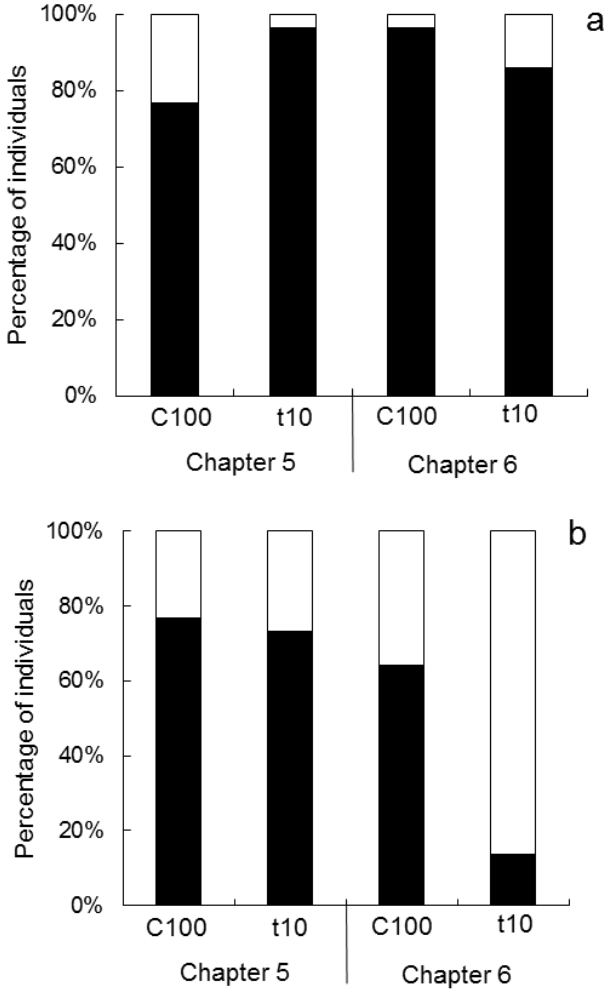

Figure 9.1: Survival (black) and death (white) of *M. jurtina* individuals after 2 days in the flight cage in the C100 and in the t10 treatment, in the test performed in 2011 and developed in chapter 5, and in the test performed in 2012 and developed in chapter 6 (data of females from Extensively managed agricultural landscape only); for a) female and b) males. Generalized Linear Model with binary distribution on the state of individual (alive or dead) at the end of the trial. Females: Chapter: $\chi^2_1 = 0.3$, $P = 0.58$; Treatment: $\chi^2_1 = 0.3$, $P = 0.58$; Chapter x Treatment: $\chi^2_1 = 12.45$, $P = 0.0004$. Males: Chapter: $\chi^2_1 = 16.16$, $P < 0.0001$; Treatment: $\chi^2_1 = 8.82$, $P = 0.003$; Chapter x Treatment: $\chi^2_1 = 6.42$, $P = 0.01$.

When resources are scarce, males no longer get enough energy income and have to rely more on their stored resources (capital). Measures of longevity were also performed in both experiments (Figure 9.2). Post-treatment longevity was lower in the t10 treatment in the first experiment (**Chapter 5**, Table 9.2 and figure 9.2), and did not differ between treatments in the second one (**Chapter 6**, Table 9.2 and Figure 9.2). In **Chapter 6**, we attributed the absence of such a difference between treatments to compensatory feeding during the post-treatment phase. As this was not observed in **Chapter 5**, we argued that a 48 h exposure to a nectar-poor environment impaired individuals as to render them unable to compete efficiently for food. Longevity was always higher after the first experiment than after the second, despite worse post-treatment conditions. This suggests that density and the level of competition in the flight cage had a significant role for the energy spent during the treatment and hence, also for the other life-history traits.

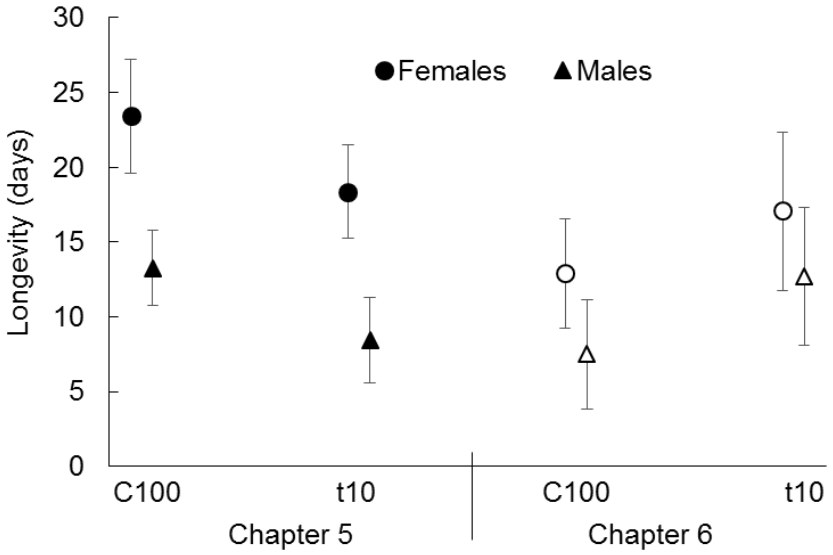

Figure 9.2: Mean longevity (± SEM) of *M. jurtina* females (circles) and males (triangles) in the C100 and t10 treatments in the experiments presented in Chapter 5 (filled symbols) and Chapter 6 (open symbols). General Linear Model on log (post-treatment longevity). Females: initial mass: $\chi^2_1 = 11.38$, $P = 0.0007$; Chapter: $\chi^2_1 = 11.12$, $P = 0.0009$; Treatment: $\chi^2_1 = 0.53$, $P = 0.47$. Males: Chapter: $\chi^2_1 = 2.07$, $P = 0.15$; Treatment: $\chi^2_1 = 0.38$, $P = 0.53$; Chapter x Treatment: $\chi^2_1 = 6.8$, $P = 0.009$.

The mechanism behind the trade-off between the different life-history traits depends on the allocation pathways of the resource pool of the organism. Under different environmental stress conditions, butterflies may shift their allocation pathways (Boggs and Niitepõld, 2014; Cahenzli and Erhardt, 2012b). Although we did not study allocation pathways in detail, some of our results suggest different trade-offs under different diets. For EL butterflies, it seems clear that a slower metabolism, together with a lower level of activity would go in the sense of favouring reproduction and survival under high food stress (t10) (Table 9.2, **Chapters 6 and 7**). IL butterflies had a higher RMR, hence needed more energy to maintain basic

functioning and probably had fewer resources to allocate to further foraging and reproduction under the same conditions of intense food stress (t10). Our results (rapid death after the experiment, very low fecundity) may suggest that essential nutrients were missing (or not present in sufficient quantity) in that treatment for females from the intensively managed agricultural landscape, whereas this was not the case for EL butterflies.

Living in a human-modified, intensively managed agricultural landscape

Intensification has deeply modified agricultural landscapes. These environmental changes represent major challenges for populations of many organisms. In order to avoid local extinction, adequate responses by rapid evolution or phenotypic plasticity are necessary (Ghalambor et al., 2007; Reznick and Ghalambor, 2001), unless conservation programs can provide complementary resources to buffer against negative impacts. Here, we discuss eco-evolutionary responses to the intensification of agriculture through phenotypic changes.

Species able to maintain populations in intensively managed agricultural landscapes are facing numerous changes compared to (semi-)natural habitats. Habitats are much more fragmented and their surface largely reduced, resource availability is lower, resource quality is different (through fertilizers for instance), plant assemblages are different and diversity is lower, microclimatic conditions are altered and population density is lower as well. Life under conditions created by intensive agriculture represents a challenge for organisms that have to cope with rarefication of resources and modification of their quality (both consumables and utilities; Dennis et al., 2003), habitat fragmentation and microclimatic changes. To face these challenges, organisms will generally modify their strategies related to resource search, life-history traits and the associated trade-offs and flight-morphology traits, amongst others. Phenotypic variation with the level of agricultural intensification may result from genetic adaptations, from phenotypic plasticity or from constraints imposed by the environmental changes (Karlsson and Van Dyck, 2005). Here, we review literature findings about changes in life-history traits, flight-related traits and metabolism related to environmental changes that are similar to the ones caused by agricultural intensification, and discuss these in relation to our results.

Eutrophication from fertilization mainly imposes constraints on growth and development of herbivores, affecting life-history traits. Fertilized plants have a higher nitrogen content than non-fertilized conspecifics (e.g. Cahenzli and Erhardt, 2012; Chen et al., 2004; Lavoie and Oberhauser, 2004), but may also differ in the concentration of other, secondary metabolites (Cahenzli and Erhardt, 2012a; Schoonhoven et al., 2005). Therefore, herbivores are generally predicted to perform better on fertilized plants, although metabolic responses of herbivores to the C/N ratio of the host plant vary among species (Throop et al., 2004), making predictions not straightforward. When reared on fertilized host plants, butterfly larvae are known to grow faster (Chen et al., 2004), but this results in adults of lower body mass (Chen et al., 2004; Lavoie and Oberhauser, 2004). In *Pieris rapae*, faster growth rates on fertilized plants came, however, with larger pupal mass (Myers, 1985). Heavier pupal or adult body mass reached by larvae fed on unfertilized host plants, or bigger amounts of ingested food are generally interpreted as an ability of compensatory feeding (Chen et al., 2004; Lavoie and Oberhauser, 2004). However, compensatory feeding (or alternatively longer development) is not always sufficient to catch up for poor host plant quality (Morehouse and Rutowski, 2010). These studies addressed ecological effects of fertilization; evolutionary responses of herbivores to fertilized plants are rarely studied. Yet, Morehouse and Rutowski (2010) showed genetic variation associated with the response to larval nutrient availability in *Pieris rapae*. Cahenzli and Erhardt (2013) showed evidence that acclimatization to the host plant could be transmitted to offspring through maternal effects. Both studies underline the possibility of selection acting on life-history traits in response to host plant quality. In our study, we found that adult butterflies from an intensively managed agricultural landscape were heavier than conspecifics from an extensively managed landscape (**Chapter 6**). A stressful (resources-poor) environment for adults, but with a good

quality (fertilized) host plants, could bring butterflies to switch toward a more capital-breeding strategy. They would consequently store more reserves at the larval stage. Our results are congruent with this hypothesis, although we did not show direct evidence as we worked with wild-caught, instead of laboratory-reared individuals. Along the same line, a study on the aphid parasitoid *Aphidius ervi* showed that small females switched their strategy from synovigeny to pro-ovigeny under stressful conditions (Ismail et al., 2012).

Microclimatic conditions during development in intensively managed, simplified agricultural landscape may also be a driver of heavier adult mass and altered growth rates at the ecological or evolutionary time scale. Although mean temperatures in spring and summer are higher in simplified agricultural landscapes than in semi-natural biotopes with stronger microclimatic buffering , convective cooling due to wind and temperature extremes are more important in open, simplified landscapes (Merckx et al., 2008), and winter temperature is lower as well. Therefore, the heavier mass that we observed for IL adults (**Chapter 6**) could be due to harsher or more variable microclimate during development. Actually, the majority of ectotherms grow slower but mature at a larger body size in colder environments, although mechanisms underpinning this relationship are not well known and may vary between species (Angilletta et al., 2004). Populations of *Papilio canadensis* from Alaska were cold-adapted and grew faster at lower temperature than conspecifics from Southern Canada (Ayres and Scriber, 1994). Hence, differences in microclimatic conditions during larval development between the two landscapes could trigger ecological and evolutionary responses. A slower growth rate also increases exposure time to killing agents such as pathogens, predators and parasites (Gotthard, 2008; Morehouse and Rutowski, 2010). For the butterfly *Aglais io* higher levels of parasitism were found in semi-natural, less fragmented woodland compared to an agricultural landscape (Serruys and Van Dyck, 2014). In a

split-brood field experiment on the related butterfly *Aglais urticae*, which also occurs in agricultural landscapes, Merckx et al. (2015) showed faster development at higher temperature, attributed to plasticity, and increased survival interpreted as reduced exposure time to killing agents. Although our observations do not indicate whether *M. jurtina* had a faster development in intensively managed landscapes, we found heavier body mass (**Chapter 6**). This aspect warrants more detailed studies.

