

Long-distance migration in butterflies and moths: a cross-species review

A master thesis



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Preface

This review is written for the Dutch Butterfly Conservation (for website see references), but is also a thesis as a part of my master programme. By writing this review, I hope to contribute to the understanding of the many facets of long-distance migration in butterflies and moths. I hope that readers will become aware of the importance of further research in this area and perhaps some readers will even find an interesting subject, in need further exploring, that they want to pursue.

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Nederlandse samenvatting (Dutch abstract)

Migratie in Lepidoptera (d.i. dag- en nachtvlinders) wordt al jarenlang bestudeerd en veelal met de focus op één soort tegelijk. Deze scriptie geeft een overzicht van het tot nu toe gedane multidisciplinaire onderzoek naar migratie van verschillende soorten vlinders en heeft als doel richting te geven aan verder onderzoek. Migratie in vlinders en insecten in het algemeen onderscheidt zich van migratie in andere diergroepen door het feit dat de migratieroute niet door één individu, maar door meerdere individuen van een reeks elkaar opvolgende generaties wordt voltooid. Dat houdt in dat de migratieroute voor iedere generatie weer anders is, wat migratie van insecten erg complex maakt. Om migratie te kunnen begrijpen, is het essentieel om alle factoren die leiden tot migratie te bestuderen. Deze factoren zijn niet alleen het trekgedrag zelf en het daaruit voortkomende migratietraject, maar ook alle eigenschappen die nodig zijn om te kunnen migreren, de genetische basis daarvan en het patroon van genexpressie waardoor de eigenschappen tot uiting komen. Ook is het belangrijk om de selectiedrukken te onderzoeken om uit te vinden hoe migratie heeft kunnen evolueren in de vlinders en wat de functie is. Tenslotte moet er onderzocht worden hoe omgevingsfactoren invloed uitoefenen op het gedrag, de genexpressie en natuurlijke selectie. De verschillende onderzoeksmethoden die worden gebruikt om trekvlinders te bestuderen, de toepassingen daarvan en de voor- en nadelen worden beschreven. Vervolgens wordt een overzicht gegeven van de huidige kennis en recente bevindingen op het gebied van migrerende vlinders. Allereerst worden de migratietrajecten van monarchvlinders (*Danaus plexippus*), distelvlinders (*Vanessa cardui*), admiraalvlinders (*Vanessa atalanta*) en gamma-uilen (*Autographa gamma*) beschreven. Daarna volgt een overzicht van het trekgedrag en de daaraan ten grondslag liggende eigenschappen en mechanismen. Hierbij wordt onder andere beschreven hoe vlinders gebruik maken van gunstige winden en hoe zij navigeren. Migratie wordt ook op genetisch vlak steeds meer onderzocht. Door genen of genexpressie van migrerende en niet-migrerende vlinders te vergelijken wordt onderzocht welke genen of genexpressiepatronen ten grondslag liggen aan trekgedrag. Migratie is erg vaak geëvolueerd in veel verschillende diergroepen en ook in de orde van de Lepidoptera is trekgedrag meerdere keren ontstaan. Daarnaast lijken veel van de tot nu toe gevonden trekeigenschappen en -genen van vlinders wijdverbreid onder insecten en zelfs onder meerdere diergroepen, wat suggereert dat migratie een uitbreiding is van bestaande eigenschappen en niet een hele nieuwe eigenschap is. Toch zijn er ook verschillen tussen verschillende soorten trekvlinders wat betreft het houden van een diapause en de mate van groepsvorming. Migratie van vlinders is groter in omvang in de jaren met hogere temperaturen, zowel wat betreft het aantal geobserveerde soorten in een bepaald gebied als het aantal individuen per soort. In verband met klimaatverandering wordt ook voor de toekomst een toename van migratie verwacht. Omdat het grootste deel van de huidige kennis is gebaseerd op slechts een klein aantal soorten, is het belangrijk om in de toekomst meer soorten te onderzoeken, zodat verschillen tussen migrerende en niet-migrerende soorten verder kan worden onderzocht. Ook het ontwikkelen van een methode om individuele trekvlinders te volgen is van groot belang, aangezien dit het bestuderen van trekgedrag in groter detail mogelijk zal maken. Tenslotte raad ik aan om navigatiegedrag te bestuderen met geïntegreerde methoden, waarbij kennis van alle verschillende kompas mechanismen wordt gecombineerd. Dit is nodig aangezien de dieren waarschijnlijk kunnen wisselen van kompas mechanisme wanneer er één geen informatie kan doorgeven of inconsistent is met de andere kompassen.

Long-distance migration in butterflies and moths: a cross-species review

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Abstract

Long-distance migration in Lepidoptera (*i.e.* butterflies and moths) has been studied for many years and mostly with a single-species focus. This review gives a cross-species overview of the latest developments in the multidisciplinary research field of Lepidopteran migration and is aimed to guide future research. The various methodologies that are used to study migratory Lepidoptera, their applications, advantages and disadvantages are discussed. Subsequently, an overview of the current knowledge and latest findings on Lepidopteran migration is given, including the migratory trajectories, migratory behaviour and its underlying traits and mechanisms. Many traits and genes studied thus far seem to be widespread among insects and some even among multiple animal taxa, supporting the evolutionary hypothesis that migration may be an extension of existing traits. Nevertheless, there are also variable migratory traits among species of migratory Lepidopterans. Lepidopteran migrations are found to increase in magnitude with higher temperatures, both regarding the number of migratory species detected and the number of individuals per species. This increase is hypothesised to continue in the future due to climate change. Since the majority of the current knowledge is based on only a few species, it is essential to include more species in future research so that the differences between migratory and non-migratory species can be investigated. Also, development of methodologies to follow individual animals along their lifetime tracks is of high priority, for it will enable to study all aspects of migratory behaviour in greater detail. Lastly, I recommend to study navigational behaviour in an integrated manner, *i.e.* combining the current knowledge on all the different compass mechanisms. Otherwise, the animals are thought to switch from one mechanism to the other in case of lacking information or inconsistency of one of the systems due to experimentation.

Keywords: migration, Lepidoptera, butterfly, moth, Monarch, *Danaus plexippus*, Painted Lady, *Vanessa cardui*, Red Admiral, *Vanessa atalanta*, Silver-Y, *Autographa gamma*, insect, monitoring, radar, migratory behaviour, wind-selection, orientation, navigation, sun compass, magnetic compass, circadian clock, juvenile hormone, genome, evolution, climate change, invasion.

Introduction

The animal kingdom is incredibly diverse and so is animal behaviour. Since ancient times, people have noticed that animals do not always spend the whole year at one area or habitat, but periodically disappear and suddenly reappear (Aristotle, reported by Berthold, 2001, p. 11; Exodus 10: 12-15). Closer inspection showed that these animals moved between different 'worlds' as the seasons progressed, a distinct type of movement that is named migration (for an overview of historic studies see Williams, 1957).

The term migration has predominantly been used with respect to the study of birds, for which *regular seasonal to-and-fro movements of the individuals between two areas* may be an appropriate definition of their migratory behaviour (adapted from Williams, 1957 and 1930). The two areas in this definition are sometimes referred to as the 'two worlds' and usually consist of a breeding area and a wintering area (Dingle and Drake, 2007). However, this definition does not include all migratory animals. For example, insects do not individually complete to-and-fro or round-trip movements. Instead, they reproduce along the way and the succession of migration of multiple generations forms a complete to-and-fro or round-trip movement (Dingle and Drake, 2007; Holland *et al.*, 2006). To include insects in the definition of migration, some researchers think it is better to define migration by describing animal behaviour during migrations instead of the outcome of migratory behaviour. An example of such a definition is: *migration is a periodic, more or less unidirectional, continued movement, assisted by the efforts of the animal, and in a direction over which it exerts a control, which results in the animal passing away from its previous daily field of operations* (Williams, 1930).

This review concerns the long-distance migration of a particular order of insects in which migratory behaviour is widespread: the Lepidoptera, that includes butterflies and moths. The life cycle of Lepidoptera consists of several stages, starting with the caterpillar stage after hatching. During the caterpillar stage, the larvae feed, grow and go through several instar stadia, separated by moults. The majority of caterpillars feed on plants, the larval food plants, which are different per species (Reppert *et al.*, 2010; Stefanescu *et al.*, 2011; Van Swaay *et al.*, 2008a). The last instar goes into the pupal stage, in which metamorphosis into the adult flying stage occurs. The duration of the development from egg to adult varies considerably between species and circumstances, from a few weeks to years (Stefanescu *et al.*, 2011; Powell, 1986; Nylin *et al.*, 1995). In contrast to the larvae, adult Lepidoptera feed on nectar (Stefanescu *et al.*, 2011; Brower, 1996). The lifespan of adults also varies greatly, from a few days to several months (Talekar and Shelton, 1993; Reppert *et al.*, 2010). Under unfavourable environmental conditions, such as droughts, cold periods, high levels of predation or parasitism, overpopulation and therewith associated resource shortage, the Lepidoptera can go into diapause, *i.e.* a state of inactivity, to wait out the harsh conditions (Masaki, 1980; Nylin *et al.*, 1995). The stage or stages of development in which Lepidoptera can go into diapause is dependent on the species (Nylin *et al.*, 1995). In a number of species, the adults migrate instead of having a diapause to avoid unfavourable conditions (Stefanescu *et al.*, 2011 and 2012).

Migratory behaviour is more common in some Lepidopteran families than in others. Migratory butterfly species are particularly common in the Pieridae, Danaidae and Nymphalidae families (Williams, 1957). In moths, migratory species are most common in the Sphingidae and Noctuidae families (Williams, 1957). The best known and best studied migratory Lepidopterans are Monarch butterflies (*Danaus plexippus*), that are famous for their massive autumn migrations in North America (Reppert *et al.*, 2010). Other well-known migrant butterfly species are the Painted Lady (*Vanessa cardui*), the Red Admiral (*Vanessa atalanta*), the Pale Clouded Yellow (*Colias hyale*), the Clouded Yellow (*Colias croceus*) and the Camberwell Beauty (*Nymphalis antiopa*) (Lempke, 1972; Dutch Butterfly Conservation Website; Van Swaay and Hensle, 2003; Van Swaay *et al.*, 2008a). Frequently observed migratory moth species are the Silver-Y moth (*Autographa gamma*), the Diamondback moth (*Plutella xylostella*), the Hummingbird Hawk moth (*Macroglossum stellatarum*) and the Black Cutworm moth (*Agrotis ipsilon*) (Dutch Butterfly Conservation Website). Although other migratory Lepidopterans are lesser known by the general public, they are present on all continents except Antarctica (Bos *et al.*, 2006; Srygley *et al.*, 2006; Lempke, 1972). Since the majority of studies are conducted on a small number of species, *i.e.* Monarch, Painted Lady, Red Admiral and Silver-Y moth, the current knowledge is strongly biased towards these species and so is the focus of this review.

Lepidopteran migrations differ from year to year. The exact migratory routes change every year (Brattström *et al.*, 2008 and 2010b; Drake *et al.*, 1995). The magnitude of Lepidopteran migrations with respect to the distances travelled differ between species and rely on environmental fluctuations (Lempke, 1956), but can be up to several thousand kilometres (Reppert *et al.*, 2010; Stefanescu *et al.* in prep.). The magnitude also changes regarding the number of insects of a particular species that

migrate during each cycle of the migration (Williams, 1957 and 1970; Lempke, 1972; Stefanescu *et al.*, in prep.). In some years, particular species of migrants may be very rare in a certain area, while they may be particularly abundant in the next year. Years of exceptional abundance of a certain migratory species are referred to as invasions and draw attention from researchers as well as civilians (Stefanescu *et al.*, in prep.). The number of migrating individuals that are necessary for a migration event to be considered an invasion differs from species to species. For rare species, a few dozens of sightings in a particular country or area may indicate an invasion, while in others it may be thousands or even millions (Lempke, 1972; Brower, 1996). Observations of large scale invasions have been reported for several centuries, but scientific studies of migratory butterflies only started in the twentieth century (Lempke, 1972). ‘The Migration of Butterflies’ by C.B. Williams (1930) is considered as the first true scientific publication on migratory butterflies. In the years following this publication, the number of studies on migration in Lepidopterans increased (Lempke, 1972). Until this day, invasions play an important role in the study of migratory butterflies and moths. During invasions, the (relative) great number of animals allow researchers to in depth study the migratory path of the species in question. Therefore, invasions are, although perhaps overrepresented in the literature (Stefanescu *et al.*, in prep.; Chapman *et al.*, 2008a), of great value to Lepidopteran studies.

