Quantifying dispersal in British noctuid moths

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Abstract

Dispersal is an important process in the ecology and evolution of organisms, affecting species' population dynamics, gene flow, and range size. Around two thirds of common and widespread British macro-moths have declined in abundance over the last 40 years, and dispersal ability may be important in determining whether or not species persist in this changing environment. However, knowledge of dispersal ability in macro-moths is lacking because dispersal is difficult to measure directly in nocturnal flying insects. This thesis investigated the dispersal abilities of British noctuid moths to examine how dispersal ability is related to adult flight morphology and species' population trends. Noctuid moths are an important taxon to study because of their role in many ecosystem processes (e.g. as pollinators, pests and prey), hence their focus in this study.

I developed a novel tethered flight mill technique to quantify the dispersal ability of a range of British noctuid moths (size range 12 – 27 mm forewing length). I demonstrated that this technique provided measures of flight performance in the lab (measures of flight speed and distance flown overnight) that reflected species' dispersal abilities reported in the wild. I revealed that adult forewing length was a good predictor of inter-specific differences in flight performance among 32 noctuid moth species. I also found high levels of intra-specific variation in flight performance, and both adult flight morphology and resource-related variables (amount of food consumed by individuals prior to flight, mass loss by adults during flight) contributed to this variation. Analysis of Rothamsted Insect Survey data and National Moth Recording Scheme data of changes in moth abundance and distribution patterns in the UK over the past 4 decades provided some evidence that dispersal ability (measures of adult forewing length) contributed to species' population trends. The analysis indicated that species with intermediate dispersal ability may be declining more than those of either high or low dispersal ability.

I conclude that the new tethered flight technique demonstrated in this thesis provides opportunities for multi-species and cross-taxon comparisons of dispersal ability. The utility of wing length as a proxy for dispersal ability may facilitate the inclusion of dispersal information into analyses for a wide range of insects. Dispersal has some effect on moth distribution and abundance changes but other factors such as habitat availability and responses to changing climate are likely to play a role.

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Declaration

I confirm that this is my own work and material from all other sources has been properly acknowledged.

Information presented in Chapter 2 is based on a paper submitted to the journal 'Methods in Ecology and Evolution':

Jones, H.B.C., Lim, K.S., Bell J.R., Hill, J.K. & Chapman, J.W. A novel automated tethered flight technique to quantify inter-specific variation in insect dispersal ability.

I designed the experiments, collected and analysed the data and co-wrote the paper.

In Chapter 5, I carried out an analysis to investigate the role of dispersal ability in the population and distribution trends of British noctuid moths, using my data from Chapters 2 and 3 in conjunction with two datasets: Abundance trends were taken from the 'State of Britain's Larger Moths 2013' (Fox et al 2013), calculated from Rothamsted Insect survey data. Distribution size and northern range margin changes were obtained from (Palmer et al., in prep) based on National Moth Recording Scheme data.

This work has not previously been presented for an award at this, or any other, University.

1 Chapter 1 – General Introduction

1.1 Dispersal

The term dispersal has been defined and used in many ways (Greenwood 1980, Clobert et al. 2001). It has been defined most simply as a spreading of individuals away from others (Begon et al. 2006). It can be defined as movement from the site of birth to the site of reproduction (natal dispersal) (Dytham 2009). However this definition does not cover the occurrence of movement between two successive breeding areas which is pertinent for many iteroparous organisms (breeding dispersal; Clobert et al. 2001). Bowler and Benton's (2005) definition best encompasses these different aspects and defines dispersal as 'any movement between habitat patches, and habitat patches as areas of suitable habitat separated in space from other such areas'. This definition does not however mention reproduction and for dispersal to be 'effective' it must result in reproduction (Greenwood 1980). Therefore the definition I will use in this thesis is that of Ronce (2007) who defined dispersal as: 'any movement of individuals or propagules with *potential* consequences for gene flow across space'.

The dispersal process can be divided into three stages; leaving the current habitat patch (emigration), the transfer time between patches (inter-patch movement) and entering and remaining in a new patch (immigration)(Bowler and Benton 2005). Conditions and processes affecting dispersal may act differently on the different stages (Barton 2001).

1.1.1 Migration and dispersal

An alternative definition of dispersal is that by Dingle (2014) who states that dispersal is 'an ecological outcome of behaviour, not behaviour itself'. Dingle argues that movement behaviours such as ranging (exploring a habitat in order to locate a suitable home range) and migration can result in dispersal, but that dispersal is a population-level process that can result from these different types of movement behaviours. Migration is defined as 'persistent and straightened-out movement effected by the animal's own locomotory exertions or by its active embarkation on a vehicle. It depends on some temporary inhibition of station-keeping responses but promotes their eventual disinhibition and recurrence' (Kennedy 1985 in Drake and Gatehouse 1995). In this definition, a 'vehicle' refers to current-borne transport (i.e.

insect movements can be aided by wind or water currents); and movements defined as 'migratory' (in contrast to searching/foraging movements) are stated to involve a suppression of the 'station-keeping' responses, which means the organism will not respond to stimuli during migration which ordinarily would arrest movement at other times (e.g. sex pheromones and host-plant odours). The key difficulty in using this behavioural definition of migration is that without complex laboratory experiments, it is typically impossible to determine the physiological state and responsiveness to stimuli of any test organism, and so in this thesis it has not proved possible to assign the flight of tethered moths to 'migratory' or 'station-keeping' (i.e. searching) categories as defined by Kennedy, Dingle and others. In this thesis, I have used the term 'dispersal' to describe the expected outcome of the flight performance results recorded by the tethered flight mills, and restrict use of the terms 'migration' and 'migrant' to those species which are known to regularly engage in long-range seasonal movements (e.g. the Silver Y moth *Autographa gamma*).

1.1.2 Introduction structure

This introduction will outline the causes, mechanisms and outcomes of dispersal, focusing on animal dispersal, and describe how dispersal is an important consideration in our current environment of climate change and habitat fragmentation. I will introduce my study group, the noctuid family of macro-moths and the motivation for studying them. I will briefly describe some aerodynamic and physiological considerations for flying animals. I will outline means of quantifying dispersal in this study group and identify possible morphological proxies for dispersal. Finally I will outline the aims of my project and the structure of the thesis.

1.2 Ecology and evolution of dispersal

1.2.1 Causes of dispersal

Proximate causes of dispersal are those factors which trigger or affect dispersal directly (i.e. the cues and conditions that the organism has experienced), and their predisposition to disperse or dispersal strategy (Starrfelt and Kokko 2012). These proximate causes include factors and characteristics of the current patch that will affect the emigration stage of dispersal, including: patch size and species' population density, resource availability, parasite and predator load, sex ratio, relatedness, and matrix habitat (or rather the habitat boundary effect of the matrix) (Bowler and

Benton 2005). At the transfer stage the individual's dispersal behaviour is likely to be heavily influenced by the matrix habitat and the search strategies and habitat cues employed by the organism. Finally at the immigration stage the characteristics of the new patch are influential, including: patch size, isolation and cues about patch quality (Bowler and Benton 2005). Ultimate causes of dispersal are processes which provide opportunities for evolution, with selection acting on fitness costs and benefits (Holekamp and Sherman 1989), including kin competition, inbreeding avoidance and habitat variability in space and time (Bowler and Benton 2005).

These causes, both proximate and ultimate, may interact with each other and understanding the complex causes of dispersal may be essential for determining the dispersal patterns of species (Ronce and Clobert 2012, Starrfelt and Kokko 2012).

1.2.2 Mechanisms of dispersal

There are two main categories of dispersal mechanism: passive dispersal and active dispersal. Passive dispersal is movement that is outside the organism's immediate control, and is usually carried out by external vectors such as gravity, wind, water currents or other organisms (Matthysen 2012). This type of dispersal is common in plants, microbes and very small invertebrates with low mobility. Active dispersal is where the organism controls its own locomotion. Most animals have the ability to walk, run, swim or fly; movement whereby the organism exerts force on its external environment and accelerates in the opposite direction (Dickinson 2000). Active dispersal may sometimes have passive elements, such as flying or swimming animals getting displaced by currents. Conversely passive dispersal has active elements, such as the timing of release of plant propagules or take-off of ballooning spiders to coincide with suitable wind conditions (Bonte et al. 2007).

1.2.3 Outcomes of dispersal

The consequences and outcomes of dispersal are what make it such an important topic of study in ecology. On an individual level, dispersal can improve fitness by removing individuals from an unfavourable habitat (e.g. high levels of inbreeding and resource depletion) to a habitat with adequate resources (i.e. food and mates; Clobert et al. 2001).

Gene flow is an important outcome of dispersal. Dispersal can decrease the likelihood of extinction of small populations by alleviating the effects of genetic drift and reducing mutation load (Tallmon et al. 2004). Dispersal can also alleviate inbreeding and the negative fitness effects of kin competition (Roze and Rousset 2005) but may contribute to outbreeding depression (Brown 1991). The increased gene flow from dispersal would be expected to prevent evolution and local adaption, (Lenormand 2002) but it can increase local adaptation (Gandon et al. 1996) and as such play a role in speciation (Barton 2001).

Dispersal has important effects on species' meta-population dynamics (Benton and Bowler 2012). The number of individuals in a population will be affected by immigration and emigration as well as birth and death rate. Immigration to a small population can rescue it from extinction and dispersal can lead to re-colonisation of patches after local extinction (Brown and Kodric-Brown 1977, Hanski 1999). Thus dispersal is important in meta-population dynamics, which are characterised by ongoing processes of extinction and colonisation in networks of connected patches (Hanski 1999). According to source-sink population dynamics, dispersal may permit some populations to persist even though they have negative growth rates (sink populations), if they are sustained by emigration from 'source' populations (i.e. intrinsic rates of increase > 0;Gundersen et al. 2001).

Many of these reasons highlighted above explain why dispersal would be advantageous and selected for in populations, but it is a costly process. There is a risk of increased mortality during the transfer stage that may be due to increased predation risk, use of non-optimal habitat, and lack of success in finding new habitat (Ronce 2007). Similarly at the immigration stage movement into suboptimal habitats that are lacking in resources, or to which the immigrant is poorly adapted, could lead to higher mortality. There are also the energetic costs associated with dispersing, as well as costs of producing the morphological features necessary to disperse (Bonte et al. 2012). These costs and benefits of dispersal may be involved in a resource allocation trade-off with other life-history traits, most prominently fecundity (Roff and Fairbairn 2001).

1.3 Dispersal, climate change and habitat fragmentation

Increasing our understanding of the processes that influence dispersal is of particular importance in an environment of climate change and habitat fragmentation (Gibbs et al. 2010b, IPCC Stocker et al. 2013, Millennium Ecosystem Assessment 2005). We would expect the distribution of species to shift to track climate change, which in the northern hemisphere means moving northwards and/or uphill as temperatures increase (Hughes 2000, Parmesan and Yohe 2003, Parmesan 2006, Hughes et al. 2007, Hargreaves and Eckert 2014). Range shifting has been demonstrated in a wide range of species spanning mammals, birds, fish, insects and other arthropods (Hickling et al. 2006, Chen et al. 2011). Insects in the order Lepidoptera have also followed this trend; a study of 35 non-migratory European butterflies revealed that 63% of species had shifted their ranges northward by 35–240km in the last century (Parmesan et al. 1999) over a time when the climate had warmed by 0.8°C. This shift is continuing, but not at a consistent rate over time (Mair et al. 2012). In order to track their bioclimate 'envelopes' efficiently, species must have a sufficient level of dispersal ability (Warren et al. 2001, Pearson and Dawson 2003), and there are signs that species distribution shifts are lagging behind the changing climate (Menendez et al. 2006, Mair et al. 2012).

The destruction, fragmentation and composition change of natural vegetation cover has been occurring for a long time, mainly due to conversion of natural habitats to agricultural land. This process leaves remnants of suitable habitats for the native biota (Saunders et al. 1991). In a landscape of increasing habitat loss, dispersal ability is hypothesised to be important for population persistence because it enables individuals to find new habitat, and evolutionary increases in dispersal ability are evident in newly-colonised meta-populations and during range expansion (Hanski 1999, Hughes et al. 2007, Berg et al. 2010). Species with high levels of mobility have been shown to have lower levels of regional decline and extinction in fragmented landscapes, whereas species with lower mobility are generally more sensitive to the effects of habitat fragmentation. Natural barriers to dispersal and fragmented habitats may prevent species from dispersing at a rate to track climate, and so distribution of suitable habitat and dispersal ability may be crucial if species are to persist and respond to climate change (Hill et al. 1999c, Warren et al. 2001, Pearson and Dawson 2003, Berg et al. 2010). The detrimental interaction of climate change and habitat destruction is likely to be a very difficult challenge for species (Thomas et al. 2004), and dispersal ability is likely to be important in influencing the long-term persistence of species (Travis 2003).

1.3.1 Implications for conservation

The dispersal ability of species can be an important force in determining whether or not populations and species persist (Hanski 1999). It has been demonstrated that species with lower dispersal abilities are more sensitive to the effects of habitat fragmentation (Tscharntke et al. 2002). Despite this, dispersal is rarely considered in a conservation context because of the difficulty in quantifying dispersal ability and the distances moved by individuals (Nathan 2005). Our limited knowledge of dispersal makes it hard to select management options that are likely to be optimal for species' conservation (Driscoll et al. 2014). This thesis addresses this lack of knowledge of dispersal for noctuid moths (Chapters 2 & 3).

As well as variation among species, dispersal ability can also vary within species (Stevens et al. 2010a). It can vary across the range of a species due to different selection pressures at distribution core versus range edge (Dytham 2009), in areas of high and low habitat disturbance (Travis and Dytham 1999) and in relation to isolation of habitat patches (Hanski 1999). There can also be individual variation in dispersal ability due to differences in sex, age, and morphological and physiological variation (Chapter 4).

1.4 Macro-moths

Insects are the most speciose taxonomic group globally and are vital to many ecosystem functions (Gullan and Cranston 2000). Estimates vary widely but generally indicate that there are ~ 800,000 described species and total insect species estimates are for upwards of 2 million species (Chapman 2009). Of these, one of the most species-rich orders is the Lepidoptera with around 160,000 species worldwide (Kristensen et al. 2007) and 2600 species recorded in Britain. In Britain there are around 900 macro-moths of which approximately 400 species belong to the family Noctuidae, which is the focus of this thesis (Waring et al. 2009).

Macro-moths are an excellent study group because of their role in ecosystems and their history of amateur and professional entomological interest, leading to good information on their ecology and distributions (Leverton 2001). Macro-moths provide a food resource for bats, birds, small mammals and other invertebrates (Merckx et al. 2009). Adult moths can be an essential food resource for bats (Vaughan 1997, Wickramasinghe et al. 2004) while larvae provide resources for insectivorous birds (Torok et al. 2004, Hogstad 2005, Visser et al. 2006). Some moth species are pollinators (Pettersson 1991, Devoto et al. 2011) and others are high profile agricultural pests (Carter 1984, Scoble 1995, Rose et al. 2000, Lammers and MacLeod 2007). The ecological diversity and species richness of macro-moths makes them good representatives of the wide range of terrestrial insects in Britain (Conrad et al. 2006). Luff and Woiwood (1995) explained why macro-moths are important indicator species of land-use and environmental change; because they are diverse, taxonomically stable, easy to monitor and vary with seasons. They are also phytophagous and hence provide information on changes in plant community composition. There have been a number of studies confirming the validity of moths as indicator species - where changes in moth species assemblages have reflected changes in the entire local community (reviewed in New 2004).

The Rothamsted Insect Survey (RIS) monitors macro-moth populations using a network of light traps around the UK, which have been sampling macro-moths consistently and continuously for 50 years (Woiwood and Gould 2008, Harrington 2014). This monitoring scheme has been acknowledged as being one of the most useful means of providing indicators of environmental change (Thomas 2005). Analyses by Conrad *et al.* (2006) and Fox *et al*. (2013)of the RIS data for 337 common and widespread British macro-moth species found that only one third had stable or increasing abundance trends during the last 35-40 years. The other two thirds of the studied macro-moth species experienced declines, many of them at a rate of greater than 30% per decade. IUCN criteria were applied to these abundance trend data and it was found that 71 species should be categorised as ‘threatened’ (Conrad et al. 2006). As a consequence of this analysis, these threatened species were subsequently added to the UK Biodiversity Action Plan to encourage research into the causes of declines, where they joined 81 other moth species (both micro- and macro-) that were considered ‘Priority Species’ for conservation effort (Parsons and Davis 2007). Similar declines in abundance to those in the UK have also been recorded in macro-moth assemblages in The Netherlands, where two thirds of 733 species declined in abundance (Groenendijk and Ellis 2011). In a nature reserve in Sweden, a fifth of local macro-moth species disappeared from the site between 1950s - 2004, with a much smaller fraction of new colonisers during this time period (Franzen and Johannesson 2007). Severe declines in the distributions of macro-moths have also been observed in Finland (Mattila et al. 2006, 2009), and changes in macro-moth distributions are also apparent in the UK (Fox et al. 2011, Fox 2012).

Due to the ecological importance of macro-moths, it is essential to understand the factors affecting their population declines. A wide range of drivers has been suggested, including habitat loss and fragmentation, climate change, agricultural intensification, urbanization and light pollution (Frank 1988, Conrad et al. 2004, Fox 2012, Bates et al. 2014). Of these factors, habitat destruction/degradation and climate change seem to be the most prominent (Conrad et al. 2004, Fox et al. 2014). As outlined above, dispersal ability of species is likely to be a key trait that determines how well species may cope with the combined pressures of habitat fragmentation and climate change. It has been shown that the mobility of Lepidoptera may determine the level of improvement in moth abundances yielded by biodiversity conservation schemes such as wide field margins, protected 'corridors' and 'stepping stones'(Merckx et al. 2009, Leidner and Haddad 2011). Thus, dispersal ability is likely to play a large role in the population dynamics of individual moth species, as has been demonstrated in butterflies (Warren et al. 2001, Pöyry et al. 2009, Burke et al. 2011, Mattila et al. 2011), but to date relatively few studies have focussed on macro-moths.

This thesis examined noctuid moths, a large and diverse macro-moth family, with species spanning a range of sizes and dispersal abilities, including trans-continental migrants (Chapman et al. 2012). It is difficult to quantify dispersal directly in macro-moths because most species are nocturnal, making it difficult to observe them flying under natural conditions. Relatively few studies have succeeded in quantifying the dispersal ability of macro-moths (but see Nieminen 1996, Betzholtz and Franzen 2011, Slade et al. 2013), but due to their indicator status, ecological importance and declining populations Betzholtz and Franzen (2011) have stated that for moths there is 'an urgent need for an increased knowledge of mobility and associated traits'.

1.5 Physiology and aerodynamics of flight

Birds, bats and insects have the benefit of flight which allows them much greater mobility and opens up new microhabitats (Gullan and Cranston 2000). Moths are winged insects and as such flight ability is key to their dispersal potential. An insect wing is a thin membrane supported by a system of tubular veins. There are two types of muscle that power the wings: direct flight muscles, which are inserted into the wing from the thorax, and indirect flight muscles, which power the wing by distorting the thorax (Chapman 1982). The work done by flight muscles to move the wings is powered by the oxidation of fuel in the form of carbohydrate (such as nectar) or fat (Pennycuick 1972). Chapter 4 investigates the effect of food resources on flight performance.

Lepidopteran wings are covered in scales which contribute to the lift but do not affect drag (Chapman 1982). Moths, like all insects, have a 'Tarsal reflex' which means that when the legs lose contact with the ground the flight muscles are activated (Gullan and Cranston 2000). This is useful when studying flight as it can be easily initiated.

To fly an organism must not only generate the thrust used to propel themselves in the chosen direction but must generate enough lift to stay aloft (Dickinson 2000). The wing is an aerofoil, as air flows over it high pressure is created below the wing and low pressure above, which generates lift. The distance a flying organism can travel is affected by the ratio between lift and drag, and the amount of fuel it is carrying to power the flight (Pennycuick 1972).