Microclimate during development may also influence adult phenotypes. In the woodland butterfly *Pararge aegeria*, which also maintains populations in agricultural landscapes, adults from populations of agricultural landscapes were furrier (Merckx et al., 2008) and darker (Berwaerts et al., 1998). Such morphological differences relate to the thermal environments in which the butterflies have to function. Moreover, at higher ambient temperature, females of this species from fragmented agricultural habitats reached higher fecundity than conspecifics from woodlands, and laid larger eggs (Karlsson and Van Dyck, 2005). This suggests adaptations to higher mean ambient temperature. Melanisation as a response to temperature during larval development (i.e. ectothermic individuals exposed to lower temperature become darker in order to increase their body temperature faster) has attracted more attention but is, however, not the only possibility. The level of pigmentation has been shown to increase with desiccation risk in *Drosophila* (Parkash et al., 2008) and more recently this has been suggested for a butterfly as well (Merckx et al., 2015).

Organisms living in fragmented habitats are expected to have better flight capacities and associated traits (e.g. Polic et al., 2014), if the inter-habitat distance is smaller than their organism maximal dispersal range (Van Dyck and Matthysen, 1999). Alternatively they may have lower flight abilities if habitat remnants are too isolated (e.g. Schtickzelle et al., 2006). For grassland butterflies in intensively managed agricultural landscapes, we

expect habitat remnants (road sides, field boundaries, hedgerows, meadows) to be close enough to one another so as to allow frequent butterfly movements. In several butterfly species, populations from fragmented landscapes invested more in flight-related morphology such as the thorax, containing the flight muscles, than conspecifics from less fragmented areas (Berwaerts et al., 1998; Hill et al., 1998; Thomas et al., 1998), suggesting adaptations towards higher mobility, or to perform at the same level under difficult conditions. Other studies reached the same conclusions based on other experimental designs and morphological measures (e.g. Hanski et al., 2004; Wang et al., 2011). In line with our predictions, we found *M. jurtina* females from populations of intensively managed agricultural landscape to have stronger flight capacities; they flew over longer distances when tested with tethered flight in a flight mill (**Chapter 7**). They were also more willing to fly and had faster flight metabolic rate, which is known to be correlated with dispersal rate in other butterflies (Niitepõld et al., 2009).

In many species, increased investment in flight may be traded-off against other life-history traits such as fecundity (Hughes et al., 2003) or longevity (Hanski et al., 2006). Under limitation of food intake, we showed that flight capacities were maintained at the cost of a shortened lifespan and a decreased fecundity (**Chapter 6**).

The resting metabolic rate (RMR) differed between the two populations. It was lower in individuals from intensive agricultural land (**Chapter 7**). In Chapter 7, we hypothesized that IL butterflies decreased their RMR as a strategy to minimize energy expenditure in a context of resource scarcity (e.g. Lighton and Bartholomew, 1988; McNab and Morrison, 1963).

Altogether, our findings point at changes in several life-history traits, flight-related traits and energy metabolism. They allow *M. jurtina* butterflies from

intensively managed landscapes to be more mobile in a context of adult resource scarcity, to save energy from maintenance and to make the most out of larval developmental conditions as by storing more resources as capital under conditions of adult resource limitation. The butterflies were, however, strongly affected by a higher food stress at the adult stage (**Chapters 6 and 7**), suggesting a cost for these changes. Hence, in our study system, the cost of higher investment in flight could rest upon lower resistance to intense food stress. The strategy could be to avoid this situation by searching actively for resources, which is facilitated by higher mobility.

Our study provides the first step to the understanding of eco-evolutionary responses to agricultural intensification in butterflies (Reznick and Ghalambor, 2005).

Conservation measures in agricultural landscapes

The main changes of agricultural intensification influencing butterflies and other flower-visiting insects include the reduced diversity and abundance of flowering plants (Potts et al., 2004; Vitousek et al., 1997), the increased field size and the resulting landscape homogenization, habitat fragmentation and the loss of biotopes (Tscharntke et al., 2005).

In Europe, agri-environmental schemes (AES) have been implemented to counteract the negative impacts of agriculture on biodiversity (EU, 2005). They typically offer financial compensation for implementing, maintaining or creating diverse management techniques (e.g. lower stocking intensity, late mowing, low input) and landscape elements (e.g. extensive field margins, hedgerows, ponds, isolated trees) intended to improve biodiversity and the general quality of environment. They may apply to a single field or to the whole farm and integrate and combine several aspects of the management and different landscape elements as well (Stoate et al., 2009). These schemes either target the global regional biodiversity, environment and ecosystem services provided by agro-ecosystems (Batáry et al., 2011; Ekroos et al., 2014), or can be more specific and target specific endangered or declining species (e.g. Perkins et al., 2011).

Efficiency of the schemes may vary considerably and also depends on the species or taxonomic group (Kleijn and Sutherland, 2003; Kleijn et al., 2006) and on the landscape context (Batáry et al., 2011; Hendrickx et al., 2007). For example, two AES that specifically targeted corncrake (*Crex crex*) in the UK also increased the abundance of late-season flowers, butterflies and bumblebees, apart from of being efficient for this target bird species, but negatively affected early forbs and springtails (Wilkinson et al., 2012).

As far as insects are concerned, their abundance, and diversity to a lesser extent, is generally enhanced by AES programs across Europe, but

endangered species need more particular attention and specific AES (Haaland and Bersier, 2011; Kleijn et al., 2006). Semi-natural grasslands are known to act as a population source for nearby intensively managed agricultural land (Öckinger and Smith, 2007b), and in some case, AES are more efficient in favouring wild bees, carabid beetles, hoverflies, true bugs and spiders, when placed in the proximity of semi-natural grasslands (Hendrickx et al., 2007; Kohler et al., 2008). On the other hand, micro and macro-moth species benefit from implementation of simple AES management prescriptions applied to relatively small areas, without considering landscape context (Fuentes-Montemayor et al., 2011).

Popular AES targeting flower-visiting insects generally consist of field margins or strips of sown wild flowers and grasses (Carvell et al., 2006; Haaland and Bersier, 2011). Authors agree on the importance of flower abundance and diversity (Potts et al., 2009; Pywell et al., 2011), extended over the whole season (including mid-and late season flowering species) and on adequate management of flowering strips (Fritch et al., 2011; Pywell et al., 2011; Woodcock et al., 2009). Moreover, linear grassy elements in the agricultural landscape increase connectivity and enhance butterfly dispersal of at least some butterfly species (Delattre et al., 2013).

In general, improving flower-visiting insect abundance and diversity in agricultural landscapes involves increasing grassy areas containing nectar and pollen sources. Grazed pastures and hay meadows that are integral parts of the agricultural landscape, even when highly productive and under intensive management, also represent potential grassy habitats. Hence, management of those productive lands is also important (Littlewood et al., 2012). Generally, the absence of inorganic fertilizers, and reduction of the intensity of cutting or grazing regimes, promote floral species richness and sward structure, increasing, in turn, the total biomass of invertebrates and food availability for higher trophic levels (Woodcock et al., 2009). In the

case of hay meadows, late mowing is in favour of insects (e.g. Orthopterans (Buri et al., 2013). Late mowing has been proved to benefit endangered butterfly species (Johst et al., 2006). If mowing is performed earlier, then it should be partial, and accompanied by an autumnal cut of the whole parcel (Pywell et al., 2011). In addition to late mowing, leaving uncut areas as refuge for the fauna is another type of AES for meadows and pastures (Braschler et al., 2009; Cizek et al., 2012; Dover et al., 2010). There is evidence for the efficiency of uncut refuge zone in orthopterans (Buri et al., 2013; Humbert et al., 2012) and other arthropods (Hossain et al., 2002; Thorbek and Bilde, 2004). We provided evidence of a concentration effect of the grassland butterfly species in the refuge zone after mowing (**Chapter 8**). However, as discussed in **Chapter 8,** the localisation of the uncut zone must be chosen carefully so as to maximize its flower abundance and diversity, and to enhance landscape connectivity if realised in very large meadows. Additionally, the size of the uncut zone must be sufficient as to sustain local populations that effectively colonize it after mowing. We showed changes in the behaviour related to increased competition for nectar resources as a result of increased insect density (**Chapter 8**). However, without the uncut zone, insects would be totally deprived of local resources, and would be forced to disperse or die.

The number, type and success of AES programs vary between countries, but in all European countries AES are usually more frequently applied in areas of extensive agriculture, that already have relatively high levels of biodiversity, but less frequently so in intensively managed landscapes where they are most needed (Kleijn and Sutherland, 2003). Belgium is no exception. For example, meadows under AES for late mowing and uncut zone are mainly found in the extensive areas, which includes our study area, and are otherwise very rare (J. Lebeau, pers. obs.).

In summary, we encourage the implementation of relatively simple measures that efficiently promote biodiversity, as the grass-and-flower strips along field margins, which are also easily understood and managed by farmers. Such schemes increase a variety of resources (flowers, seeds, grasses, nesting sites) for farmland fauna (insects, birds, small mammals) and also serve as corridors to promote dispersal and movement within the intensively managed landscape (Delattre et al., 2013). Botanical diversity of the strips should be as diverse as possible, both within strips (α diversity) and between strips (β diversity) and they should offer resources throughout the season (Pywell et al., 2004). Ideally, they should be relatively extensively managed.

Perspectives

Throughout this PhD-thesis, we showed several clear patterns of life-history consequences of changes in nectar quality and quantity, and of traits that varied between populations of extensively and intensively managed agricultural landscapes. Now it is warranted to analyse in detail the mechanisms underpinning these patterns.

Radiotracers (e.g. ^{14} C, ^{3}H incorporated into food of a specific life stage) are useful tools to study allocation pathways between larval and adult acquired resources for reproduction (Boggs, 1997). Identifying the origin of nutrients used for fecundity under different nectar regimes, associated with respirometry to get an insight in energy expenditure for the different functions, offer an interesting perspective in this context. Moreover, allocation pathways could differ between populations of butterflies (either IL or EL), indicating differences in physiological traits between the populations of different landscape type. If IL butterflies would use more of their larval resources for reproduction, independently of adult food income, this would confirm the hypothesis of a switch towards a higher capital breeding life style in IL *M. jurtina* butterflies.

In **Chapter 6**, we showed that butterflies were capable of compensatory feeding in most cases. We argued that a longer nectar treatment would affect even stronger life-history traits and that compensatory feeding could conceal effects of nectar quality and/or quantity reduction. Hence, longer exposure to our nectar treatments would offer an interesting follow-up approach in the framework of life-history consequences of adult food sources in butterflies.

Discriminating between environmental effects, phenotypic plasticity and genetic adaptations as mechanisms causing the observed differences between IL and El butterflies would require additional experimental work.

Common-garden experiments would allow to identify the heritable part of traits variation (Berwaerts et al., 2008), whereas experiments using split-brood design would additionally test for phenotypic plasticity and environmental effects on the measured phenotypic traits (Merckx and Van Dyck, 2006).

Flight capacity is determined by a combination of physiological processes (energetic costs of flight) (Niitepõld and Hanski, 2013), morphological characters (e.g. wing loading) (Hill et al., 1998), biomechanical mechanisms (kinematics; Berwaerts et al. 2006) and behaviour (Skórka et al., 2013). The more detailed analysis of these different components of flight capacity between IL and EL butterflies would allow further mechanistic comprehension of the adaptations of butterflies to life in agricultural landscapes.

In this PhD-thesis we showed differences between *M. jurtina* butterflies from intensively and extensively managed landscapes in the way they deal with different adult resource levels and what this means for their behaviour and life-history. Developing an eco-evolutionary perspective on such changes would be a very interesting next step. For example, it would be interesting to test the effect of different host plant species and quality on larvae life-history traits. Moreover, understanding evolutionary mechanisms of organisms in agricultural areas would allow better thinking and planning of conservation measures such as agri-environmental schemes (Littlewood et al., 2012).

In the Anthropocene, organisms increasingly have to face human-induced changes, at an ever growing rate. These changes represent multiples challenges for organisms, to which they must respond by adjusting strategies and phenotypes to avoid extinction. Urbanisation, global warming, deforestation, pollution and biological invasions for example are

just as many major changes due to human activities. Understanding changes in organisms in response to one may enlighten comprehension of changes in response to others and help thinking and planning conservation measures.

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References

Alm, J., Ohnmeiss, T.E., Lanza, J., Vriesenga, L., 1990. Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia 84, 53–57.

Andersson, S., Dobson, H.E.M., 2003. Behavioral foraging responses by the butterfly *Heliconius melpomene* to *Lantana camara* floral scent. J. Chem. Ecol. 29, 2303–2318.

Andersson, S., Nilsson, A.L., Groth, I., Bergstrom, G., 2002. Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. Bot. J. Linn. Soc. 140, 129–153.