To fully understand migration, it is necessary to study all the factors that contribute to this complex behaviour. Researchers have developed a conceptual model of migration that shows how four basic components, *i.e.* the migration arena, the migratory trajectory, the migration syndrome and the genetic complex, are connected by processes (Adapted from Drake *et al.*, 1995 and Dingle and Drake, 2007) (Figure 1). The migration arena stands for the environment in which migration takes place. The migratory trajectory is the spatiotemporal and demographic development of the population that results from migration. Although relatively small in body size, the migratory trajectories of butterflies and moths are no less extensive when compared to migrating animals from different taxa (Reppert *et al.*, 2010; Stefanescu *et al.* in prep.). The relatively short lifespan of Lepidopterans make that long-distance migration in these insects is multigenerational. Therefore, the migratory routes are different for each successive generation, which makes Lepidopteran migration even more complex and puzzling (Dingle and Drake, 2007; Holland *et al.*, 2006). How are these animals able to find their way? They cannot have learned the migratory path from their parents or their own experience as is sometimes the case in other species, nor is it likely that they learn it from other conspecifics, because migrating Lepidopterans are all inexperienced and often migrating in too low densities for social communication (Riley, 1989 cited in Reynolds *et al.*, 2010b). Therefore, the answer apparently lies within the individual migrants itself. Migratory behaviour must have a genetic basis, which is somehow activated and influenced by the environment (Dingle and Drake, 2007; Brower, 1996; Lempke, 1972). Individual migratory Lepidoptera have a set of traits that enable migratory behaviour, which together form the migration syndrome (Figure 1). The migration syndrome encompasses traits such as mobility and the ability to navigate, which enable the process of migratory behaviour that leads to the migratory trajectory. The genetic complex is the set of genes that form the basis for the migration syndrome through the process of gene expression. The connection between the migratory trajectory or the outcome of migration and the genetic complex is the process of natural selection. It should be noted here that natural selection acts upon the outcome of individual migratory behaviour and not on the outcome of the

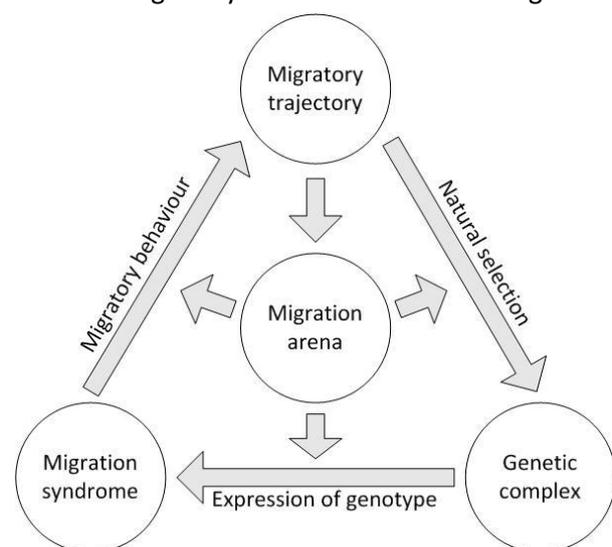


Figure 1 Conceptual model of a migratory system. The circles represent the four components and the arrows the processes that connect the components. (Adapted from Drake *et al.* 1995 and Dingle and Drake 2007)

population as a whole. All of the processes in the model, *i.e.* natural selection, gene expression and migratory behaviour are influenced by the environment, the migration arena. The migrants, in turn, also influence the migratory arena, because the migration trajectory defines, per definition, what the geographic range of the migration arena is. This model gives a clear overview of components and processes that are involved in animal migration, which makes it very suitable for categorising existing studies in order to gain insight of what has been studied and subsequently for giving direction to future studies of the migratory system.

Gaining knowledge on migratory Lepidopterans is not only of biological significance, but also of great conservational and economic importance (Sparks *et al.*, 2007; Chapman *et al.*, 2002 and 2011). Conservation is regrettably an increasingly important reason to study Lepidopteran migration, as more and more species become threatened with extinction (Van Swaay *et al.*, 2006; Van Swaay and Warren, 1999). The conservational aspects do not solely imply the migratory species itself, but also sedentary insect species that have a similar niche (Sparks *et al.*, 2007). The economic interests are also very significant, since migratory Lepidopterans are often agricultural pest species (Sparks *et al.*, 2007; Chapman *et al.*, 2002 and 2011; Talekar and Shelton, 1993). For example, caterpillars of the Diamondback moth (*Plutella xylostella*) feed on important crop species, *i.e.* cruciferous plants such as cabbage, broccoli and cauliflower, and management of this species was estimated to cost one billion US dollars per year in 1993 (Talekar and Shelton, 1993). Economically, it is important to not only gain knowledge on migration itself, but also on the species' invasion dynamics. This information is essential to develop a reliable forecasting method for invasions of pest species, so that management strategies can be anticipated (Chapman *et al.*, 2002 and 2011).

In this review, I try to give an up-to-date overview of the studies conducted on migratory Lepidopterans so far. I will describe the methodologies that are used in this research area and integrate studies of different species to discuss the current knowledge on migrant Lepidopterans, so that the developments in the field and the possibilities for future research become clear. I will use the conceptual model of the migratory system (Figure 1) as a guide to review the different aspects of migration.

Methodologies in migrant Lepidopteran studies

As Ovaskainen and colleagues (2008) pointed out, there is no single method to study all aspects of migration. Over the years, multiple methodologies have been developed to aid the study of migratory Lepidopterans. The methodology used in any particular study is dependent on the research question and is also frequently a combination of multiple approaches. Here, I aim to describe these approaches, give insight into how each method can be employed and discuss their advantages and disadvantages. The methodologies are in order from purely observational to more invasive and also technically more demanding approaches.

Observational methods

a. Opportunistic data collection

The oldest method is to opportunistically record data of migrant butterflies and moths. This approach can result in anecdotal evidence, which may not be strong scientifically, but can lead to important findings or inspire a more reliable scientific study. To aid the collection of opportunistic data, online surveys were created where the general public can voluntarily enter observations of different animal species (for example: www.butterfly-recording.eu, observado.org and www.telmee.nl). The access of the general public to these datasets give great advantages. The most important advantage is that it provides a wealth of information to researchers. Moreover, the possibility for the general public to actively engage in data collection in their own surroundings creates awareness, which is very important for the conservation of Lepidopterans, especially considering the endangered status of many butterflies and moths (Van Swaay *et al.*, 2006; Van Swaay and Warren, 1999). The majority of the observations are automatically verified by a computer, that

determines the probability of the observation by comparing the location and time of observation to what is known about the species. Unlikely observations of species outside their normal range or on unlikely times are further investigated by experts of the species in question (Telme website). Since this system is based on indirect information, there is an inevitable uncertainty in its results. Moreover, in case of the highly mobile migratory species, the conditions for automatic verification regarding the range are more flexible when compared to sedentary species. However, migratory species are often very recognisable and observations of these species are considered to be trustworthy (A. Vliegthart, personal communication).

b. Butterfly Monitoring Schemes

In order to study Lepidopterans in a scientifically sound manner, multiple organisations have been founded that aim to assess regional and national trends in butterfly and moth abundance per species using standardised sampling methods (Van Swaay *et al.*, 2008b). The first national butterfly monitoring scheme was founded in 1976 in the United Kingdom and, since then, other European countries followed, bringing the number of active monitoring schemes to 18 in 2012 (Van Swaay *et al.*, 2008b; Butterfly Conservation Europe website). In 2004 the Butterfly Conservation Europe was founded, an umbrella organisation that aims to stimulate and coordinate conservation activities of butterflies, moths and their habitats by operating through its 45 network partners from all over Europe (Van Swaay *et al.*, 2008b; Butterfly Conservation Europe website).

The schemes that work with the Butterfly Conservation Europe all use the same method to collect data on butterfly and moth abundance (Van Swaay *et al.*, 2008b). This method, developed by the British scheme, is to count the animals along fixed transects of about 1 kilometre in length. All butterflies within 2.5 metres alongside the path where the observer is walking and up till 5 meter in front and above of the observer are recorded. The data collection period is between March and October on days with favourable weather for butterflies to fly. The data from all schemes together are referred to as Butterfly Monitoring Scheme (BMS) records, which are not only used to estimate the population densities and trends for the different species, but also to study the relationship between species abundances with environmental factors, such as climate change, land use and nutrient load (Van Swaay *et al.*, 2008b). Moreover, the BMS data can be used for (back)tracking migratory species (Van Swaay *et al.*, 2008b; Stefanescu *et al.*, in prep.). An example of using BMS data to track migratory species is given by Stefanescu *et al.* (in prep.). In this study, the average weekly densities of the Painted Lady butterflies (*Vanessa cardui*) were used to track the movement through Europe during the invasion of 2009.

Although this system may sound ideal, it also has some disadvantages. Because the numbers of migratory Lepidoptera fluctuate dramatically between years, long-term trends in abundance are not very informative. Furthermore, the number of transects differ between schemes, ranging from a few to hundreds per country (Van Swaay *et al.*, 2008b). The number of visits to the different transects also differ greatly between the separate schemes, *i.e.* from weekly in some countries to three times a year in others, which results in a geographical bias in the data (Van Swaay *et al.*, 2008b; Stefanescu *et al.*, in prep.). Moreover, differences in observer experience, habitat type of the transects and overrepresentation of special protected areas, containing rare species, can bias the data (Van Swaay *et al.*, 2008b). To minimize the bias, the data can be weighed by habitat type, region and protection status of the region during the data analysis. Inherent to this method is that only the day-active low-flying insects or the ones that sit on low vegetation or the ground are recorded. All night-active moths and high-flying day-active Lepidopterans, which include a lot of migrants, are mostly missed.

Trapping methods

a. Ground trapping

Since the night-active Lepidopterans are very difficult if not impossible to observe by eye, researchers use night-vision equipment and light traps to study them (Chapman *et al.*, 2002; Baker, 1985 and 1987; Gatehouse, 1997). These traps have light bulbs to lure moths. Usually, multiple traps are set for the night and emptied the following morning (Baker, 1987). The caught individuals, up to

1000 individuals per trap, are examined (in the case of migrants usually for abundance) or used as a subject for experiments (Chapman *et al.*, 2002; Baker, 1985 and 1987). Also, if the individuals are marked, released and recaptured, it is possible to estimate population sizes or individual trajectories (which will be discussed in the next section). It has been found, however, that nocturnally migrating moths do not always fly towards artificial light, as they do not always fly straight to the moon, which is what the light bulb represents. Therefore, only a subset of nocturnal active moths, that currently orient towards the moon, would be caught in a light trap (Baker, 1987).

b. Catch-Mark-Release-Resight

Another possibility to study movement is by catching and marking individuals, release them and try to find them back (Von Roer, 1970; Urquhart and Urquhart, 1976; Baker, 1985). It is in essence the same methodology as the ringing of birds (Lempke, 1972). For Lepidopterans, this method has mostly been used to study short distance movements, but also proved to be useful to study large-distant migration (Cant *et al.*, 2005; Von Roer, 1970; Urquhart and Urquhart, 1976). From the recaptured individuals it is possible to analyze, among other things, their past pathway and their migration speed (Davis and Garland, 2004). However, catching, handling and marking can potentially affect the behaviour of the insects and it is best to test any effects thereof prior to the study. Moreover, it may be very challenging and labour-intensive to find the marked individuals again, especially when the subjects are high-flying migrants that can displace hundreds of kilometres per day. The percentage of recaptures at sites more than 100 km from the release site may be less than 0.1 % (Brower, 1996). Therefore, the chances to succeed in such studies will be highest if a large number of individuals is marked and researchers work together with a lot of (foreign) colleagues, so that, if someone recaptures a marked individual, the findings have higher chances to return to the researcher (Lempke, 1972). Also, in studies of long-distance migration, it may be essential to already know the migration trajectory in order to know where to search for the marked individuals (as Urquhart and Urquhart, 1976). As Monarchs are known to migrate to a very specific overwintering area, this method may be very useful to study the long-distance migratory behaviour of this species (Davis and Garland, 2004). However, for other species with less predictable destinations this method may be impractical. Based on the difficulty of recapturing marked animals and the lack of recent studies that use this method for species other than Monarchs I think this method is dated and generally not used to study long-distance migratory Lepidoptera anymore. Instead of marking individuals, it is possible to use naturally occurring markers, such as certain atomic isotopes in the bodies, to study the animals' origin or pathway (Brattström *et al.*, 2010). The use of natural markers will be discussed in a later section on biochemical studies.

Techniques to study high-flying insects

To observe insects that fly several tens of metres from the ground in wooded areas, it is very useful to conduct observations from platforms above forest canopy (Greenbank *et al.*, 1980). However, this approach would only be useful to study diurnal insects. Also, for most long distance flights, as is the case in migrant species, Lepidoptera often utilize the fast winds several hundred metres until 2 or 3 kilometres above the ground to increase their velocity and displacement (Chapman *et al.*, 2011; Talekar and Shelton, 1993; Stefanescu *et al.*, 2007). At these heights, it is impossible to observe them with the naked eye from the earth's surface. For heights up to 300-500 meter it is possible to use a binocular to study diurnal migrants (Brower, 1996). For higher flying diurnal insects, observations can be done from planes or gliders (Brower, 1996). In addition, researchers have developed multiple techniques to study high-flying insects that are suitable to study both diurnally and nocturnally migrating Lepidopterans.

a. Aerial netting

In some studies, a balloon-supported net was used to sample the abundance of a particular species in a particular layer of the atmosphere (Chapman *et al.*, 2002) (Figure 2). Such balloons are tethered and filled with helium. It is possible to equip balloons with devices to control whether the net is open

or closed, to prevent contamination with lower flying insects than was aimed for (Chapman *et al.*, 2002; Farrow and Dowse, 1984). Another possibility is to use a blimp or kite instead of a balloon (Sanders *et al.*, 2011; Farrow and Dowse, 1984). A disadvantage of tethered kites and balloons is that they cannot reach further than a few hundred metres above ground and they are impractical with strong winds (Chapman *et al.*, 2002). To sample at greater heights, it is also possible to equip an airplane with nets (Chapman *et al.*, 2002; Greenbank *et al.*, 1980). All of these approaches are very useful to check the insect species and their abundances that are present in particular atmosphere layers. However, aerial netting is expensive, labour-intensive and limited by air traffic regulations (Chapman *et al.*, 2002 and 2003). All of the above cited aerial netting studies on Lepidoptera are on moth species and it is plausible that this method is not useful to study butterflies, as these have good visual capacities and may fly around the nets.

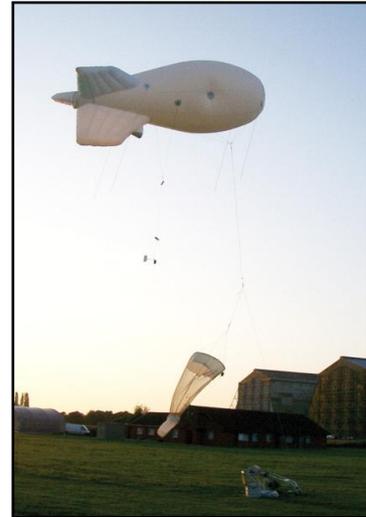


Figure 2 Aerial netting with a helium-filled blimp (Sanders *et al.*,

b. Radar observations

Another approach to study high-flying insects is the use of radar technique. This non-invasive technique was first used in 1968 to study locust, moth and butterfly migration in West Africa (Schaefer, 1969 cited by Chapman *et al.*, 2004 and Chapman *et al.*, 2011). The early entomological radars were so called X-band scanning radars, which could give information about target speed and direction (Schaefer, 1976 cited by Chapman *et al.*, 2011). These radars were mostly used from the ground, but could also be mounted on ships and airplanes (Chapman *et al.*, 2011; Schaefer, 1979; Greenbank *et al.*, 1980; Gatehouse, 1997). In the years following, several new radar techniques were invented, which eventually led to the development of the vertical-looking radar (VLR) (The Radar Entomology Website). In contrast to the manually operating X-band scanning radar, the VLR required little labour and was the first radar to automatically, remotely and continuously record aerial insect activity in 1990 (Chapman *et al.*, 2003, 2004, 2011; Beerwinkle *et al.*, 1995). The first VLRs could give information on insect densities and speed on several heights and wing-beat frequencies of the targets, which is used to identify the targets' species (Drake *et al.*, 2002; Smith *et al.*, 1993). This technology allows entomologists to gather large quantities of data and study the migratory behaviour of insects in great detail (Chapman *et al.*, 2011). Until this day, the majority of entomological studies of aerial insect activity using radars is conducted with VLRs (Chapman *et al.*, 2011).