Basic fluid dynamics apply to all flying and swimming organisms. Due to their relatively small size, insects experience lower 'Reynolds numbers'. This means that the fluid (air) that they fly in is very viscous for them, their flight can be compared to swimming in molasses (Dudley 2001). Unlike birds both bats and insects generate a leading-edge vortex on their wings, which increases the amount of lift they experience (Ellington et al. 1996, Muijres et al. 2008). Finally, insects, unlike the birds and bats, are ectothermic and thus heavily constrained by temperature (Nation 2002).

1.6 Quantifying dispersal ability in Lepidoptera

There are many approaches to quantifying dispersal ability that have been applied to Lepidoptera and other insects. Some methods permit dispersal or flight ability to be recorded *in vivo,* including field observations of flight performance to quantify flight speed, time in flight and habitat boundary crossing behaviour (Shreeve 1981, Kallioniemi et al. 2014). Vertical-looking entomological radar can be used to record the direction, heading, speed and sometimes wing-beat frequency of individual moths migrating at high altitude (Chapman et al. 2011).

Cage experiments allow insects to fly naturally but within a constrained area to permit observation of flight propensity, and behaviour at habitat boundaries (Norberg et al. 2002, Hanski et al. 2006, Turlure et al. 2011, Wang et al. 2011). Using the harmonic radar technique (Chapman et al. 2011), in which a tagged insect is tracked by radar, allows detailed investigation of local foraging movements, but this technique is not suitable for longer-range dispersive movements. Observations of vagrants i.e. a species observed in an area where its host plants are not present, are an effective way of capturing differences in dispersal ability among species (Cook et al. 2001, Stevens et al. 2010b). Observation can also be used to classify moths as 'highly mobile' if they occur on islands distant from other islands or mainland areas where there are resident populations (Betzholtz and Franzen 2013). Similarly, expert opinion has been shown to be a reliable way of assessing butterfly dispersal ability that correlates well with less subjective assessments (Cowley et al. 2001, Komonen et al. 2004, Burke et al. 2011), but it has been noted that this tends to reflect migration tendencies rather than dispersal ability (Stevens et al. 2010b).

Multisite mark-release-recapture (MRR) is a commonly-used method for assessing dispersal ability. Data from recaptures can be used to calculate various measures of dispersal of populations and individuals, including the mean daily displacement and dispersal fraction or frequency (proportion of recaptures that were in in a different patch to first capture)(see Stevens et al. 2010b for a review of MRR studies in butterflies). The dispersal kernels of populations can also be calculated, which is the probability distribution of the distance travelled by any individual (Nathan et al. 2012). MRR using butterfly nets has been used to quantify dispersal frequency in day-flying moths (Franzen and Nilsson 2012) and light traps and/or sugar-bait traps have also been used to quantify dispersal distances and dispersal frequencies in nocturnal moths (Nieminen et al. 1999, Merckx et al. 2009, Slade et al. 2013). MRR is widely used and has yielded much information on dispersal in butterflies and increasingly in moths, but dispersal distances can be biased by the study area size (Schneider et al. 2003, Franzen and Nilsson 2007) making the development of alternative and more reliable methods important.

Genetic structure of populations can also be used to infer rates of successful dispersal based on gene flow (resulting in reproduction). F_{ST} is a measure of the genetic correlation between individuals in a subpopulation relative to the entire population (Holsinger and Weir 2009), and has been used to calculate the relative mobility of butterfly species (Stevens et al. 2010b). However, as with MRR studies, this technique is sensitive to the spatial extent of sampling and there are concerns that it is difficult to distinguish between the effects of genetic drift and the effects of gene flow (i.e. to detect successful dispersal) (Stevens et al. 2010b, Baguette et al. 2013).

Laboratory studies provide an opportunity to study flight and dispersal under controlled conditions, which can be important for ectothermic animals such as macro-moths whose activity may be greatly affected by abiotic conditions. A variety of laboratory techniques have been developed to measure flight ability, including methods for measuring free-flying insects on ‘air-treadmills’ or vertical flight chambers (Kennedy and Booth 1963, Blackmer et al. 2004), as well as methods involving tethering individuals (Dingle 1965). Tethered flight mills have been used successfully for a wide variety of insects including flies (Kaufmann et al. 2013), beetles (Moriya 1995, Van Dam et al. 2000, Dubois et al. 2009, Taylor et al. 2010), leafhoppers/true-bugs (Taylor et al. 1992, Zhou et al. 2003), lacewings (Liu et al. 2011), grasshoppers (Kent and Rankin 2001), honeybees (Brodschneider et al. 2009), woodwasps (Bruzzone et al. 2009), butterflies (Berwaerts et al. 2002), and moths (Gatehouse and Hackett 1980, Murata and Tojo 2004, Hashiyama et al. 2013). Insects can be tethered in various ways to assess orientation, flight propensity and flight duration. This topic is covered in greater detail in Chapter 2, where a novel tethered flight apparatus developed for this project is described.

It is also possible to measure flight metabolic rate as a means of quantifying dispersal ability. Metabolic rate can be quantified by measuring CO_2 production as the insect is flown in a sealed container (Haag et al. 2005, Niitepold et al. 2009). The ATP/ADP ratio of insect tissues that are flash frozen immediately after flight activity can be measured, with a higher ratio indicating greater ability to sustain activity (Hanski et al. 2004). The aerobic capacity of insect flight muscles has been measured by Cytochrome-c Oxidase concentration and mitochondria size and amount (Rauhamäki et al. 2014). Similarly the presence of certain alleles of the gene encoding for phosphoglucose isomerase (*pgi*) enzyme have been linked to elevated flight metabolic rate and longer flight distance (Watt et al. 1983, Haag et al. 2005, Niitepold et al. 2009). These molecular methods provide useful quantification of flight ability but are outside of the scope of this study.

1.7 The relationship between morphology and dispersal ability

Biologists have long been interested in the link between morphological features and flight, as a means of better understanding and predicting movement capabilities (Dixon 1892, cited in Hein et al. 2012). In addition, due to the difficulty in measuring dispersal directly, morphological traits can act as useful proxies for movement capability (Sekar 2012). A list of morphological features that have been associated with dispersal ability is shown in Table 1.1 and these features are explained further in Chapter 3.

Intra-specific variation in dispersal ability and morphological traits is expected to occur in moths because there are high levels of intra-specific variation in flight morphology in butterflies (Stevens et al. 2010a). This is explored in Chapter 4.

Table 1.1. *Morphological features that have been shown in literature to be related to dispersal ability*

Morphological feature	Relationship with dispersal ability	References
Body size	Dispersal ability/distance moved increases with size	(Kuussaari et al. 1996, Nieminen et al. 1999, Greenleaf et al. 2007, Jenkins et al. 2007, Hein et al. 2012) but see (Skorka et al. 2013)
Wing length/Wing span	Dispersal ability/flight speed increases with wing length/span	(Dudley 1990, Berwaerts et al. 2002, Burke et al. 2011, Sekar 2012, Stevens et al. 2012, Skorka et al. 2013)
Wing aspect ratio - higher value means longer,narrower wings.	Dispersal ability/distance moved increases with aspect ratio	(Winkler and Leisler 1992, Yao and Zhang 2001, Hughes et al. 2007, Bowlin and Wikelski 2008, DeVries et al. 2010) but see (Hill et al. 1999a)
Wing loading - higher values mean greater weight for the size of the wings	Longer and faster flights with increasing wing loading	(Betts and Wootton 1988, Berwaerts et al. 2002)
Thorax width (and proportional thorax width) -contains flight muscles	Dispersal ability increases with thorax width	(Dempster 1991, Hill et al. 1999a, Hill et al. 1999c)
Thorax mass (and proportional) -contains flight muscles	Dispersal ability increases with thorax mass	(Chai and Srygley 1990, Srygley and Chai 1990, Berwaerts et al. 2002)

1.8 Project aims

Dispersal ability of British macro-moths may determine whether or not their species persist in a changing environment, but knowledge of individual species dispersal abilities is severely lacking. The main aim of the thesis is to quantify the dispersal ability of noctuid moths, deduce which morphological features are good predictors of flight performance, and investigate the links between dispersal capacity and population trends.

In this thesis I quantify the dispersal ability of a number of noctuid macro-moth species. This study was achieved by the development of a new tethered flight technique, and in Chapter 2 I answer the question of whether flight performance on the mills is representative of natural flight ability by comparing the flight performance of moth species on flight mills with expert opinion. In Chapter 3, I explore the relationship between adult flight morphology and flight performance on the tethered flight mills, to answer the questions:

- Are morphological features useful predictors of tethered flight performance?
- If so, which morphological features are the best predictors of flight performance and how much of the variation in flight performance can they explain?

In Chapter 4, I ask whether additional variation in tethered flight performance can be explained by moth sex, age and resource availability. In Chapter 5, I use measures of dispersal ability (tethered flight performance) and morphological features from Chapter 3 to investigate the role of dispersal ability in the population dynamics and distribution changes of noctuid moths in Britain, to test whether moth with greater dispersal ability are faring better than less mobile species.

2 Chapter 2 – A novel automated tethered flight technique to quantify inter-specific variation in insect dispersal ability.

2.1 Abstract

Dispersal plays a crucial role in many aspects of species' life histories, yet is often difficult to measure directly. This is particularly true for many insects, especially nocturnal species (e.g. moths) that cannot be easily observed under natural field conditions. Consequently, over the past five decades, laboratory tethered flight techniques have been developed as a means of estimating insect flight duration and speed. Here I describe novel apparatus that allows the comparison of flight ability in a wide range of insect body sizes and types. The novelty arises because previous designs were only suitable for studying a single species (typically migrant pests). Such multi-species comparisons are important for obtaining a better understanding of dispersal for insect population dynamics and range shifts as well as parameterising mathematical models.

Here I describe a new laboratory tethered flight technique that automatically records flight duration and speed of individual insects. The new rotational tethered flight mill has very low friction and the arm to which flying insects are attached is extremely lightweight while remaining rigid and strong, permitting both small and large insects to be studied. The apparatus is compact and thus allows many individuals to be studied simultaneously under controlled laboratory conditions. I demonstrate the performance of the apparatus by using the mills to assess the flight capability of 24 species of British noctuid moths, ranging in size from 12 – 27 mm wing length (~0.04-0.66g body mass). I validated the new technique by comparing tethered flight data with existing information on dispersal ability of noctuids from the published literature and expert opinion.

Measures of total distance flown overnight and maximum flight speed – computed from tethered flight data – characterised the main differences in flight mill activity among the study species. Values for these tethered flight variables were in agreement with existing knowledge of dispersal ability in these species, supporting the use of this new method to quantify dispersal in insects. Importantly, this new technology opens up the potential to investigate genetic and environmental factors affecting insect dispersal among a wide range of species.

2.2 Introduction

Dispersal is a key facet of species' ecology and evolution, and it has profound effects on population dynamics, gene flow, species distribution and range size (Clobert et al. 2001, Bowler and Benton 2005, Lester et al. 2007, Stevens et al. 2010b). Dispersal, and these ecological characteristics influenced by it, can affect the evolution of life-history traits and speciation (Ronce 2007). Increasing our understanding of dispersal is of particular importance in an environment of accelerating climate change and habitat fragmentation (Hughes et al. 2007, Gibbs et al. 2010b) because dispersal is important for range shifting (Hill et al. 1999b, Pearson and Dawson 2003) and meta-population dynamics (Hanski et al. 2000). However, obtaining direct measures of dispersal ability can be challenging, making it important to develop new tools for measuring species' flight capability.

Insects play key roles in ecosystems (e.g. as predators, prey, pests and pollinators), and also demonstrate wide variation in dispersal ability (from highly sedentary species to continental-wide migrants). Dispersal is important in many aspects of insect life history (e.g. finding mates, new food sources and breeding habitats, and escaping crowded conditions), but direct measures of dispersal are often difficult to obtain. In addition, field studies using mark-and-capture methods can be confounded by environmental factors and biased by study methods (Schneider et al. 2003). By contrast, the advantages of lab studies are that they provide opportunities to study dispersal under controlled and experimental conditions. Over the past 50 years, a variety of laboratory techniques has been developed to measure flight ability, including methods for measuring free-flying insects (Kennedy and Booth 1963) as well as methods involving tethering individuals (Dingle 1965). Insects can be tethered in ways that allow them to change their orientation, which is useful for studies of seasonal migration direction (Mouritsen and Frost 2002, Nesbit et al. 2009). Tethered flight may also allow insects to repeatedly take-off and land and thus assess propensity for flight (Gatehouse and Hackett 1980). Insects can also be tethered on a flight mill that allows them to fly round in a circle to assess flight duration (Chambers et al. 1976, Beerwinkle et al. 1995). Here I extend these previous methods, and describe a new tethered flight apparatus. The unique features of the method are: roundabout-style apparatus allowing flight distance, duration and speed to be quantified on the same individual insect; compact multiple units allowing many individuals to be recorded simultaneously; very low friction bearings and magnetic suspension system to minimize the degree of friction associated with turning the arm during flight; and a lightweight but rigid tethered flight arm, allowing a wide range of species to be flown (from min 10 mm, to max 40 mm wing length). The system for attachment of the insect to the flight mill by a rigid wire handle attached to the top of the thorax allows for ease of handling, facilitating weighing and feeding and minimising stress to the insect during preparation. This system records flight distance to the nearest 10 cm and is updated every 5 seconds, providing the most fine scale speed data currently available. I have also tested and assessed the bespoke software developed for downloading and summarising flight data.

Here I describe the new apparatus which was designed to quantify flight ability in the lab, and illustrate its capabilities by using it to examine differences in flight ability of 24 species of British noctuid moths. This family was chosen because it includes species with a wide range of different dispersal abilities (including continental migrants), body sizes, and life histories, and so illustrates the potential of the apparatus. First I describe the new tethered flight mill system. Secondly I explore the different flight measures that are recorded, and assess how these data characterise moth flight behaviour and dispersal ability. Finally the data gathered by the new apparatus is validated by demonstrating that differences in flight mill performance reflect differences in dispersal abilities under natural conditions. I discuss how these direct measures of flight ability could be used to better understand dispersal ability across a wide range of insect species.

2.3 Methods

2.3.1 Tethered flight mills and their operation

An illustration of a flight mill is shown in Figure 2.1. Each mill consists of a lightweight arm suspended between two magnets. This magnet suspension provides an axis with very little resistance, so even relatively weak fliers can turn the mill successfully. The arm is very lightweight but suitably rigid due to a unique construction method (Patent: Lim et al. 2013). The insect is attached to one end of the arm as shown in Figure 2.1b and flies in a circular trajectory with a circumference of 50 cm. A disk with a banded pattern is attached to the axis so that it turns with the arm, and a light detector detects the movement of the bands to record the distance flown and the flight speed. The tethered flight mill system currently has 16 channels (arms) allowing 16 individual insects to be flown simultaneously (but could easily be extended to include more channels). Flight data are automatically downloaded to a computer. The embedded microcontroller board records the distance flown by the insect to the nearest 10 cm and updates the computer with the distance travelled in five second intervals, an example of the raw data generated is shown in Appendix A.

2.3.2 Preparing insects for study

Noctuid moths captured in light-traps on site at Rothamsted Research, Harpenden (51.809° N, -0.356° W) during summer 2013 were used in flight mill trials. Visual inspection of wing wear was used to ensure only recently-emerged adults were flown, to constrain variation in flight according to adult age. Following Thomas (1983), wing wear was assessed on a four point scale; fresh (4), good (3), poor (2) and worn (1); only stage three and four individuals were tested (Appendix B).

Individuals were stored in a domestic fridge during the day. Moths were removed from the fridge and restrained under netting, scales were removed from the upper surface of the thorax using sticky tape, and then 'handles' were attached with contact adhesive (Appendix B), before being returned to the fridge. This system of having a short handle attached to the moth facilitates weighing and feeding prior to the insect being attached to the flight mills. This attachment also enables moths to be flown on successive nights with minimal stress to the animal (but this was not done in these trials). About two hours prior to flight, moths were removed from the fridge, weighed, and then given 20% honey solution *ad libitum*. They were then re-weighed to verify feeding and attached to the flight mill with a piece of paper to hold on to and left until the lights were switched off at 21:00 BST. Each moth was flown on only one night. The flight mills were housed in a controlled environment insectary room at 18°C and 18L: 6D, which is equivalent to midsummer in the UK. Lights were gradually dimmed during the one hour before and after the night-time dark period to simulate dawn and dusk.

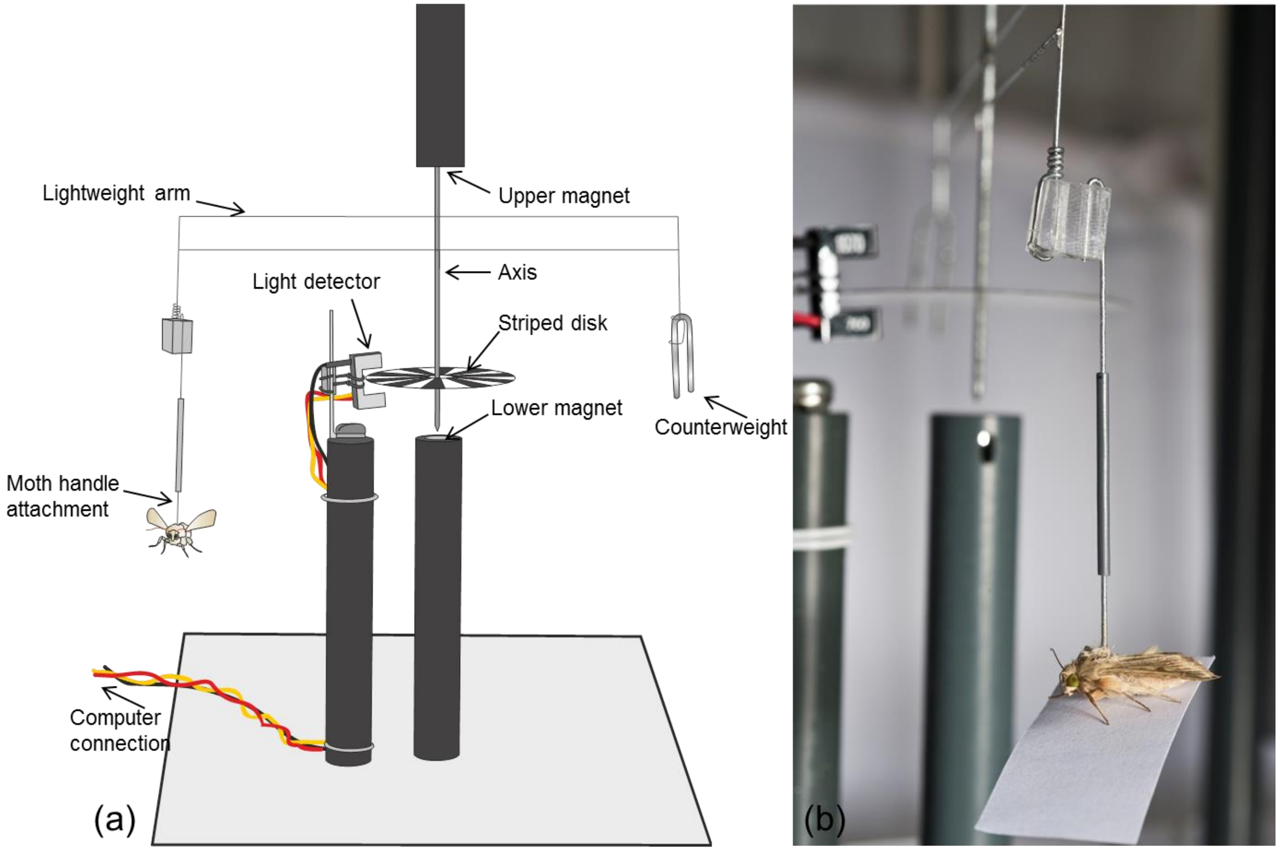

Figure 2.1. *Tethered flight mills.* ***a.*** *Labelled diagram of an individual flight mill.* ***b.*** *Close up of the method of attaching the moth to the flight mill. Flight mills are patented (UK Patent Application No. 1314415.9).*

2.3.3 Analysing flight data

Data recorded by the flight mills are measures of distance flown (m), time spent flying (secs), and flight speed (m/sec) (Table 2.1). These data can be used to analyse measures of distance, duration and speed of specific flights (e.g. the first flight of the night, or the longest flight), and derive additional variables. Flight data for each individual moth for each 8 hour flight period (from beginning of 'dusk' to end of 'dawn') were processed using a script written in Matlab (The MathWorks Inc. 2012b) to extract the beginning and end time of each individual flight and calculate each flight's duration, distance and average speed. The maximum speed (calculated from the greatest distance travelled in any five second interval) is also extracted. These flight data were then processed in R (R Core Team 2013)to extract a total of 16 tethered flight variables (listed in Table 2.1). Multivariate analyses were carried out to examine which of these tethered flight variables were the most biologically informative. A matrix of pair-wise correlations was constructed and Principal Components Analysis used to ascertain levels of redundancy among variables. Canonical Variate Analysis determined which tethered flight variables were best for distinguishing between species.