Angilletta, M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a puzzle. Integr. Comp. Biol. 44, 498–509.

Asher, J., Warren, M., Fox, R., Harding, P., Jeffcoate, G., Jeffcoate, S., 2001. The Millenium Atlas of Butterflies in Britain and Ireland. Oxford University Press, Oxford.

Aviron, S., Herzog, F., Klaus, I., Schüpbach, B., Jeanneret, P., 2011. Effects of wildflower strip quality, quantity, and connectivity on butterfly diversity in a Swiss arable landscape. Restor. Ecol. 19, 500–508.

Aviron, S., Jeanneret, P., Schüpbach, B., Herzog., F., 2007. Effects of agri-environmental measures, site and landscape conditions on butterfly diversity of Swiss grassland. Agric. Ecosyst. Environ. 122, 295–304.

Awmack, C.S., Leather, S.R., 2002. Host plant quality and fecundity in herbivorous insects. Annu. Rev. Entomol. 47, 817–844.

Ayres, M.P., Scriber, M.J., 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera : Papilionidae). Ecol. Monogr. 64, 465–482.

Baguette, M., Van Dyck, H., 2007. Landscape connectivity and animal behavior: functional grain as a key determinant for dispersal. Landsc. Ecol. 22, 1117–1129.

Baker, H.G., 1975. Sugar concentrations in nectars from hummingbird flowers. Biotropica 7, 37–41.

Baker, H.G., 1977. Non-sugar chemical constituents of nectar. Apidologie 8, 349–356.

Baker, H.G., Baker, I., 1973. Amino-acids in nectar and their evolutionary significance. Nature 241, 543–545.

Baker, H.G., Baker, I., 1975. Studies of nectar-constitution and pollinator-plant coevolution, in: Gilbert, L.E., Raven, P.H. (Eds.), Coevolution of Animals and Plants. University of Texas Press, Austin, pp. 100–140.

Baker, H.G., Baker, I., 1977. Intraspecific constancy of floral nectar amino acid complements. Bot. Gaz. 138, 183–191.
Baker, H.G., Baker, I., 1983. Floral nectar sugars constituents in relation to pollinator type, in: Jones, C.E., Littel, R.J. (Eds.), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York, p. 141.
Baker, H.G., Baker, I., 1986. The occurence and significance of amino acids in floral nectar. Plant Syst. Evol. 151, 175–186.
Baker, H.G., Baker, I., 1990. The predictive value of nectar chemistry to the recognition of polinator types. Isr. J. Bot. 39, 157–166.
Balmer, O., Erhardt, A., 2000. Consequences of succession on extensively grazed grasslands for Central European butterfly communities: rethinking conservation practices. Conserv. Biol. 14, 746–757.
Barker, R.J., Lehner, Y., Kunzman, M.R., 1980. Pesticides and honey bees: nectar and pollen contamination in alfalfa treated with dimethoate. Arch. Environ. Contam. Toxicol. 9, 125–133.
Batáry, P., Báldi, A., Kleijn, D., Tscharntke, T., 2011. Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. Proc. Biol. Sci. 278, 1894–1902.
Bauerfeind, S.S., Fischer, K., 2005. Effects of food stress and density in different life stages on reproduction in a butterfly. Oikos 11, 514–524.
Bauerfeind, S.S., Perlick, J.E.C., Fischer, K., 2009. Disentangling environmental effects on adult life span in a butterfly across the metamorphic boundary. Exp. Gerontol. 44, 805–811.
Beenakkers, A.M.T., Van der Horst, D.J., Van Marrewijk, W.J. a., 1984. Insect flight muscle metabolism. Insect Biochem. 14, 243–260.
Behmer, S.T., 2009. Insect herbivore nutrient regulation. Annu. Rev. Entomol. 54, 165–187.
Behmer, S.T., Simpson, S.J., Raubenheimer, D., 2002. Herbivore foraging in chemically heterogeneous environments: Nutrients and secondary metabolites. Ecology 83, 2489–2501.
Benton, T.G., Bryant, D.M., Cole, L., Crick, H.Q.P., 2002. Linking agricultural practice to insect and bird populations: a historical study over three decades. J. Appl. Ecol. 39, 673–687.
Benton, T.G., Vickery, J. A., Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends Ecol. Evol. 18, 182–188.
Berger, D., Olofsson, M., Gotthard, K., Wiklund, C., Friberg, M., 2012. Ecological Constraints on Female Fitness in a Phytophagous Insect. Am. Nat. 180, 464–480.
Bergerot, B., Fontaine, B., Julliard, R., Baguette, M., 2011. Landscape variables impact the structure and composition of butterfly assemblages along an urbanization gradient. Landsc. Ecol. 26, 83–94.

Bernays, E.A., Chapman, R.F., 1994. Host-plant selection by phytophagous insects. Springer Science & Business Media.
Berwaerts, K., Aerts, P., Van Dyck, H., 2006. On the sex-specific mechanisms of butterfly flight: Flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria*. Biol. J. Linn. Soc. 89, 675–687.
Berwaerts, K., Matthysen, E., Van Dyck, H., 2008. Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: a quantitative genetic assessment. Evolution. 62, 2525–2533.
Berwaerts, K., Van Dyck, H., 2004. Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria*. Oecologia 141, 536–545.
Berwaerts, K., Van Dyck, H., Van Dongen, S., Matthysen, E., 1998. Morphological and genetic variation in the speckled wood butterfly (*Pararge aegeria* L.) among differently fragmented landcsapes. Netherlands J. Zool. 48, 241–253.
Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313, 351–354.
Bink, F.A., 1992. Ecologische atlas van de dagvlinders van Noordwest-Europa. Schuyt & Co. Uitgevers en Importeurs., Haarlem.
Blake, R.J., Woodcock, B.A., Westbury, D.B., Sutton, P., Potts, S.G., 2010. New tools to boost butterfly habitat quality in existing grass buffer strips. J. Insect Conserv. 15, 221–232.
Boggs, C.L., 1988. Rates of nectar feeding in butterflies: effects of sex, size, age and nectar concentration. Funct. Ecol. 2, 289–295.
Boggs, C.L., 1992. Resource allocation: exploring connections between foraging and life history. Funct. Ecol. 6, 508–518.
Boggs, C.L., 1997. Dynamics of reproductive allocation from juvenile and adult feeding : radiotracer studies. Ecology 78, 192–202.
Boggs, C.L., 2009. Understanding insect life histories and senescence through a resource allocation lens. Funct. Ecol. 23, 27–37.
Boggs, C.L., Freeman, K.D., 2005. Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia 144, 353–61.
Boggs, C.L., Gilbert, L.E., 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206, 83–84.
Boggs, C.L., Niitepõld, K., 2014. Insights from stable isotopic tracers on reproductive allocation under stress. Integr. Comp. Biol. 54, 880–889.

Boggs, C.L., Ross, C.L., 1993. The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). Ecology 74, 433–441.
Bonnet, X., Bradshaw, D., Shine, R., 1998. Capital versus income breeding: An ectothermic perspective. Oikos 83, 333–342.
Boratyński, Z., Koteja, P., 2010. Sexual and natural selection on body mass and metabolic rates in free-living bank voles. Funct. Ecol. 24, 1252–1261.
Boyd, I.L., Arnould, J.P.Y., Barton, T., Croxall, J.P., 1994. Foraging behaviour of Antarctic fur seals during periods of contrasting prey abundance. J. Anim. Ecol. 63, 1–6.
Braby, M.F., Jones, R.E., 1995. Reproductive patterns and resource allocation in tropical butterflies : influence of adult diet and seasonal phenotype on fecundity, longevity and egg size. Oikos 72, 189–204.
Brakefield, P.M., 1982a. Ecological studies on the butterfly *Maniola jurtina* in Britain. I. Adult behaviour, microdistribution and dispersal. J. Anim. Ecol. 51, 713–726.
Brakefield, P.M., 1982b. Ecological studies on the butterfly *Maniola jurtina* in Britain. II. Population dynamics: the present position. J. Anim. Ecol. 51, 727–738.
Brakefield, P.M., 1987. Geographical variability in , and temperature effects on , the phenology of *Maniola jurtina* and *Pyronia tithonus* (Lepidoptera , Satyrinae) in England and Wales. Ecol. Entomol. 12, 139–148.
Brakefield, P.M., van Noordwijkl, A.J., 1985. The genetics of spot pattern characters in the meadow brown butterfly *Maniola jurtina* (Lepidoptera : Satyrinae). Heredity (Edinb). 54, 275–284.
Braschler, B., Marini, L., Thommen, G.H., Baur, B., 2009. Effects of small-scale grassland fragmentation and frequent mowing on population density and species diversity of orthopterans: a long-term study. Ecol. Entomol. 34, 321–329.
Briscoe, A.D., Chittka, L., 2001. The evolution of color vision in insects. Annu. Rev. Entomol. 46, 471–510.
Brown, J.S., 1988. Patch use as an indicator of habitat preference, predation risk, and competition. Behav. Ecol. Sociobiol. 22, 37–47.
Buri, P., Arlettaz, R., Humbert, J.-Y., 2013. Delaying mowing and leaving uncut refuges boosts orthopterans in extensively managed meadows: Evidence drawn from field-scale experimentation. Agric. Ecosyst. Environ. 181, 22–30.
Burrows, M.T., Hughes, R.N., 1990. Variation in growth and consumption among individuals and populations of gogwhelks, *Nucella lapillus*: a

link between foraging behaviour and fitness. J. Anim. Ecol. 59, 723–742.
Burton, T., Killen, S.S., Armstrong, J.D., Metcalfe, N.B., 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proc. R. Soc. B 278, 3465–3473.
Butet, A., Michel, N., Rantier, Y., Comor, V., Hubert-Moy, L., Nabucet, J., Delettre, Y., 2010. Responses of common buzzard (*Buteo buteo*) and Eurasian kestrel (*Falco tinnunculus*) to land use changes in agricultural landscapes of Western France. Agric. Ecosyst. Environ. 138, 152–159.
Butler, S.J., Brooks, D., Feber, R.E., Storkey, J., Vickery, J. A., Norris, K., 2009. A cross-taxonomic index for quantifying the health of farmland biodiversity. J. Appl. Ecol. 1154–1162.
Butler, S.J., Vickery, J.A., Norris, K., 2007. Farmland biodiversity and the footprint of agriculture. Science 315, 381–384.
Cahenzli, F., Erhardt, A., 2012a. Host plant defence in the larval stage affects feeding behaviour in adult butterflies. Anim. Behav. 84, 995–1000.
Cahenzli, F., Erhardt, A., 2012b. Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121, 1417–1423.
Cahenzli, F., Erhardt, A., 2012c. Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia 169, 1005–1014.
Cahenzli, F., Erhardt, A., 2013a. Transgenerational acclimatization in an herbivore − host plant relationship. Proc. Natl. Acad. Sci. 280, 20122856.
Cahenzli, F., Erhardt, A., 2013b. Nectar amino acids enhance reproduction in male butterflies. Oecologia 171, 197–205.
Carroll, S.P., Hendry, A.P., Reznick, D.N., Fox, C.W., 2007. Evolution on ecological time-scales. Funct. Ecol. 21, 387–393.
Carvell, C., Meek, W.R., Pywell, R.F., Goulson, D., Nowakowski, M., 2006. Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. J. Appl. Ecol. 44, 29–40.
Chambers, P., Sword, G., Angel, J.E., Behmer, S., Bernays, E.A., 1996. Foraging by generalist grasshoppers: two different strategies. Anim. Behav. 52, 155–165.
Chen, Y., Lin, L., Wang, C., Yeh, C., Hwang, S., 2004. Responses of two *Pieris* species to fertilization of a host plant. Zool. Stud. 43, 778–786.
Choutt, J., Turlure, C., Baguette, M., Schtickzelle, N., 2011. Parasitism cost of living in a high quality habitat in the bog fritillary butterfly. Biodivers. Conserv. 20, 3117–3131.