In addition to the standard VLR, an additional modified version was invented in 1991 (Smith *et al.*, 1993). The modified version did not use a stationary radar beam as the earlier entomological radars did, but a beam that nutated (*i.e.* wobbled) about a vertical axis and had a continuously rotating plane of polarisation (Smith *et al.*, 1993). In addition to insect density and speed, this feature allowed the radar to give insight into the body shape and mass of the target, which greatly improved the species identification of the targets (Smith *et al.*, 1993; Drake *et al.*, 2002). Furthermore, this VLR system obtains information on the direction of movement and the body orientation of the targets, which can be used to study flight behaviour (Smith *et al.*, 1993; Chapman *et al.*, 2004, 2008a, 2008b, 2010 and 2011). Gaining knowledge on flight behaviour is especially valuable for the study of long-distance migrant Lepidopterans, since their behaviour can give us insight into how they orient and what environmental cues might be involved (Chapman *et al.*, 2008a and 2008b).

Unfortunately, with the nutating VLR version it is no longer possible to calculate wing-beat frequency as is possible with the stationary VLR version (Drake *et al.*, 2002). It is possible however, to temporarily turn off the dipole polarisation and nutation of the beam in order to get stationary beam information, that can be used to calculate wing-beat frequency (Drake *et al.*, 2002). Since radars are not readily available, this implies that researchers have to choose which of the two operating modes they want to use at any given time and they can thus not use both approaches to measure

parameters in the same individuals (Drake *et al.*, 2002). What operating mode or combination thereof is used ultimately depends on the research question.

Although the identification of target species has improved greatly in VLRs, when compared to earlier entomological radars, a lot of studies still rely on additional data from other sources such as aerial netting or ground trapping networks to confirm the identity of the species (Chapman *et al.*, 2002 and 2004). Furthermore, VLRs can only record insects that are flying higher than approximately 150-200 metres of the ground (Chapman *et al.*, 2004; Drake *et al.*, 2002). That means high-flying insects flying lower than 150-200 metres above the ground have to be studied in another way. Under good circumstances it may be possible to observe them by eye. Another possibility may be to use another radar technique, the harmonic radar.

The harmonic radar has first been used entomologically to study walking beetles in the mid-eighties (Mascanzoni and Wallin, 1986 cited in Chapman *et al.*, 2011). This technique uses a tiny electronic diode, which is glued or taped (using double-sided sticky foam) to the target insect, that reflects microwave beams emitted by a portable radar device. The tag on the insect does not require a battery and thus allows minimisation of the device (Riley *et al.*, 1996). In 1996 the device was small enough to work on a variety of flying insect species, including bumble bees, honey bees, butterflies, and moths (Riley *et al.*, 1996; Roland *et al.*, 1996). The harmonic radar can detect targets at distances of several hundred metres and does not get swamped by signals emitted from vegetation, as would be a problem for other radars (Chapman *et al.*, 2003). However, vegetation can still form a barrier that can cause the tags to be undetectable (Chapman *et al.*, 2004 and 2011). In 2004, the harmonic radar was first used to study the flight paths of butterflies (Cant *et al.*, 2005). The tags did not seem to hinder or affect the insects' behaviour (Riley *et al.*, 1996; Cant *et al.*, 2005). Among the five butterfly species studied by Cant and colleagues (2005) were the Red Admiral (*Vanessa atalanta*) and the Painted Lady (*Vanessa cardui*), both well-known migrants. Although both species are known for migrating hundreds of metres above the ground, they are also reported to migrate close to the earth's surface (Scott, 1992; Stefanescu *et al.*, in prep.; Mikkola, 2003b; Williams, 1970). It was found that the flight paths of the migrant species, flying close to the ground, were much faster and very straight when compared to local sedentary species and it is possible that the migrant butterflies were indeed migrating at the time (Cant *et al.*, 2005). Harmonic radars may thus be used to study low-flying migrant Lepidoptera, although it may be challenging to follow them at longer distances.

Experimental studies

a. Behavioural experiments

Besides observational and trapping studies of Lepidopterans, they are also studied in experiments, both in the field and in laboratories. Experiments are frequently used to study the mechanisms of navigation. For example, to find out whether and how the geomagnetic cues influence the behaviour, researchers have reversed the magnetic field or applied a strong magnetic pulse to confuse the possibly existing magnetic navigational system in the Lepidoptera (Srygley *et al.*, 2006). To find out whether the location of the sun is used for navigation, researchers use clock-shift experiments (Oliveira *et al.*, 1998). Subsequently, to find out if the mechanisms for a solar compass might be in the antennae of butterflies, researchers have surgically removed the antennae (Merlin *et al.* 2009). After these experimental procedures, the insects are often released to visually observe their headings and record the compass bearings (Oliveira *et al.*, 1998; Srygley *et al.*, 2006), but they are also frequently observed tethered in an experimental flight tunnel or flight simulator (Merlin *et al.*, 2009; Nesbit *et al.*, 2009). Certainly, there are many more experiments on migratory Lepidoptera, but since my aim is to describe what different categories of methodologies have been used, describing all of them is out of the scope of this article.

b. Biochemical studies

As mentioned in the section of trapping methods, the capture-mark-release-resight approach often yields little information, because the re-encounter rates are too low. As an alternative, it is also possible to use natural occurring markers. For example, Brattström and colleagues (2010) sampled

hydrogen isotopes the wings of Red Admiral butterflies as a biogeochemical marker to determine their natal area, which was very useful in studying their migratory trajectory. Alternatively, Gregg and colleagues (2001) compared pollen carried on moths' probosces between moths that just arrived after migration and moths from the presumed source area to investigate the origin of the migrants.

A relatively new field in the study of migration is that of genetics. The analysis of particular regions of the genome can be used to investigate the genetic structure of populations (Brattström *et al.*, 2010b). Also, by comparing genetic sequences of migrant and sedentary Lepidopteran species, it is possible to study the genes that are part of the migratory syndrome and the evolution of migration. Very recently, the Monarch (*Danaus plexippus*) genome was the first to be sequenced completely, which will undoubtedly be of great value for current and future studies of migration (Zhan *et al.*, 2011).

Other methodologies

In addition to studying adult migrating Lepidopterans, studying non-migrating adults, for example foraging, mating, egg-laying, overwintering adults also greatly contributes to our knowledge of the species and its trajectory (Stefanescu *et al.*, 2011b; Urquhart and Urquhart, 1976; Brower, 1996). The same is true for studying larvae and eggs (Stefanescu *et al.*, 2011b and 2012; Brower, 1996). Also, studying the migrants' host plants and natural enemies helps to understand migration (Brower, 1996; Stefanescu *et al.*, 2012).

Knowledge of migratory Lepidopterans

Using the above described methodologies, researchers have gained a lot of knowledge on Lepidopteran migration and a review is timely. By integrating information from multiple studies using different methodologies, I give an overview of the current knowledge on migratory Lepidopterans. The conceptual model of the migratory system (Figure 1 on page 6) will be used to structure the overview. The studies of a migratory species basically always begin with either the migratory trajectory or the migratory behaviour, because this is how migratory species are noticed in the first place. People may for example notice unidirectional flights or sudden appearances or disappearances of certain species (Williams, 1930). When the ranges of migratory animals are known, in depth studies of their migratory behaviour is possible. Subsequently, to find out why the animals behave as they do, researchers search for environmental factors that influence the behaviour, migratory traits and the genetic basis thereof. This knowledge is then used to investigate how and why migratory behaviour may have evolved. The above described progression of research topics will be used in this overview, which will start with the migratory trajectory and migratory behaviour and then focus on the migration syndrome, the genetic complex and what is known about the evolution of the migratory system.

Migratory trajectory

The migratory trajectory is a great starting point for any research on migrant butterflies and moths. However, it is also an aspect that is poorly known for most species. Current technologies do not provide methodologies to follow individual animals along their lifetime track and so the only solution is to rely on observations or measurements of as many individuals of the population as possible on multiple locations and on relatively short distances. Then all the information must come together to construct the trajectories.

More often than not, knowledge on important elements of a species' migratory trajectory is lacking. For a lot of species, it is unknown where they are overwintering and in what stadium of their development (Stefanescu *et al.*, in prep.). Also, one half of the migratory trajectory is usually less obvious to human observers than the other. For example, researchers observed the Painted Lady butterflies (*Vanessa cardui*) flying from southern to northern Europe in spring, but they never noticed any return flights later in the year, which led them to think that the summer population in

Northern and Central Europe did not return south in autumn (Stefanescu *et al.*, in prep.). It was thought that this part of the population was the result of overpopulation in the South and that they would die in the winter, a so called Pied piper effect (McNeil, 1987 cited in Stefanescu *et al.*, in prep.). It was later that researchers discovered the Painted Lady butterflies could also migrate hundreds of metres above the ground, aided by fast winds, and flew back south out of sight of researchers (Stefanescu *et al.*, 2007 and in prep.). In this chapter, I focus on a few species of which the migratory trajectory is well studied, the North American Monarch butterfly (*Danaus plexippus*) and the European populations of the Painted Lady butterfly (*Vanessa cardui*) and the Red Admiral (*Vanessa atalanta*). In addition, I describe our current knowledge of the migratory trajectory of one of the best studied moth species, the Silver-Y moth (*Autographa gamma*).

The **Monarch** butterfly migratory trajectory is known in great detail (Figure 3 and 4). In North America, two distinct populations are known, the population west of the Rocky Mountains and the population east of these mountains (Reppert *et al.*, 2010). The famous massive autumn migrations are seen in the eastern population. In autumn, when the obligate larval food plant (milkweed) starts to wither and the temperatures are cooling, the migration southward begins (Reppert *et al.*, 2010). Individual Monarchs as far north as South-Canada start their journey south, which can be up to 4000 km (Reppert *et al.*, 2010; Zhu *et al.*, 2008; Brower, 1996). During the autumn migration the Monarch butterflies become highly gregarious and, as they move further south and the North American landmass becomes narrower, the density of the migrants increases markedly, up to the point where millions of individuals migrate together, or at least in high densities (Brower, 1996; Williams, 1957; Reppert *et al.*, 2010). At night, Monarchs stop migrating and overnight in trees. If the weather is favourable on the following morning, they resume the migration, but if it is not, they may stay for several days (Brower, 1996). Such interruptions of the migrations are called stopovers and it is thought that the butterflies rest and forage to regain energy (*i.e.* roosting) (Davis and Garland, 2004). Sometimes, Monarchs are observed to migrate despite of bad weather conditions. In these cases, they usually fly close to the ground using powered flight (*i.e.* active flapping of wings) (Brower, 1996). Nevertheless, Monarchs are thought to predominantly migrate by soaring on favourable winds, judging from observations and the fact that the butterfly's wings do usually not show heavy wear once they have arrived at the wintering grounds (Brower, 1996). All of the Monarchs in the autumn migration are headed to an area of approximately 800 km² in the mountains of Central Mexico to overwinter in a state of inactivity, called diapause (Reppert *et al.*, 2010). During this diapause, they occasionally fly to drink or cool off, but otherwise they remain inactive (Brower, 1996). In spring, the same individuals break diapause, reproduce and fly north, where they lay eggs on newly emerging milkweed plants. Then, the next (spring) generation continues to repopulate North America. There are at least two more spring/summer generations of Monarch butterflies, that spread over North America and Canada, before the migration south begins for the autumn generation again (Reppert *et al.*, 2010; Brower, 1996). The degree of migration is very different between the spring/summer and autumn generation. The autumn generation is clearly highly migratory, but the spring/summer generation may also be following the progressive northerly increase of milkweed, the larval food plant (Brower, 1996). Recent studies also shown that the spring/summer butterflies have less oriented flight when compared to the autumn butterflies (Zhu *et al.*, 2009). Also, in contrast to spring/summer butterflies, the autumn butterflies are in reproductive diapause, which lasts until spring, and they are longer lived than the spring/summer generations (Zhu *et al.*, 2009; Herman and Tatar, 2001). All of these findings resulted in a distinction between summer and migratory Monarch butterflies, which is subject to current studies that aim to find the differences between these two types of Monarchs (Reppert *et al.*, 2010; Zhu *et al.*, 2009).



Figure 3 Monarch butterfly (*Danaus plexippus*). The sources of this illustration is given in the list of references.