The computer software records flight activity data every 5 seconds, but very short flights may not represent 'true' flights. Thus I examined the consequences of excluding short flights based on 13 thresholds of minimum flight length, from 5 seconds to 5 minutes. All analyses were repeated based on these 13 different cut-off thresholds to see whether definitions of what comprised 'true' flight affected the conclusions.

Table 2.1. *Measured and derived tethered flight performance variables extracted from flight mill data. Raw data are distance, duration, average speed and maximum speed of individual flights ≥10 seconds.*

Tethered flight variable	Definition	Units	PCA label
Total distance	Sum of distance covered by all flights	metres	Distance 1
Total duration	Sum of duration of all flights	seconds	Duration 1
Number of flights	Count of flights	numeric	NumFlights
Average flight distance	Mean of distances of flights	metres	Distance 2
Average flight duration	Mean of duration of flights	seconds	Duration 2
Average flight speed	Mean of the speeds of individual flights (calculated as distance/duration)	metres/sec	Speed 1
Maximum speed attained	Greatest distance attained in any 5 second interval/5 – of the whole night	metres/sec	Speed 2
First flight distance	Distance of first flight of the night	metres	Distance 3
First flight duration	Duration of first flight of the night	seconds	Duration 3
First flight average speed	Speed of first flight of the night(calculated as distance/duration)	metres/sec	Speed 3
First flight max speed	Greatest speed attained in any 5 second interval of the first valid flight	metres/sec	Speed 4
Furthest flight distance	Distance travelled in the flight of greatest distance of the whole night	metres	Distance 4
Longest flight distance	Distance travelled in the flight of greatest duration of the whole night	metres	Distance 5
Longest flight duration	Duration of the flight with greatest duration	seconds	Duration 4
Longest flight average speed	Speed of the flight with greatest duration(calculated as distance/duration)	metres/sec	Speed 5
Longest flight max speed	Greatest speed attained in any 5 second interval of the flight of greatest duration	metres/sec	Speed 6

2.3.4 Validating flight mill data

In order to test the assumption that tethered flight performance reflects natural dispersal behaviour in the wild, all study species were assigned to a mobility category based on two sources of information. First, I examined Rothamsted Insect Survey suction trap (Macaulay et al. 1988) data on the occurrence of moths in traps 12.2 m above the ground over the period 2000-2009 (Wood et al. 2009). I used the presence of a study species in the top 25% of all species caught 12.2 m above the ground to infer a strong likelihood of the study species to engage in long distance dispersal (Wood et al. 2009). Secondly, I carried out a survey asking experts to classify the study species according to whether species were relatively sedentary, mobile or very mobile (Appendix C). In order to assess how 'correlated' these two measures were, the gamma statistic was calculated in Genstat. The gamma statistic is a way of assessing the agreement between two variables measured using ordinal scales (Castellan 1988). This indicated that the two scores were weakly associated with one another (gamma=1.883 p=0.06). A Mann-Whitney U test demonstrated that species that were present in the suction traps had significantly higher ranks of expert score (U=32.5, p=0.024). The two sources of information were then combined to place the 24 study species into three categories: 'low', 'medium', and 'high' mobility (Table 2.2). An ANOVA was used to compare tethered flight variables among moth species assigned to these three mobility categories.

2.4 Results

2.4.1 Characterising dispersal ability with tethered flight

Males were more likely to be caught in light-traps than females, and so sample sizes were higher for males (495) than females (122). Given that there is likely to be intra-specific variation in flight between males and females (Berwaerts et al. 2006), and in order to maximise the number of species studied, all analyses are based on males only. In order to obtain robust measures for species, and to account for intra-specific variation in flight, I only included species with ≥8 individuals flown (hence 456 individuals were measured in total, median 15 individuals per species). Flight data for 24 species were included in the analyses (Table 2.2). There was no evidence of a bimodal distribution of flight durations (Figure 2.2), and so no logical place to set the threshold for 'true' flights versus 'noise'. The different cut-off points generated total distance data for each species that were very highly correlated (Appendix D) providing no support for excluding any of the flight data from subsequent analysis. However, because flight duration is, in the current set-up, always rounded up to the nearest 5 seconds by the recording equipment, any small movement by an insect on the mill would be recorded as a flight of 5 seconds. Thus I analysed all data for flights of 10 seconds or longer.

Table 2.2. *Summary table of 24 individual moth species flown on tethered flight mills. All individuals are males. Mobility category assigned by summing scores from suction trap data and expert survey. One point was assigned if species were in the top 25% of species caught in Rothamsted Insect Survey (RIS) suction traps (mean yearly catch over period 2000-2009). Expert opinion is the mean value of responses where 5 experts were asked to assign species to categories of low (0), medium (1) and high (2) mobility (see Appendix C). 'Score' sums these two methods of classification and mobility category was assigned according to thresholds: ≤1 = Low, >1 to ≤2 = Medium and >2 = High.*

Species	**N flown**	**Suction trap score**	**Expert opinion**	**Score**	**Mobility category**
Agrotis exclamationis	18	1	1	2.0	Medium
Agrotis puta	8	1	1.2	2.2	Medium
Amphipoea oculea	11		0.8	0.8	Low
Amphipyra pyramidea	14		0.8	0.8	Low
Apamea monoglypha	39	1	1.4	2.4	High
Autographa gamma	13	1	2	3.0	High
Axylia putris	14		0.6	0.6	Low
Hoplodrina alsines	13		0.8	0.8	Low
Hoplodrina ambigua	13		1.4	1.4	Medium
Hydraecia micacea	23		0.6	0.6	Low
Lacanobia oleracea	16		0.6	0.6	Low
Mesapamea didyma	10	1	0.8	1.8	Medium
Mesapamea secalis	16	1	0.8	1.8	Medium
Mythimna impura	11		0.8	0.8	Low
Mythimna pallens	19		0.8	0.8	Low
Noctua comes	26		1.2	1.2	Medium
Noctua janthe	13		1.4	1.4	Medium
Noctua pronuba	37	1	2	3.0	High
Ochropleura plecta	20		1	1.0	Low
Omphaloscelis lunosa	16		1	1.0	Low
Phlogophora meticulosa	10	1	1.6	2.6	High
Xestia c-nigrum	59	1	1.4	2.4	High
Xestia triangulum	12		0.8	0.8	Low
Xestia xanthographa	25	1	0.8	1.8	Medium

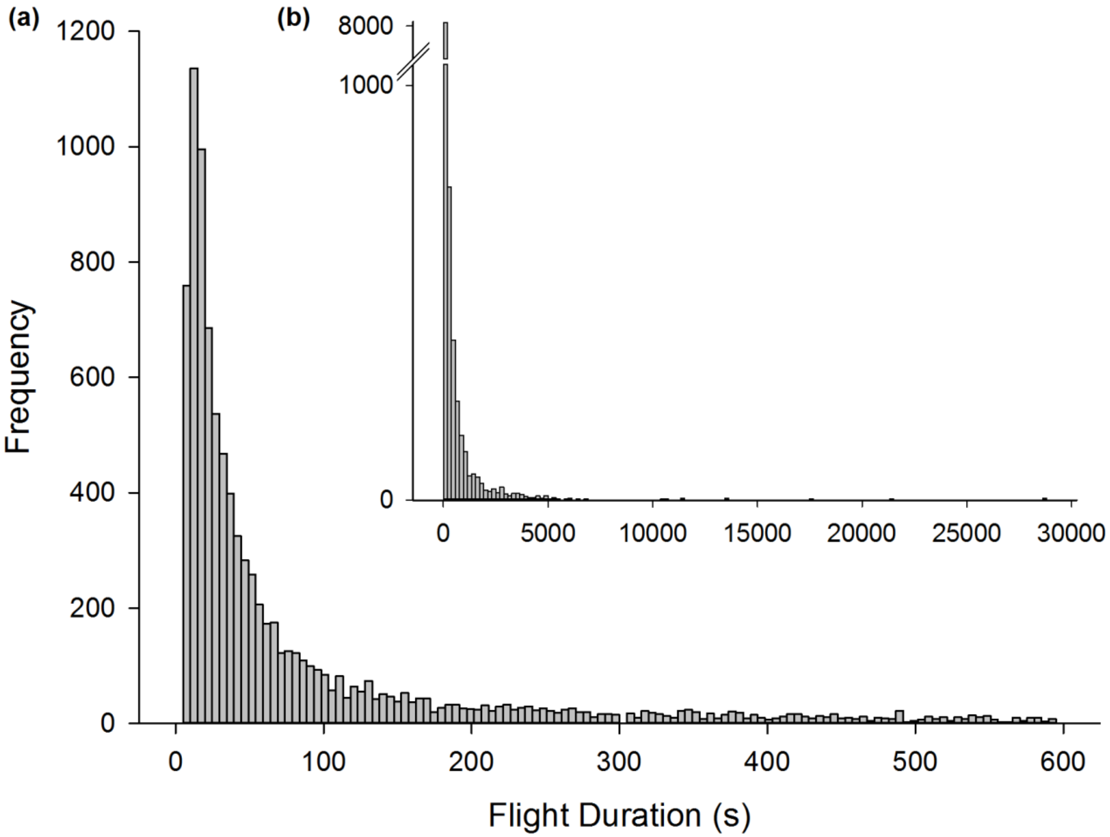

Figure 2.2. *Histograms of the frequency of flight durations derived by the tethered flight mills across all noctuid individuals studied.* ***a.*** *Flights up to ten minutes in duration.* ***b.*** *Inset graph shows all flights, with the y axis broken to show the tail of the frequency distribution more clearly. NB the axis break means that values for bins 2-6 are curtailed (frequency values of ~750,400,250,150,100).*

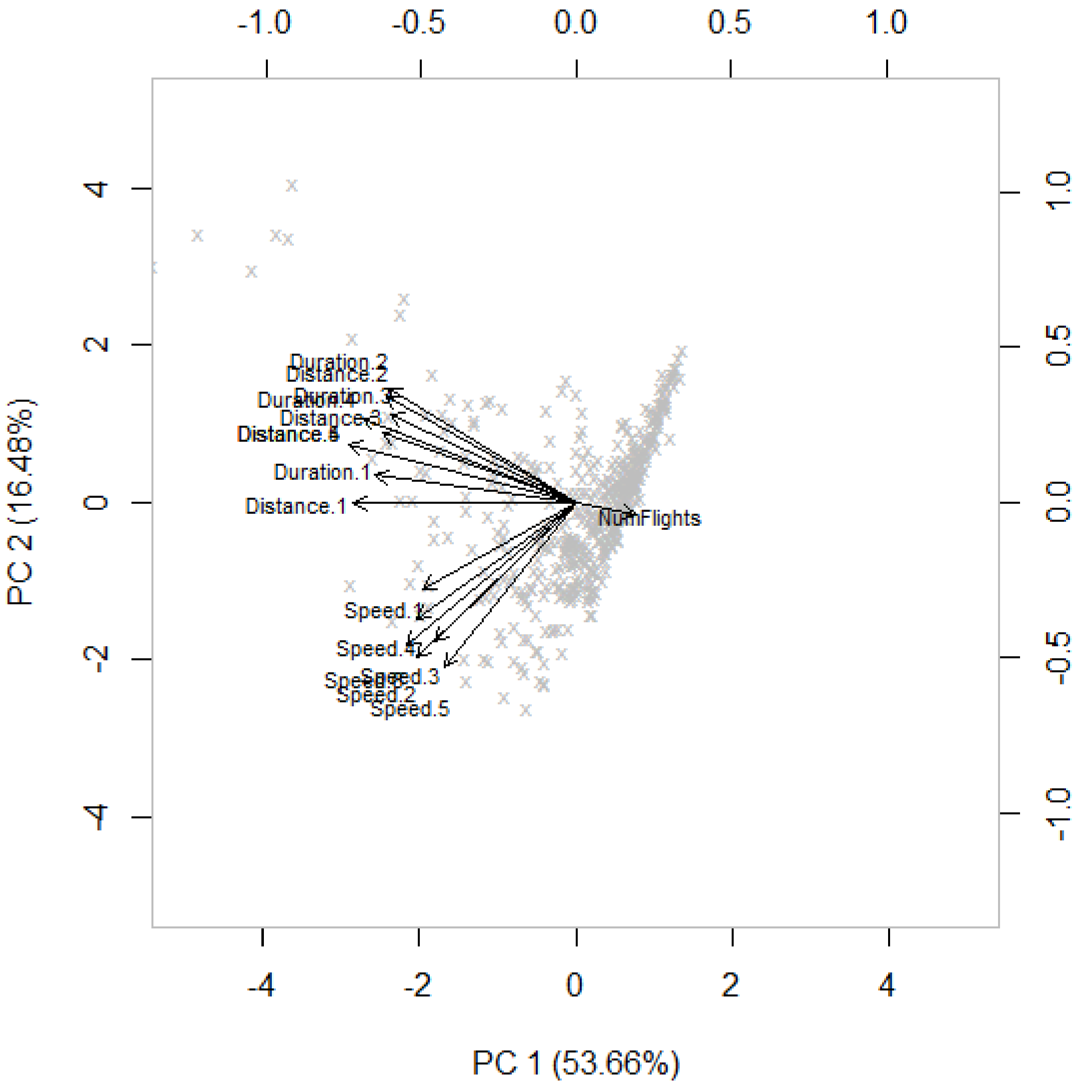

Figure 2.3. *Principal components analysis biplot of the 16 tethered flight mill variables listed in Table 2.1. The two first principal components are plotted with the proportion of variance explained by each component printed next to the axes label which together explain >70% of variation in the data. Crosses indicate the 456 male individuals in the data set; the top and right axes show principal component scores of the individuals. The arrows indicate the principal component loadings of the different tethered flight variables.*

All tethered flight variables (as outlined in Table 2.1) were highly correlated (Appendix F) and a Principal Components Analysis confirmed redundancy in measures (Figure 2.3), but that measurement of flight distance/duration and flight speed characterise different aspects of dispersal. A Canonical Variates Analysis (Appendix F) indicated that measures of flight speed best distinguished among moth study species. Thus I concluded that the two most informative variables for the study species were maximum flight speed attained in any 5 second interval of the night and total distance flown overnight.

2.4.2 Validating flight mill data

Moth individuals were assigned to three mobility categories (low, medium and high) according to their species scores in Table 2.2. Mobility category had a significant effect on both flight distance and speed (total distance: $F_{2,21} = 8.69$, $P = 0.002$; Maximum speed: $F_{2,21} = 4.61$, $P = 0.022$; Figure 2.4). A Tukey post-hoc test confirmed that the medium and low mobility groups had significantly shorter total distances than the high group, but there was no significant difference between the medium and low groups. Similarly for maximum flight speed, the low and high mobility groups were significantly different; however the medium group was not significantly different from either. Information on total distance and maximum speed are plotted as boxplots (Figure 2.4).

This analysis was also carried out for the two mobility measures separately (suction trap score and expert opinion from Table 2.2). In both these versions of the categories the higher mobility group had higher mean total distance flown, but there were no significant differences between categories in maximum speed (this analysis is outlined in detail in Appendix E).

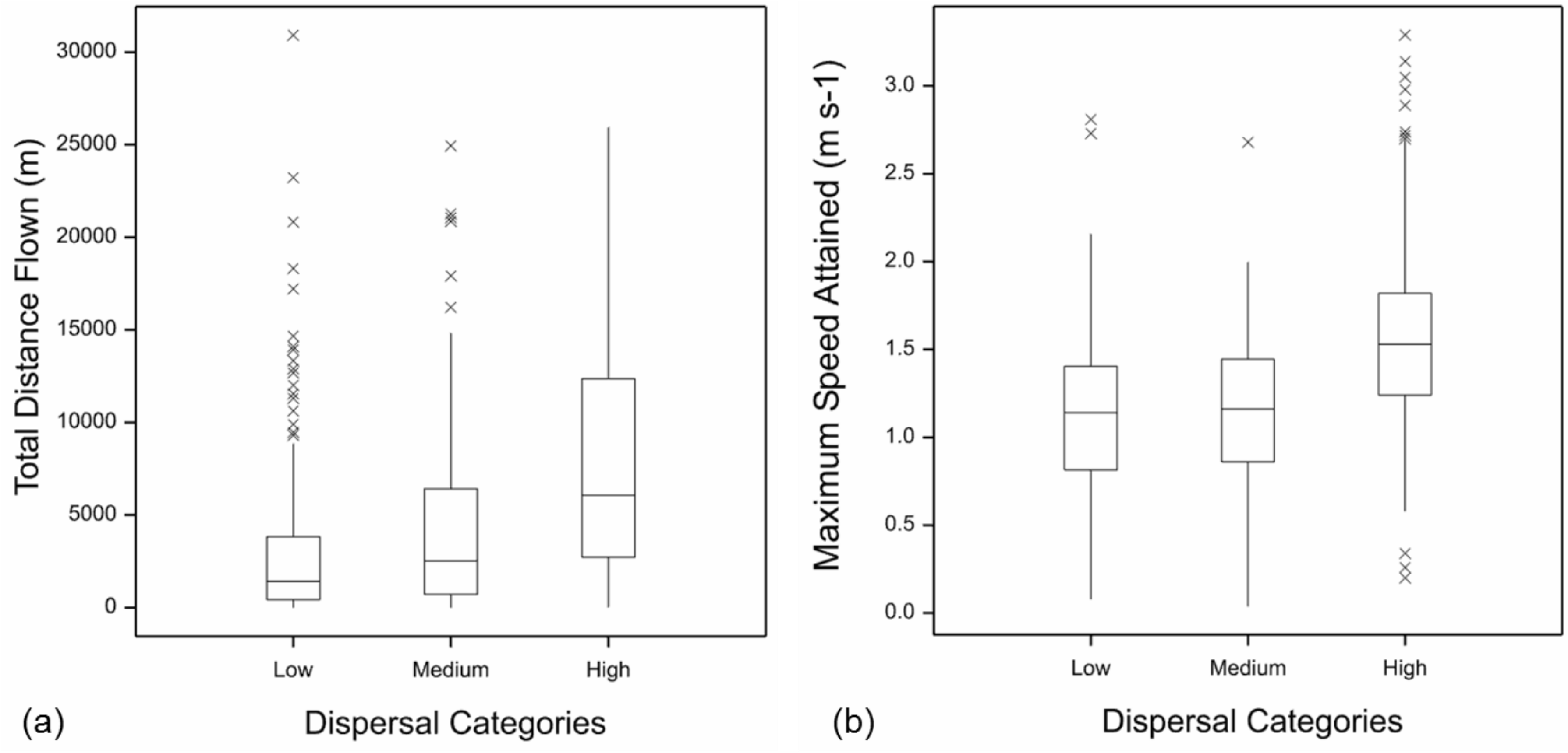

Figure 2.4. *Boxplots showing **(a)** total distance flown and **(b)** maximum speed attained on tethered flight mills of 456 individuals assigned to three dispersal categories according to their species (Table 2.2). Boxes span the interquartile range of values, with the line dissecting the box indicating the median. Whiskers extend to 1.5 times the interquartile range beyond the quartiles. Beyond this outliers are plotted as a cross.*

2.5 Discussion

In this study I present a detailed explanation of the function and methodology associated with a new tethered flight system that has enabled me to fly a wide variety of moth species (Patent pending: Lim *et al.* 2013). While other studies have used tethered flight mills to examine intra-specific variation in flight performance, e.g. in relation to sex, population, age and levels of sexual maturity (Mcanelly 1986, Schumacher et al. 1997, Berwaerts et al. 2006, Taylor et al. 2010), they lacked the technical capability to compare flight performances across a range of species.