Cizek, O., Zamecnik, J., Tropek, R., Kocarek, P., Konvicka, M., 2012. Diversification of mowing regime increases arthropods diversity in species-poor cultural hay meadows. J. Insect Conserv. 16, 215–226.
Clausen, H.D., Holbeck, H.B., Reddersen, J., 2001. Factors influencing abundance of butterflies and burnet moths in the uncultivated habitats of an organic farm in Denmark. Biol. Conserv. 98, 167–178.
Comba, L., Corbet, S.A., Hunt, L., Warren, B., 1999. Flowers, nectar and insect visits: evaluating British plant species for pollinator-friendly gardens. Ann. Bot. 83, 369–383.
Conradt, L., Bodsworth, E.J., Roper, T.J., Thomas, C.D., 2000. Non-random dispersal in the butterfly *Maniola jurtina*: implications for metapopulation models. Proc. R. Soc. B 267, 1505–1510.
Conradt, L., Roper, T.J., 2006. Nonrandom movement behavior at habitat boundaries in two butterfly species : implications for dispersal. Ecology 87, 125–132.
Conradt, L., Roper, T.J., Thomas, C.D., 2001. Dispersal behaviour of individuals in metapopulations of two British butterflies. Oikos 95, 416–424.
Conradt, L., Zollner, P.A., Roper, T.J., Frank, K., Thomas, C.D., 2003. Foray search: an effective systematic dispersal strategy in fragmented landscapes. Am. Nat. 161, 905–915.
Corbet, S.A., 2000. Butterfly nectaring flowers: butterfly morphology and flower form. Entomol. Exp. Appl. 96, 289–298.
Cormont, A., Malinowska, A.H., Kostenko, O., Radchuk, V., Hemerik, L., WallisDeVries, M.F., Verboom, J., 2010. Effect of local weather on butterfly flight behaviour, movement, and colonization: significance for dispersal under climate change. Biodivers. Conserv. 20, 483–503.
Cowley, M.J.R., Thomas, C.D., Roy, D.B., Wilson, R.J., Gutiérrez, D., Bulman, C.R., Quinn, R.M., Moss, D., Gaston, K.J., 2001. Density – distribution relationships in British butterflies. I. The effect of mobility and spatial scale. J. Anim. Ecol. 70, 410–425.
Creed, E.R., Dowdeswell, W.H., Ford, E.B., McWhirter, K.G., 1959. Evolutionary studies on *Maniola jurtina*: The English Mainland, 1956-57. Heredity (Edinb). 13, 363–391.
Croxton, P.J., Hann, J.P., Greatorex-Davies, J.N., Sparks, T.H., 2005. Linear hotspots? The floral and butterfly diversity of green lanes. Biol. Conserv. 121, 579–584.
Dahlström, A., Lennartsson, T., Wissman, J., Frycklund, I., 2008. Biodiversity and traditional land use in South-Central Sweden: the significance of management timing. Environ. Hist. Camb. 14, 385–403.
Dapporto, L., Bruschini, C., Baracchi, D., Cini, A., Gayubo, S.F., González, J.A., Dennis, R.L.H., 2009. Phylogeography and counter-intuitive inferences

in island biogeography: evidence from morphometric markers in the mobile butterfly *Maniola jurtina* (Linnaeus) (Lepidoptera, Nymphalidae). Biol. J. Linn. Soc. 98, 677–692.
Darwin, C., 1876. The effects of cross-and self-fertilization in the animal kingdom. Murray, London.
Davies, N.B., 1978. Territorial defence in the speckled wood butterfly (*Pararge aegeria*): The resident always wins. Anim. Behav. 26, 138–147.
De Vos, J.M., Joppa, L.N., Gittleman, J.L., Stephens, P.R., Pimm, S.L., 2014. Estimating the normal background rate of species extinction. Conserv. Biol. 29, 452–462.
Delattre, T., 2010. Influence de la structure du paysage et es condition smétéorologiques sur le comportement de dispersion de *Maniola jurtina* (Lepidotera: Nymphalidae, L.) dans un agroécosystème bocager. Thèse de doctorat, Université de Rennes 1.
Delattre, T., Burel, F., Humeau, A., Stevens, V.M., Vernon, P., Baguette, M., 2010. Dispersal mood revealed by shifts from routine to direct flights in the meadow brown butterfly *Maniola jurtina*. Oikos 119, 1900–1908.
Delattre, T., Vernon, P., Burel, F., 2013. An agri-environmental scheme enhances butterfly dispersal in European agricultural landscapes. Agric. Ecosyst. Environ. 166, 102–109.
Demotte, R., Lutgen, B., 2008. Arrêté du Gouvernement Wallon relatif à l'octroi de subventions agro-environnementales (M.B. du 17/06/2008, p.31162).
Dennis, R.L.H., 2004. Butterfly habitats, broad-scale biotope affiliations, and structural exploitation of vegetation at finer scales: the matrix revisited. Ecol. Entomol. 29, 744–752.
Dennis, R.L.H., Shreeve, T.G., 1988. Hostplant-habitat structire and the evolution of butterfly mate-locating behaviour. Zool. J. Linn. Soc. 94, 301–318.
Dennis, R.L.H., Shreeve, T.G., Van Dyck, H., 2003. Towards a functional resource-based concept for habitat : a butterfly biology viewpoint. Oikos 102, 417 – 426.
Dennis, R.L.H., Shreeve, T.G., Van Dyck, H., 2006. Habitats and resources: the need for a resource-based definition to conserve butterflies. Biodivers. Conserv. 15, 1943–1966.
DeWitt, T.J., Sih, A., Wilson, D.S., 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. 13, 77–81.
Djawdan, M., Rose, M.R., Bradley, T.J., 1997. Does selection for stress resistance lower metabolic rate ? Ecology 78, 828–837.

Donald, P.F., Sanderson, F.J., Burfield, I.J., van Bommel, F.P.J., 2006. Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990–2000. Agric. Ecosyst. Environ. 116, 189–196.
Dover, J.W., 1996. Factors affecting the distribution of satyrid butterflies on arable farmland. J. Appl. Ecol. 33, 723–734.
Dover, J.W., 1997. Conservation headlands: Effects on butterfly distribution and behaviour. Agric. Ecosyst. Environ. 63, 31–49.
Dover, J.W., Rescia, A., Fungariño, S., Fairburn, J., Carey, P., Lunt, P., Dennis, R.L.H., Dover, C.J., 2010. Can hay harvesting detrimentally affect adult butterfly abundance? J. Insect Conserv. 14, 413–418.
Dover, J.W., Settele, J., 2008. The influences of landscape structure on butterfly distribution and movement: a review. J. Insect Conserv. 13, 3–27.
Dowdeswell, W., Fisher, R., Ford, E., 1949. The quantitative study of populations in the Lepidoptera. Heredity 3, 67–84.
Dowdeswell, W.H., 1961. Experimental studies on natural selection in the butterfly, *Maniola jurtina*. Heredity 16, 39–52.
Dowdeswell, W.H., 1981. The life of the meadow brown. Heinemann, London.
Ekroos, J., Olsson, O., Rundlöf, M., Wätzold, F., Smith, H.G., 2014. Optimizing agri-environment schemes for biodiversity, ecosystem services or both? Biol. Conserv. 172, 65–71.
Elliott, S.E., Irwin, R.E., Adler, L.S., Williams, N.M., 2008. The nectar alkaloid, gelsemine, does not affect offspring performance of a native solitary bee, *Osmia lignaria* (Megachilidae). Ecol. Entomol. 33, 298–304.
Erhardt, A., Baker, I., 1990. Pollen amino acids-an additional diet for a nectar feeding butterfly? Plant Syst. Evol. 169, 111–121.
Erhardt, A., 1991. Nectar sugar and amino acid preference of *Battus philenor* (Lepidopteran Papilionidae). Ecol. Entomol.
Erhardt, A., 1992. Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). Oecologia 90, 581–585.
Erhardt, A., 1995. Ecology ad conservation of alpine Lepidoptera, in: Pullin, A. (Ed.), Ecology and Conservation of Butterflies. Chapman & Hall, London, pp. 258–276.
Erhardt, A., Mevi-Schütz, J., 2009. Adult food resources in butterflies, in: Settele, J., Shreeve, T.G., Konvicka, M., Van Dyck, H. (Eds.), Ecology of Butterflies in Europe. Cambridge University press, Cambridge, pp. 9–16.

Erhardt, A., Rusterholz, H., 1998. Do Peacock butterflies (*Inachis io* L .) detect and prefer nectar amino acids and other nitrogenous compounds ? Oecologia 117, 536–542.
EU, 2005. Agri-environment Measures, Overview on General Principles, Types of Measures, and Application.
Feber, R.E., Smith, H.G., Macdonald, D.W., 1996. The effects on butterfly abundance of the management of uncropped edges of arable fields. J. Appl. Ecol. 33, 1191–1205.
Feest, A., Van Swaay, C., Van Hinsberg, A., 2014. Nitrogen deposition and the reduction of butterfly biodiversity quality in the Netherlands. Ecol. Indic. 39, 115–119.
Feinsinger, P., 1976. Organization of a tropical guild of nectarivorous birds. Ecol. Monogr. 46, 257–291.
Feng, R., Isman, M.B., 1995. Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. Experientia 51, 831–833.
Fewell, J.H., 1988. Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. Behav. Ecol. Sociobiol. 22, 401–408.
Fichefet, V., Barbier, Y., Baugnée, J.-Y., Dufrêne, M., Goffart, P., Maes, D., Van Dyck, H., 2008. L'Atlas des papillons de jour de Wallonie (1985-2007). Service Public de Wallonie, Direction générale de l'Agriculture, des Ressources Naturelles et de l'Environnement.
Field, R.G., Gardiner, T., Mason, C.F., Hill, J., 2006. Agri-environment schemes and butterflies: the utilisation of two metre arable field margins. Biodivers. Conserv. 16, 465–474.
Fischer, K., Beinlich, B., Plachter, H., 1999. Population structure, mobility and habitat preferences of the violet copper *Lycaena helle* (Lepidoptera: Lycaenidae) in Western Germany: implications for conservation. J. Insect Conserv. 3 , 43–52.
Fischer, K., Fiedler, K., 2000. Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. Oecologia 124, 235–241.
Freeman, E.C., Worthington, R.D., Jackson, M.S., 1991. Floral nectar sugar compositions of some South and Southeast Asian species. Biotropica 23, 568–574.
Fritch, R.A., Sheridan, H., Finn, J.A., Kirwan, L., Huallacháin, D.Ó., 2011. Methods of enhancing botanical diversity within field margins of intensively managed grassland: a 7-year field experiment. J. Appl. Ecol. 48, 551–560.
Fuentes-Montemayor, E., Goulson, D., Park, K.J., 2011. The effectiveness of agri-environment schemes for the conservation of farmland moths: assessing the importance of a landscape-scale management approach. J. Appl. Ecol. 48, 532–542.

García-barros, E., 2000. Egg size in butterflies (Lepidoptera : Papilionoidea and Hesperiidae): a summary of data. J. Res. Lepitoptera 35, 90–136.
Gardener, M.C., Gillman, M.P., 2002. The taste of nectar – a neglected area of pollination ecology. Oikos 98, 552–557.
Gardner, W.R., Ehlig, C.F., 1965. Physical Aspects of the Internal Water Relations of Plant Leaves. Plant Physiol. 40, 705–710.
Gegear, R.J., Laverty, T.M., 1995. Effect of flower complexity on relearning flower-handling skills in bumble bees. Can. J. Zool. 73, 2052–2058.
Gegear, R.J., Manson, J.S., Thomson, J.D., 2007. Ecological context influences pollinator deterrence by alkaloids in floral nectar. Ecol. Lett. 10, 375–82.
Gegear, R.J., Thomson, J.D., 2004. Does the flower constancy of bumble bees reflect foraging economics? Ethology 110, 793–805.
Geister, T.L., Lorenz, M.W., Hoffmann, K.H., Fischer, K., 2008. Adult nutrition and butterfly fitness: effects of diet quality on reproductive output, egg composition, and egg hatching success. Front. Zool. 5 (10).
Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21, 394–407.
Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl. Anim. Behav. Sci. 63, 269–287.
Gibbs, M., Van Dyck, H., Breuker, C.J., 2012. Development on drought-stressed host plants affects life history, flight morphology and reproductive output relative to landscape structure. Evol. Appl. 5, 66–75.
Goffart, P., Waeyenbergh, M., 1994. Exigences écologiques et gestion des populations de deux papillons des prairies humides ardennaises : Le Cuivré et le Nacré de la bistorte (*Lycaena helle*, *Proclossiana eunomia*). Cah. Réserves Nat. 7, 21–29.
Gotthard, K., 2008. Adaptive growth decisions in butterflies. Bioscience 58, 222–230.
Gottlieb, D., Keasar, T., Shmida, A., Motro, U., 2005. Possible foraging benefits of bimodal daily activity in *Proxylocopa olivieri* (Lepeletier) (Hymenoptera: Anthrophoridae). Environ. Entomol. 34, 2–4.
Goulson, D., 1993. Allozyme variation in the butterfly, *Maniola jurtina* (Lepidoptera: Satyrinae) (L.): evidence for selection. Heredity 71, 386–393.