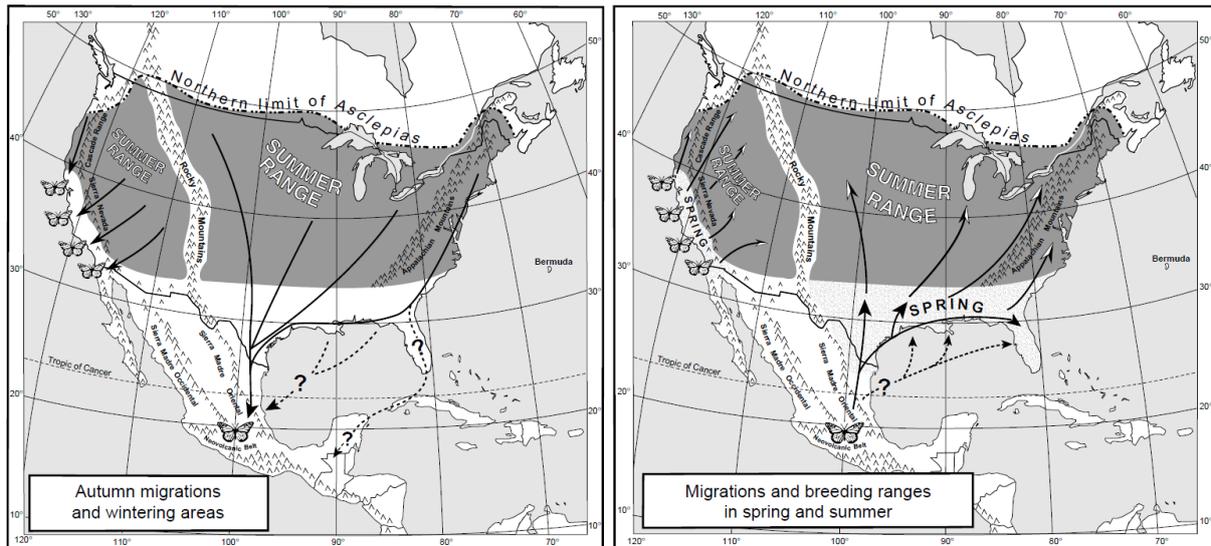


Figure 4 Left picture: autumn migration trajectories of Western and Eastern Monarch populations in North America. Right picture: Spring and summer trajectories of western and eastern Monarch populations in North America. (Brower, 1996)

The butterflies of the population west of the Rocky Mountains are less well studied, but they migrate as well, along different, shorter, migratory trajectories (Reppert *et al.*, 2010). In autumn, these butterflies migrate south-westward and overwinter in protected roosts along the Pacific coast and they repopulate the land west of the Rocky mountains in spring (Reppert *et al.*, 2010; Brower, 1996). As in the eastern population, the overwintering site of the western population is highly localised and the same locations are used every year (Brower, 1996).

From North America the Monarchs have spread to other continents and today Monarchs are also found in Australia, parts of Asia and the Canary Islands in Europe (Lempke, 1972). However, migratory behaviour has not been studied in these populations.

The **Painted Lady** is very cosmopolitan, ranging on all continents except South America, where the butterflies are very rare or absent, and Antarctica (Bos *et al.*, 2006; Williams, 1970) (Figure 5). In most regions, however, this species is unable to survive cold winters and the presence of the species is a result of annual migrations (Williams, 1970). Their migrations have been studied the most in Europe/Africa (Palearctic) and to some extent in North America (Nearctic). Although it is long known that Monarchs migrate at great heights aided by wind currents, it was only recently that the same was confirmed for the Painted Lady (Stefanescu *et al.*, 2007 and in prep.). In contrast to the Monarchs, the Painted Lady butterflies are a continuously-brooded species, *i.e.* they reproduce throughout the year, and do not have a diapause (Stefanescu *et al.*, in prep.). The migratory trajectory of the Painted Lady in the western Palearctic has been studied in great detail. Stefanescu and colleagues (in prep.) were able to study the butterflies during the major invasion on 2009 and reported the annual migratory trajectory of each subsequent generation. In early spring, butterflies that have developed in Northern Africa during the winter migrate Northward and repopulate Southern Europe, where they reproduce. The second generation subsequently colonizes Central and Northern Europe later in the spring. They have even been observed above the Arctic circle as far as Spitsbergen (Svalbard Insects website). Butterflies of the third summer generation migrate south again, some all the way to Western Africa and some until Central or Southern Europe to produce a fourth generation, that will then migrate south in autumn. Consequently, the numbers of butterflies in North-western Africa are increasing in the



Figure 5 Painted Lady butterfly (*Vanessa cardui*). The source of this illustration is given in the list of references.

autumn. At the same time, the Northwest African population increases due to migration from more southern populations in Africa. The African part of the migratory trajectory is less clear, but it is known that a fifth generation is produced in the winter, which may migrate towards the south. Lastly, a sixth generation that emerges in late winter migrates to the areas in North-western Africa to complete the circle and their offspring will recolonise Europe in the following spring. In addition, part of the North African population migrates to the south in spring to exploit resources there in the same way the other part of the populations exploit resources on the Northern Hemisphere (Williams, 1970). This part of the migration has not been studied much.

In North America, the migratory movements of the Painted Lady are also studied, although in less detail when compared to Europe. The studies suggests the migratory path in North America is very similar to that in Europe (Williams, 1970). The Nearctic Painted Lady butterflies overwinter predominantly in Mexico, where they breed throughout the year, their northward spring/summer migration can extent up to Southern Canada (*i.e.* migration of almost 5000 kilometres) and they migrate back south again in autumn (Williams, 1970). The exact routes of each subsequent generation has not yet been described, but is presumably similar as in Europe.

The **Red Admiral** (*Vanessa atalanta*) is an Holarctic butterfly species (*i.e.* occurring in the northern continents) (Stefanescu *et al.*, 2001; Brattström *et al.*, 2008) (Figure 6). As is found in other migratory species on the Northern Hemisphere, Red Admirals migrate north in spring and summer and south in autumn (Stefanescu *et al.*, 2001; Mikkola, 2003a and 2003b; Brattström *et al.*, 2010a). In the Palaearctic, they overwinter in the Mediterranean region (both South Europe and North Africa), where they keep reproducing during the winter and larvae keep developing during the winter, although slowly (Stefanescu *et al.*, 2001; Brattström *et al.*, 2010a). When this generation emerges in spring, they start migrating north (Stefanescu *et al.*, 2001). There are one or two further summer generations that continue to recolonise Northern Europe as well as higher elevations in mountains (Stefanescu *et al.*, 2001; Brattström *et al.*, 2008). These findings suggest that Red Admirals, like the painted ladies, do not go into diapause (Stefanescu *et al.*, 2001). However, there are multiple reports on the presence of the butterflies in Northern Europe in mid-winter and too early in spring to be newly emerged migrants (summarized in Brattström *et al.*, 2008), that suggest the Red Admirals have been hibernating in the north. Nevertheless, hibernating in the Northern Europe is probably a less important overwintering strategy than migrating south and overwintering around the Mediterranean sea (Brattström *et al.*, 2010a).

Recent studies using biogeochemical markers suggest that Red Admirals in Western Europe may be isolated from the migrants in North-Eastern Europe and both parts of the population may migrate along different trajectories (Brattström *et al.*, 2010a). This migratory division may be due to topography effects on behaviour of the Red Admirals, channelling them on either side of the Alps and subsequently along coastlines (Brattström *et al.*, 2010a). However, genetic analysis of the European butterflies could not confirm this theory. Although two distinct genotype clusters have been found, they did not coincide with different study areas (Brattström *et al.*, 2010b). Further studies are thus needed to unravel the possible migratory division in Red Admiral populations in Europe.

As for the Asian and North American part of Red Admiral migration, very little is known about the migration trajectories there.

The **Silver-Y moth** (*Autographa gamma*) is one of the best studied migratory moths (Figure 6). Nevertheless, we know



Figure 6 Top picture: Red Admiral (*Vanessa atalanta*); Bottom picture: Silver-Y moth (*Autographa gamma*). The sources of these illustrations are given in the list of references.

little of its migratory trajectory. It is an Holarctic species with for humans important crops such as cabbage, tomato and potato as host plants, which makes it an agricultural pest species (Hill, 1987, p. 444). In the Palaearctic, these moths are known to migrate north in spring were they can produce two to three generations before they migrate back South (Hill, 1987, p. 444; Hill and Gatehouse, 1992; Chapman *et al.*, 2008a and 2008b). They are thought to overwinter in North Africa or the Middle East (Hill, 1987, p. 444; Hill and Gatehouse, 1992).

Although all of the above described trajectories are roughly comprised of northward and southward migration, this is the only observed migratory pattern in Lepidopterans. The Camberwell Beauty (*Nymphalis antiopa*) and Eastern Bath White (*Pontia edusa*), for example, are known to migrate between Eastern and Western Europe (Van Swaay and Hensle, 2003; Van Swaay *et al.*, 2008a; Bos *et al.*, 2006).

Migratory behaviour

The behaviour of migrating Lepidoptera differs from that of non-migrating Lepidoptera in that migrants are initially not distracted by stimuli that indicate a favourable environment, *i.e.* they do not respond to flowers, host plants or mates (Dingle and Drake, 2007; Drake *et al.*, 1995). However, for some species, migratory behaviour is episodic (Gatehouse, 1994). Nocturnal moths, for example, are known to take shelter during the day and the diurnal Monarchs take shelter during the night (Gatehouse, 1997; Brower, 1996; Davis and Garland, 2004). For other day-active Lepidopterans it is not very clear whether they take shelter at night, because they are also frequently observed to continue migrating at night (*e.g.* Stefanescu *et al.*, 2007; Lempke, 1972). Besides taking shelter, migrants are sometimes observed to interrupt their flight to feed (Gatehouse, 1997). Although these interruptions allow the animals to temporarily forage and rest, they do not completely lift the inhibition on responsiveness to stimuli that are associated with resources and do not terminate the migratory behaviour (Gatehouse, 1994 and 1997).

Another marked difference between migrating and non-migrating Lepidopterans is that the migrants' flying track is much more straightened-out when compared to non-migrants (Drake *et al.*, 1995; Cant *et al.*, 2005). Migrant Lepidopterans fly in a definite, seasonally favourable, direction, whether during the day or night, over land or over sea (Williams, 1957; Gatehouse, 1997) and simultaneously migrating individuals of the same species in a particular area all fly in that same direction (Brower, 1996; Gatehouse, 1997; Mikkola, 2003b; Stefanescu *et al.*, in prep.). For a lot of the migrant species studied so far, the migration direction shifts about 180° between spring and autumn (Chapman *et al.*, 2008b; Stefanescu *et al.*, in prep.; but see Brower, 1996).

Nevertheless, migrants may occasionally deviate from the seasonally favourable direction because of obstacles. If the obstacles are rather small, for example a building or trees, they are reported to fly over them rather than around them (Williams, 1957), but in case of large topographic obstacles, such as mountains or large bodies of water, they are observed to adjust their direction (Davis and Garland, 2002; Brattström *et al.*, 2008 and 2010a). When migrant Lepidopterans encounter large bodies of water, they are reported to follow coastlines, which can result in deviation from the seasonal favourable direction (Brattström *et al.*, 2008). For example, in their southward autumn migration, Palaearctic Red Admiral butterflies migrate westward along the coastline in Finland until they eventually cross the sea at its narrowest point to reach Denmark and fly further south from there (Brattström *et al.*, 2008). This behaviour potentially originates from the avoiding of large bodies of water as much as possible (Brattström *et al.*, 2008). The mechanism used to select the narrowest water bodies has not been studied, but it is hypothesised that the insects can either detect the closest land on the other side or the water width as indicated by the linearly polarised light that is reflected from the water surface (Brattström *et al.*, 2008). Ultimately, however, deviations from the seasonally favourable direction are found to be temporarily (Brattström *et al.*, 2010a; Brower, 1996).

Since the beginning of the migrant Lepidopteran studies, the focus was on low-flying migrants. The layer in which these insects fly is close to the earth's surface and is called the Flight Boundary Layer (FBL). In the FBL, wind speeds are typically lower than the self-propelled flight speed of the

Lepidopterans, which enables the insects to fly in any direction (Chapman *et al.*, 2011; Srygley and Dudley, 2008; Drake and Farrow, 1988). Although there was anecdotal evidence for insect migration at great heights (*e.g.* Williams, 1970; Mikkola, 2003b), the part of insects involved was underestimated for a long time (and it probably still is), because the phenomenon is mostly unobservable to the human eye. Today, evidence is accumulating for an increasing number of Lepidopteran species that migrate above the FBL at heights of hundreds up to 3000 metres above the ground (Stefanescu *et al.*, 2007 and 2011; Mikkola, 2003a and 2003b; Scott, 1992; Williams, 1970; Chapman *et al.*, 2008a, 2008b, 2010).

The most important difference between migration at high and low altitudes is the migration speed. At great heights, the insects can take advantage of the fast wind streams for rapid transportation at a low energy cost (*i.e.* 50-70 kilometres per hour) (Chapman *et al.*, 2011; Brower, 1996). However, as the wind speeds at these heights are many times more than the self-propelled flight speed of the Lepidopterans, one would think that the insects were helplessly blown in the direction of the wind, which must frequently be an unfavourable direction (Williams, 1970; Chapman *et al.*, 2011). Airborne migration thus seems very risk-prone strategy when compared to migrating within the FBL, where the insects have full control over their displacement direction, but at a higher energy cost.

Discoveries since the recognition of windborne migration have shown that Lepidopteran migratory behaviour is actually much more complicated, less dependent on the wind and thus less risk-prone than first assumed. Migratory behaviour is not only the result of environmental factors, but also of morphological, biochemical, physiological and behavioural traits, which together make up the migration syndrome (Drake and Gatehouse, 1995).

Migration syndrome

The migration syndrome is the total set of traits that make migration possible. Here, I aim to give an overview of the current knowledge on the Lepidopteran migration syndrome and the mechanisms thereof. The ability to migrate is in the first place dependent on morphological traits that influence mobility (*e.g.* wings and muscles) (Drake and Gatehouse, 1995), which is out of the scope of this review. In addition to mobility, migrants have behavioural and endocrinological mechanisms that influence migration. Here, I will describe behavioural mechanisms that influence the migration, including wind selection, orientation and navigation. Then I will describe the current knowledge on hormonal influences on migration.

a. Wind selection and orientation

The species that utilize fast wind streams for airborne migration do not just migrate in wind streams of any direction, but they select favourable winds. The Silver-Y moths (*Autographa gamma*), for example, are found to migrate during northerly winds in autumn, when they migrate south, and southerly winds in spring, when they migrate north (Chapman *et al.*, 2008a and 2008b). During these studies, the wind blew out in all compass directions in equal frequency and so the moths had somehow selected the favourable winds (Chapman *et al.*, 2008a and 2008b) (Figure 7). Similarly,

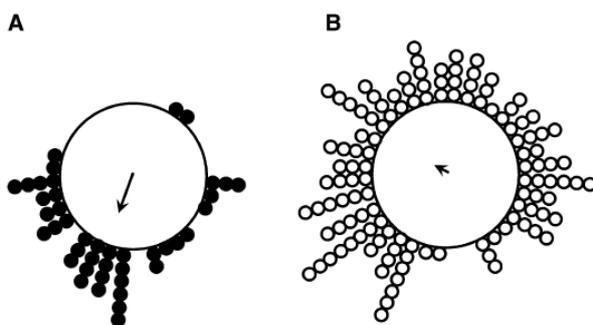


Figure 7 Example of wind selection during high-altitude autumn migration of the Silver-Y moth. The large circles represent compasses and the small circles in the periphery are mean observed directions in migration events. Arrows indicate the mean observed direction of all data.