2.5.1 Data generated by flight mills

The apparatus collects flight data in 5 second intervals, and I explored whether there was an appropriate place to draw a threshold at which flights could be classified as ‘noise’ or ‘true’ flights. The analyses generally indicated that there was no obvious threshold or change point; flight duration frequency followed a smooth curve indicating there were no abrupt behavioural differences in activity. Changing this threshold over the range 5 to 300 seconds did not change the pattern of the total distances flown among individuals, or qualitatively alter the conclusions about relationships with flight ability among the different study species. Thus I took a conservative approach to analyse all data from flights > 5 seconds.

Many different measures of flight performance can be obtained from the tethered flight mills, but my analyses show that only a few variables are necessary to summarize tethered flight ability in the study moth species. Most derived tethered flight variables are highly correlated, e.g. lengths of the first or longest flight are correlated with other measures of duration and distance. I conclude that flight data collected during tethered flight can be defined into three broad types of measurement; measurements of flight distance/duration, measurements of flight speed, and the number of flights (Figure 2.3). Thus my subsequent analyses focus on two measures that encompass these different aspects of flight ability; total distance flown and maximum flight speed, but a different set of tethered flight variables could be more relevant to other taxa. Insects fly for many reasons (e.g. avoiding predators, migrating, foraging, mate finding) and these different types of flight activity may require different flight behaviours (speed, manoeuvrability, endurance), many of which are recorded by the flight mills. Total distance flown provides a useful comparison to distinguish between species and individuals in relation to which have greater propensity to disperse and fly further in the field (Taylor et al. 2010), whereas measures of speed provide information on flight power of moths.

2.5.2 Flight mill validation

I have shown that the tethered flight data are representative of natural flight ability of species, and that species placed in the high mobility category (such as *Noctua pronuba* and *Autographa gamma;* group mean = 8178m*)* had mean flight distances 2.5 times that of species in the low mobility category (such as *Axylia putris* and *Hydraecia micaea*; group mean = 3263m). This is unsurprising as four of the five species in the 'high' mobility group (*A. gamma*, *N. pronuba, Phlogophora meticulosa* and *Xestia c-nigrum*) are known or suspected to be regular migrants (Waring et al. 2009). This is in contrast with the least mobile species, for which there is very little published information on dispersal ability. All the study species are noctuids and are relatively mobile compared with some other macro-moths (e.g. geometrids), but nonetheless there is variation in dispersal ability among the study species which was evident in flight mill data. In addition, there was some lack of consensus by experts on which moths belonged in the 'low' and 'medium' categories (Appendix C) highlighting the lack of knowledge on flight ability in many non-migrant species, and probably explaining why the analyses were generally less capable of distinguishing between the low and medium group compared with the high group. I conclude that the tethered flight mills are therefore an important new tool to elucidate dispersal ability in a wider range of species.

2.5.3 Potential for flight mill system

The tethered flight mills provide a platform to explore the relationship between measures of dispersal ability (such as flight speed and duration), and physiological, genetic and environmental factors that promote or inhibit flight. Insects can be flown after being caught from the wild, enabling assessment of the amount of variation in dispersal ability present in wild populations. Insects can also be flown having been reared under controlled conditions, which enables the effects of food availability, climate and disease levels during development on dispersal propensity to be assessed. The 'handle' by which the moths are attached to the mill is small and light compared to many other set-ups, enabling moths to be flown on sequential nights, and therefore age-related changes in flight behaviour can be easily quantified. Genetic and epigenetic factors affecting dispersal ability can also be assessed and compared across species. In addition, more robust quantification of dispersal ability would help greatly in the parameterisation of ecological models, such as those for metapopulation dynamics, pest management and disease transmission (Dwyer and Elkinton 1995, Hanski et al. 2004).

In addition to the flight mill apparatus outlined in this chapter, flight mills with longer arm lengths have been tested (data not shown) for large, powerfully-flying species such as the European hornet (*Vespa crabro)*, hawk moths (Sphingidae), bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*); and flight mills with extremely small and lightweight arms that have been used to quantify the flight ability of small, weak-flying insects including brown planthoppers (*Nilaparvata lugens)* and mosquitoes (*Aedes aegypti)*. Calibration methods that will enable the comparison of distances flown on different arm types are currently being developed.

I conclude that the new tethered flight apparatus provides a robust technique to assess the flight ability of insects. This tethered flight technique has enabled me to quantify the flight propensities of a wide range of different species of noctuid moth. This new tethered flight mill system opens up the potential to quantify the dispersal abilities of a much wider range of species for which current knowledge of dispersal is lacking, and to address a plethora of scientific questions about factors affecting insect dispersal.

3 Chapter 3 – Adult flight morphology is related to flight ability in a multi-species comparison of noctuid moths

3.1 Abstract

Dispersal is fundamental to many ecological processes but is difficult to quantify directly. Direct measures of dispersal are therefore unavailable for many species, especially insect species that e.g. fly at night and/or are too small for studies using tagging technologies. In this chapter, laboratory tethered flight techniques were used to assess whether easy-to-obtain indirect measures of dispersal based on adult morphology provide reliable measures of flight ability in noctuid moths.

Measures of forewing length (a measure of overall adult size), wing shape and thorax characteristics best summarised differences in flight morphology among 32 noctuid moth study species (median of 13 males measured per species). Tethered flight performance (total distance flown overnight, and maximum flight speed) increased with increasing forewing length, and proportional thorax mass explained an additional significant but small amount of variation. However, there was considerable intra-specific variation, and measures of morphology accounted for only ~30% of variation in flight performance among individuals across all species. By contrast, flight morphology measures were much better predictors of inter-specific variation in flight performance, explaining ~76% of the variation in flight performance among species.

In conclusion, adult morphology, especially measures of adult size, provide a good measure of dispersal ability, especially when distinguishing among species. The potential to use wing length measures to quantify dispersal ability across a wide range of noctuid moth species, and possibly other insects, will allow better understanding of factors affecting population dynamics and distribution trends in this taxonomic group, which contains species that are important pollinators, prey and pests.

3.2 Introduction

3.2.1 Dispersal

Dispersal is an important process in the ecology and evolution of organisms, affecting population dynamics, gene flow, species distributions and range size (Clobert et al. 2001, Bowler and Benton 2005, Lester et al. 2007, Stevens et al. 2010b). Quantifying dispersal is particularly important in an environment of accelerating climate change and habitat fragmentation (Hughes et al. 2007, Gibbs et al. 2010b) because of its importance for range shifting (Hill et al. 1999b, Pearson and Dawson 2003) and meta-population dynamics (Hanski et al. 2000).

However, obtaining direct measures of dispersal ability can be challenging, especially for flying animals. The movements of large birds can be recorded by attaching GPS satellite transmitters to directly record distances flown (Bridge et al. 2011, Klaassen et al. 2014). Smaller birds and bats can be tracked by other types of active tags that record the animal's position, but the tags either have to be recovered (Stutchbury et al. 2009), or come within range of a receiver (Sapir et al. 2014). Telemetry and harmonic radar techniques hold promise for directly tracking larger insects (Chapman et al. 2011, Kissling et al. 2014), but the range over which signals can be detected (typically a maximum of only a few hundred metres) restricts their use for studying longer dispersal events. In any case, the majority of insects are much too small to carry any sort of tracking device, and thus other measures of assessing dispersal must be used.

Mark-release-recapture (MRR) is a commonly used method for assessing dispersal ability, but field studies using this method can be confounded by environmental factors, are usually limited in study area extent relative to potential dispersal events, and are biased by study area size (Schneider et al. 2003). Cage experiments allowing insects to fly naturally but within a constrained area, and tracking individuals by following them, can assess flight propensity and behaviour at habitat boundaries (Turlure et al. 2011, Wang et al. 2011) but not long distance movements. By contrast with field studies, laboratory studies allow investigations of dispersal propensity under controlled experimental conditions. Techniques and equipment used in the lab include: vertical flight chambers (Kennedy and Booth 1963, Blackmer et al. 2004), forced flight cages (Gibbs et al. 2010a) and tethered flight (Dingle 1965). This study builds on these lab studies and uses a novel tethered flight technique (described in Chapter 2) to measure flight duration, distance and speed in noctuid moths.

3.2.2 Relating dispersal to morphology

Biologists have long been interested in the link between morphological features and flight, in order to better understand and predict movement capabilities (Dixon 1892, cited in Hein et al. 2012). In addition, due to the difficulty in measuring dispersal directly, morphological traits can act as useful proxies for movement capability (Sekar 2012). For example, across a selection of walking, swimming and flying animals, maximum migration distance was found to increase with body mass (Hein et al. 2012). There is evidence that greater body size is correlated with greater dispersal ability in flying animals such as birds, bats, bees, butterflies and moths (Norberg and Rayner 1987, Kuussaari et al. 1996, Paradis et al. 1998, Nieminen et al. 1999, Greenleaf et al. 2007). However this relationship starts to break down at larger sizes, because the energetic cost of flight increases rapidly with increasing mass (Pennycuick 1975, Hein et al. 2012).

Flight is dependent on wings, and so many wing measures are indicative of flight ability. Wing length is an important correlate of flight speed and dispersal in butterflies (Dudley 1990, Sekar 2012, Stevens et al. 2012) and dispersal distance and probability of dispersal in birds (Skjelseth et al. 2007). Wing aspect ratio, a measure of wing shape, may also be important: a higher aspect ratio indicates longer, narrower wings which are thought to allow for longer faster flights. Migrant Lepidoptera and birds tend to have higher aspect ratios (Winkler and Leisler 1992, Yao and Zhang 2001, Bowlin and Wikelski 2008, DeVries et al. 2010), which indicates that high aspect ratios may also be associated with greater dispersal capability. Wing loading is an evaluation of the relationship between body mass and wing area: the heavier an organism is for the size of its wings, the higher its wing loading. The relationship between wing loading and flight is not clear-cut, because greater wing-loading may increase the cost of long distance flight, but is balanced by the need to carry adequate fuel supplies (Pennycuick 1972, Kvist et al. 2001). In Lepidoptera, higher wing loading may allow longer and faster flights whereas lower wing loadings are associated with slower flights and hovering (Betts and Wootton 1988, Berwaerts et al. 2002).

For insects, thorax mass and shape are also important morphological traits because insect flight muscles are contained within the thorax. Fast-flying butterflies tend to have relatively wider thoraxes, greater thoracic mass and greater thorax mass relative to total mass (Chai and Srygley 1990, Srygley and Chai 1990), and this has also been shown on an intra-specific level (Berwaerts et al. 2002). Relatively larger and/or wider thoraxes are also associated with butterflies at newly colonized sites and expanding range boundaries, and relatively smaller or narrower thoraxes are associated with range centre and isolated populations (Dempster 1991, Hill et al. 1999a, Hill et al. 1999c). Therefore, there is evidence that morphological traits are indirectly related to flight but data are lacking explicitly linking morphology to dispersal ability. Additionally, most morphology measures apart from size have been explored in butterflies, and macro-moths despite being more speciose are under-studied.

This study examined moths from the Noctuidae because it is a large and diverse macro-moth family, with species spanning a range of sizes and containing moths with a range of dispersal abilities, including some trans-continental migrants (Chapman et al. 2012). This study aims to:

- Quantify dispersal ability in study species using tethered flight mills,
- Examine a range of adult morphology measures, inspired by the literature and existing evidence of their important role in flight, and examine which of these measures are the best predictors of tethered flight performance.
- Test the hypothesis that body size is a strong predictor of flight ability, with other morphological measures explaining additional variation in flight performance.

3.3 Methods

3.3.1 Study species and measuring tethered flight performance

Noctuid moths captured in light-traps during summer 2013 were used in flight mill experiments and to measure morphology. Visual inspection of wing wear was used to ensure only recently-emerged adults were flown, to constrain the variation in age. Following Thomas (1983), wing wear was assessed on a four point scale; fresh (4), good (3), poor (2) and worn (1); and only stage 3 and 4 individuals were tested (Appendix B).

Individuals were stored in a domestic fridge during the day (~4°C). Moths were removed from the fridge, scales removed from their thoraces using tape and 'handles' attached with contact adhesive before being returned to the fridge. About 2 hours prior to flight, moths were removed from the fridge, weighed, and then given 20% honey solution *ad libitum*. They were then re-weighed to verify feeding. Moths were attached to the tethered flight mills in the evening with a piece of paper to hold on to and left for lights-off. Each moth was flown on only one night. The flight mills were housed in a controlled environment insectary room at 18°C and under an 18L: 6D photoperiod (timed to overlap with outside daylight), which is equivalent to midsummer in the UK. Lights were gradually turned on and off 1 hour before and after night to simulate dawn and dusk.

As described in detail in Chapter 2, tethered flight mills consist of a lightweight arm suspended between two magnets (Figure 2.1) (Patent pending: Lim et al. 2013). The moth is attached to one end of the arm and its flight pushes the arm around, so that the moth flies on a circular trajectory of circumference 50 cm. A disk with a striped pattern is attached to the axis, and turns with the arm and a light detector detects the movement of the stripes to record the distance flown by the moth and its flight speed. The flight mills measure distance flown to the nearest 10 cm, and flight activity is recorded in 5 second increments. The tethered flight mill system has 16 channels (arms) allowing 16 individual moths to be flown simultaneously and flight data are automatically downloaded to a computer. Data for each individual for each 8 hour flight period were processed using Matlab and R to extract 16 flight performance variables (Table 2.1). All flights > 5 secs were analysed (Chapter 2, section 2.4.1).

3.3.2 Flight morphology measurement

After detachment from the flight mill in the morning, moths were weighed and then placed in a freezer to kill them. Individuals were removed from the freezer and air dried for 2 weeks, before morphological measures were taken as illustrated in Figure 3.1.

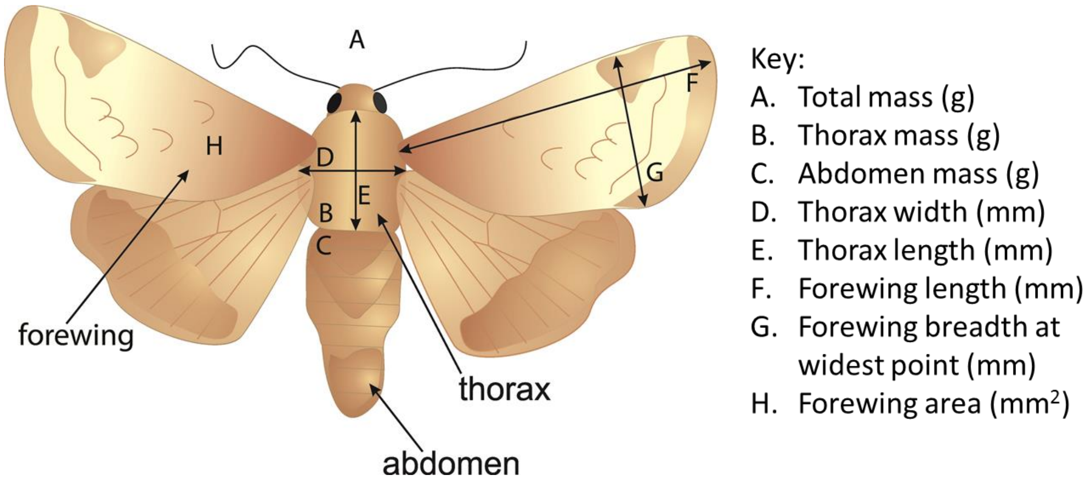

Figure 3.1. *Illustration of morphological measurements taken from dried moth specimens after flight on tethered flight mills.*

Table 3.1. *Results of repeatability assessment of measurement. Morphological measures (as illustrated in Figure 3.1) were taken three times from a sample of 25 individuals. Error calculated as the mean value of the repeated measures/the range of the three measures.*

	Morphological measurement	**Error (%)**
A	Total mass (g)	1.8
B	Thorax mass (g)	5.7
C	Abdomen mass (g)	4.7
D	Thorax width (mm)	9.0
E	Thorax length (mm)	8.7
F	Wing length (mm)	3.9
G	Wing breadth (mm)	2.5
H	Forewing area (mm2)	0.8

Moths were first weighed (using a Sartorius R200D balance that is accurate to 0.00001 g) and then thorax, abdomen and wings separated with scalpel and tweezers and weighed separately. The wings were placed between two microscope slides and secured with tape. Thorax width and length and forewing length and breadth were measured with callipers. Forewing area was measured using image analysis software (MARVIN digital seed analyser; GTA Sensorik GmbH). A repeatability assessment of the accuracy of measurements was carried out on a sample of 25 individuals, morphological measures were taken three times and the error calculated as the mean of the measures/the range of the three measures, expressed as a percentage (following Van Dyck et al. 1998). The morphological measures all had error values <10%, and most were much lower (Table 3.1).

A number of morphological characteristics were calculated from these measures. Wing aspect ratio was calculated as (4*forewing length^2)/forewing area (Berwaerts et al. 2006). Also, wing loading (total dry mass/forewing area), proportional thorax mass (thorax mass/total body mass) and proportional abdomen mass (abdomen mass/total body mass) were calculated. Finally thorax shape was calculated as thorax width/thorax length as an assessment of the relative broadness of the thorax, where a value greater than one indicates a thorax wider than it is long and a value less than one a thorax that is longer than it is wide.

3.3.3 Multivariate analyses of flight and morphology data sets

Multivariate analyses were carried out to examine which of the tethered flight variables and morphology variables were the most biologically informative and to explore redundancy in the two datasets. For each of these two sets of data, 16 tethered flight variables and 14 morphological variables, a matrix of pair-wise correlations was constructed and Principal Components Analysis (PCA) performed. I used PCA to select a smaller number of variables that encompassed the range of variation in the two data sets. PCA is a data reduction process that extracts a smaller number of independent factors from a larger number of correlated variables. Separately for the morphology and flight data sets, I included all variables into a PCA and selected a number of variables by visual inspection of the PCA biplots. This process was used to select a minimal number of variables for morphology and flight ability to use in the next stages of analysis.

3.3.4 *Individual level relationship between flight and morphology*

In order to examine which morphological variables were good predictors of flight performance, linear mixed effects (LME) models were fitted to tethered flight variables using restricted maximum likelihood (REML), assuming a Gaussian distribution. This was carried out using variables selected by the multi-variate analyses above, with tethered flight variables as the response variables and morphology variables as the explanatory variables (fixed effects). Tethered flight variables were transformed where necessary to ensure they conformed to a normal distribution. Model parameters were estimated within R statistical modelling language using the library lmerTest (v2.06, R Core Team 2013).

Species, genus, and mill identity were included in models as random factors to account for the variation between mills in the flight mill apparatus and for differences among species or genera (with species nested within genus). AIC (Akaike Information Criterion) was used to compare different models, with the model with the lowest value being the 'best' (Burnham and Anderson 2002). AIC summarises how well a model fits and penalizes additional parameters, thus selecting the most parsimonious best-fitting model. The random effects structure for all models was first optimised using intercept-only models to see which combination of random effects created a model with the lowest AIC (or the simplest model if it was not significantly different to the one with the lowest AIC when tested by ANOVA).

The full fixed effect models included all the morphological variables (explanatory variables) selected by the multivariate analyses, and were carried out separately for each flight variable (response variables) selected by the PCA. In order to remove morphological variables which did not contribute significantly to predicting the response, reverse model selection was used; the full models were run and the fixed terms with the smallest non-significant ($p>0.05$) t-values were successively removed (Zuur et al. 2009). Model checking of the residuals and fitted values were assessed and an estimated R^2 was derived to quantify fixed and random term contributions (Bell et al. 2012).