Goulson, D., Ollerton, J., Sluman, C., 1997a. Foraging strategies in the small skipper butterfly, *Thymelicus flavus*: when to switch? Anim. Behav. 53, 1009–1016.
Goulson, D., Stout, J.C., Hawson, S.A., 1997b. Can flower constancy in nectaring butterflies be explained by Darwin's interference hypothesis? Oecologia 112, 225–231.
Goulson, D., Wright, N.P., 1995. Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) 9, 215–219.
Goverde, M., Erhardt, A., 2003. Effects of elevated CO 2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera , Satyridae). Glob. Chang. Biol. 9, 74–83.
Grill, A., Cerny, A., Fiedler, K., 2013. Hot summers, long life: egg laying strategies of *Maniola* butterflies are affected by geographic provenance rather than adult diet. Contrib. to Zool. 82, 27–36.
Grill, A., Schtickzelle, N., Cleary, D.F.R., Nève, G., Menken, S.B.J., 2006. Ecological differentiation between the Sardinian endemic *Maniola nurag* and the pan-European *M. jurtina*. Biol. J. Linn. Soc. 89, 561–574.
Grundel, R., Pavlovic, N.B., Sulzman, C.L., 2000. Nectar Plant Selection by the Karner Blue Butterfly *Lycaeides melissa samuelis* at the Indiana Dunes National Lakeshore. Am. Midl. Nat. 144, 1–10.
Gullan, P.J., Cranston, P.S., 2005. The Insects: An Outline of Entomology, 3rd ed. Blackwell Publishing Ltd.
Haaland, C., Bersier, L.F., 2011. What can sown wildflower strips contribute to butterfly conservation?: An example from a Swiss lowland agricultural landscape. J. Insect Conserv. 15, 301–309.
Habel, J.C., Rödder, D., Stefano, S., Meyer, M., Schmitt, T., 2010. Strong genetic cohesiveness between Italy and North Africa in four butterfly species. Biol. J. Linn. Soc. 99, 818–830.
Haenke, S., Scheid, B., Schaefer, M., Tscharntke, T., Thies, C., 2009. Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscapes. J. Appl. Ecol. 46, 1106–1114.
Hainsworth, F.R., Hamill, T., 1993. Foraging rules for nectar: food choices by painted ladies. Am. Nat. 142, 857–867.
Handford, P.T., 1973a. Patterns of variation in a number of genetic systems in *Maniola jurtina*: the boundary region. Proc. R. Soc. B 183, 265–284.
Handford, P.T., 1973b. Patterns of variation in a number of genetic systems in *Maniola jurtina* : the Isles of Scilly. Proc. R. Soc. B 183, 285–300.
Hanski, I., Breuker, C.J., Scho, K., Setchfield, R., Nieminen, M., 2002. Population history and life history influence the migration rate of female Glanville fritillary butterflies. Oikos 1, 87–97.

Hanski, I., Erälahti, C., Kankare, M., Ovaskainen, O., Sirén, H., 2004. Variation in migration propensity among individuals maintained by landscape structure. Ecol. Lett. 7, 958–966.
Hanski, I., Saastamoinen, M., Ovaskainen, O., 2006. Dispersal-related life-history trade-offs in a butterfly metapopulation. J. Anim. Ecol. 75, 91–100.
Hanski, I., Saccheri, I., 2006. Molecular-level variation affects population growth in a butterfly metapopulation. PLoS Biol. 4, 719–726.
Hawlena, D., Schmitz, O.J., 2010. Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. Proc. Natl. Acad. Sci. 107, 15503–15507.
Hendrickx, F., Maelfait, J.-P., Van Wingerden, W., Schweiger, O., Speelmans, M., Aviron, S., Augenstein, I., Billeter, R., Bailey, D., Bukacek, R., Burel, F., Diekötter, T., Dirksen, J., Herzog, F., Liira, J., Roubalova, M., Vandomme, V., Bugter, R., 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. J. Appl. Ecol. 44, 340–351.
Hendry, A.P., Farrugia, T.J., Kinnison, M.T., 2008. Human influences on rates of phenotypic change in wild animal populations. Mol. Ecol. 17, 20–29.
Hill, C.J., 1989. The effect of adult diet on the biology of butterflies. 2. The common crow butterfly ,*Euploea core corinna*. Oecologia 81, 258–266.
Hill, J.K., Thomas, C.D., Lewis, O.T., 1998. Flight morphology in fragmented populations of a rare British butterfly, *Hesperia comma*. Biol. Conserv. 87, 277–283.
Hill, P.S.M., Hollis, J., Wells, H., 2001. Foraging decisions in nectarivores: unexpected interactions between flower constancy and energetic rewards. Anim. Behav. 62, 729–737.
Hirsch, M., Pfaff, S., Wolters, V., 2003. The influence of matrix type on flower visitors of *Centaurea jacea* L. Agric. Ecosyst. Environ. 98, 331–337.
Hoffmann, A.A., Willi, Y., 2008. Detecting genetic responses to environmental change. Nat. Rev. Genet. 9, 421–432.
Holland, J.M., Luff, M.L., 2000. The effects of agricultural practices on Carabidae in temperate agroecosystems. Integr. Pest Manag. Rev. 5, 109–129.
Hossain, Z., Gurr, G.M., Wratten, S.D., Raman, A., 2002. Habitat manipulation in Lucerne *Medicago sativa*: arthropod population dynamics in harvested and “refuge” crop strips. J. Appl. Ecol. 39, 445–454.
Hughes, C.L., Hill, J.K., Dytham, C., 2003. Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. Proc. R. Soc. B 270, S147–S150.

Humbert, J.-Y., Ghazoul, J., Richner, N., Walter, T., 2012. Uncut grass refuges mitigate the impact of mechanical meadow harvesting on orthopterans. Biol. Conserv. 152, 96–101.
Humbert, J.-Y., Ghazoul, J., Sauter, G.J., Walter, T., 2010. Impact of different meadow mowing techniques on field invertebrates. J. Appl. Entomol. 134, 592–599.
Humbert, J.-Y., Ghazoul, J., Walter, T., 2009. Meadow harvesting techniques and their impacts on field fauna. Agric. Ecosyst. Environ. 130, 1–8.
Ismail, M., Vernon, P., Hance, T., Pierre, J.S., van Baaren, J., 2012. What are the possible benefits of small size for energy-constrained ectotherms in cold stress conditions? Oikos 121, 2072–2080.
Issacs, R., Tuell, J.K., Fiedler, A.K., Gardiner, M.M., Landis, D.A., 2009. Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. Front. Ecol. Environ. 7, 196–203.
Jackson, S., Nicolson, S.W., 2002. Xylose as a nectar sugar : from biochemistry to ecology 131, 613–620.
Janz, N., Bergström, A., Sjögren, A., 2005. The role of nectar sources for oviposition decisions of the common blue butterfly P*olyommatus icarus*. Oikos 109, 535–538.
Janz, N., Nylin, S., Wedell, N., 1994. Host plant utilization in the comma butterfly: sources of variation and evolutionary implications. Oecologia 99, 132–140.
Jennersten, O., 1984. Flower visitation and pollination effinciency of some North European butterflies. Oecologia 63, 80–89.
Jervis, M.A., Boggs, C.L., 2005. Linking nectar amino acids to fitness in female butterflies. Trends Ecol. Evol. 20, 585–7.
Jocque, M., Field, R., Brendonck, L., De Meester, L., 2010. Climatic control of dispersal-ecological specialization trade-offs: A metacommunity process at the heart of the latitudinal diversity gradient? Glob. Ecol. Biogeogr. 19, 244–252.
Joern, A., Provin, T., Behmer, S.T., 2012. Not just the usual suspects: Insect herbivore populations and communities are associated with multiple plant nutrients. Ecology 93, 1002–1015.
Johst, K., Drechsler, M., Thomas, J., Settele, J., 2006. Influence of mowing on the persistence of two endangered large blue butterfly species. J. Appl. Ecol. 43, 333–342.
Jones, E.C., Buchmann, S.L., 1974. Ultraviolet floral patterns as functional orientation cues in hymenopterous polination systems. Anim. Behav. 22, 481–495.
Jönsson, I.K., 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78, 57–66.

Kalarus, K., Skorka, P., Nowicki, P., 2013. Resource use in two contrasting habitat types raises different challenges for the conservation of the dryad butterfly *Minois dryas*. J. Insect Conserv. 17, 777–786.
Karasov, W.H., Martinèz del Rio, C., 2007. Physiological ecology: how animals process energy, nutrients and toxins. Princeton University Press, Princeton, New-Jersey.
Karl, I., Lorenz, M.W., Fischer, K., 2007. Energetics of reproduction : consequences of divergent selection on egg size, food limitation, and female age for egg composition and reproductive effort in a butterfly. Biol. J. Linn. Soc. 91, 403–418.
Karlsson, B., 1994. Feeding habits and change of body composition with age in three nymphalid butterfly species. Oikos.
Karlsson, B., Van Dyck, H., 2005. Does habitat fragmentation affect temperature-related life-history traits? A laboratory test with a woodland butterfly. Proc. R. Soc. B 272, 1257–1263.
Kennedy, C.M., Lonsdorf, E., Neel, M.C., Williams, N.M., Ricketts, T.H., Winfree, R., Bommarco, R., Brittain, C., Burley, A.L., Cariveau, D., Carvalheiro, L.G., Chacoff, N.P., Cunningham, S.A., Danforth, B.N., Dudenhöffer, J.H., Elle, E., Gaines, H.R., Garibaldi, L.A., Gratton, C., Holzschuh, A., Isaacs, R., Javorek, S.K., Jha, S., Klein, A.M., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L., Neame, L.A., Otieno, M., Park, M., Potts, S.G., Rundlöf, M., Saez, A., Steffan-Dewenter, I., Taki, H., Viana, B.F., Westphal, C., Wilson, J.K., Greenleaf, S.S., Kremen, C., 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol. Lett. 16, 584–599.
Kerns, D.L., Gaylor, M.J., 1992. Insecticide resistance in field populations of the cotton aphid (Homoptera: Aphididae). J. Econ. Entomol. 85, 1–8.
Kessler, D., Baldwin, I.T., 2007. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. Plant J. 49, 840–854.
Kitahara, M., Sei, K., Fujii, K., 2000. Patterns in the structure of grassland butterfly communities along a gradient of human disturbance: further analysis based on the generalist/specialist concept. Popul. Ecol. 42, 135–144.
Kleijn, D., Baquero, R.A., Clough, Y., Díaz, M., De Esteban, J., Fernández, F., Gabriel, D., Herzog, F., Holzschuh, A., Jöhl, R., Knop, E., Kruess, A., Marshall, E.J.P., Steffan-Dewenter, I., Tscharntke, T., Verhulst, J., West, T.M., Yela, J.L., 2006. Mixed biodiversity benefits of agri-environment schemes in five European countries. Ecol. Lett. 9, 243–254.
Kleijn, D., Sutherland, W.J., 2003. How effective are European agri-environment schemes in conserving and promoting biodiversity? J. Appl. Ecol. 40, 947–969.