A: Mean displacement direction of the moths during migration events.

B: Mean wind direction at 300 m above ground level during migration period.

The contrast between figures A and B shows that the majority of migration events occurred on nights with northerly winds. (Chapman *et al.*, 2008a)

Monarch butterflies (*Danaus plexippus*), Red Admiral butterflies (*Vanessa atalanta*) and Painted Lady butterflies (*Vanessa cardui*) are also found to migrate at great heights on favourable winds (Mikkola, 2003a and 2003b; Stefanescu *et al.*, in prep.; Reppert *et al.*, 2010; Brower, 1996). The selecting of favourable winds is less important for insects flying in the Flight Boundary Layer, where the wind has less influence on the insects' displacement direction, and accordingly, butterflies and moths have not been found to select winds from favourable directions at these low elevations (Brattström *et al.*, 2008).

Species that are reported to be airborne migrants are always reported to migrate within the FBL as well (Stefanescu *et al.*, 2007). These species thus combine both strategies. It has been found that butterflies use the high-flying strategy on days with favourable weather and wind conditions, *i.e.* tailwinds, and the low-flying strategy on days with less favourable weather or headwinds (Mikkola, 2003b; Davis and Garland, 2002 and 2004; Williams, 1970). It has also been suggested that the initial phase of migration in such species is windborne and the second phase is to fly within the FBL and regain responsiveness to stimuli from favourable habitats (Stefanescu *et al.*, 2007; Gatehouse 1994). In some cases, like the migration of the Painted Lady (*Vanessa cardui*) in Europe, the initial phase of airborne migration may be linked to the fact that this species has to cross a large body of water (*i.e.* the Mediterranean sea) at the start of their migration. In support of this theory, butterflies have been reported to gain height before crossing a large body of water, which is thought to be a safety strategy (Brattström *et al.*, 2008). Further research is needed to investigate if this theory might be true. In both cases, it seems that migrant Lepidopterans do not automatically use the airborne strategy, but only if the circumstances are favourable or require this strategy.

High-flying migratory insects not only select favourable wind directions, they are also found to select the fastest wind flows (Aralimarad *et al.*, 2011; Reynolds *et al.*, 2009; Drake and Farrow, 1988). During the day, the part of the Earth's atmosphere closest to the Earth (*i.e.* planetary boundary layer) is influenced by solar radiation that causes convective upcurrents and subsequently circulation of the atmosphere (Gatehouse, 1997). At night-time, these circulations cease and the Earth's surface cools down. When the Earth's surface is cooler than the atmosphere, the latter forms stable layers, which differ in wind speed and direction (Gatehouse, 1997; Drake and Farrow, 1988). Interestingly, when the atmosphere is layered like this, the distribution of nocturnally migrating insects in the atmosphere is layered as well. The highest concentration of insects is found in the fastest and often warmest layer (Figure 8) (Gatehouse, 1997; Aralimarad *et al.*, 2011; Reynolds *et al.*, 2009; Reynolds *et al.*, 2010a).

In addition to flying in the same narrow layer of the atmosphere, the airborne migrants are also found to have the same orientation (Gatehouse, 1997). As they select winds from favourable directions, the orientation is usually close to the downwind direction (Reynolds *et al.*, 2010a and 2010b; Chapman *et al.*, 2011), which is thought to be highly adaptive as the insects can add their own self-propelled speed to the air speed and increase their displacement speed (Chapman *et al.*, 2011). However, it also occurs that all insects are oriented at a certain angle (usually no more than 90°) to the downwind direction (Gatehouse, 1997; Chapman *et al.*, 2008a, 2008b and 2010). The latter occurs when the wind direction is sub-optimal and deviates from the seasonally favourable direction (Chapman *et al.*, 2008a and 2008b). By orienting and flying at a correction angle to the wind, the insects are able to bias their displacement direction towards the seasonally preferred direction and correct for wind drift (Chapman *et al.*, 2008a, 2008b, 2010; Gatehouse, 1997; Brower, 1996; Brattström *et al.*, 2008) (Figure 9).

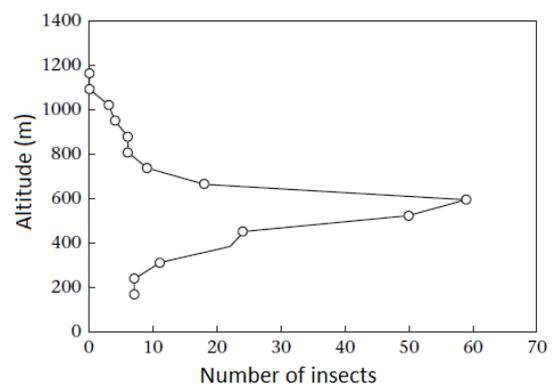


Figure 8 Example of a layered profile in the vertical distribution of insects. Here the insects are concentrated at approximately 600 metres above the ground. Adapted from Aralimarad *et al.* 2011: Vertical Looking Radar data from July 13th, 2006 between 22:00 and 22:06 hours in the UK.

To explain the layering and common orientation of the airborne insects, wind-mediated mechanisms have been proposed. It might be possible for the insects to use atmospheric turbulence as a cue to select the favourable layer in the nightly atmosphere (Aralimarad *et al.*, 2011; Reynolds *et al.*, 2009). By actively following the vertical gusts of turbulence, the insects would in theory concentrate in the layer that is most stable, *i.e.* the layer with the least turbulence (Reynolds *et al.*, 2009). This is also the layer with the fastest and usually warmest wind flows (Reynolds *et al.*, 2009). In order to orient in the downwind direction, the animals would have to be able to perceive the direction of the wind while flying in that wind flow. However, when the air around the insects is moving just as fast as they are, they cannot feel the direction. Therefore, the key is in asymmetries of the wind, horizontal turbulence. Horizontal turbulence causes fluctuations in the wind's velocity, which are usually aligned with the wind direction (Reynolds *et al.*, 2010a). If the insects are aligned with the wind direction, the turbulence gusts of wind should be equally fast on both lateral sides of the body, but if they are faster on one side of the insects body than it is on the other, it would indicate that the insects are not aligned with the wind direction (Reynolds *et al.*, 2010a). For this theory to be possible, the insects would have to be able to detect almost negligible turbulence asymmetries, which requires a yet to be discovered mechanism. The antennae are hypothesised to play a role in detecting wind flows, since the Johnston's organ, which are located in the basal segments of the antennae, are found to respond to small movements of the antennae (Sane *et al.*, 2007).

However, wind-mediated mechanisms cannot explain all of the migratory behaviour that is seen in Lepidopterans. It does not explain how the insects 'know' in which direction they should migrate and in which direction they are actually moving. Therefore, it cannot account for the selection of winds from favourable directions and wind drift correction. They must have a more sophisticated navigational mechanism.

b. Navigation

Migratory Lepidopterans are thought to have a map sense, which enables them to determine the direction of their destination (Reppert *et al.*, 2010; Merlin *et al.*, 2012). There are different types of map senses, a partial and a complete map sense. Whereas the partial map sense only provides information on direction of the destination, the complete map sense also provides information on the relative position of the migrant with respect to its destination (Reppert *et al.*, 2010; Merlin *et al.*, 2012). It is not certain whether the Lepidopteran migrants have the partial or the complete map sense and the map sense, in general, is poorly understood.

In addition to a map sense, migrants must have an internal compass to orient in the right direction (Reppert *et al.*, 2010; Merlin *et al.*, 2012; Gould, 1998). In contrast to the map sense, an

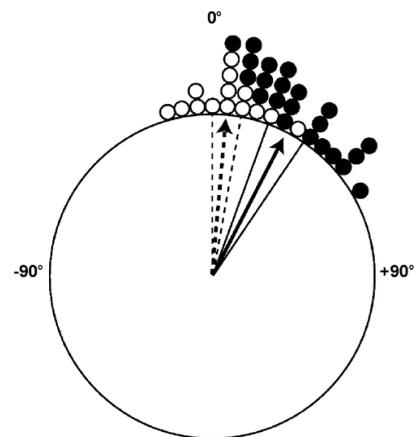


Figure 9 Example of wind drift compensation during high-altitude migration of the Silver-Y moth. The circles in the periphery indicate the mean correction angle of the moths during migration events. A correction angle of 0° indicates that the mean displacement and heading direction are identical. Positive correction angles indicate that the moths compensate for wind drift by adjusting their heading away from their displacement direction towards the seasonally preferred direction. Negative correction angles indicate that the moths adjusted their headings away from the seasonally preferred direction. Open circles indicate migration events in which the mean displacement direction differed < 20° from the seasonally preferred direction. The dotted arrow represents the mean correction angle during these events, which is not significantly different from 0°. The filled circles indicate migration events in which the mean displacement direction differed > 20° from the seasonally preferred direction. The solid arrow represents the mean correction angle for these events, which is significantly different from 0°. Therefore, the moths compensate for wind drift when their displacement direction differs > 20° from the seasonally preferred direction, by adjusting their heading towards the seasonally preferred direction. (Chapman *et al.*, 2008a)

increasing number of studies are aimed at Lepidopteran compasses. For a compass to work, there has to be a reference point, an environmental cue used to deduce the orientation or bearing. Environmental cues that have been studied as potential reference points for internal Lepidopteran compasses are the sun, the moon, the stars, polarised light or the Earth's magnetic field (Merlin *et al.*, 2012).

The best studied compass is the **sun compass**, which is thought to be the most important compass for day-active Lepidopteran migrants (Reppert *et al.*, 2010; Merlin *et al.*, 2012; Nesbit *et al.*, 2009). Theoretically, the least complicated strategy to use the sun as navigation for southward migration on the Northern Hemisphere would be to simply follow the sun (Nesbit *et al.*, 2009). This is obviously not the most efficient strategy, since the sun is a moving target. To efficiently use the sun for navigation, the animals must compensate for the sun's movement across the sky during the day, which requires a sense of time. To find out if and how the sun was used for navigation, the orientation behaviour of the butterflies was observed with respect to the sun. The results showed that the butterflies did not simply follow the sun or flew at a certain angle to the sun, as their heading was not correlated to the sun's position (Nesbit *et al.*, 2009). Further experiments used clock shifts, in which butterflies were kept under altered light-dark conditions, to investigate whether the time of the day influenced the orientation. Individuals with a shifted time sense were found to migrate in a significantly shifted direction when compared to the control butterflies. The direction of the shift supported the use of a time-compensated sun compass (Oliveira *et al.*, 1998; Mouritsen and Frost, 2002). Clock-shift experiments provided behavioural evidence for the use of a time-compensated sun compass in multiple species of butterflies (Reppert *et al.*, 2010; Merlin *et al.*, 2012; Oliveira *et al.*, 1998; but see Nesbit *et al.*, 2009) (Figure 10).

If the insects use the sun for navigation, they would not be able to migrate on cloudy days. However, researchers have found that butterflies could still orient towards the seasonally favourable direction, even though the sun was not visible (*e.g.* Srygley *et al.*, 2006, but see Nesbit *et al.*, 2006). Therefore, the butterflies apparently use another cue for their navigational system. If the sky is partially clouded, the solar azimuth (*i.e.* the horizontal angle of the sun) can also be derived from scattered sunlight that induces polarised sunlight and spectral gradients. The angle of the polarisation, the so called e-vector, can be used to deduce the position of the sun (Reppert *et al.*, 2004 and 2010). However, the information from e-vectors is ambiguous and butterflies are found to fly in exact opposite directions when only exposed to polarised light (Reppert *et al.*, 2004). It has been suggested that spectral gradients might be used to solve the ambiguity of e-vector information, since wavelengths of light close to the sun are longer than those further away from the sun (Heinze and Reppert 2011). However, there is no evidence for the use of spectral gradients for navigation and the behavioural evidence for polarised sunlight as a directional cue in butterflies is contradictory (Reppert *et al.*, 2004 and 2010; Srygley and Dudley, 2008; Stalleicken *et al.*, 2005).

The underlying mechanism of the time-compensated sun compass has been studied extensively in one species of long-distance migratory

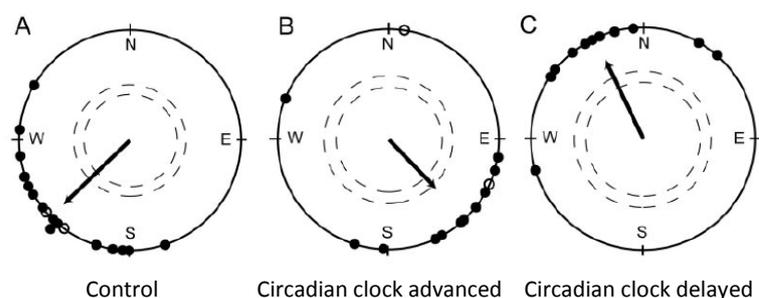


Figure 10 Example of a clock shift experiment in Monarchs. The large circles represent compasses and the small circles in the periphery show mean orientations of individual tested butterflies. The arrows indicate the mean of all data.

A: Control group. Photoperiod: 7 a.m. - 7 p.m. Butterflies oriented toward southwest, the seasonally preferred direction.

B: Circadian clock advanced 6 hours. Photoperiod: 1 a.m. - 1 p.m. Butterflies shifted orientation anti-clockwise.