LMEs assess linear effects, but relationships between flight and morphology may not be linear. To investigate potential non-linear relationships, generalized additive mixed models (GAMMs) were used. GAMMs could not be used for the initial model selection process because the random effects structure (of mill identity and species nested within genus) did not leave sufficient degrees of freedom to fit the large number of fixed effects to be examined. In addition, it is not possible to get fixed effect coefficients in GAMMs. Thus GAMMS were carried out after variable selection by LMEs. The package 'mgcv'(Wood 2011) was used to fit Gaussian models to flight response variables using REML (R Core Team 2013). The random and fixed effects structures used were those from the LME models. The explanatory variables were the morphological variables selected by the LME reverse model selection, using splines ('smoothing') to fit curves to data to allow non-linear relationships with the response variables to be computed (Zuur et al. 2009). GAMMs provide outputs in the form of the significance of the fixed effects in the model, an adjusted R^2 value for the model, and the estimated degrees of freedom (edf) which are an indication of the degree of non-linearity in relationships. When edf = 1, this indicates a linear relationship, with edf values greater than one indicating increasing non-linearity. Model checking for homogeneity of variances and normality of residuals was carried out with gam.check and the results plotted with vis.gam.

3.3.5 Inter-specific relationship between flight and morphology

Analyses above were carried on individual data. In order to make broad conclusions about the relationship between morphology and flight ability across the Noctuidae, data were also analysed by species' mean values (by averaging data from all individuals per species and computing the arithmetic mean value). Species mean values were calculated for flight and morphological variables, and relationships explored using linear regressions weighted by sample size for each species (transformed pre-calculation of the mean where necessary to ensure variables were normally distributed).

3.4 Results

3.4.1 Study species

Males were more likely to be caught in light-traps than females (males = 495 individuals; females = 122) across the 32 species (median = 16 individuals per species; 13 males, 3 females). Previous studies have shown intra-specific variation in flight between male and female Lepidoptera (Berwaerts et al. 2006), and so all subsequent analyses in this chapter were performed on males only. Therefore 495 individuals across 32 species were examined (Table 3.2).

3.4.2 Multivariate analyses of flight and morphology data sets

Both the data sets of tethered flight variables and morphological variables contained variables that were highly correlated (Figures 3.2a & 3.2c) and PCA confirmed redundancy in measures in both data sets (Figures 3. 2b & 3.2d).

Among the flight performance variables, measurement of flight distance and flight speed characterised different aspects of dispersal as shown by their separation in Figure 3.2c. Thus the two most informative variables for these study species were maximum flight speed attained in any 5 second interval of the night and total distance flown overnight (8 hours). The data for all individuals for these two variables are shown in Figure 3.3. The noctuid species that flew the furthest was the Copper Underwing (*Amphipyra pyramidea*) with a mean distance of 12,350 m, and one individual flying a distance of 30,914 m. The species that flew the least was the Shuttle-shaped Dart (*Agrotis puta*) with a mean distance of 597 m. The fastest noctuid species was the Dark Arches (*Apamea monoglypha*) with a mean speed of 2.1 m/s. All four species known to be migrants (*Phlogophora meticulosa*, *Noctua pronuba*, *Autographa gamma* and *Xestia c-nigrum*) (Waring et al. 2009; Chapman et al. 2010) had flight speeds above the average for all study species, with speeds of above 1.16 m/s. The two selected tethered flight variables are, however, correlated with each other (Figure 3.4; R^2 = 71.1%).

Morphological variables were also very highly correlated with each other, especially those pertaining to size, such as measurements of total mass, thorax width, wing length and wing area (Figure 3.2b). The PCA confirmed this, and all measures of mass, length and breadth of body parts were closely clustered (Figure 3.2d). Separate from this cluster were measures of proportional abdomen mass and proportional thorax mass, which are understandably at opposite ends of the same axis, because these two body parts constitute around 80% of moth total dry mass. Abdomen mass and wing loading were separate from the main cluster of data points, but close together, and so only one of these variables was included in further analysis. Finally forewing aspect ratio and thorax shape were independent of other variables.

Table 3.2. *Summary table of 32 moth species flown on tethered flight mills. Only data for males were analysed in this Chapter.*

Species	**Common name**	**Number of females**	**Number of males**
Agrotis exclamationis	Heart & Dart	6	18
Agrotis puta	Shuttle-shaped Dart	5	8
Amphipoea oculea	Ear moth	0	11
Amphipyra pyramidea	Copper Underwing	7	14
Apamea anceps	Large Nutmeg	1	8
Apamea monoglypha	Dark Arches	2	39
Apamea sordens	Rustic Shoulder-knot	4	5
Autographa gamma	Silver Y	14	13
Axylia putris	The Flame	3	14
Charanyca trigrammica	Treble Lines	0	8
Cosmia trapezina	The Dun-bar	2	4
Hoplodrina alsines	Uncertain	3	13
Hoplodrina ambigua	Vine's Rustic	1	13
Hydraecia micacea	Rosy Rustic	1	23
Lacanobia oleracea	Bright-line Brown-eye	4	16
Mesapamea didyma	Lesser Common Rustic	0	10
Mesapamea secalis	Common Rustic	0	16
Mythimna impura	Smoky Wainscot	4	11
Mythimna pallens	Common Wainscot	5	19
Noctua comes	Lesser Yellow Underwing	16	26
Noctua janthe	Lesser Broad-bordered Yellow Underwing	8	13
Noctua pronuba	Large Yellow Underwing	12	37
Ochropleura plecta	Flame Shoulder	4	20
Oligia fasciuncula	Middle-barred Minor	0	2
Oligia latruncula	Tawny Marbled Minor	0	1
Oligia strigilis	Marbled Minor	0	7
Omphaloscelis lunosa	Lunar Underwing	2	16
Phlogophora meticulosa	Angle Shades	6	10
Thalophila matura	Straw Underwing	0	4
Xestia c-nigrum	Setaceous Hebrew Character	5	59
Xestia triangulum	Double Square-spot	2	12
Xestia xanthographa	Square-spot Rustic	5	25

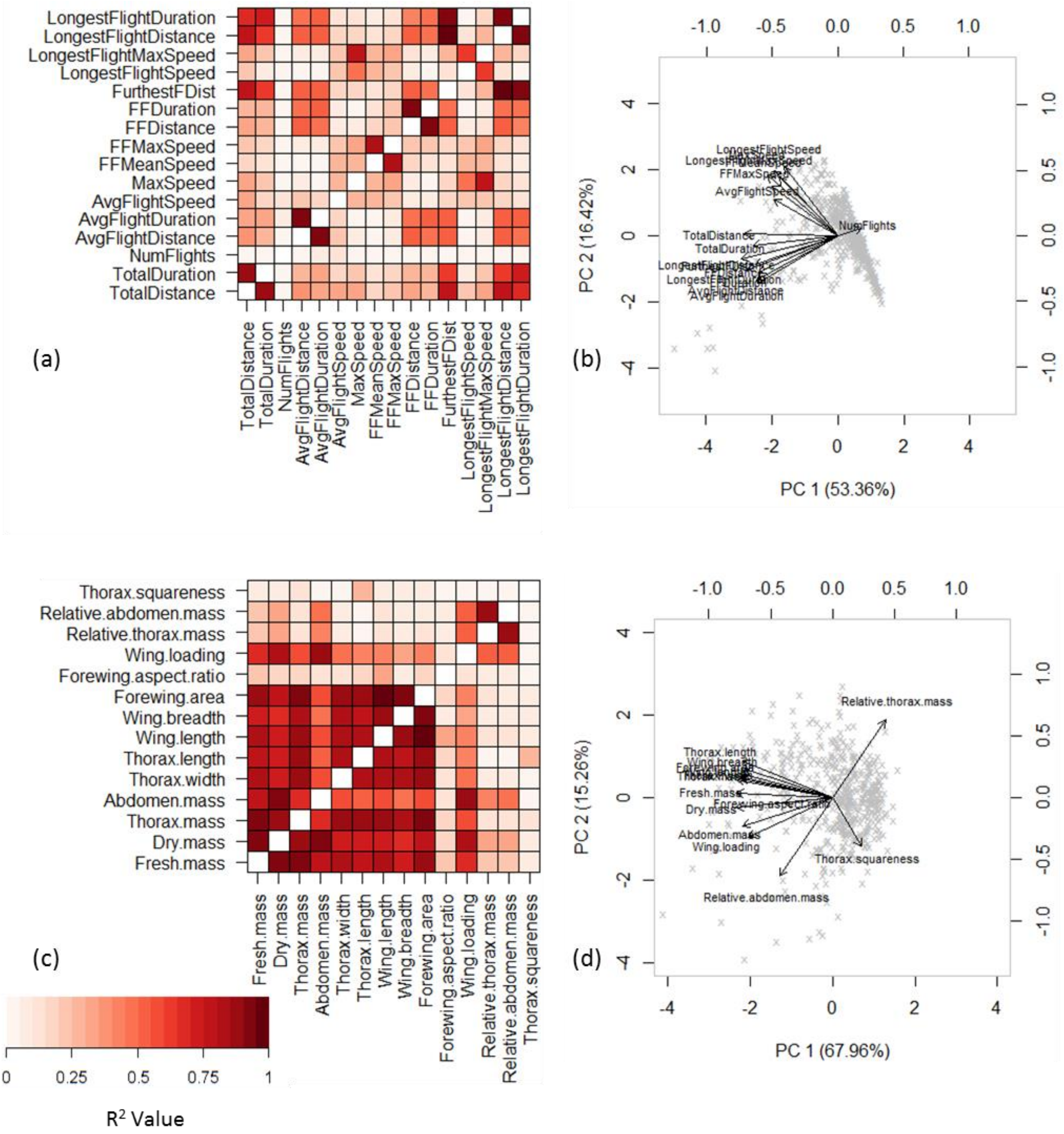

Figure 3.2. *Multivariate exploratory analyses of tethered flight* ***(a & b)*** *and morphological* ***(c & d)*** *variables taken from 495 male noctuid moths.* ***(a & c)*** *Matrix of pair-wise correlations.* ***(b & d)*** *Principal components analysis biplots. The two first principal components are plotted with the proportion of variance explained by each component printed next to the axes label which together explain >70% of variation in the data. Crosses indicate the 495 male individuals in the data set; the top and right axes show principal component scores of the individuals. The arrows indicate the principal component loadings of the different tethered flight variables.*

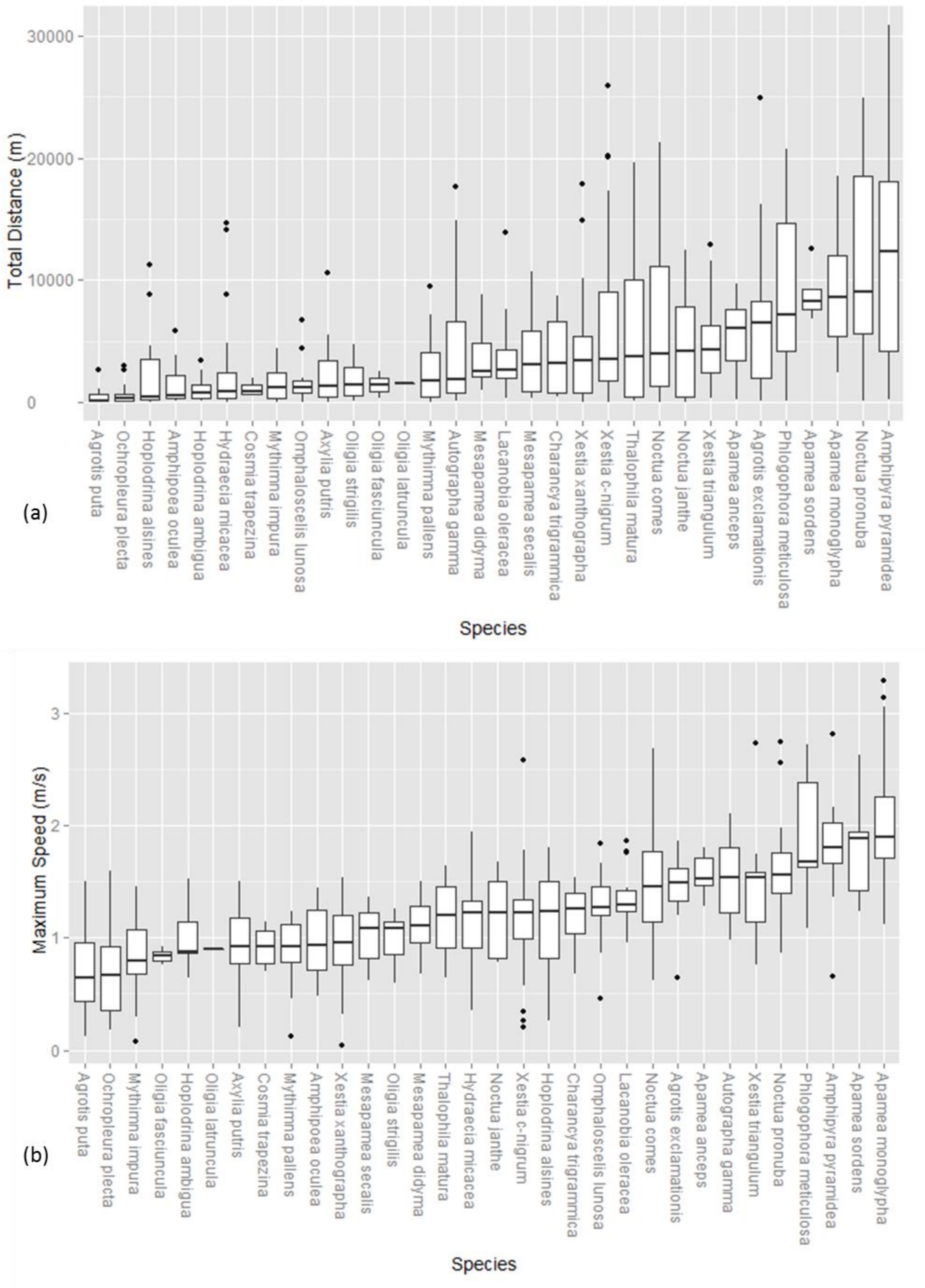

Figure 3.3. *Boxplots of (a) total distance flown overnight and (b) maximum speed attained on tethered flight mills by male individuals from 32 noctuid species, arranged by median for each species. Bar represents median, box is interquartile range and whisker extends to 1.5* the interquartile range from the box. Points outside this are outliers.*

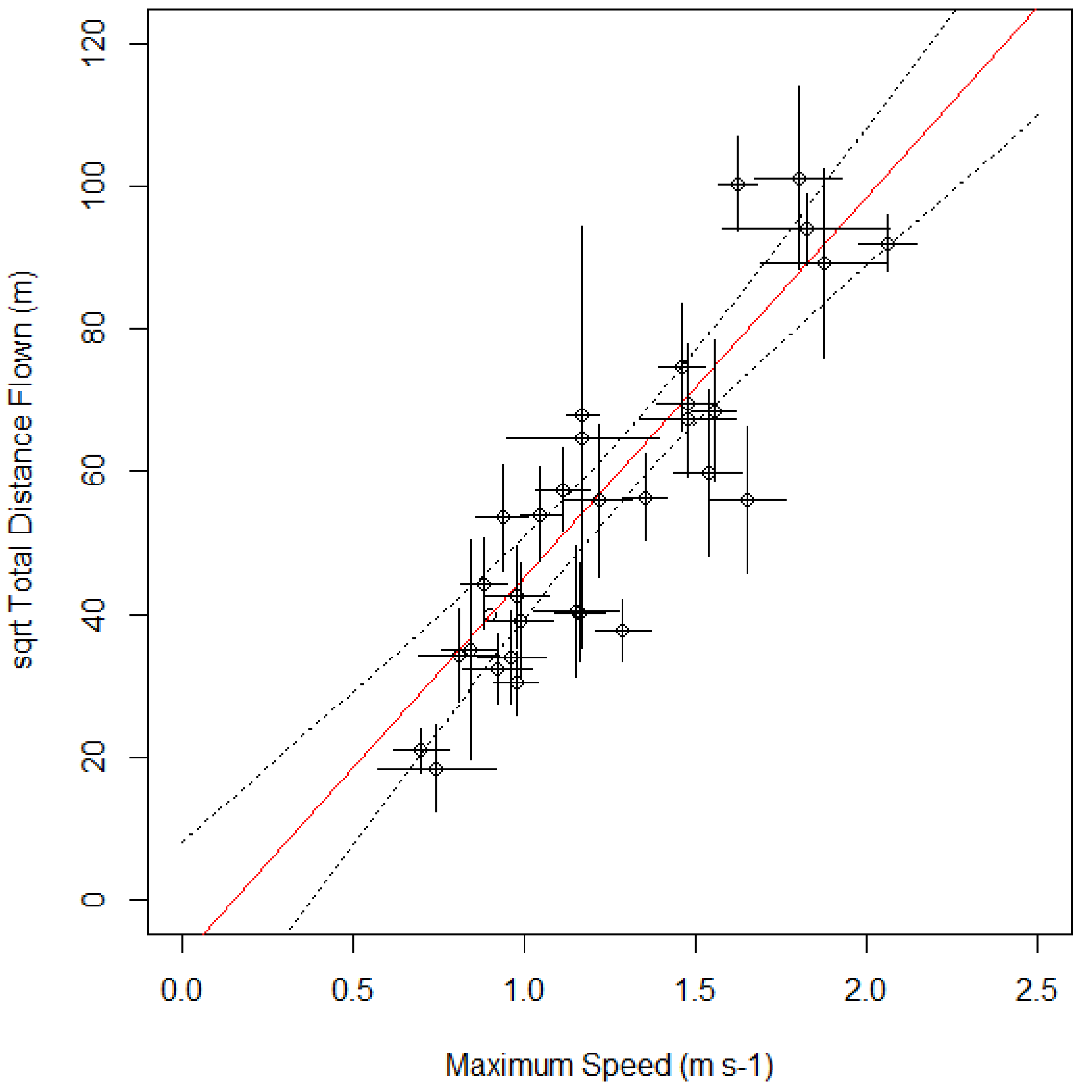

Figure 3.4. *Relationship between tethered flight variables 'maximum speed attained' and 'total distance flown overnight' for 32 noctuid moth species, analysing mean data for species. Regression analyses was weighted by species sample size. Intercept = -8.162; coefficient of maximum speed = 53.344;* R^2 *= 71.1%. (Regression is shown by solid red lines with dotted lines showing 95% confidence intervals. Circles denote species means ± SE.*

Based on the PCA outputs, analyses of relationships between morphology and flight examined total distance flown overnight and maximum flight speed as the response variables and wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio as explanatory morphology variables.

3.4.3 Individual-level relationships between flight and morphology

Total distance flown overnight and maximum speed attained were the two responses that were explored using separate model frameworks. In both models, the random effects structure was first optimised using intercept only models to see which combination of random effects created a model with the lowest AIC. This led to the conclusion that species identity and mill number were required for the model of total distance flown, and species nested within genus and mill number were required for the model of maximum flight speed.

The full fixed effects for both models comprised: wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio. The linear mixed effects (LME) reverse selection resulted in only two morphological variables (wing length and proportional thorax mass) remaining in the final models (Table 3.3), with positive relationships between both of these morphological variables and flight performance. In other words, flight speed and flight distance increased with increasing forewing length and larger proportional thorax mass. For both flight variables, wing length was a predictor with higher significance than proportional thorax mass (total distance: wing length $t_{33.2}$= 10.879, $P<0.001$, proportional thorax $t_{300.1}$= 2.239, $P=0.026$; max speed: wing length $t_{41.5}$= 10.032, $P<0.001$, proportional thorax $t_{389.8}$= 2.715, $P=0.007$). A unit change (1 mm) of wing length yielded a 41.0±0.36 m increase in total distance flown (back transformed) and 0.095±0.009 m/s increase in speed. Thus larger individual moths flew further and faster, with additional variation explained by proportional thorax mass; for any given wing length (size) of moth, those whose thorax comprised a greater proportion of their mass flew further and faster but this effect was smaller and had higher standard errors than that of wing length (Table 3.3).