Kohler, F., Verhulst, J., Van Klink, R., Kleijn, D., 2008. At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes? J. Appl. Ecol. 45, 753–762.
Kralj-Fišer, S., Schuett, W., 2014. Studying personality variation in invertebrates: Why bother? Anim. Behav. 91, 41–52.
Krauss, J., Steffan-dewenter, I., Tscharntke, T., 2003. How does landscape context contribute to effects of habitat fragmentation on diversity and population density of butterflies ? J. Biogeogr. 30, 889–900.
Krenn, H.W., 2010. Feeding mechanisms of adult Lepidoptera: structure, function, and evolution of the mouthparts. Annu. Rev. Entomol. 55, 307–327.
Krenn, H.W., Penz, C.M., 1998. Mouthparts of Heliconius butterflies (Lepidoptera : Nymphalidae): A search for anatomical adaptations to pollen-feeding behavior. Int. J. Insect Morphol. Embryol. 27, 301–309.
Krenn, H.W., Zulka, K.P., Gatschnegg, T., 2001. Proboscis morphology and food preferences in nymphalid butterflies (Lepidoptera: Nymphalidae). J. Zool. 254, 17–26.
Kulfan, J., Štrbová, E., Zach, P., 2012. Effect of vegetation and management on occurrence of larvae and adults of generalist *Maniola jurtina* L. (Lepidoptera) in meadow habitats. Polish J. Ecol. 60, 601–609.
Lack, A.J., 1982a. Competition for pollinators in the ecology of *Centaurea scabiosa* L. and *Centaurea nigra* L. II. Observations on nectar production. New Phytol. 91, 309–320.
Lack, A.J., 1982b. Competition for pollinators in the ecology of *Centaurea scabiosa* L. and *Centaurea nigra* L. III. Insect visits and the number of successful pollinations. New Phytol. 91, 321–339.
Lavoie, B., Oberhauser, K.S., 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environ. Entomol. 33, 1062–1069.
Lázaro, A., Lene, A., Aase, T.O., Totland, Ø., 2011. Relationships between densities of previous and simultaneous foragers and the foraging behaviour of three bumblebee species. Ecol. Entomol. 36, 221–230.
Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. Proc. Natl. Acad. Sci. 105, 2498–2503.
Lemke, T.O., 1984. Foraging ecology of the long-nosed bat, *Glossophaga soricina*, with respect to resource availability. Ecology 65, 538–548.
Lewis, A.C., 1989. Flower constancy in *Pieris rapae*, the Cabbage Butterfly. J. Anim. Ecol. 58, 1–13.
Lighton, J.R., Bartholomew, G.A., 1988. Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: Effects of temperature,

body mass, group size, and humidity. Proc. Natl. Acad. Sci. 85, 4765–4769.
Lima, S.L., Valone, T.J., Caraco, T., 1985. Foraging-efficiency-predation-risk trade-off in the grey squirrel. Anim. Behav. 33, 155–165.
Littlewood, N.A., Stewart, A.J.A., Woodcock, B.A., 2012. Science into practice - how can fundamental science contribute to better management of grasslands for invertebrates? Insect Conserv. Divers. 5, 1–8.
Loos, J., Kuussaari, M., Ekroos, J., Hanspach, J., Fust, P., Jackson, L., Fischer, J., 2015. Changes in butterfly movements along a gradient of land use in farmlands of Transylvania (Romania). Landsc. Ecol. 30, 625–635.
Maes, D., Van Dyck, H., 2001. Butterfly diversity loss in Flanders (north Belgium): Europe's worst case scenario? Biol. Conserv. 99, 263–276.
Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., Brooks, R.C., 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr. Biol. 18, 1062–1066.
Mallet, J., 1989. The evolution of insecticide resistance: Have the insects won? Trends Ecol. Evol. (Personal Ed. 4, 336–340.
Marden, J.H., 1989. Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. Physiol. Zool. 62, 505–521.
Marini, L., Fontana, P., Scotton, M., Klimek, S., 2007. Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. J. Appl. Ecol. 45, 361–370.
Marshall, E.J.P., Moonen, A.C., 2002. Field margins in northern Europe: their functions and interactions with agriculture. Agric. Ecosyst. Environ. 89, 5–21.
Marshall, L.D., McNeil, J.N., 1989. Spermatophore Mass as an Estimate of Male Nutrient Investment: A Closer Look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Funct. Ecol. 3, 605-612.
Matlack, G.R., 1993. Microenvironment variation within and among forst edge sites in the Eastern United States. Biol. Conserv. 66, 185–194.
Mattila, A.L.K., Hanski, I., 2014. Heritability of flight and resting metabolic rates in the Glanville fritillary butterfly. J. Evol. Biol. 27, 1733–1743.
May, P.G., 1992. Flower selection and the dynamics of lipid reserve in two nectarivorous butterflies. Ecology 73, 2181–2191.
May, R.M., Hassell, M.P., Anderson, R.M., Tonkyn, D.W., 1981. Density dependence in host-parasitoid models. J. Anim. Ecol. 50, 855–865.
McCollin, D., Moore, L., Sparks, T., 2000. The flora of a cultural landscape: Environmental determinants of change revealed using archival sources. Biol. Conserv. 92, 249–263.

McGavin, G.C., 2001. Essential Entomology. an order-by-order introduction. Oxford University Press, Oxford.
McLaughlin, A., Mineau, P., 1995. The impact of agricultural practices on biodiversity. Agric. Ecosyst. Environ. 55, 201–212.
McNab, B.K., Morrison, P., 1963. Body temperature and metabolism in subspecies of *Peromyscus* from arid and mesic environments. Ecol. Monogr. 33, 63–82.
Mcpeek, M.A., Holt, R.D., 1992. The evolution of dispersal in spatially and temporally varying environments. Am. Nat. 140, 1010–1027.
Meek, B., Loxton, D., Sparks, T., Pywell, R., Pickett, H., Nowakowski, M., 2002. The effect of arable field margin composition on invertebrate biodiversity. Biol. Conserv. 106, 259–271.
Melvin, R.G., Van Voorhies, W.A., Ballard, J.W.O., 2007. Working harder to stay alive: Metabolic rate increases with age in *Drosophila simulans* but does not correlate with life span. J. Insect Physiol. 53, 1300–1306.
Mennechez, G., Petit, S., Schtickzelle, N., Baguette, M., 2004. Modelling mortality and dispersal : consequences of parameter generalisation on metapopulation dynamics. Oikos 106, 243–252.
Menz, M.H.M., Phillips, R.D., Winfree, R., Kremen, C., Aizen, M.A., Johnson, S.D., Dixon, K.W., 2011. Reconnecting plants and pollinators: Challenges in the restoration of pollination mutualisms. Trends Plant Sci. 16, 4–12.
Merckx, T., Serruys, M., Van Dyck, H., 2015. Anthropogenic host plant expansion leads a nettle-feeding butterfly out of the forest: consequences for larval survival and developmental plasticity in adult morphology. Evol. Appl. in press.
Merckx, T., Van Dongen, S., Matthysen, E., Van Dyck, H., 2008. Thermal flight budget of a woodland butterfly in woodland versus agricultural landscapes: An experimental assessment. Basic Appl. Ecol. 9, 433–442.
Merckx, T., Van Dyck, H., 2002. Interrelations among habitat use , behavior, and flight-related morphology in two cooccurring satyrine butterflies , *Maniola jurtina* and *Pyronia tithonus*. J. Insect Behav. 15, 541–561.
Merckx, T., Van Dyck, H., 2006. Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. Oikos 113, 226–232.
Merckx, T., Van Dyck, H., Karlsson, B., Leimar, O., 2003. The evolution of movements and behaviour at boundaries in different landscapes: a common arena experiment with butterflies. Proc. Biol. Sci. 270, 1815–1821.
Mevi-Schütz, J., Erhardt, A., 2002. Can *Inachis io* detect nectar amino acids at low concentrations ? Physiol. Entomol. 27, 256–260.

Mevi-Schütz, J., Erhardt, A., 2003. Effects of nectar amino acids on fecundity of the wall brown butterfly (*Lasiommata megera* L.). Basic Appl. Ecol. 4, 413–421.
Mevi-Schütz, J., Erhardt, A., 2003. Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). Ecology 84, 2788–2794.
Mevi-Schütz, J., Erhardt, A., 2004. Mating frequency influences nectar amino acid preference of *Pieris napi*. Proc. Natl. Acad. Sci. 271, 153–158.
Mevi-Schütz, J., Erhardt, A., 2005. Amino acids in nectar enhance butterfly fecundity: a long-awaited link. Am. Nat. 165, 411–419.
Molleman, F., 2010. Puddling : from natural history to understanding how it affects fitness 107–113.
Molleman, F., Ding, J., Wang, J.-L., Brakefield, P.M., Carey, J.R., Zwaan, B.J., 2008. Amino acid sources in the adult diet do not affect life span and fecundity in the fruit-feeding butterfly *Bicyclus anynana*. Ecol. Entomol. 33, 429–438.
Morehouse, N.I., Rutowski, R.L., 2010. Developmental responses to variable diet composition in a butterfly: the role of nitrogen, carbohydrates and genotype. Oikos 119, 636–645.
Munro, H.N., 1951. Carbohydrates and fats as factors in protein utilization and metabolism. Physiol. Rev. 31, 449–488.
Murphy, D.D., 1984. Butterflies and their nectar plants : the role of the checkerspot butterfly *Euphydryas editha* as a pollen vector. Oikos 43, 113–117.
Murphy, D.D., Launer, A.E., Ehrlich, P.R., 1983. The role of adult feeding in egg production and population dynamics of the checkerspot butterfly Euphydryas editha. Oecologia 56, 257–263.
Myers, J.H., 1985. Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly *Pieris rapae*. J. Anim. Ecol. 54, 193–204.
Nicolson, S.W., Thornburg, R.W., 2007. Nectar Chemistry, Nectaries and Nectar. Springer Netherlands.
Niitepõld, K., 2010. Genotype by temperature interactions in the metabolic rate of the Glanville fritillary butterfly. J. Exp. Biol. 213, 1042–8.
Niitepõld, K., Hanski, I., 2013. A long life in the fast lane: positive association between peak metabolic rate and lifespan in a butterfly. J. Exp. Biol. 216, 1388–97.
Niitepõld, K., Perez, A., Boggs, C.L., 2014. Aging, life span, and energetics under adult dietary restriction in Lepidoptera. Physiol. Biochem. Zool. 87, 684–94.

Niitepõld, K., Smith, A.D., Osborne, J.L., Reynolds, D.R., Carreck, N.L., Martin, A.P., Marden, J.H., Ovaskainen, O., Hanski, I., 2009. Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. Ecology 90, 2223–2232.

Norberg, U.L.F., Enfja, K., 2002. Habitat exploration in butterflies – an outdoor cage experiment. Evol. Ecol. 16, 1–14.

O'Brien, D.M., Boggs, C.L., Fogel, M.L., 2004. Making eggs from nectar : the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. Oikos 105, 279–291.

O'Brien, D.M., Fogel, M.L., Boggs, C.L., 2002. Renewable and nonrenewable resources : Amino acid turnover and allocation to reproduction in Lepidoptera. Proc. Natl. Acad. Sci. 99, 4413–4418.

Öckinger, E., Smith, H.G., 2007a. Asymmetric dispersal and survival indicate population sources for grassland butterflies in agricultural landscapes. Ecography 30, 288–298.

Öckinger, E., Smith, H.G., 2007b. Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. J. Appl. Ecol. 44, 50–59.

Odendaal, F.J., Turchin, P., Stermitz, F.R., 1989. Influence of host-plant density and male harassment on the distribution of female *Euphydryas anicia* (Nymphalidae). Oecologia 78, 283–288.

Ômura, H., Honda, K., 2005. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. Oecologia 142, 588–596.

Ômura, H., Honda, K., Hayashi, N., 1999. Chemical and chromatic bases for preferential visiting by the cabbage butterfly, *Pieris rapae*, to rape flowers. J. Chem. Ecol. 25, 1895–1906.

Oster, G., Heinrich, B., 1976. Why do bumblebees major? A mathematical model. Ecol. Monogr. 46, 129–133.

Ouin, A., Aviron, S., Dover, J., Burel, F., 2004. Complementation/supplementation of resources for butterflies in agricultural landscapes. Agric. Ecosyst. Environ. 103, 473–479.

Ouin, A., Martin, M., Burel, F., 2008. Agricultural landscape connectivity for the meadow brown butterfly (*Maniola jurtina*). Agric. Ecosyst. Environ. 124, 193–199.

Owen, D.F., Smith, D.A. S., 1990. Interpopulation variation and selective predation in the meadow brown butterfly, *Maniola jurtina* (L.) (Lepidoptera: Satyridae) in the Canary Islands. Biol. J. Linn. Soc. 39, 251–267.

Pan, M.L., Telfer, W.H., 2001. Storage hexamer utilization in two lepidopterans: differences correlated with the timing of egg formation. J. Insect Sci. 1, 1–8.