C: Circadian clock delayed 6 hours. Photoperiod: 1 p.m. - 1 a.m. Butterflies shifted orientation clockwise.

The direction and magnitude of these shifts are in line with the use of a time-compensated sun compass. (Adapted from Mouritsen and Frost, 2002)

butterflies, the Monarch. The solar azimuth is thought to be detected by the main retina and polarised light by specialised photoreceptors in the dorsal rim of the compound eye (Reppert *et al.*, 2004 and 2010; Zhan *et al.*, 2011; Heinze and Reppert, 2011). Butterfly and moth vision is based on three photoreceptor classes, that are sensitive to UV, blue and green light (Stavenga and Arikawa, 2006). In addition to photoreceptors, spectral filters were found that modify the spectral sensitivity of the receptors. Red filters can enable the insects to perceive the colour red. Such filters have been found in several butterflies, including Monarchs, and are hypothesized to be present in other butterfly species as well (Stavenga and Arikawa, 2006). In the dorsal rim area, where cells are sensitive to polarised light, only UV-sensitive receptors have been found (Stavenga and Arikawa, 2006). Therefore, Lepidopterans are thought to use polarised light of UV wavelengths for navigation. The sun compass itself is presumed to be located in the central complex, an area in the middle of the Monarch brain, where information from both eyes is integrated and neurons are sensitive to both polarised and unpolarised light (Merlin *et al.*, 2012; Reppert *et al.*, 2010; Heinze and Reppert, 2011). Moreover, individual neurons are sensitive to both polarised and unpolarised light, showing that information from both the sun itself and scattered sunlight is integrated in a very early stage of the neural circuit (Heinze and Reppert, 2011). However, butterflies do not need information from polarised light if the sun itself is visible (Stalleicken *et al.*, 2005). Also, neuronal responses to unpolarised light spots were greater than to polarised light (Heinze and Reppert, 2011). Therefore, polarised light may not be used as a compass cue, but rather as a reference point to the solar azimuth (Stalleicken *et al.*, 2005). In addition, the sensitivity of neurons to unpolarised light of multiple wavelengths suggests that butterflies use the sun itself, not the spectral gradient, for navigation (Heinze and Reppert, 2011). Therefore, the solar azimuth is thought to be the most important skylight cue for butterfly navigation.

In addition to the sun compass itself, the time-compensated sun compass needs a circadian clock to compensate for the sun's movement. Two distinct circadian clock mechanisms have been found. One of the clocks is thought to be located in four cells in the dorsolateral region of either side of the brain. The rhythm of this clock is determined by a negative transcriptional and translational feedback loop of several core clock proteins, among which two types of cryptochrome proteins (Reppert *et al.*, 2010; Merlin *et al.*, 2009 and 2012). Neurons that express the core clock proteins connect the clock area with the sun compass area in the brain, which suggested that the sun compass and the brain clocks form the mechanism for the time-compensated sun compass. However, more recent studies discovered a second clock mechanism in the antennae. The antennal clocks work in the same way as the brain clocks, except that they are directly dependent on sunlight shining on the antennae and work independent of the brain. Experimental studies show how important the antennal clocks are for navigation. Butterflies with surgically removed antennae did not show oriented flight at all and butterflies with opaque black painted antennae oriented in a shifted direction, consistent with desynchronised free-running antennal clocks (*i.e.* clocks that drift from the normal cycle) (Merlin *et al.*, 2009). In both of these experiments, the brain clocks were intact and working appropriately. These findings suggest the antennal clocks, and not the brain clocks, are an important part of the time-compensated sun compass (Merlin *et al.*, 2009). For this mechanism to work, there must be a neural connection between the antennae and the brain, which is currently under investigation (Reppert *et al.*, 2010; Heinze and Reppert, 2011). However, the brain clocks are still thought to be involved as well, as clock-shift experiments had an effect on the flight direction of butterflies with black painted antennae (Merlin *et al.*, 2009). This reaction to the clock shift could not have been due to the antennal clocks and it was thus probably attributable to the brain clocks (Merlin *et al.*, 2009). It has been hypothesised that the brain clocks are involved in time-compensation for diurnal changes in polarised light angles due to solar elevation, whereas the antennal clocks are for time-compensation of diurnal changes in the solar azimuth (Heinze and Reppert, 2011).

When all the skylight information is processed, it must be integrated and used to direct the motor system. This part of the neural circuit has not yet been studied extensively. However, Heinze and Reppert (2011) have found a neuron on the presumed output side of the sun compass that is connected to a large part of the lateral accessory lobe. Therefore, this part of the brain is thought to

be involved in the integration of information and the connection to the motor circuits that induce migratory behaviour (Heinze and Reppert, 2011).

At times when the sky is completely overcast and butterflies cannot use a compass based on skylight cues, they are still able to orient appropriately (Reppert *et al.*, 2010; Merlin *et al.*, 2012). A possibility to navigate under these circumstances is to use the **Earth's magnetic field for navigation** as is reported for other species of long-distance migrating animals such as birds (Reppert *et al.*, 2010). The magnetic field changes in its polarity, inclination angle and intensity depending on the location and can provide consistent information for both an internal compass and a map sense (Reppert *et al.*, 2010). A directional system based on the Earth's magnetic field does not require any time compensation as is the case for a sun compass and is not affected by overcast skies (Merlin *et al.*, 2012; Srygley and Dudley, 2008). To study the presence of a magnetic navigational system insects were exposed to strong magnetic pulses or a reversed magnetic field, that would disrupt respectively reverse their orientation if they used the magnetic field for their direction (Srygley *et al.*, 2006; Merlin *et al.*, 2012; Reppert *et al.*, 2010; Mouritsen and Frost, 2002; Stalleicken *et al.*, 2005). The outcomes are contradictory and further research is necessary to find out whether Lepidopteran migrants use then Earth's magnetic field for navigation.

There are two proposed mechanisms, that possibly coexist, for a magnetic compass in Lepidopterans (Merlin *et al.*, 2009; Reppert *et al.*, 2010). The first is based on small magnetic particles, magnetite, that could sense the magnetic field as an inbuilt compass needle (Kirschvink *et al.*, 2001). Such magnetic particles are indeed present in Monarchs (Merlin *et al.*, 2009; Reppert *et al.*, 2010). The second magnetoreception mechanism is based on a chemical reaction which is dependent on light (Phillips and Borland 1992). The same cryptochrome proteins that are involved in the circadian clocks can potentially function as light-dependent magnetoreceptors, as homologues proteins in *Drosophila melanogaster* have that function and the Monarch cryptochromes can rescue the magnetic compass function in cryptochrome-deficient *Drosophila melanogaster* (Gegeer *et al.*, 2008 and 2010). This light-dependent chemical reaction may require a particular wavelength of light, as is found in other species (Phillips and Borland 1992). This requirement was not taken into account in the designs of all studies and the Plexiglas coverings, used to simulate overcast skies, may block essential wavelengths of light (Mouritsen and Frost, 2002; Stalleicken *et al.*, 2005). Therefore, the contradictory evidence for the use of a magnetic compass may be the result of inadequate methodologies and further studies are necessary to investigate this navigational approach in Lepidopterans.

For nocturnally migrating Lepidopterans may also be possible to use cues in the night sky for navigation, *i.e.* the moon, polarised moonlight and stars. This possibility has thus far not been explored much, but Chapman and colleagues (2008a) reported that the silver-Y moths oriented in the appropriate direction while the moon was below the horizon and they doubt whether moths are visually capable of using a stellar compass.

All in all, Lepidopteran navigational mechanisms are far from unravelled. It is well possible that the different compasses coexist, since they are not mutually exclusive, and they may even be integrated. It has been hypothesised that the various compasses are used to calibrate each other (Chapman *et al.*, 2008a; Baker, 1987; Gould, 1998). Migrant Lepidopterans may thus have a combination of compasses and the relative importance of each compass most likely depends on the circumstances and the accessibility of the various cues (Merlin *et al.*, 2012). The integration of all these mechanisms could also explain the difficulty in studying one of the compasses. If one of the compasses is blocked or somehow modified, the insects could perceive the inconsistency between the information from the different mechanisms and switch to one of their other compasses. I think the best way to study this complicated navigational system is to integrate the different approaches and investigate the mechanisms simultaneously (*e.g.* simulate the position of the sun, the polarised sunlight and the geomagnetic field simultaneously and then experiment with one of them at the time).

Besides maps, compasses and wind-mediated mechanisms, it may also be possible for the migrants to use **visual cues**, **olfactory cues** and **social communication** for their navigation. They may

use landmarks or the optomotor reaction when they fly in the Flight Boundary Layer. With the optomotor response the apparent motion of the ground is perceived as an optic flow and can be used to estimate speed and displacement. Through this mechanism and the use of landmarks, the insects can detect wind drift and subsequently control for it (Srygley and Dudley, 2008). For high-flying or nocturnally migrating insects this mechanism is not probable due to visual difficulties (Chapman *et al.*, 2011; Reynolds *et al.*, 2010a). In case of specific destinations, such as the wintering area in Monarchs, landmarks may also serve to recognise the location of the destination. Alternatively, migrants may use olfactory cues to determine that they have arrived at their destination. In case of Monarchs, the scent of the unique pine forest in which the butterflies overwinter may play a role in finding their destination (Reppert *et al.*, 2010). Also, although Lepidopteran migrants mostly migrate solitary in low densities, especially when they migrate above the FBL, it may be possible to use social communication (*e.g.* through sounds or pheromones) when the densities of certain species are high enough, which most likely occurs more easily within the FBL (*e.g.* Monarch butterflies are highly gregarious in their autumn migration) (Reppert *et al.*, 2010; Riley, 1989 cited in Reynolds *et al.*, 2010b; Gatehouse, 1994; Brower, 1996).

c. Migration initiation and termination

Two very basic questions remain: 'what triggers migratory behaviour?' and 'when is it stopped?'. For migratory behaviour to stop a complete disinhibition of the insects' responsiveness to stimuli from favourable environments is required (Gatehouse, 1997). It is likely that the insects use a map sense to determine their destination once they have arrived (Reppert *et al.*, 2010; Merlin *et al.*, 2012). The underlying mechanisms of this disinhibition and map sense is unknown. To trigger migratory behaviour, there must be some environmental cue or cues. Possible cues for migration are for example the withering of the larval food plants, the shortening of days or cooling temperatures; all signs of a deteriorating environment, which will soon be unsuitable as a habitat (Reppert *et al.*, 2010). Cooling temperatures in autumn on the Northern Hemisphere may also forecast northerly winds that are used to migrate south (Wikelski *et al.*, 2006). Accordingly, dragonflies are found to migrate on the days that follow nights that were colder than the night before. They did not migrate on days following nights with increasing temperature (Wikelski *et al.* 2006). In addition, large concentrations of migratory Lepidoptera are associated with the passage of cold fronts, in front of which warmer air flows transport the insects (Brower, 1996; Drake and Farrow, 1988). Perhaps these high concentrations are the result of convergent air flows, but it may also be possible for Lepidoptera to forecast cold fronts, possibly by sensing a change of the humidity, and use this as a cue to initiate migration (Drake and Farrow, 1988). Therefore, temperature or humidity may serve as a cue for favourable winds. Conversely, it may also be possible for Lepidoptera to take off for migration regardless of the wind direction and only stay aloft with favourable winds (Chapman *et al.*, 2011). Therefore, favourable winds itself may be the cue for migration. In addition, adverse conditions such as droughts, cold periods, high population pressure and subsequent food competition, high levels of parasitism and predation are thought to initiate migration (Shields, 1974; Stefanescu *et al.*, 2011 and 2012; Holland *et al.*, 2006). It has been hypothesised that the brain clocks play a role in initiating migration by detecting decreasing day length (Zhu *et al.*, 2008; Reppert *et al.*, 2010). However, evidence on precise environmental cues that trigger migration is yet to be found (Reppert *et al.*, 2010). In addition to external cues that trigger migration, there is of course the internal state of the insect that must be ready for migration.

d. Endocrinological mechanisms

Most species do not reproduce until after their migration (*e.g.* Painted Lady, Monarch, Red Admiral) (Stefanescu *et al.*, 2001; Zhu *et al.*, 2009; Stefanescu *et al.*, in prep.; Gatehouse, 1994 and 1997). This phenomenon has been studied in Monarch butterflies. From the time of emergence as an adult until after migration they are in so called reproductive diapause (Zhu *et al.*, 2009). Monarchs are unique, because they have a non-reproductive migratory autumn generation, but also reproductively active non-migratory summer generations (Zhu *et al.*, 2009; Herman and Tatar, 2001). This feature makes

them ideal subjects for studies that investigate what factors cause migration. Not only do migratory Monarchs not reproduce, they are not capable of reproduction, as the reproductive glands of both sexes are not fully developed until after their migration (Herman, 1975). The reproductive organs in non-migratory summer Monarchs, on the other hand, start to develop and enlarge from the moment of emergence (Herman 1975). This reproductive diapause and development has been found to be regulated by hormonal levels of the juvenile hormone (JH) (Herman, 1975). A similar regulation by JH has also been found in the Painted Lady (Herman and Dallmann, 1981). Reproductive development in summer Monarchs requires JH (Herman, 1975) and the JH deficiency in autumn butterflies causes their reproductive arrest (Zhu *et al.*, 2009). In addition to differences in the reproductive activity, the migratory generation is longer lived than the summer generations (Herman and Tatar, 2001). Whereas the summer generations probably live around two to five weeks, migratory Monarchs live several months from early autumn until spring (Herman and Tatar, 2001). This increased life span is also caused by a JH deficiency and may be intrinsic to the reproductive diapause, as reproductive behaviour increases mortality and developed reproductive organs require nutrients that can otherwise be invested in somatic maintenance (Herman and Tatar, 2001). Removal of the corpora allata gland (allatectomy), where JH is produced, causes increased longevity and arrest of reproductive development (Herman, 1975 and Herman and Tatar, 2001). Alternatively, adding JH to migrant Monarchs reduces longevity (Herman 2001) and causes reproductive development (Herman, 1975; Zhu *et al.*, 2009), but cannot disrupt migratory flight behaviour in already migrating individuals (Zhu *et al.*, 2009). Therefore, additional factors must be involved in maintaining migratory flight behaviour. Whether JH is required to initiate migratory behaviour is unknown (Zhu *et al.*, 2009). To further investigate the relation between JH and migratory behaviour and to find other factors that may influence migratory behaviour, researchers have recently compared gene expression patterns between summer and migratory autumn Monarchs (Zhu *et al.*, 2008 and 2009; Zhan *et al.*, 2011).