Table 3.3. *Summary of linear mixed effects (LME) models of moth morphology traits predicting flight performance. Response variables were (square-root) total distance flown overnight (8 hours) and maximum speed attained. Full fixed effects models included wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio. The order of removal for reverse model selection was the same in analyses of total distance and maximum speed: [thorax shape, wing loading and then forewing aspect ratio]. *P < 0.05; **P < 0.01; ***P < 0.001.*

Model	Final fixed effects	Fixed effect coeff ±se	df	t-value	Estimated R^2 for fixed model	Estimated R^2 for random effect
Total distance	Constant	-78.1 (17.6)	113.0	-4.450***	28.33%	Species:3.8% Mill:9.2%
	Wing length	6.4(0.6)	33.2	10.879***		
	Proportional thorax mass	67.7(30.3)	300.1	2.239*		
Maximum speed	Constant	-0.769 (0.251)	121.5	-3.068**	36.9%	Species:4.7%
	Wing length	0.095(0.009)	41.5	10.032***		Genus:3.8% Mill:2.9%
	Proportional thorax mass	1.081(0.398)	389.8	2.715**		

Table 3.4. *Summary of generalised additive mixed effects (GAMM) models of morphology data predicting flight performance. Response variables were (square-root) total distance flown overnight (8 hours) and maximum speed attained (in any 5 sec interval). Explanatory variables were wing length and thorax mass as a proportion of total mass. Both fixed effects were included in the model as smoothers (i.e. non-linear terms). *P < 0.05; **P < 0.01; ***P < 0.001.*

Model	Smoothed fixed effects	Estimated df	F statistic	Adjusted R^2 for fixed model
Total distance	Wing length	1.75	116.755***	30.1%
	Proportional thorax mass	1	7.005**	
Maximum speed	Wing length	4.6	69.02***	43.3%
	Proportional thorax mass	1	13.72***	

Having fitted LMEs, potential non-linearity of the relationship between the morphological and flight variables was explored using GAMMs (Table 3.4 & Figure 3.5). This revealed significant non-linear relationships between wing length and maximum speed, and to a lesser extent between wing length and total distance (max speed: edf=4.6, F=69.02, P<0.001; total distance: edf=1.75, F=116.76, P<0.001). By contrast, the relationships between proportional thorax mass and flight speed and duration were linear, as shown by the estimated degrees of freedom (edf) values of 1 (max speed: edf=1, F=13.72, P<0.001; total distance: edf=1, F=7.01, P=0.008). It is inappropriate to directly compare R^2 values between the LME models and the GAMM models as they are calculated by different methods but the proportion of variance explained appears to be roughly similar between the two model types for both total distance and maximum speed (Tables 3. 2 & 3.3). Therefore, while the non-linear relationship between wing length and flight performance is significant and provides an insight into the relationship between morphology and flight, it does not give notably greater R^2 values in models.

Further inspection of the non-linear relationship between wing length and maximum flight speed (Figures 3.5 b and d) showed that while maximum speed increased with wing length, there was apparently an ‘optimum’ wing length of ~24mm where speed levelled off then started to decline in the largest individuals. Figures 3.5 a and b illustrate the smaller effect size of proportional thorax mass compared with wing length; the gradient of both flight variables rises more steeply along the x axis (wing length) than the y axis (proportional thorax mass), and the slopes in Figures 3.5 e and f are not as steep as Figures 3.5 c and d.

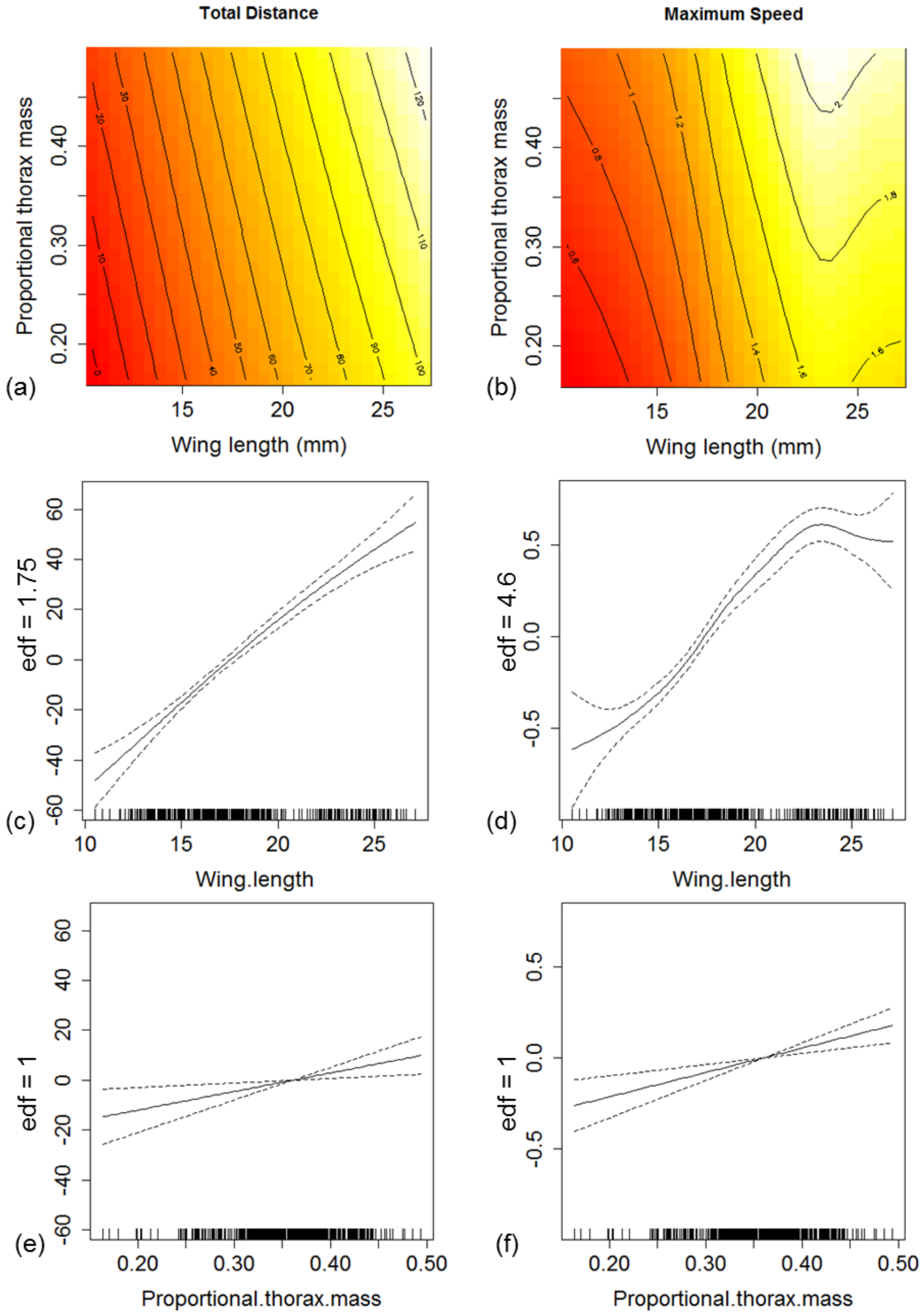

Figure 3.5. *Plots showing how morphological variables predict flight performance (fixed components of GAMMs).* ***(a, c, e)*** *are total distance flown overnight (square-root) and* ***(b, d, f)*** *maximum speed attained.* ***(a & b)*** *show surface plots of the combined smoothed (i.e. non-linear) fixed effects of wing length and proportional thorax mass on the flight performance variables; paler yellow colour indicates higher values of flight performance and contour lines show those values.* ***(c-f)*** *show the shape of the relationship between the morphological fixed effect and response, as estimated smoother effects with 95% confidence intervals. In these plots the y axis is a centred calibration parameter so only the estimated degrees of freedom (edf) are shown. An edf of one indicates a linear relationship and >1 indicates increasing non-linearity. The x axis has two components the tick marks indicate numerical values and above those 'rug' plots show the individuals with the corresponding value of the morphological variable.*

3.4.4 Inter-specific relationships between flight and morphology

In order to examine inter-specific relationships between morphology and flight ability across the Noctuidae, regressions of tethered flight variables and morphological variables were carried out based on species' mean values, weighted by sample size (N) for that species. Adjusted R^2 values for these regressions are shown in Table 3.5. The best predictor of tethered flight performance using these mean data was wing length, with an adjusted R^2 value of 79.0% for total flight distance and 72.9% for maximum flight speed. Proportional thorax mass, by contrast to individual analyses above, was not a good predictor, with adjusted R^2 values of only 7.9% for total distance and 3.6% for maximum speed. Adding additional morphological variables into the regression between wing length and total distance flown did not increase the R^2 value from 79%.

Table 3.5. *Regression analysis of morphological variables predicting tethered flight performance using mean data for species. Adjusted R^2 values of linear models weighted by sample size for each species.*

Morphological Variable	**R^2 value for morphological variable against flight performance variable:**	
	Total distance (square-root)	**Max speed**
Forewing length	79.0	72.9
Proportional thorax mass	7.9	3.6
Wing loading	37.6	33.4
Thorax shape	29.2	26.2
Forewing aspect ratio	21.7	25.7

The linear regressions between wing length and total distance flown, and wing length and maximum speed are plotted in Figure 3.6. These yield formulae to predict the flight performance of noctuid species:

Square-root total flight distance = -40.494 + wing length (mm) * 5.739 (Eqn. 1)

Maximum flight speed = -0.272 + wing length (mm) * 0.088 (Eqn. 2)

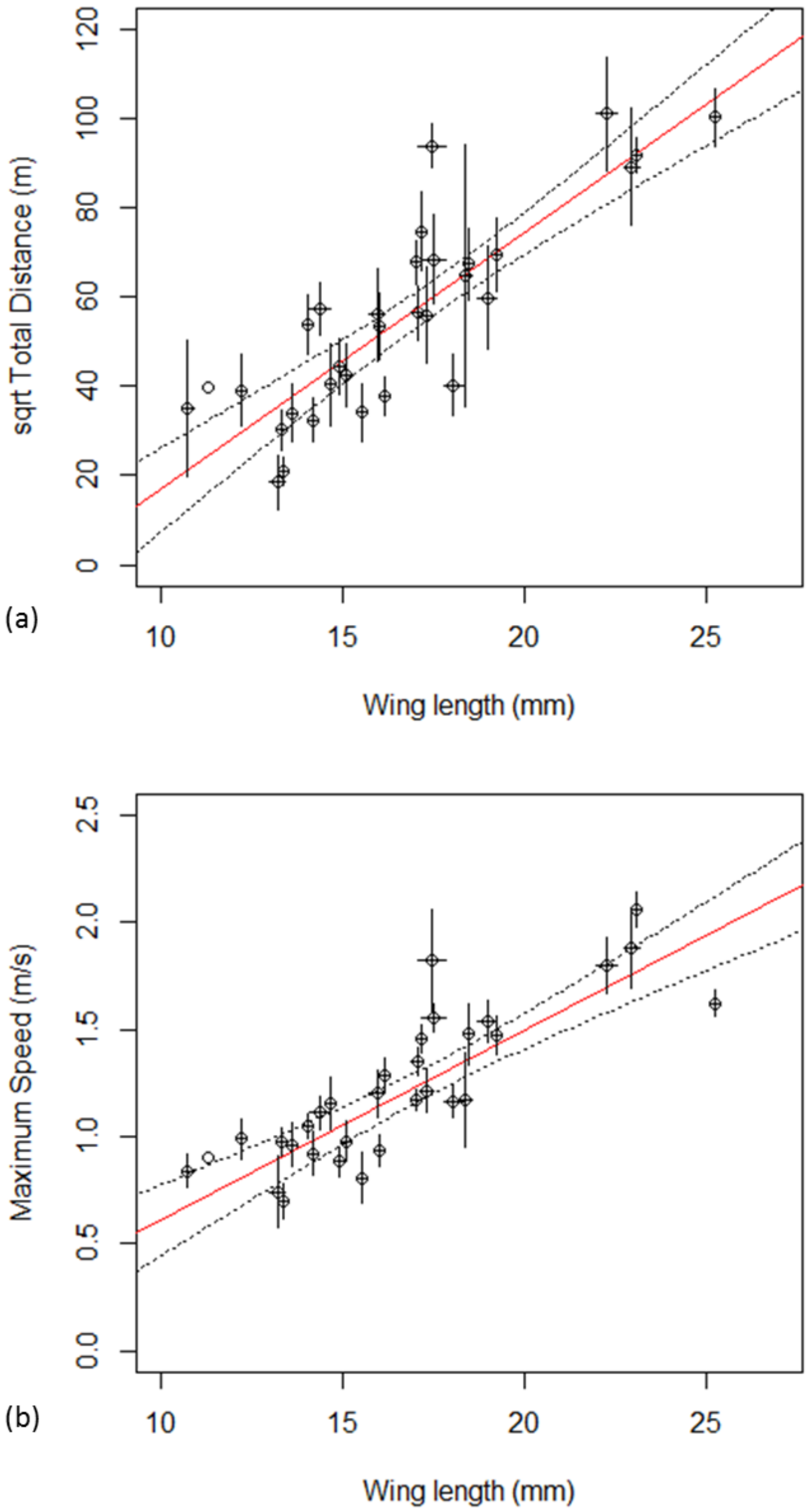

Figure 3.6. *Relationship between flight performance variables and wing length for 32 noctuid moth species, analysing mean data for species. Regression analyses examined wing length in relation to* ***(a)*** *square-root total distance flown* ***(b)*** *maximum speed, and analyses were weighted by sample size. (a) Total distance: Intercept = -40.494; coefficient of wing length = 5.739; R^2 = 79.0%. (b) Maximum speed: Intercept = -0.274; coefficient of wing length = 0.088; R^2 = 72.9%. Regression is shown by solid red lines with dotted lines showing 95% confidence intervals. Circles denote species means ± SE.*

3.5 Discussion

3.5.1 Dispersal ability in noctuid moths

A total of 32 noctuid species were studied, and flight performance, quantified using tethered flight mills varied considerably both within and between species. Moths flown in this study were caught in light-traps, and this could have constrained which species were included, as some species are not attracted or caught in light-traps (Waring et al. 2009, Merckx and Slade 2014), and the least dispersive may not fly far enough to be trapped. Further studies could use other trapping methods to increase the range of species studied. Nonetheless, light-trapping is a standard method for sampling moths and samples the majority of species in a locality.

Total distance flown overnight varied among species from ~600 m to over 12 km (Figure 3.1), and maximum flight speeds varied from 0.7 m/s to ~2 m/s. Total distance flown had higher levels of intra-specific variation compared with maximum speed, which may reflect varying motivation to fly among individuals, in addition to their ability to fly, that could be due to behavioural and physiological factors such as fuel levels and available cues. These aspects are considered in the next Chapter.

It is worth considering how the distances and speeds obtained on the flight mills reflect distances and speeds of natural flight. The maximum speeds in this study were lower than estimated wild speeds of 3 - 4 m/s (Chapman et al. 2008), but this is understandable as the moths must overcome the friction and weight of the flight mill arm and individuals may also be compensating for flying in a circular trajectory. Total distance flown in the lab is harder to translate into natural distances flown as there are two opposing factors affecting tethered flight: the increased effort involved with pushing the mill versus lack of cues for landing and inability to land.

Results from the study support the idea that the flight mills are however very valuable in comparing different groups of flying insects, such as the different species in this study. Studies comparing tethered flight to free flight have found that the differences between study groups are reflected across the two methods (Taylor et al. 2010, Blackmer et al. 2004) further supporting the conclusions of this study

3.5.2 Redundancy among flight and morphology measures

A total of 16 different flight variables were obtained from the tethered flight mills, but multivariate analyses indicate that only two were necessary to summarize differences in flight performance. Most tethered flight variables were highly correlated, e.g. lengths of the first or longest flight were correlated with other measures of duration and distance. Therefore two variables were selected to move into later analyses, total distance flown and maximum flight speed.

The 14 different measurements of flight morphology were also highly correlated with each other and formed clusters in a PCA (Figure 3.2). Therefore, of variables pertaining to size (measures of mass, wing and thorax dimensions), only one variable was selected to include into the subsequent analyses, to summarize overall size of insects. A size variable was expected to be a good predictor of dispersal because size has commonly been shown to be related to dispersal (Hein et al. 2012). Wing length was selected from among these different size variables because it is very easy to measure, even on live insects, and the information is also readily available from identification guides (Waring et al. 2009), making this a useful measure in any future study. Multivariate (PCA) analyses were used in this study to minimise the redundancy among morphological variables by selecting just five variables to explore in relation to flight performance. Thus the variables that were examined were: wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio. This covered features of the wings, thorax and overall size.

3.5.3 Predicting flight performance from adult morphology- individual level

Linear mixed effects (LME) modelling reduced the number of morphological variables included in analyses predicting flight performance to two; wing length and proportional thorax mass. Individuals with longer wings flew further and faster. Proportional thorax mass was also included as a significant positive term in the model, but with a weaker and noisier relationship. The generalized additive mixed models (GAMMs) further clarified the details of this relationship (Figure 3.5), providing strong evidence for the usefulness of wing length as a proxy for dispersal ability in moths. This supports findings for butterflies (Sekar 2012), and confirms that noctuid moths exhibit an increase in distance moved with increasing body size as anticipated from many other studies (Hein et al. 2012) and a previous study of moths (Nieminen et al. 1999). There was also evidence that proportional thorax mass in addition to wing length is a predictor of flight distance and speed, albeit to a lesser extent than forewing length. This is likely due to thorax mass being principally composed of flight muscles that power the wings (Srygley and Chai 1990), and so individuals with more flight muscles may fly further and faster.

Using morphological variables it was possible to predict a greater proportion of the variance in maximum flight speed (43.3%) than total distance flown (30.1%) (Table 3.3). This may be an indication that morphology is more of a constraint to flight power (as measured by maximum speed) than it is to total distance flown. The shape of the relationship between wing length and maximum speed (Figure 3.5e) could be due to physiological constraints. One would expect a plateau in speed above a certain size, as other trade-offs come into play such as the need to carry more fuel to power flight combined with rapid consumption of fuel due to greater mass (Pennycuick 1972). The lower predictive power of morphology in relation to total distance flown compared with flight speed may reflect the complexity of behavioural and physiological factors that affect the propensity of individuals to fly; including motivation and developmental history. In this study, I controlled for sex effects by only studying males. However, the insects were caught from the wild, rather than reared under controlled conditions, which means many details about their history and were not known. Although individuals in this study had their age constrained somewhat by selection of individuals with the least wing wear, their precise age was unknown and wing wear may not always be a good indicator of age. Flight performance tends to decline with age, especially upon sexual maturity and mating (Colvin and Gatehouse 1993a, McNeil et al. 1995). By flying moths showing relatively little wing wear, it is likely that moths in this study were young and still at their most dispersive. The individuals' access to nutrition before capture and developmental (larval) history were also not known but adult food resources can also influence flight activity (Gatehouse and Hackett 1980). Larval food resources such as which plants the larvae feed on and how much food is available to them can affect their lipid reserves and therefore flight activity (Itoyama et al. 1999, Sakamoto et al. 2004, Boggs and Freeman 2005). These differences may contribute to the extremely high levels of intra-specific variation recorded on the flight mills, and may translate to variation among individuals in flight propensity in nature.

3.5.4 Predicting flight performance from adult morphology- species level

By contrast to analyses of individuals, analyses of mean values for species revealed that morphology was an extremely strong predictor of flight performance among species (Table 3.4 and Figure 3.6). Forewing length explained 79% of the variation in total distance flown and 73% of maximum speed among species. These strong relationships suggest that easily obtainable measures of wing length could be used to predict flight performance in species other than those studied here. Here noctuid moths ranging from 12 – 27 mm wing length were studied, which covers a good range of sizes of noctuids (from 7 mm e.g. Least Minor, *Photedes captiuncula* or *S*mall marbled, *Eublemma purpurina* to 48mm, the migrant Clifden Nonpareil, *Mormo maura*; Waring et al 2009). However it would be useful to study a greater number of larger species to see if the apparent plateauing of flight speed with wing length shown found in larger individuals is also apparent at an inter-specific level. Further work should also elucidate whether the relationship between wing length and flight performance is present in other macro-moth families, for example moths with similar body plans such as the Arctiidae and Notodontidae might be expected to show broadly similar relationships between morphology and flight as shown here, while Geometridae have different wing placement and flying styles and so may not follow the same trend.