Parkash, R., Rajpurohit, S., Ramniwas, S., 2008. Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. J. Insect Physiol. 54, 1050–1056.
Parsons, A., Newman, J., Penning, P., 1994. Diet preference of sheep: effects of recent diet, physiological state and species abundance. J. Anim. Ecol. 63, 465–478.
Peet, R.K., 1974. The measurement of species diversity. Annu. Rev. Ecol. Syst. 5, 285–307.
Peet, R.K., 1975. Relative Diversity Indices. Ecology 56, 496–498.
Pellet, J., 2008. Seasonal variation in detectability of butterflies surveyed with Pollard walks. J. Insect Conserv. 12, 155–162.
Pellmyr, O., 2003. Yuccas, Yucca Moths, and Coevolution: A Review. Ann. Missouri Bot. Gard. 90, 35–55.
Pellmyr, O., Krenn, H.W., 2002. Origin of a complex key innovation in an obligate insect-plant mutualism. Proc. Natl. Acad. Sci. 99, 5498–5502.
Perkins, A.J., Maggs, H.E., Watson, A., Wilson, J.D., 2011. Adaptive management and targeting of agri-environment schemes does benefit biodiversity: a case study of the corn bunting *Emberiza calandra*. J. Appl. Ecol. 48, 514–522.
Petanidou, T., 2007. Ecological and evolutionary aspects of floral nectars in mediterranean habitats, in: Nicolson, S.W., Nepi, M., Pacini, E. (Eds.), Nectaries and Nectar. Springer Netherlands, pp. 343–375.
Pijpe, J., Brakefield, P.M., Zwaan, B.J., 2008. Increased life span in a polyphenic butterfly artificially selected for starvation resistance. Am. Nat. 171, 81–90.
Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M., Sexton, J.O., 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science 344, 1246752.
Pivnick, K.A., Mcneil, J.N., 1985. Effects of nectar concentration on butterfly feeding : measured feeding rates for *Thymelicus lineola* (Lepidoptera : Hesperiidae) and a general feeding model for adult Lepidoptera. Oecologia 66, 226–237.
Plepys, D., Ibarra, F., Francke, W., Lofstedt, C., 2002. Odour-mediated nectar foraging in the silver Y moth, *Autographa gamma* (Lepidoptera : Noctuidae): behavioural and electrophysiological responses to floral volatiles. Oikos 99, 75–82.
Pohl, N.B., Van Wyk, J., Campbell, D.R., 2011. Butterflies show flower colour preferences but not constancy in foraging at four plant species. Ecol. Entomol. 36, 290–300.

Polic, D., Fiedler, K., Nell, C., Grill, A., 2014. Mobility of ringlet butterflies in high-elevation alpine grassland: effects of habitat barriers, resources and age. J. Insect Conserv. 18, 1153–1161.
Pollard, E., Yates, T.J., 1993. Monitoring Butterflies for Ecology and Conservation. Chapman and Hall, London.
Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353.
Potts, S.G., Vulliamy, B., Roberts, S., Toole, C.O., Dafni, A., Ne, G., Willmer, P.G., 2004. Nectar resource diversity organises flower-visitor community structure Entomol. Exp. Appl. 113, 103–107.
Potts, S.G., Woodcock, B.A., Roberts, S.P.M., Tscheulin, T., Pilgrim, E.S., Brown, V.K., Tallowin, J.R., 2009. Enhancing pollinator biodiversity in intensive grasslands. J. Appl. Ecol. 46, 369–379.
Pryke, S.R., Samways, M.J., 2001. Width of grassland linkages for the conservation of butterflies in South African afforested areas. Biol. Conserv. 101, 85–96.
Pulliam, R.H., 1988. Sources, sinks and population regulation. Am. Nat. 132, 652–661.
Pyke, G.H., 1984. Optimal forgaing theory: a critical review. Annu. Rev. Ecol. Syst. 15, 523–575.
Pywell, R.F., Meek, W.R., Hulmes, L., Hulmes, S., James, K.L., Nowakowski, M., Carvell, C., 2011. Management to enhance pollen and nectar resources for bumblebees and butterflies within intensively farmed landscapes. J. Insect Conserv. 15, 853–864.
Pywell, R.F., Warman, E.A., Sparks, T.H., Greatorex-Davies, J.N., Walker, K.J., Meek, W.R., Carvell, C., Petit, S., Firbank, L.G., 2004. Assessing habitat quality for butterflies on intensively managed arable farmland. Biol. Conserv. 118, 313–325.
R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Raubenheimer, D., Simpson, S.J., 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. J. Exp. Biol. 206, 1669–1681.
Reece, J., Urry, L.A., Meyers, N., Cain, M.L., Wasserman, S.A., Minorsky, P. V., Jackson, R.B., Cooke, B.N., 2011. Campbell Biology, 8th ed, Campbell Biology. Pearson Higher Educarion, AU.
Reinhold, K., 1999. Energetically cost behaviour and the evolution of resting metabolic rate in insects. Funct. Ecol. 13, 217–224.
Reznick, D.N., Ghalambor, C.K., 2001. The population ecology of contemporary adaptations: What empirical studies reveal about the

conditions that promote adaptive evolution. Genetica 112-113, 183–198.
Reznick, D.N., Ghalambor, C.K., 2005. Selection in nature: Experimental manipulations of natural populations. Integr. Comp. Biol. 45, 456–462.
Roark, A.M., Bjorndal, K.A., 2009. Metabolic rate depression is induced by caloric restriction and correlates with rate of development and lifespan in a parthenogenetic insect. Exp. Gerontol. 44, 413–419.
Robinson, R.A., Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. J. Appl. Ecol. 39, 157–176.
Rodrigues, D., Weiss, M.R., 2012. Reward Tracking and Memory Decay in the Monarch Butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae). Ethology 118, 1122–1131.
Roeder, K.A., Behmer, S.T., 2014. Lifetime consequences of food protein-carbohydrate content for an insect herbivore. Funct. Ecol. 28, 1135–1143.
Rosin, Z.M., Myczko, Ł., Sko, P., Sparks, T.H., Tryjanowski, P., 2011. Butterfly responses to environmental factors in fragmented calcareous grasslands. J. Insect Conserv. 16, 321–329
Rusterholz, H.P., Erhardt, A., 1997. Preferences for nectar sugars in the peacock butterfly, *Inachis io*. Ecol. Entomol. 22, 220–224.
Rusterholz, H.P., Erhardt, A., 2000. Can nectar properties explain sex-specic flower preferences in the Adonis blue butterfly L*ysandra bellargus*? Ecol. Entomol. 25, 81–90.
Rusterholz, H.P., Erhardt, A., 1998. Effects of elevated CO2 on flowering phenology and nectar production of nectar plants important for butterflies of calcareous grasslands. Oecologia 113, 341–349.
Saarinen, K., Valtonen, A., Jantunen, J., Saarnio, S., 2005. Butterflies and diurnal moths along road verges: Does road type affect diversity and abundance? Biol. Conserv. 123, 403–412.
Sánchez-Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non-target organisms. Jounral Environ. Anal. Toxicol. S:4, 1–11.
Sang, A., Teder, T., 2011. Dragonflies cause spatial and temporal heterogeneity in habitat quality for butterflies. Insect Conserv. Divers. 4, 257–264.
SAS, 2012. SAS/STAT 12.1 User's Guide. SAS Institute Inc., Cary NC.
Scali, V., 1971. Imaginal diapause and gonadal maturation of *Maniola jurtina* (Lepidoptera: Satyridae) from Tuscany. J. Anim. Ecol. 40, 467–472.
Scali, V., Masetti, M., 1973. The population structure of *Maniola jurtina* (Lepidoptera: Satyridae): the sex-ratio control. J. Anim. Ecol. 42, 773–778.

Scaraffia, P.Y., Wells, M.A., 2003. Proline can be utilized as an energy substrate during flight of *Aedes aegypti* females. J. Insect Physiol. 49, 591–601.
Schaffer, W.M., Jensen, D.B., Hobbs, D.E., Gurevitch, J., Todd, J.R., Schaffer, M.V., 1979. Competition, foraging energetics, and the cost of sociality in three species of bees. Ecology 60, 976–987.
Scherr, S.J., Mcneely, J.A., 2008. Biodiversity conservation and agricultural sustainability : towards a new paradigm of 'ecoagriculture' landscapes. Philos. Trans. R. Soc. Lond. B. 363, 477–494.
Schmitt, T., Röber, S., Seitz, A., 2005. Is the last glaciation the only relevant event for the present genetic population structure of the meadow brown butterfly *Maniola jurtina* (Lepidoptera : Nymphalidae)? Biol. J. Linn. Soc. 85, 419–431.
Schneider, C., Dover, J., Fry, G.L.A., 2003. Movement of two grassland butterflies in the same habitat network : the role of adult resources and size of the study area. Ecol. Entomol. 28, 219–227.
Schoonhoven, L.M., Van Loon, J.J., Dicke, M., 2005. Insect-Plant Biology, 2nd ed. Oxford University Press, Oxford.
Schtickzelle, N., Mennechez, G., Baguette, M., 2006. Dispersal depression with habitat fragmentation in the bog fritillary butterfly. Ecology 87, 1057–1065.
Schultz, C.B., Dlugosch, K.M., 1999. Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. Oecologia 119, 231–238.
Schultz, C.B., Franco, A.M.A., Crone, E.E., 2012. Response of butterflies to structural and resource boundaries. J. Anim. Ecol. 81, 724–734.
Schweiger, O., Maelfait, J.P., Van Wingerden, W., Hendrickx, F., Billeter, R., Speelmans, M., Augenstein, I., Aukema, B., Aviron, S., Bailey, D., Bukacek, R., Burel, F., Diekötter, T., Dirksen, J., Frenzel, M., 2005. Quantifying the impact of environmental factors on arthropod communities in agricultural landscapes across organizational levels and spatial scales. J. Appl. Ecol. 42, 1129–1139.
Scrimgeour, G.J., Culp, J.M., 1994. Feeding while evading predators by a lotic mayfly: linking short-term foraging behaviours to long-term fitness consequences. Oecologia 100, 128–134.
Serruys, M., Van Dyck, H., 2014. Development, survival, and phenotypic plasticity in anthropogenic landscapes: trade-offs between offspring quantity and quality in the nettle-feeding peacock butterfly. Oecologia 176, 379–387.
Shapiro, S.S., Wilk, M.B., 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika 52, 591–611.

Sharp, M.A., Parks, D.R., Ehrlich, P.R., 1974. Plant resources and butterfly habitat selection. Ecology 55, 870–875.
Shaw, M.R., Stefanescu, C., Van Nouhuys, S., 2009. Parasitoids of European butterflies, in: Settele, J., Shreeve, T.G., Konvicka, M., Van Dyck, H. (Eds.), Ecology of Butterflies in Europe. Cambridge University press, Cambridge.
Shiraishi, A., Kuwabara, M., 1970. The effects of amino acids on the labellar hair chemosensory cells of the fly. J. Gen. Physiol. 56, 768–782.
Sibly, R.M., Winokur, L., Smith, R.H., 1997. Interpopulation variation in phenotypic plasticity in the speckled wood butterfly, *Pararge aegeria.* Oikos 78, 323–330.
Sih, A., 1980. Optimal behavior: can foragers balance two conflicting demands? Science 210, 1041–1043.
Sih, A., Bell, A., Johnson, J.C., 2004. Behavioral syndromes: An ecological and evolutionary overview. Trends Ecol. Evol. 19, 372–378.
Sinclair, B.J., Bretman, A., Tregenza, T., Tomkins, J.L., Hosken, D.J., 2011. Metabolic rate does not decrease with starvation in *Gryllus bimaculatus* when changing fuel use is taken into account. Physiol. Entomol. 36, 84–89.
Singer, M.C., Thomas, C.D., Parmesan, C., 1993. Rapid human-induced evolution of insect–host associations. Nature 366, 681–683.
Singer, M.S., Bernays, E.A., Carrière, Y., 2002. The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. Anim. Behav. 64, 629–643.
Skórka, P., Nowicki, P., Lenda, M., Witek, M., Śliwińska, E.B., Settele, J., Woyciechowski, M., 2013. Different flight behaviour of the endangered scarce large blue butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) within and outside its habitat patches. Landsc. Ecol. 28, 533–546.
Smart, S.M., Firbank, L.G., Bunce, R.G.H., Watkins, J.W., 2000. Quantifying changes in abundance of food plants for butterfly larvae and farmland birds. J. Appl. Ecol. 37, 398–414.
Sparks, T.H., Parish, T., 1995. Factors affecting the abundance of Butterflies in field boundaries in swavesey fens, Cambridgeshire, UK. Biol. Conserv. 73, 221–227.
Steigenga, M.J., Fischer, K., 2009. Fitness consequences of variation in developmental temperature in a butterfly. J. Therm. Biol. 34, 244–249.
Stephens, P.A., Boyd, I.L., Mcnamara, J.M., Houston, A.I., 2009. Capital breeding and income breeding: their meaning, measurement and worth. Ecology 90, 2057–2067.

Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J., Baguette, M., 2012. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol. Lett. 15, 74–86.

Stevens, V.M., Turlure, C., Baguette, M., 2010. A meta-analysis of dispersal in butterflies. Biol. Rev. 85, 625–642.