Gene expression and the genetic complex

The genetic complex of the migration system encompasses all the genetic information that underlies the traits of the migration syndrome. To unravel which genes are part of the genetic complex and what gene expression patterns lead to migration, researchers investigated the difference between summer and autumn generations of Monarchs. Since summer and autumn Monarchs belong to the same species, but differ in their migratory behaviour, genes that differ in their expression levels between these generations are hypothesised to be part of the genetic complex. Researchers were particularly interested in the gene expression patterns in the brain, as the focus of molecular research has recently been on the neural control of migratory behaviour (*i.e.* the role of the juvenile hormone and the mechanism of circadian clocks and compasses). In order to study gene expression in the brain, mRNA was extracted from brain tissue and used to reveal sequences of genes that were being expressed in Monarch brain cells at the time of the experiment (*i.e.* the transcriptome of the brain) (Zhu *et al.*, 2008). By means of microarray analysis, expression levels of these genes can be compared between summer and migratory Monarchs (Zhu *et al.*, 2008 and 2009; Zhan *et al.*, 2011). Using this approach, 62 genes were found that differ in expression between summer and autumn butterflies. Part of these genes, 23, had expression patterns that correlated with JH activity and the reproductive state of the butterflies (Zhu *et al.*, 2009). The remaining 39 genes had expression patterns that correlated with migratory flight behaviour independent from JH activity (Zhu *et al.*, 2009; Zhan *et al.*, 2011). Now that the sequences of these genes are known, further investigation is needed to study how they play a role in migration. The first step in this process is to investigate the identity of the genes. By comparing the cDNA sequences to sequences of other species in online genetic databases, it is possible to find homologues genes. Since homologues genes are often comparable in function, this search can yield information on the possible functions of the Monarch genes (Zhu *et al.*, 2008). Very recently, the whole genome of Monarchs was sequenced, which further helped in the process of identifying the genes that differed in expression between migratory and non-migratory butterflies (Zhan *et al.*, 2011). Also, the genome revealed all the genes that are

involved in the biosynthesis pathway of JH and it was found that the deficiency of JH in migrant Monarchs had different underlying mechanisms between the sexes. Whereas the biosynthesis itself is suppressed in males, a higher turnover rate of JH is found in females (Zhan *et al.*, 2011). The contribution of the revealed genes to migration and the environmental or epigenetic factors that influence their expression can now be studied in more detail (Reppert *et al.*, 2010). In addition to intraspecific comparison of gene expression patterns and identifying genes that are possibly responsible for migration, comparing the Monarch genome to other species can yield information on evolution of Lepidopteran long-distance migration.

Evolution

Besides the Monarch butterfly genome only one other Lepidopteran genome has been sequenced, which is that of the non-migratory domesticated Silk moth (*Bombyx mori*) (Zhan *et al.*, 2011). When compared to other insects, it was found that the Monarch and the Silk moth had a relatively large number of unique sequences and also lacked a relatively large number of sequences that were present in other insects (Figure 11). Therefore, the order of Lepidoptera is thought to be more derived than other insect orders (Zhan *et al.*, 2011). Also, because of the relatively large genetic differences between the Monarch and the Silk moth and the relatively short time since these species diverged, the Lepidoptera seem the fastest evolving insect order known to date (Zhan *et al.*, 2011). Although, these findings give information on Lepidopteran evolution, they do not necessarily elucidate the evolution of long-distance Lepidopteran migration.

One would think that migration evolved because of its benefits (Holland *et al.*, 2006). The most often reported benefit in vertebrates is the ability for animals to follow and take advantage of seasonally available resources (Holland *et al.*, 2006). The same is hypothesised to be true for insects (Drake and Farrow, 1988; Chapman *et al.*, 2011, but see Holland *et al.*, 2006). Alternatively, escaping from inhospitable environments may be the selective factor for migration in insects. Species that do not go into diapause to survive unfavourable conditions have to migrate in order to survive (Stefanescu

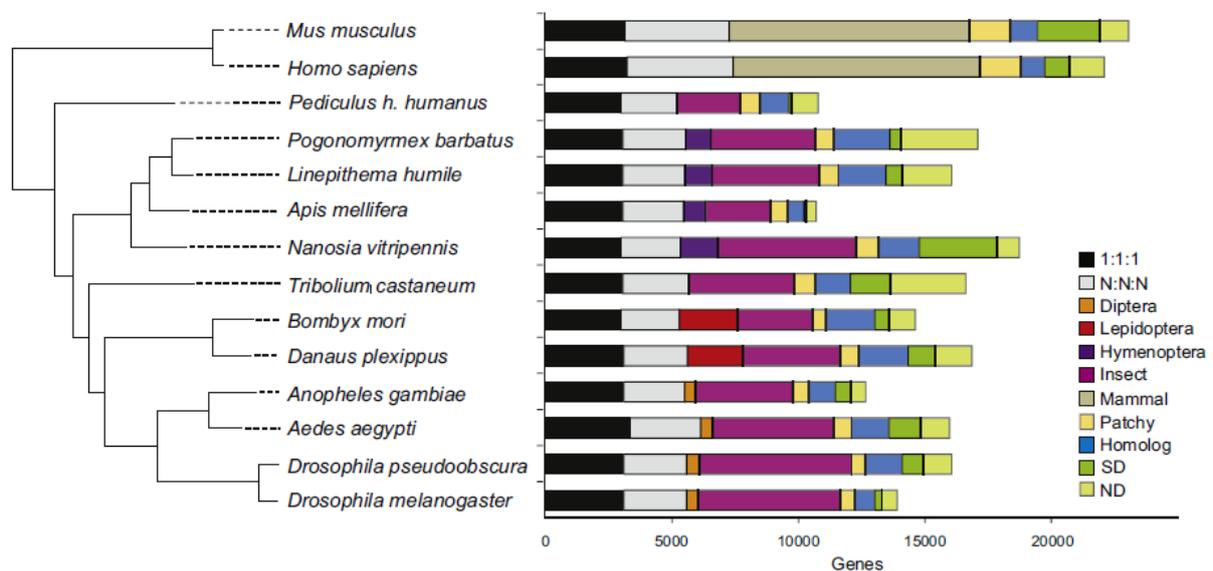


Figure 11 Phylogeny for twelve insect species, including Monarchs (*Danaus plexippus*) and Silk moths (*Bombyx mori*), and two mammal species as outgroup. The bar diagram shows the different types of orthologues genes (*i.e.* homologues genes by vertical descent) found in the genomes of each species. 1:1:1 and N:N:N are categories of universal genes. The phylogenetic tree is based on genes from the 1:1:1 group. The Diptera, Lepidoptera and Hymenoptera categories indicate orthologs that are specific to these insect orders. The number of genes that is specific to Lepidoptera is relatively large when compared to the other insect orders. The categories insect and mammal indicate insect- respectively mammalian-specific orthologs. The Lepidoptera lack a part of the insect-specific orthologs that are present in other insect orders. Patchy indicates orthologs that are present in at least one mammal and one insect species. Homolog indicate homologues genes of which orthology is not detected. SD are species-specific duplications and ND are species-specific genes. (Zhan *et al.*, 2011)

et al., 2012; Holland *et al.*, 2006). An environment may be unfavourable because of climatic factors that can cause droughts or cold periods, shortage of the larval food plant or nectar holding plants and high levels of predation (Shields, 1974; Stefanescu *et al.*, 2007). Furthermore, avoiding parasites may also be a contributing factor for migration to evolve. As Lepidopteran host species stay in a certain area, the populations of their parasitoids start to build up. By migrating to parasitoid-low areas, they can avoid a lot of parasitism (Stefanescu *et al.*, 2012). Following favourable environments and avoiding unfavourable environments would both result in the same migratory pattern and are not mutually exclusive. To find out if migration is indeed adaptive and why, one would have to measure reproductive success, which is not possible, given that we currently do not have the methodologies to follow individual Lepidopterans along their lifetime tracks.

Regardless of the adaptiveness of migration, comparing genetic information between migratory and non-migratory species of different taxa can give a lot of insight into how different aspects of migration most likely have evolved. As for long-distance migration in general, it has evolved repeatedly in so many animals from many taxa, that it is thought to be a relatively simple step in evolution (Alerstam 2006; Dingle and Drake, 2007; Gatehouse, 1997). Even within Lepidoptera, migration apparently evolved numerous times, as closely related species are both migrants and sedentary species. Migratory behaviour may be an extension of non-migratory behaviour (Alerstam, 2006; Dingle and Drake, 2007). For example, the ability to fly is an essential trait for long-distance Lepidopteran migration, but it is already present in all Lepidopterans and used for non-migratory behaviour such as foraging.

Several findings show that other essential characteristics and genes for long-distance migration are also widespread among animal taxa. For example, the neural pathway to detect polarised light is found to be similar to the migratory Desert locust (*Schistocerca gregaria*) (Heinze and Reppert, 2011). Since Monarchs and Desert locust are distantly related insects, this finding suggests that a lot of insects have the ability to detect polarised light and probably have a sun compass (Heinze and Reppert, 2011). Also, the magnetic particles, that could be used to detect the Earth's magnetic field, are found in organisms ranging from bacteria to higher vertebrates and are therefore thought to be present in all animals (Kirschvink *et al.*, 2001). Furthermore, among the core proteins of the Monarch circadian clocks are two cryptochrome proteins, CRY1 and CRY2 (Zhu *et al.*, 2005). CRY1 is a photoreceptor protein that resembles the cryptochrome proteins of *Drosophila*. CRY2, on the other hand, is a transcriptional repressor that resembles the proteins of circadian clocks in mice (*Mus musculus*) (Zhu *et al.*, 2005; Reppert *et al.*, 2010). The discovery of the vertebrate-like CRY proteins in the Monarch clock, which were later also found in other insects orders, suggests the molecular clockwork, that is needed for a time-compensated sun-compass, is also widespread among animals (Zhu *et al.*, 2005).

These findings support the hypothesis that migration is an extension of non-migratory traits. All of the migratory traits seem widespread under many animal taxa. Now the question is about the nature of this extension. As the generational difference in migratory behaviour in Monarchs suggest, migration may be in gene expression differences, rather than actual differences in genes (Zhu *et al.*, 2008 and 2009; Zhan *et al.*, 2011). What causes these differential expression patterns? Perhaps only one or a few mutations can cause the differences in gene expression that are needed for migration.

Cross-species migration comparisons

Having described the migration for some of the best studied Lepidopterans, it is interesting to look at the patterns that exist across species. I will first describe similarities and differences among different species of migratory Lepidoptera and then compare migration between Lepidoptera and other migratory insects and birds.

Comparisons among migratory Lepidoptera

Most Lepidoptera species that are described in this review migrate north in spring and south in autumn on the Northern Hemisphere (*i.e.* Monarch, Painted Lady, Red Admiral, Silver-Y moth) (Reppert *et al.*, 2010; Stefanescu *et al.*, in prep and 2001; Chapman *et al.*, 2008a and 2008b). As described in the section on evolution, it was found that several migratory traits and genes are shared among many insects or even among multiple animal taxa. Therefore, different species of migratory Lepidoptera are thought to have migratory traits in common. Some of these traits were also found in behavioural studies. For example, multiple species were found to migrate with a time-compensated sun compass and many flew at high elevations when the weather conditions were favourable and close to the Earth's surface when the conditions were unfavourable for migration (Reppert *et al.*, 2010; Merlin *et al.*, 2012; Oliveira *et al.*, 1998; Mikkola, 2003b; Davis and Garland, 2002 and 2004; Williams, 1970).

Interestingly, some species also share similar population dynamics. It was found that the Painted Lady, the Silver-Y moth and the Rush Veneer (*Nomophila noctuella*) are often abundant simultaneously (Williams 1970; Stefanescu *et al.*, in prep.). This simultaneous abundance is probably due to shared environmental effects, such as favourable temperatures that allow the build up of a large source population in the overwintering area (Stefanescu *et al.*, in prep.; Williams 1970; Sparks *et al.*, 2005). Also, a positive trend was found between invasions of the Painted Lady in Europe and North America (Williams, 1970) and similar patterns have been reported between different subspecies of migratory hawk-moths, *i.e.* *Celerio lineata lineate* and *Celerio lineata livornica*, occurring in North America respectively Europe. (Williams 1957). The populations on the two continents do not have the same source population or source area and the simultaneous invasions are most likely caused by a common climatic factor (Williams 1970). This would imply that Lepidopteran population dynamics are influenced by transcontinental climatic patterns (Williams 1970). Interestingly, the Painted Lady source areas in North Africa and Mexico are on the same latitude and have a similar dry climate (Williams, 1970). In addition to higher temperatures in the source area, favourable weather conditions along the migratory trajectory, such as favourable winds, are thought to influence the magnitude of migration in certain areas (Gatehouse, 1994). Conversely, unfavourable conditions in the source area, such as aridity, overcrowding or parasitism, may also induce migration (Gatehouse 1994; Stefanescu *et al.*, 2012). However, for all of these factors to cause an invasion, there must also have been a period of favourable weather earlier in the season, so that the population is large enough to cause an invasion in the first place.

In addition to the similarities between species, there are also differences. First, whereas the spring migrations are most obvious in most species, it is the autumn migration that is most apparent in Monarchs (Stefanescu *et al.*, in prep.; Mikkola, 2003a and 2003b; Reppert *et al.*, 2010). Furthermore, whereas Monarchs go into diapause to survive the winter Painted Lady butterflies stay active throughout the year and Red Admirals are thought to use both strategies (Reppert *et al.*, 2010; Stefanescu *et al.*, in prep. and 2001; Brattström *et al.*, 2008 and 2010a). Also, whereas a lot of migrant Lepidoptera are thought to migrate solitary, especially above the Flight Boundary Layer where the wind disperses the animals, Monarchs are very gregarious (Gatehouse, 1994; Riley, 1989 cited in Reynolds *et al.*, 2010b; Brower, 1996; Mikkola, 2003b).