In conclusion, in Noctuid moths, morphology was a good predictor of individual flight performance, and a much better predictor of flight performance among species. The individual-level modelling showed that there was an effect of body size (wing length) as well as proportional thorax mass. The inter-specific modelling showed that size (wing length) alone could predict 79% of variation in flight distance, and 73% of maximum speed. These results add to the body of literature showing that animal body size is a good predictor of dispersal across a wide range of taxa (Paradis et al. 1998, Norberg & Raynor 1987, Greenleaf et al. 2007, Kuussaari et al. 1996). These findings imply that measures of flight performance can be extrapolated to a wider range of noctuid species than studied here, providing a robust quantification of dispersal ability. This inter-specific quantification of dispersal ability provides a valuable tool to explore the role of dispersal ability in the current population trends of moth species under a changing environment. The next chapter will explore the high levels of intra-specific variation in flight performance highlighted in this chapter, especially how physiology and food consumption affect flight, and I will also explore differences in flight ability between sexes.

4 Chapter 4 – Inter- and Intra-specific variation in flight performance and relationships with moth physiological status and morphology

4.1 Abstract

Individuals vary in their propensity to disperse. This variation can have important effects on both the fitness of these individuals and the viability of their populations. Selection for greater dispersal ability may principally act upon morphological and physiological features, and in this chapter I examine how sex, age, levels of food consumption and morphological differences among individuals affect variation in flight capability of moths on tethered flight mills.

Previously I have shown (Chapter 3) that key morphological features (wing length and relative thorax mass) explained ~30% of variation in flight performance (total distance flown overnight and maximum speed attained) in multi-species analyses. In this Chapter, I found that physiological measures related to flight fuel (food consumed prior to flight and body mass lost by individuals during flight) were also important predictors of flight performance in multi-species analyses (N = 30 species), albeit their effect was smaller than the effects of morphological factors. Inclusion of these two resource-related measures into models increased the amount of variation explained from 28.5% to 35.0% for distance flown, and from 35.1% to 35.6% for maximum speed when compared with models including only morphological variables. However, including a proxy for moth age (wing wear) into models did not explain any additional variation, and contrary to expectations, there were no detectable differences between males and females in flight performance.

I focussed on a smaller number of species (N = 5) that had large sample size. When separate analyses were carried out for these species the models indicated that the effects of flight morphology were generally less important and varied considerably among the five species. In contrast to the multi-species analyses, resource-related variables sometimes explained high levels of variation (up to 54%) in some species.

In conclusion, the inclusion of resource-related variables can explain an additional 0.5-6.5% of variation in flight performance in multi-species models compared to models containing only morphological variables. However single species analyses

showed that high levels of variation in flight performance can more often be explained by the physiological variables measured here, but there still remains a large proportion of variation to be explained.

4.2 Introduction

Variation in dispersal ability among individuals of a species can have important effects on the fitness of those individuals and the viability of their populations or species as a whole (Clobert et al. 2001, Hanski 1999). This is especially relevant in an environment of accelerating climate change and habitat fragmentation (Hughes et al. 2007, Gibbs et al. 2010b) where dispersal ability may determine how well species shift their ranges to track climate (Warren et al. 2001). Very high levels of intra-specific variation can hinder species specific quantification of dispersal (Stevens et al. 2010a), and so a better understanding of the scale of individual variation may help in multi-species studies. Therefore it is necessary to understand what may drive intra-specific variation in dispersal.

4.2.1 Variation in dispersal within a species

Heterogeneity in dispersal ability may occur across the distribution range of a species, due to the different costs and benefits associated with dispersal for individuals in core and margin areas (Dytham 2009), expanding range edges (Simmons and Thomas 2004) and in areas of high and low habitat disturbance (Travis and Dytham 1999). The greater likelihood of dispersive individuals to found new populations, combined with the heritability of dispersive traits means we might expect to find more dispersive individuals in newer populations compared to older populations (Hanski et al. 2004, Parmesan 2006). These dispersive types are most detectable in wing-dimorphic species, such as bush crickets, where more recently founded populations typically have higher proportions of long-winged (macropterous) individuals than the older populations which are dominated by short-winged (brachypterous) individuals (Niemelä and Spence 1991, Thomas et al. 2001). Proportions of macropterous individuals are also expected to be greater in highly disturbed habitat (Denno et al. 1996). More subtle differences in flight morphology such as larger adult mass and greater proportional thorax mass (i.e. flight muscle mass) have been found in individuals from range edge/recently colonized areas compared to continuously occupied areas (Hill et al. 1999a, Hughes et al. 2003). Metabolic and genetic differences between recently colonized/range edge populations and core/well connected populations have also been detected (Hanski et al. 2004, Haag et al. 2005).

Dispersal ability is a key driver of meta-population dynamics (Clobert et al. 2001). Selection for high dispersal ability would be expected to enhance the persistence of meta-populations through colonization of empty habitat patches and genetic enrichment of occupied patches (Hanski 1999). However, high levels of dispersal might lead to local extinction if meta-populations become too fragmented resulting in increased risk of mortality associated with dispersing to unfavourable areas. Similarly, it is expected that within an isolated patch, selection would favour lower dispersal ability (Hanski 1999). Therefore selection pressure on dispersal ability is predicted to vary according to patch size, resource availability and connectivity within the meta-population (Hill et al. 1996). It has been shown that patch area and connectivity of habitat patches can be good predictors of movement (Hanski et al. 2000). However there are other sources of variation at play, for example two meta-populations of the bog fritillary butterfly (*Proclossiana eunomia*), despite occupying highly fragmented landscapes, had large differences in dispersal patterns (Mennechez et al. 2004). These high levels of variability in dispersal may themselves enable long-term persistence of meta-populations (Leimar and Norberg 1997, Hawkes 2009), if patterns of patch size and connectivity vary over time.

As mentioned above, this variation in dispersal ability within the distribution of a species may occur by various mechanisms including selection on morphological features of flight ability. It may be a product of phenotypic plasticity in dispersal or it may also arise as a result of selection on different physiological, metabolic, age and sex related differences in flight performance.

4.2.2 Variation in dispersal due to sex

Sex biased dispersal is common across organisms, with evolutionary explanations including minimization of inbreeding and different costs and benefits to dispersal for each sex (Lambin et al. 2001). Birds tend to have female-biased dispersal (i.e. the females carry out the majority of dispersal for the species) whereas mammals tend to have male-biased dispersal (Greenwood 1980). In invertebrates both male-biased and female-biased dispersal has been reported (Bowler and Benton 2005). For example, female-biased dispersal has been detected in damselflies and butterflies

(Kuussaari et al. 1996, Beirinckx et al. 2006) and male biased dispersal in milkweed beetles and soil mites (Lawrence 1987, Bowler and Benton 2009). Among moths there as contrasting evidence for females or males dispersing more. There are species in which female moths have shown greater flight performance than males (Hughes and Dorn 2002, Luo et al. 2002). High levels of female flight performance have typically been shown in migratory species, and among non-migratory macro-moths there is some indication that males may be more mobile than females. Males are more frequently caught in light traps (Williams 1939, Altermatt et al. 2009). This may be because male eyes are more sensitive to light (Meyer-Rochow and Lau 2008) or because males show greater flight activity than females, as the males search for females to mate with (Scoble 1995), and hence males are more likely to encounter traps. In addition sometimes there is extreme sexual dimorphism in dispersal, for example the winter moth (Operophtera brumata), where the females are wingless (Begon et al. 2006). In summary there is no clear evidence for sex-biased dispersal across the macro-moths but some species may exhibit signs of it.

4.2.3 Variation in dispersal due to age

The role of age in dispersal has been well-studied in migratory moth species, but has not been investigated in non-migratory species of moths. Laboratory studies have shown that overall flight performance tends to decline with age, especially upon sexual maturity and mating (Armes and Cooter 1991, Colvin and Gatehouse 1993a, McNeil et al. 1995). Some studies show an initial increase in flight performance in the days immediate after eclosion (Armes and Cooter 1991, Luo et al. 2002), followed by a decline in flight performance, usually with the onset of sexual maturity (Armes and Cooter 1991, Colvin and Gatehouse 1993b, but see Sappington and Showers 1992, Luo et al. 1995). The decline in flight performance after sexual maturity has been attributed to a physiological trade-off between reproduction and flight (Johnson 1963, Han and Gatehouse 1993), whereby internal resources can be re-allocated away from the flight muscles to the reproductive organs (Stjernholm et al. 2005).

4.2.4 Variation in dispersal due to resource availability

Animals that are in better condition may be better able to disperse (Lawrence 1987, Anholt 1990). This is not surprising if better nutritional resources can provide energy to power prolonged flight, and reduce dispersal mortality (Bowler and

Benton 2009). Greater resource availability has been shown to increase flight activity across a range of species (Wanner et al. 2006, Kaufmann et al. 2013). Lepidoptera acquire resources during two life stages, larval and adult. Larval food resources such as which plants the larvae feed on and how much food is available to them can affect their lipid reserves and therefore flight activity (Gunn and Gatehouse 1993, Itoyama et al. 1999, Sakamoto et al. 2004, Boggs and Freeman 2005). Adults can consume nectar from flowers to increase their sugar resources, and adult food resources have also been shown to influence the active period of flight activity (Colvin and Gatehouse 1993b), inter-patch movement (Schneider et al. 2003) and flight distance (Kaufmann et al. 2013). Similarly inadequate nectar availability can affect flight activity, lifespan and other life-history traits (Boggs 2009, Van Dyck et al. 2014).

In chapter 3 the effect of adult morphology on flight performance (total distance flown overnight and maximum speed) was investigated across male individuals of 32 species of noctuid moths. This chapter will test whether sex, age, and resource-related variables, in addition to morphology, have an effect on tethered flight mill performance. The chapter also tests whether similar relationships exist within individual species, as appear in the cross-species analysis. The prediction is that morphology will not prove to be as significant a predictor of variation in flight performance within species, and age and resource related variables may be more important. This prediction is motivated by the high levels of intra-specific variation in flight performance, compared to relatively low levels of variation in the morphological measures within species. The aims of this chapter are:

- To test the hypothesis that tethered flight performance differs between the sexes
- To explore if additional variation in flight performance can be explained by resource and age-related variables compared to morphology alone.
- To test whether the morphological, age and resource-related variables have similar ability to predict flight performance within species as they do in cross species analysis. High levels of intra-specific variation mean the prediction is for age and resource-related variables to be of importance.

4.3 Methods

Wild caught noctuid moths were flown on tethered flight mills during summer 2013 and the variables ‘total distance flown overnight’ and ‘maximum speed attained’ were selected to summarise flight performance (Chapters 2 & 3). Morphological variation was measured from all individuals flown on flight mill, of which ‘forewing length’, ‘proportional thorax mass’, ‘wing loading’, ‘thorax shape’ and ‘forewing aspect ratio’ were selected. These variables were previously shown to span the full range of adult morphological features without being correlated with each other (see Chapters 2 & 3 and Table 4.1 for how these flight and morphology variables were measured). In addition to the morphological variables, three physiological and age-related variables were recorded. ‘Wing wear’ was assessed on a four point scale following Thomas (1983): fresh (4), good (3), poor (2) and worn (1) but generally only stage 3 and 4 individuals were selected for tethered flight experiments. Moths were allowed to feed on 20% honey solution and body mass was recorded pre-feeding, post-feeding and post-flight to enable the calculation of features of resource use and availability (using a Sartorius R200D balance that is accurate to 0.00001 g). ‘Proportional food consumed’ is a measure of how much honey solution the moth consumed as a proportion of its body mass and provides information on how much carbohydrate energy resource the moth has gained. ‘Proportional body mass change during flight’ is a measure of how much of the individuals pre-feeding mass was lost i.e. how much of its internal resources such as body fat, previously obtained nectar, water and muscle mass was used during flight. These variables and how they are calculated are summarised in Table 4.1.

Table 4.1. *Morphological, physiological and age-related measures taken from individual noctuid moths flown on tethered flight mills.*

Variable	Definition
Forewing length	Length of forewing from wing apex to base (mm)
Proportional thorax mass	Thorax mass/total body mass. Proportion of mass occupied by thorax
Wing loading	Total dry mass/forewing area. Higher values indicate a heavier an organism is for the size of its wings.
Thorax shape	Thorax width/thorax length. (Values >1 indicates a thorax wider than it is long, values <1 a thorax that is longer than it is wide)
Forewing aspect ratio	(4*forewing length^2)/forewing area. Higher aspect ratio indicates longer, narrower wings.
Wing wear	Proxy for age. Assessed on a four point scale; fresh (4), good (3), poor (2) and worn (1)
Proportional food consumed	(Post-feed mass - pre-feed mass) / pre-feed mass. Honey solution consumed (g) before tethered flight as a proportion of initial total mass.
Proportional body mass change during flight	(Post-flight mass - pre-feed mass) / pre-feed mass. Pre-feed mass change following tethered flight as a proportion of initial total mass. Positive value indicates gain in mass and negative value loss in mass compared to pre-feed.

4.3.1 Variation in flight performance due to the effect of sex, age and resource–related variables in a cross species analysis

In order to test whether there was a significant effect of sex on flight performance, linear mixed effects (LME) models were fitted using restricted maximum likelihood (REML), assuming a Gaussian distribution. Models were checked to see that the underlying assumptions of normality were met, transformation of the response was used when these assumptions were breached. Model parameters were estimated within R statistical modelling language using the library lmerTest (v2.06, R Core Team 2013). All linear mixed effects (LME) models in this chapter were created using the same methods outlined in detail in chapter 3.

The two tethered flight variables, total distance flown overnight and maximum speed, were used as the response variable. The random effects structure was determined using intercept only models as in Chapter 3, to select which of mill number and date created a model with the lowest AIC (or the simplest model if it was not significantly different to the one with the lowest AIC when tested by ANOVA). Species and sex and the interaction between them were added as explanatory variables, and the significance of these variables to predict flight performance examined in order to detect if there were differences in flight performance due to sex.

In Chapter 3, I investigated which morphological variables best predicted flight performance in male noctuids using LME models, and showed that wing length and proportional thorax mass together explain ~28% of the variation in total distance flown and ~37% of the variation in maximum speed on the tethered flight mills. In order to examine whether these morphological variables were also good predictors of flight performance in both males and females, the same analysis was repeated including both sexes of the same species (or species aggregates) analysed in Chapter 3 (Table 4.1. Models were fitted separately for total distance and maximum speed as response variables, with forewing length, proportional thorax mass, wing loading, thorax shape and forewing aspect ratio as explanatory variables (fixed effects). Random effects were selected for inclusion into models from genus, species, sex and mill number using intercept-only models to see which combination of random effects created a model with the lowest AIC (or the simplest model if it was not significantly different to the one with the lowest AIC when tested by ANOVA). In order to remove variables which did not contribute significantly to predicting the response, reverse model selection was used; the full models were run and the fixed terms with the smallest non-significant ($p>0.05$) t-values were successively removed (Zuur et al. 2009). Model checking of the residuals and fitted values were assessed and an estimated R^2 was derived to quantify fixed and random term contributions (Bell et al. 2012).

To investigate whether age and resource-related variables could increase the amount of variation explained, models were fitted as described above but additionally including the age and resource-related variables. Thus, in addition to the five morphology variables in the full fixed effects model, proportional food consumed, proportional body mass change during flight and wing wear were included. Reverse model selection was then repeated, to see which of the eight variables remained as significant predictors of flight performance.

4.3.2 Variation in flight performance due to the effect of sex, age and resource–related variables in a within species analysis

I wanted to test whether the general trends of morphology, age and resource-related variables predicting flight performance in cross species analyses were true within species. Therefore separate LMEs were fitted for each explanatory variable, including only the chosen variable and species as interacting terms. A significant interaction term indicates that the relationship between the morphological variable and the flight performance variable differs between species. The random effects used were selected from among sex, date and mill number using intercept-only models separately for total distance and maximum speed. This led to the use of mill number as a random effect.

Next, in order to explore the ability of morphology, age and resource-related variables to predict flight performance within species, separate models were run for the five species with >25 individuals (*Apamea monoglypha, Autographa gamma, Noctua pronuba, Xestia c-nigrum and Xestia xanthographa*). This analysis was done to see if the same morphological and physiological variables that predicted flight performance on an inter-specific level also predicted flight performance variation within species. All five morphological variables and three physiological variables were included in the full fixed effect model. The random effects used were selected from among sex, date and mill number using intercept-only models for each model separately.

4.4 Results

For this chapter *Mesapamea spp.* and *Oligia spp.* were aggregated in order to allow females from these species to be included, as only males were identified to species level. This means 617 individuals from 30 species are included (median 16 individuals per species), for which individual values for flight performance are shown in Figure 4.1 and sample sizes and summary statistics in Table 4.2.