Stjernholm, F., Karlsson, B., 2006. Reproductive expenditure affects utilization of thoracic and abdominal resources in male *Pieris napi* butterflies. Funct. Ecol. 20, 442–448.

Stjernholm, F., Karlsson, B., Boggs, C.L., 2005. Age-related changes in thoracic mass : possible reallocation of resources to reproduction in butterflies. Biol. J. Linn. Soc. 86, 363–380.

Stoate, C., Báldi, A., Beja, P., Boatman, N.D., Herzon, I., van Doorn, A., de Snoo, G.R., Rakosy, L., Ramwell, C., 2009. Ecological impacts of early 21st century agricultural change in Europe--a review. J. Environ. Manage. 91, 22–46.

Stoate, C., Boatman, N.D., Borralho, R.J., Rio Carvalho, C., de Snoo, G.R., Eden, P., 2001. Ecological impacts of arable intensification in Europe. J. Environ. Manage. 63, 337–365.

Suarez, R.K., 2000. Energy metabolism during insect flight : biochemical design and physiological performance. Physiol. Biochem. Zool. 73, 765–771.

Suarez, R.K., Darveau, C.-A., Welch, K.C., O'Brien, D.M., Roubik, D.W., Hochachka, P.W., 2005. Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. J. Exp. Biol. 208, 3573–3579.

Suggitt, A.J., Gillingham, P.K., Hill, J.K., Huntley, B., Kunin, W.E., Roy, D.B., Thomas, C.D., 2011. Habitat microclimates drive fine-scale variation in extreme temperatures. Oikos 120, 1–8.

Sutton, M.A., Mason, K.E., Sheppard, L.J., Sverdrup, H., Haeuber, R., Hicks, W.K. (Eds.), 2014. Nitrogen deposition, critical loads and biodiversity. Springer, Berlin.

Svärd, L., Wiklund, C., 1989. Mass and production rate of ejaculates in relation to monandry / polyandry in butterflies. Behav. Ecol. Sociobiol. 24, 395–402.

Tabashnik, B.E., 1982. Responses of pest and non-pest *Colias* butterfly larvae to intraspecific variation in leaf nitrogen and water content. Oecologia 55, 389–394.

Tallowin, J.R.B., Smith, R.E.N., Goodyear, J., Vickery, J.A., 2005. Spatial and structural uniformity of lowland agricultural grassland in England : a context for low biodiversity. Grass Forage Sci. 60, 225–236.

Tammaru, T., Haukioja, E., 1996. Capital breeders and income breeders among Lepidoptera: consequences to population dynamics. Oikos 77, 561–564.

Telang, A., 2001. How female caterpillars accumulate their nutrient reserves. J. Insect Physiol. 47, 1055–1064.
Thomas, C.D., Hill, J.K., Lewis, O.T., 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. J. Anim. Ecol. 67, 485–497.
Thomas, J.A., 2005. Monitoring Change in the Abundance and Distribution of Insects Using Butterflies and Other Indicator Groups. Philos. Transcations Biol. Sci. 360, 339–357.
Thorbek, P., Bilde, T., 2004. Reduced numbers of generalist arthropod predators after crop management. J. Appl. Ecol. 41, 526–538.
Thornber, C., Stachowicz, J.J., Gaines, S., 2006. Tissue type matters: Selective herbivory on different life history stages of an isomorphic alga. Ecology 87, 2255–2263.
Throop, H.L., Holland, E. A., Parton, W.J., Ojima, D.S., Keough, C. a., 2004. Effects of nitrogen deposition and insect herbivory on patterns of ecosystem-level carbon and nitrogen dynamics: Results from the CENTURY model. Glob. Chang. Biol. 10, 1092–1105.
Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. Science 292, 281–284.
Tolman, T., Lewington, R., 1999. Guide des papillons d'Europe et d'Afrique du Nord, 2007th ed. Delachaux et Niestlé, Paris.
Torres, C., Galetto, L., 2002. Are nectar sugar composition and corolla tube length related to the diversity of insects that visit Asteraceae flowers? Plant Biol. 4, 360–366.
Traynier, R.M.M., 1979. Long-term changes in the oviposition behaviour of the cabbage butterfly, *Pieris rapae*, induced by contact with plants. Physiol. Entomol. 4, 87–96.
Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Carsten, T., 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. Ecol. Lett. 8, 857–874.
Tudor, O., Dennis, R.L.., Greatorex-Davies, J.., Sparks, T.., 2004. Flower preferences of woodland butterflies in the UK: nectaring specialists are species of conservation concern. Biol. Conserv. 119, 397–403.
Turlure, C., 2009. Habitat from a butterfly's point of view : how specialist butterflies map onto ecological resources.
United Nations, Department of Economics and Social Affairs, P.D., 2013. World Population Prospects. The 2012 Revision. Volume I: Comprehansive tables.
Vahed, K., 1998. The function of nuptial feeding in insects : a review of empirical studies. Biol. Rev. 73, 43–78.

Valtonen, A., Saarinen, K., 2005. A highway intersection as an alternative habitat for a meadow butterfly : effect of mowing , habitat geometry and roads on the ringlet (*Aphantopus hyperantus*). Ann. Zool. Fenn. 42, 545–556.

Valtonen, A., Saarinen, K., Jantunen, J., 2006. Effect of different mowing regimes on butterflies and diurnal moths on road verges. Anim. Biodivers. Conserv. 29, 133–148.

Van Dyck, H., Baguette, M., 2005. Dispersal behaviour in fragmented landscapes: Routine or special movements? Basic Appl. Ecol. 6, 535–545.

Van Dyck, H., Baguette, M., 2012. Dispersal and habitat fragmentation in invertebrates — examples from widespread and localized butterflies, in: Clobert, J., Baguette, M., Benton, T.G., Bullock, J.M. (Eds.), Dispersal Ecology and Evolution. Oxford University Press, Oxford, pp. 413–419.

Van Dyck, H., Matthysen, E., 1999. Habitat fragmentation and insect flight : a changing " design " in a changing landscape ? Trends Ecol. Evol. 14, 172–174.

Van Dyck, H., Regniers, S., 2010. Egg spreading in the ant parasitic butterfly, *Maculinea alcon*: from individual behaviour to egg distribution pattern. Anim. Behav. 80, 621–627.

Van Dyck, H., Van Strien, A.J., Maes, D., Van Swaay, C.A.M., 2009. Declines in common, widespread butterflies in a landscape under intense human use. Conserv. Biol. 23, 957–965.

Van Marrewijk, W.J.A., van den Broek, A.T.M., Beenakkers, A.M.T., 1980. Regulation of glycogenolysis in the locust fat body during flight. Insect Biochem. 10, 675–679.

Van Swaay, C., Warren, M., Loïs, G., 2006. Biotope Use and Trends of European Butterflies. J. Insect Conserv. 10, 189–209.

Van Swaay, C.A.M., Van Strien, A.J., Harpke, A., Fontaine, B., Stefanescu, C., Roy, D.B., Maes, D., Kühn, E., Ounap, E., Regan, E., Svitra, G., Prokofev, I., Heliölä, J., Settele, J., Pettersson, L., Botham, M., Musche, M., Titeux, N., Cornish, N., Leopold, P., Julliard, R., Verovnik, R., Öberg, S., Popov, S., Collins, S., Goloshchapova, S., Roth, T., Brereton, T., Dorset, L., Warren, M., 2013. The European Grassland Butterfly Indicator: 1990–2011. Luxembourg.

Vande Velde, L., Schtickzelle, N., Van Dyck, H., 2012. Effect of larval food stress on male adult behaviour, morphology and reproductive investment in the butterfly *Pararge aegeria*. Evol. Ecol. 27, 221–234.

Vande Velde, L., Van Dyck, H., 2013. Lipid economy, flight activity and reproductive behaviour in the speckled wood butterfly: On the energetic cost of territory holding. Oikos 122, 555–562.

Vanreusel, W., Van Dyck, H., 2007. When functional habitat does not match vegetation types : a resource-based approach to map butterfly habitat. Biol. Conserv. 135, 202–211.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, 4th Editio. ed. New York.
Vickery, J. A., Tallowin, J.R., Feber, R.E., Asteraki, E.J., Atkinson, P.W., Fuller, R.J., Brown, V.K., 2001. The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. J. Appl. Ecol. 38, 647–664.
Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.
WallisDeVries, M.F., Poschlod, P., Willems, J.H., 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe : integrating the requirements of flora and fauna. Biol. Conserv. 104, 265–273.
Wallisdevries, M.F., Van Swaay, C. a M., Plate, C.L., 2012. Changes in nectar supply: A possible cause of widespread butterfly decline. Curr. Zool. 58, 384–391.
Wang, R., Ovaskainen, O., Cao, Y., Chen, H., Zhou, Y., Xu, C., Hanski, I., 2011. Dispersal in the Glanville fritillary butterfly in fragmented versus continuous landscapes: Comparison between three methods. Ecol. Entomol. 36, 251–260.
Warren, M.S., Hill, J.K., Thomas, J.A., Asher, J., Fox, R., Huntley, B., Roy, D.B., Tefler, M.G., Jeffcoate, S., Harding, P., Jeffcoate, G., Willis, S.G., Greatorex-Davies, J.., Moss, D., Thomas, C.D., 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. Lett. to Nat. 414, 65–69.
Waser, N.M., 1986. Flower constancy: definition, cause, and measurement. Am. Nat. 127, 593–603.
Watanabe, M., Hirota, M., 1999. Effects of sucrose intake on spermatophore mass produced by male swallowtail butterfly P*apilio xuthus* L. BioOne 16, 55–61.
Watanabe, M., Sato, K., 1993. A Spermatophore Structured in the Bursa Copulatrix of the Small White *Pieris rapae* (Lepidoptera, Pieridae) During Copulation, and It's Sugar Content. J. Res. Lepid. 32, 26–36.
Watt, W.B., Chew, F.S., Snyder, L.R.G., Watt, A.G., Rothschild, D.E., 1977. Population structure of pierid butterflies. Oecologia 27, 1–22.
Watt, W.B., Hoch, P.C., Mills, S.G., 1974. Nectar resource use by *Colias* butterflies - Chemical and visual aspects. Oecologia 14, 353–374.

Watts, D.P., 1998. Long-term habitat use by mountain gorillas (*Gorilla gorilla beringei*). 1. Consistency, variation, and home range size and stability. Int. J. Primatol. 19, 651–679.
Weeda, E., de Kort, C.A.D., Beenakkers, A.M.T., 1980. Oxidation of proline and pyruvate by flight muscle mitochondria of the Colorado beetle, *Leptinotarsa decemlineata say*. Insect Biochem. 10, 305–311.
Weimerskirch, H., Doncaster, C.P., Cuenot-Chaillet, F., 1994. Pelagic seabirds and the marine environment: foraging patterns of wandering albatrosses in relation to prey availability and distribution. Proc. R. Soc. B Biol. Sci. 255, 91–97.
Weiss, M.R., 1997. Innate colour preferences and flexible colour learning in the pipevine swallowtail. Anim. Behav. 53, 1043–1052.
West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.
Wheeler, D., 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41, 407–431.
Wickman, P.-O., 1988. Dynamics of mate-searching behaviour in a hilltopping butterfly, *Lasiommata megera* (L.): the effects of weather and male density. Zool. J. Linn. Soc. 93, 357–377.
Wiegmann, D.D., Wiegmann, D.A., Waldron, F.A., 2003. Effects of a reward downshift on the consummatory behavior and flower choices of bumblebee foragers. Physiol. Behav. 79, 561–566.
Wilkinson, N.I., Wilson, J.D., Anderson, G.Q.A., 2012. Agri-environment management for corncrake *Crex crex* delivers higher species richness and abundance across other taxonomic groups. Agric. Ecosyst. Environ. 155, 27–34.
Wilson, J.D., Morris, A.J., Arroyo, B.E., Clark, S.C., Bradbury, R.B., 1999. A review of the abundance and diversity of invertebrate and plant foods of granivorous birds in northern Europe in relation to agricultural change. Agric. Ecosyst. Environ. 75, 13–30.
Woodcock, B.A., Potts, S.G., Tscheulin, T., Pilgrim, E., Ramsey, A.J., Harrison-Cripps, J., Brown, V.K., Tallowin, J.R., 2009. Responses of invertebrate trophic level, feeding guild and body size to the management of improved grassland field margins. J. Appl. Ecol. 46, 920–929.
Yang, Y., Joern, A., 1994. Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. Physiol. Entomol. 19, 75–82.
Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression Models for Count Data in R. J. Stat. Softw. 27.