Comparing migration of Lepidoptera and other animal species

On a larger scale, long-distance migration in Lepidoptera is not unlike that of other insect and even bird species. Dragonflies and birds also generally migrate north in spring and south in autumn (Wikelski *et al.*, 2006; Alerstam *et al.*, 2011; Richardson, 1998). Dragonflies, birds and butterflies all accumulate fat before migrating (Wikelski *et al.*, 2006) and make stopovers to refuel their energy supply and avoid unfavourable winds (Wikelski *et al.*, 2006; Richardson, 1998; Ma *et al.*, 2011; Davis and Garland, 2004; Mikkola 2003b; Stefanescu *et al.*, in prep.; Williams, 1970). As in Lepidopterans, migratory birds compensate for wind drift by adjusting their headings and they fly lower to minimise the effects of opposing winds, although not as close to the Earth's surface as Lepidoptera (Richardson, 1998; Chapman *et al.*, 2008a and 2008b; Mikkola, 2003b; Davis and Garland, 2002 and

2004; Williams, 1970). In comparison with insects, birds are less selective of favourable winds (Alerstam *et al.*, 2011; Grönroos *et al.*, 2012). They are generally found to start migrating with tailwinds, but have the flight capacity to migrate during headwinds as well, although at a higher metabolic cost (Richardson *et al.*, 1998; Alerstam *et al.*, 2011; Grönroos *et al.*, 2012). Although the flight speed of birds is several times greater when compared to Lepidopterans, migrating passerine birds and Silver-Y moths were found to migrate at the same ground speed (Alerstam *et al.*, 2011). While passerines were not very selective of favourable winds and flew on their own flapping power, the moths selected fast wind streams to soar on. Therefore, despite of their differences, passerines and moths had similar migratory performances.

Other shared aspects of migratory flights between insects and birds relate to topography. Birds, dragonflies and butterflies are found to follow topographical lines, such as valleys and rivers (Brattström *et al.*, 2008; Richardson, 1998; Wikelski *et al.*, 2006). When butterflies and birds encounter a large body of water, they follow the coastline (Brattström *et al.*, 2008; Richardson, 1998). This behaviour may enable the animals to find the narrowest body of water so that crossing the water is less risky (Brattström *et al.*, 2008). Birds and dragonflies have also been observed to fly a few kilometres above a large bay, turn and fly in the opposite, seasonally unfavourable, direction. They are thought to follow the coastline further inland to cross the bay or the river that flows to the bay further upstream at a narrower point (Wikelski *et al.*, 2006).

Like Monarchs, but unlike most other Lepidopterans, birds often migrate in groups (*i.e.* flocks) and to very specific areas (Berthold, 2001; Alerstam, 1990; Alerstam *et al.*, 2011). Although birds generally complete the migratory trajectory multiple times in their lifetime and can possibly recall the trajectory from their own memory or follow experienced conspecifics, they can also find their way on their own (Wiltschko and Wiltschko, 2003). To navigate, birds are shown to use a map and similar compass mechanisms as suggested for Lepidopterans, including compasses based on the sun, polarised sunlight, stars and geomagnetic cues (Wiltschko and Wiltschko, 2003; Alerstam and Gudmundsson, 1999). The navigational system in the closer related locusts is also similar to Lepidopteran systems. The sun compasses of the Monarch and Desert locust (*Schistocerca gregaria*) have both been studied extensively and found to be highly homologues (Heinze and Reppert, 2011). The anatomy of the brain and neuronal circuits of the sun compasses in these species are very similar (Heinze and Reppert, 2011 and 2012). However, there is a marked difference in the neural response to skylight cues between the butterflies and locusts. As described in an earlier section, the Monarch's neural response to various wavelengths of light is indifferent (Merlin *et al.*, 2012). In contrast, locusts do differentiate between these wavelengths, which provides them with the possibility to use the spectral gradient of scattered sunlight to solve the ambiguity of polarised light cues (Pfeiffer and Homberg, 2007). These findings led to the hypothesis that Monarchs use the solar azimuth itself as main skylight cue for navigation, whereas locusts use both the solar azimuth and scattered sunlight (Merlin *et al.*, 2012).

In addition to similarities between insects and birds, they also differ on a crucial aspect. As mentioned earlier, insect migration is multigenerational whereas birds complete the migratory trajectory individually and possibly many times in one lifetime (Dingle and Drake, 2007; Holland *et al.*, 2006). Therefore, migratory trajectories of insects change for each generation and require a different, presumably more complex, underlying mechanism when compared to birds. In addition, whereas birds have a particular diet throughout their lives, Lepidopteran larvae and adults have different food sources (Reppert *et al.*, 2010; Stefanescu *et al.*, 2011; Van Swaay *et al.*, 2008a; Brower, 1996), which makes the requirements for favourable environments more complex for Lepidoptera.

Climate change and conservation

In line with the earlier mentioned causal factors of invasions, it was found that the migration for many Lepidopteran species increased, regarding the number of individual migrants per species, in years of increased temperature (Sparks *et al.*, 2005). This relation between the climate and

population dynamics is also visible in the effects of climate change. It was found that the number of migrant Lepidoptera species was positively correlated with climate change in the UK (Sparks *et al.*, 2007). The higher temperatures are hypothesised to be linked with larger source populations in the spring, dryer southern regions in the summer on the Northern Hemisphere and a northward shift in source areas, all of which can cause increased migration to be detected in northern parts of the migratory trajectories (Sparks *et al.*, 2005).

Climate change affects many if not all species and many animals share similar effects thereof. The increasing temperature leads to a poleward shift of the warmer climate (Devictor *et al.*, 2012). In accordance with this geographical shift in temperature, the animal ranges on the Northern Hemisphere also shift north, including that of butterflies and birds (Devictor *et al.*, 2012). However, since the temperature shift is greater than the shifts in the ranges of butterflies and birds, these animals are delayed in their response to the climate change and have so called climatic debts (Devictor *et al.*, 2012). Furthermore, the rate at which the birds shift their range is lower than that of butterflies, which is thought to be the effect of the shorter generation time of the insects (Devictor *et al.*, 2012). This difference in range shift between birds and butterflies causes a trophic mismatch, as many birds eat caterpillars, and is of great conservational concern (Devictor *et al.*, 2012). This mismatch can thus be problematic for birds that cannot reach their prey, but positive for Lepidoptera, as some of their predators cannot reach them. However, what is positive for Lepidopterans, can at the same time be an economic problem in case of agricultural pest species (Sparks *et al.*, 2005). For endangered Lepidoptera species, climate change can thus lead to an ethical conflict between conservation and the economy. The methodologies used in this study by Devictor and colleagues (2012) do not distinguish between different species of Lepidoptera and the results may be different for migrant butterflies. As migrant Lepidopterans are highly mobile species, they are thought to be better able to adjust their ranges northward when compared to sedentary species (Pöyry *et al.*, 2009). The climatic debt may thus be smaller for migrant butterflies and, consequently, I expect the mismatch between migrant Lepidoptera and their bird predators to be greater than calculated by Devictor and colleagues (2012). However, a shift to non-migratory insect species on the birds' menu may make the extent of this mismatch less obvious.

The effects of climate change are also different for specialist and generalist species and it is generally found that specialist species are more susceptible to change than generalist species (Heikkinen *et al.*, 2010; Sparks *et al.*, 2007). Since specialists are dependent on specific environments, they cannot adjust to other circumstances as well as generalists species. Since the majority of migrant Lepidopterans are generalists, the conservational concern is mostly with sedentary species (Stefanescu *et al.*, 2011a). Nevertheless, there is also concern for migrants, as climate change causes droughts that affect specialists as well as generalists (Stefanescu *et al.*, 2011a). Furthermore, intensified land-use results in habitat loss for grassland species, which also include migratory species (Stefanescu *et al.*, 2011a).

Future prospects of research on migratory Lepidoptera

The future studies on migratory butterflies and moths are mainly dependent on developments and improvements of methodologies and what species are included in the studies.

The monitoring schemes are used to gather abundance data from many diurnal low-flying species of Lepidoptera. By further increasing the number of countries and regions that take part in this monitoring, it is possible to greatly enhance the network's coverage (Van Swaay *et al.*, 2008b). Such expansion of the network would imply an improved ability to study the migrants' movements and forecast invasions. Moreover, more data on the occurrence of species helps to determine whether species are at risk of extinction and can be used to adjust management actions to aid the conservation of the species. These trends are mainly important for sedentary species, as fluctuations in migratory species are not very informative. In addition to expanding the network, there is great potential for improvement of the monitoring in the manner in which transects are chosen and

sampled. Transects have to be selected carefully so that they represent the fauna of a larger area and any bias therein needs to be correctly adjusted for during analysis of the data (Van Swaay *et al.*, 2008b). These rather simple adjustments in data collection and analysis can significantly improve the reliability of the data.

The field of radar entomology is also wide open for improvements. With increasingly affordable equipment, it may be possible to install more radars to start a radar network, which would improve the ability to follow migrants along their trajectories (Chapman *et al.*, 2011). Also, the technology is continuously further developed and it may soon be possible to use VLRs that can sample from the ground up, without missing the first 150 metres (Chapman *et al.*, 2011). Comparing radar measurements of more species will enhance the identification of radar target species (Chapman *et al.*, 2011). Lastly, further studies that investigate how meteorological radars can be used for entomological research are very important, since meteorological radars cover entire countries and would yield an enormous amount of data (Chapman *et al.*, 2011; Shamoun-Baranes *et al.*, 2010).

In addition to radar, radio telemetry is also used to study animals at a distance (Wikelski *et al.*, 2006; Rowley and Alford, 2007). As in the harmonic radar technique, radio telemetry requires a device attached to the target animal and a receiver (Wikelski *et al.*, 2006; Riley *et al.*, 1996). Instead of radar, radio waves are used to indicate the position of the animals. When the two techniques are compared, they yield the same data, but radio telemetry tagged animals were easier to find again (Rowley and Alford, 2007). Whereas the harmonic radar can best be used at distances of a few hundred meters, radio waves can be detected from space (Chapman *et al.*, 2003; Wikelski *et al.*, 2006). Therefore, radio telemetry is preferred over harmonic radar technique (Rowley and Alford, 2007). Unfortunately, radio telemetry has a very significant disadvantage; it requires a battery in the device attached to the animals. The batteries make the radio telemetry devices much heavier than the devices used in harmonic radar technique (*i.e.* 300 g respectively 1.5 g) (Wikelski *et al.*, 2006; Riley *et al.*, 1996). Therefore, target animals must have a minimum size and weight for this method to work. The radio telemetry technique has already been used to study common green darner dragonflies (*Anax junius*). These dragonflies, that weigh on average 1.2 g and are known to carry large prey or mates, did not seem to have difficulty flying with the 300 g device (Wikelski *et al.*, 2006). However, most Lepidoptera and the species described in this review weigh less and do not normally carry heavy loads, certainly not while migrating. Further minimisation of the device, so that it can be used to study Lepidopterans, will make an important difference for future studies. Since the radio waves can be detected in space, the use of satellites would enable GPS tracking of individual insects (Wikelski *et al.*, 2006).

Studying individual insects would improve all research areas that are described in this review. It would be possible to study the exact lifetime migratory tracks of butterflies and moths, disentangle possible population differences in trajectories, study what factors influence migratory flight behaviour, migration initiation and termination and it might even be possible to determine the location of post-migratory individuals in time to observe oviposition and use this to study fitness and the adaptiveness of migration.

The current knowledge is concentrated on only a few species and in particular the Monarch. By including more species of both migrating and non-migrating Lepidoptera, it is possible to find out what traits, genes or gene expression patterns differentiate migratory species and how long-distance migration may have evolved. Knowledge on more species is also essential for conservational and economic purposes. As in Monarchs, the Painted Lady may also have generations that differ in their migratory behaviour. The winter generations of the Painted Lady in Africa do not seem to have predictable directional migrations as the spring and summer generations do (Stefanescu *et al.*, in prep.). However, most studies on the Painted Lady and migratory Lepidoptera in general are conducted in Europe and North America. Further research is thus needed to study the African part of the Painted Lady migratory trajectory (Stefanescu *et al.*, in prep.). If there is indeed a difference between generations, comparison among them may be of great value to further studies of Lepidopteran migration. Gathering data in other continents is also important for other species to yield a better understanding of migratory trajectories and ranges.

For the study of orientation and navigation, the next steps are to investigate mechanisms to sense turbulence and to identify magneto receptors (Merlin *et al.*, 2009; Reppert *et al.*, 2010; Reynolds *et al.*, 2010a; Chapman *et al.*, 2011). Also, the neural connection between antennal clocks and the brain is yet to be discovered and the output pathways of the clocks and the sun compass must be further investigated anatomically and functionally (Heinze and Reppert, 2011). Above all, it is important to use an integrative approach to study navigational capacities, as the animals are thought to switch from compass mechanism when one of the compasses does not yield information or is non-consistent with the other compasses due to experimentation (Merlin *et al.*, 2012).

In sum, the priorities for future research are to study more species on more continents, develop methodologies to track individual animals along their lifetime tracks and to study navigational capacities in an integrated manner.

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Illustrations

Illustration of a Painted Lady butterfly on the front page and in figure 5: Kristin Vigander, 2006. Available at: www.kristvi.com (Accessed June 3, 2012)

Illustration of a Monarch butterfly in figure 3: Darlyne A. Murawski. Available at: fineartamerica.com (Accessed June 27, 2012)

Illustration of a Red Admiral butterfly in figure 6: Tom Hermansson Snickars, 2006. Available at: www.ambipropect.com/lepidoptera/nymphalidae/vanessa_atalanta.htm (Accessed June 27, 2012)

Illustration of a Silver-Y moth in figure 6: Marcos Veiga. Available at: www.cartinafinland.fi (Accessed July 1, 2012)