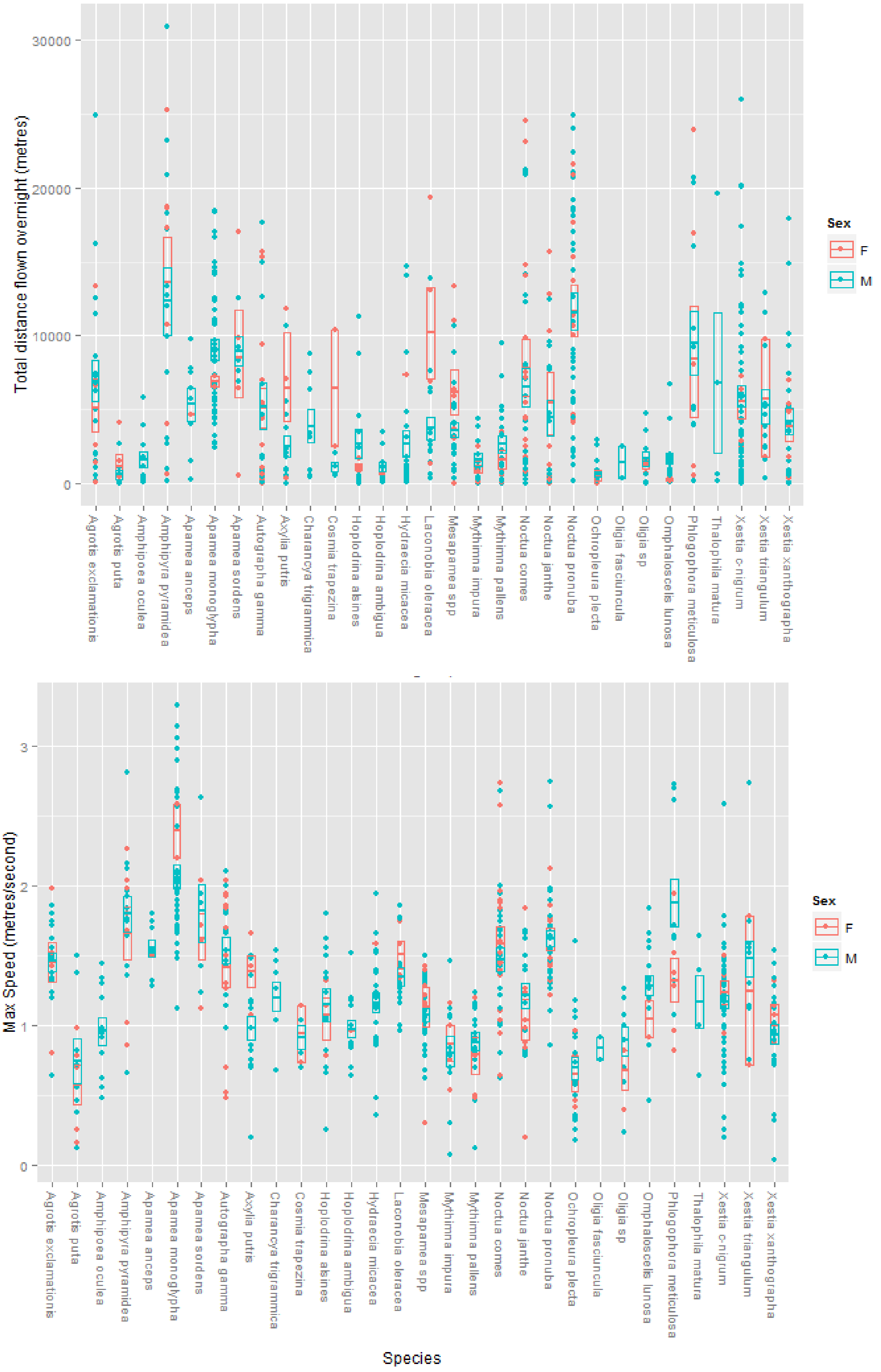

Figure 4.1. *Dotplots of* ***(a)*** *total distance flown overnight and* ***(b)*** *maximum speed attained on tethered flight mills by individuals from 30 noctuid species, arranged alphabetically. Points show each individual, boxes show median and interquartile range for each sex within species.*

Table 4.2. *Summary table of moth species flown on tethered flight mills. Species mean data for two flight performance variables and the standard error.*

				Total Distance		**Maximum Speed**	
Species	**Sample size**	**No females**	**No males**	**Mean**	**SE**	**Mean**	**SE**
Agrotis exclamationis	24	6	18	6487.1	1210.4	1.46	0.06
Agrotis puta	13	5	8	831.7	348.5	0.68	0.12
Amphipoea oculea	11	0	11	1580.2	557.1	0.96	0.10
Amphipyra pyramidea	21	7	14	12770.3	1926.3	1.75	0.11
Apamea anceps	9	1	8	5302.7	1016.9	1.55	0.06
Apamea monoglypha	41	2	39	8930.1	700.1	2.08	0.08
Apamea sordens	9	4	5	8725.1	1503.4	1.73	0.15
Autographa gamma	27	14	13	5155.7	1063.5	1.47	0.09
Axylia putris	17	3	14	3172.2	886.9	1.05	0.09
Charancya trigrammica	8	0	8	3876.3	1163.2	1.20	0.11
Cosmia trapezina	6	2	4	2884.2	1526.7	0.93	0.08
Hoplodrina alsines	16	3	13	2364.4	825.7	1.14	0.10
Hoplodrina ambigua	14	1	13	1124.7	288.4	0.97	0.06
Hydraecia micacea	24	1	23	2842.3	864.5	1.18	0.07
Laconobia oleracea	20	4	16	5041.9	1131.9	1.38	0.06
Mesapamea spp	33	7	26	4136.6	574.2	1.08	0.05
Mythimna impura	15	4	11	1460.3	356.2	0.82	0.09
Mythimna pallens	24	5	19	2459.7	505.1	0.86	0.06
Noctua comes	42	16	26	7024.4	1093.6	1.52	0.08
Noctua janthe	21	8	13	4846.8	1123.1	1.15	0.09
Noctua pronuba	49	12	37	11614.6	1034.7	1.62	0.05
Ochropleura plecta	24	4	20	606.3	162.8	0.69	0.07
Oligia fasciuncula	2	0	2	1463.9	1078.5	0.84	0.08
Oligia spp.	12	3	9	1550.7	405.3	0.84	0.09
Omphaloscelis lunosa	18	2	16	1535.2	380.6	1.26	0.07
Phlogophora meticulosa	16	6	10	9109.5	2012.5	1.67	0.15
Thalophila matura	4	0	4	6797.1	4536.4	1.17	0.22
Xestia c-nigrum	64	5	59	5875.5	701.4	1.18	0.05
Xestia triangulum	14	2	12	5326.5	1062.4	1.45	0.13
Xestia xanthographa	30	5	25	4136.9	806.8	0.96	0.06

4.4.1 *Variation in flight performance due to sex*

Of the 617 individuals flown 132 were females and 496 males, a median of 4 females and 13 males per species. Sex was not a significant predictor of either total distance flown or maximum flight speed attained on flight mills (Table 4.3), indicating that there were no significant differences between males and females in flight performance.

Table 4.3. *Summary of linear mixed effects (LME) models of the effect of sex on tethered flight performance. Response variables were (square-root) total distance flown overnight (8 hours) and maximum speed attained on flight mills. Fixed effects included were species, sex and the interaction between them. The degrees of freedom are generated by a Satterthwaite-type approximation (Gałecki and Burzykowski 2013).*

Model	Fixed effect	Numerator df	Denominator df	F value	P value	Estimated R^2 for fixed model	Estimated R^2 for random effect
Total distance	Species	29	558.97	8.0161	<0.01	28.7%	Mill number 10.6%
	Sex	1	559.86	1.4191	0.234		
	Species *Sex	25	560.42	0.8442	0.685		
Maximum speed	Species	26	560.28	11.0032	<0.01	43.9%	Mill number 2.9%
	Sex	1	562.23	0.1235	0.726		
	Species *Sex	25	563.39	0.8798	0.635		

4.4.2 *Variation in flight performance due to the effect of morphology, age and resource–related variables a cross species analysis*

There was very little difference between the models generated using data from both sexes and the models using just males in Chapter 3. Wing length was a good predictor of both flight variables in both analyses. Including both males and females, a unit change (1 mm) of wing length yielded a 46.2±0.36 m increase in total distance flown (back transformed) which was very similar to the 41.0±0.36 m effect in the males only analysis in Chapter 3. Similarly, a 1mm change of wing length yielded a

0.090±0.009 m/s increase in speed compared with the males only analysis of 0.095±0.009 m/s. In the analysis of males only, proportional thorax mass was a significant explanatory variable for both speed and distance (in addition to forewing length), whereas in the analysis of data for both males and females, proportional thorax mass was only a significant predictor of maximum speed. Forewing aspect ratio was the second significant predictor of total distance flown in analyses of both sexes, but not for male-only data. Overall the models for both sexes and for males accounted for similar amounts of variation in the data set for total distance flown overnight (males only: 28.3%, both sexes: 28.5%) and for maximum speed attained (males only: 36.9%, both sexes: 35.1%). In summary, the inclusion of both sexes rather than just males made very little difference to which morphological variables significantly predicted flight performance and how much variation was explained, except to make forewing aspect ratio a significant predictor of total distance flown rather than proportional thorax mass.

The inclusion of resource but not age-related variables into models resulted in additional variation being explained in flight performance data compared with models with only morphology variables included. For total distance flown, both proportional food consumed and proportional body mass change during flight were significant terms, in the model resulting model R^2 values increasing from 28.5% to 35% (Table 4.4). For maximum speed attained, proportional food consumed and proportional body mass change were again significant terms, but proportional thorax mass was not, and the model R^2 value only slightly increased from 35.1% to 35.6% with the inclusion of physiological variables. Both flight performance variables had a positive relationship with proportional food consumed, meaning moths that consumed more honey solution for their size flew further and faster. Proportional body mass change had a negative relationship with both flight performance variables, inferring that moths which used more of their body mass (e.g. fat reserves), flew longer distance and faster. These two physiology variables are positively correlated with each other (R^2 =36%).

Table 4.4. *Summary of linear mixed effects (LME) models of moth morphology traits predicting flight performance on tethered flight mills of moth individuals from both sexes. Response variables were (square-root) total distance flown overnight (8 hours) and maximum speed attained. Full fixed effects models included wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio for the morphology models and these plus wing wear, proportional food consumed and proportional body mass change during flight for the morphology & physiology models.*

	Model	**Final fixed effects**	**Fixed effect coeff ±se**	**df**	**t value**	**P value**	**Estimated R^2 for fixed model**	**Estimated R^2 for random effect**
Morphology	Total distance	Constant	-6.6 ±22.8	96.5	-0.291	0.772	28.5%	Species :3.6% Genus:1.3% Mill: 9.0%
		Wing length	6.8 ±0.6	37.4	10.629	<0.001		
		Forewing aspect ratio	-4.1±2.0	122.0	-2.11	0.037		
	Maximum speed	Constant	-0.610±0.217	113.1	-2.817	0.006	35.1%	Species :5.1% Genus:3.9% Mill: 2.4%
		Wing length	0.090±0.009	46.1	10.106	<0.001		
		Relative thorax mass	0.852±0.324	580.6	2.629	0.009		
Morphology & Physiology	Total distance	Constant	-8.7±21.6	92.2	-0.402	0.689	35.0%	Species :3.7% Genus:0.0% Mill: 7.6%
		Wing length	7.1±0.6	33.9	12.254	<0.001		
		Forewing aspect ratio	-5.3±1.8	109.1	-2.879	0.005		
		Food consumed (proportional)	28.6±5.3	583.9	5.381	<0.001		
		Body mass change (proportional)	-100.1±14.3	601.9	-7.034	<0.001		
	Maximum speed	Constant	-0.376±0.163	54.6	-2.306	0.025	35.6%	Species :6.4% Genus:4.4% Mill: 2.4%
		Wing length	0.086±0.0096	49.3	9.521	<0.001		
		Food consumed (proportional)	0.351±0.068	618.7	5.16	<0.001		
		Body mass change (proportional)	-0.825±0.181	622.1	-4.551	<0.001		

4.4.3 Variation in flight performance due to the effect of morphology, age, sex and resource–related variables in a within species analysis

There were no significant interactions between any morphological variables examined and species for 'total distance flown' models (Table 4.5.a). For maximum speed models, only wing wear had a significant interaction with species, but this was borderline ($F_{28,561} = 1.5002$, $P = 0.049$) (Table 4.5.b). These results indicate that relationships between morphology and flight performance generally do not differ between species.

Models run for the five species separately did show varying results (Table 4.6) and most did not include the wing length variable that dominated the cross-species analyses. The R^2 for the models varied widely between 4.2 and 54.2%. These intra-specific models were adequate but less robust than the cross species models according to diagnostic plots, probably as a result of low sample size. Also, for all of the individual species models the random effect selection did not yield sex as a useful random effect. This compounds the analysis indicating that there is no difference between the sexes in flight performance.

The hypothesis that morphology is less important on an intra-specific level compared to in the cross species at predicting flight performance, does seem to hold true; of the five species only two, *Xestia c-nigrum* and *Autographa gamma*, had morphological variables remaining significant in the model. The other three, *Xestia xanthographa, Noctua pronuba* and *Apamea monoglypha* had only resource-related variables as significant predictors; proportional food consumed and/or proportional body mass change during flight. Despite its extreme importance in the cross-species analysis, wing length was only a significant predictor of flight performance for *Xestia c-nigrum.* The more subtle morphological measure of proportional thorax mass came out as a significant predictor of flight performance for both flight variables of *Xestia c-nigrum* and maximum speed of *Autographa gamma.* These two species also both had wing wear as a significant predictor of maximum speed. Wing wear runs on a scale from one to four where four is perfect wings (indicating a recently emerged individual), so this positive effect indicates that younger individuals flew faster in these two species.

Table 4.5. *Summary of linear mixed effects (LME) models of moth morphology traits interacting with species to predict flight performance on tethered flight mills. Response variables were (sqrt) total distance flown overnight (8 hrs) and maximum speed attained. Degrees of freedom are generated by a Satterthwaite-type approximation (Gałecki and Burzykowski 2013).*

a.Total distance

Morphological variable	Fixed effects	NumDF	DenDF	F value	P value
Wing length	Species	29	556.56	1.0536	0.391
	Wing length	1	557.38	3.968	0.047
	Species:Wing length	29	556.56	1.101	0.329
Proportional thorax mass	Species	29	555.69	1.18087	0.238
	Proportional thorax mass	1	556.62	0.09386	0.759
	Species:Proportional thorax mass	29	555.76	0.92203	0.586
Wing loading	Species	29	555.67	1.44454	0.064
	Wing loading	1	556.96	0.69503	0.405
	Species:Wing loading	29	555.73	1.09863	0.332
Thorax shape	Species	29	554.85	1.29227	0.143
	Thorax shape	1	556.66	0.06635	0.797
	Species:Thorax shape	29	554.86	1.02372	0.433
Forewing aspect ratio	Species	29	556.2	0.84199	0.705
	Forewing aspect ratio	1	555.82	0.95954	0.328
	Species:Forewing aspect ratio	29	556.2	0.80824	0.753
Proportional food consumed	Species	29	555.9	5.2911	<0.001
	Prop food consumed	1	555.91	0.1378	0.711
	Species:Prop food consumed	29	556.84	1.0842	0.351
Proportional body mass change	Species	29	554.86	8.3817	<0.01
	Prop body change	1	558.55	12.8057	0.000
	Species:Prop body change	29	557.09	0.8869	0.639
Wing wear	Species	29	557.4	1.2943	0.141
	Wing wear	1	555.6	0.9509	0.330
	Species:Wing wear	28	557.52	1.2556	0.173

b. Maximum speed

Morphological variable	Fixed effects	NumDF	DenDF	F value	P value
Wing length	Species	29	560.04	1.2401	0.183
	Wing length	1	562.14	2.1803	0.140
	Species:Wing length	29	560.05	1.2361	0.186
Proportional thorax mass	Species	29	558.95	0.98818	0.485
	Proportional thorax mass	1	560.83	0.16952	0.681
	Species:Proportional thorax mass	29	559.12	0.70918	0.871
Wing loading	Species	29	560.34	1.58328	0.028
	Wing loading	1	562.68	0.08273	0.774
	Species:Wing loading	29	560.48	1.1517	0.269
Thorax shape	Species	29	558.17	1.6481	0.019
	Thorax shape	1	561.05	0.0674	0.795
	Species:Thorax shape	29	558.22	1.1687	0.251
Forewing aspect ratio	Species	29	560.36	0.92953	0.574
	Forewing aspect ratio	1	559.76	0.48518	0.486
	Species:Forewing aspect ratio	29	560.38	0.91994	0.589
Proportional food consumed	Species	29	558.29	6.5036	<0.01
	Prop food consumed	1	558.6	1.6537	0.199
	Species:Prop food consumed	29	560.18	1.4615	0.058
Proportional body mass change	Species	29	555.57	15.4774	<0.01
	Prop body change	1	562.67	0.9281	0.336
	Species:Prop body change	29	559.9	1.2982	0.139
Wing wear	Species	29	560.69	1.8473	0.005
	Wing wear	1	556.51	2.3783	0.124
	Species:Wing wear	28	560.99	1.5002	0.049

Table 4.6. *Summary of linear mixed effects (LME) models of moth morphology traits predicting flight performance of moth individuals within 5 species with >25 sample size (Table 4.1). Response variables were (square-root) total distance flown overnight (8 hours) and maximum speed attained. Full fixed effects models included wing length, proportional thorax mass, wing loading, thorax shape, and forewing aspect ratio, wing wear, proportional food consumed and proportional body mass change during flight.*

Species	Model	Final fixed effects	Fixed effect coeff ± se	df	T value	P value	Estimated R^2 for fixed model	Estimated R^2 for random effect
Xestia c-nigrum	Total distance	Constant	-237.7 ± 120.3	54.84	-1.976	0.053	4.2%	Mill: 35.5%
		Wing length	12.2 ± 6.0	54.69	2.017	0.049		
		Proportional thorax mass	291.3 ± 127.2	56.83	2.291	0.026		
	Maximum speed	Constant	-4.605 ± 1.413	59.34	-3.258	0.002	17.7%	Mill: 6.7%
		Wing length	0.201 ± 0.065	59.8	3.065	0.003		
		Proportional thorax mass	3.548 ± 1.359	59.85	2.611	0.011		
		Wing wear	0.298 ± 0.134	56.02	2.226	0.030		
Xestia xanthographa	Total distance	Constant	19.6 ± 11.8	26.69	1.664	<0.001	54.2%	Mill: 1.92%
		Prop food consumed	81.8 ± 24.7	23	3.309	0.003		
		Prop body change	-385.4 ± 62.0	26.58	-6.218	<0.001		
	Maximum speed	Constant	0.968 ± 0.059	27.991	16.288	<0.001	11.3%	Mill: 0%
		Prop body change	-1.694 ± 0.702	27.991	-2.412	0.023		
Noctua pronuba	Total distance	Constant	90.3 ± 7.2	24.52	12.591	<0.001	8.9%	Mill: 11.6%
		Prop body change	-180.2 ± 76.9	46.8	-2.344	0.023		
	Maximum speed	Constant	1.517 ± 0.066	32.57	22.9	<0.001	5.0%	Date: 20.4%
		Prop food consumed	0.521 ± 0.218	42.07	2.388	0.022		
Apamea monoglypha	Total distance	Constant	75.0 ± 8.0	38	9.325	<0.001	7.0%	Mill: 0%
		Prop food consumed	32.6 ± 16.6	38	1.965	0.057		
		Prop body change	-129.6 ± 53.9	38	-2.405	0.021		
	Maximum speed	Constant	1.871 ± 0.115	38.99	16.23	<0.001	7.9%	Mill: 0%
		Prop food consumed	0.630 ± 0.268	38.99	2.35	0.024		
Autographa gamma	Total distance	Constant	365.6 ± 146.3	17.65	2.499	0.023	35.0%	Mill: 47.4%
		Forewing aspect ratio	-25.3 ± 10.8	16.73	-2.348	0.031		
		Prop food consumed	69.0 ± 19.7	22.99	3.51	0.002		
		Prop body change	-217.8 ± 44.8	16.93	-4.866	<0.001		
	Maximum speed	Constant	-6.639 ± 2.482	21.694	-2.675	0.014	20.0%	Date: 16.0%
		Proportional thorax mass	9.499 ± 3.353	21.693	2.833	0.010		
		Wing loading	5840.690 ± 1983.042	22.993	2.945	0.007		
		Wing wear	0.540 ± 0.218	20.724	2.481	0.022		

For *Xestia c-nigrum* only a very small proportion (4.2%) of the variation in flight performance was explained by the morphological variables. This could be due to the fact that this species is suspected to be a partial migrant, and the high levels of variation in distance flown dependant on whether an individual was a migrant phenotype or not rather than any of the variables measured here. This is in contrast to *Xestia xanthographa*, which is a closely related species but one which was classified as 'medium' mobility by the expert survey in Chapter 2 (whereas *X c-nigrum* was classified as 'high'mobility). For this lower mobility species resource-related variables alone could explain 54.2% of the variation in flight performance.

4.5 Discussion

4.5.1 Variation in dispersal due to sex

Despite the commonly found differences between the sexes in flight performance there was no significant difference between the sexes or between sexes within species for total distance flown and maximum speed. It is possible that any effect would not be easily detectable in this study as the sample size of females was low for the majority of species (median 4 females per species compared to median 13 males). However in one of the species for which an individual analysis was performed (*Autographa gamma*) there were 14 females and 13 males and no significant effect of sex was detected. An alternative explanation is that because mostly only stage 3 or 4 individuals were flown in the study, the age range was restricted to sexually immature or unmated individuals, and that the sexes do not differ in flight performance until after sexual maturity (Armes and Cooter 1991).

However it is possible that there is not a clear sexual bias to dispersal in moths, and that findings depend on species studied. For example, some studies of Lepidoptera report greater dispersal in males (Gu and Danthanarayana 1990, Nieminen 1996) but others in females (Kishava et al. 1967, Kuussaari et al. 1996, Hughes and Dorn 2002, Berwaerts et al. 2006). In addition, differences in dispersal between sexes can be complex; for example in the butterfly *Euphydryas aurinia* males had longer average lifetime movement but females were more likely to undertake long distance dispersal (Zimmermann et al. 2011). Another example are the closely related butterflies *Boloria aquilonaris* and *B. eunomia*, in which adjustment of flight speed at habitat boundaries was different between sexes, but the relationship was in a different direction in each species (Turlure et al. 2011). Finally some studies show no differences between males and females in movement or flight performance (Schumacher et al. 1997, Breuker et al. 2007), similar to the present study. Thus, I conclude that the lack of difference due to sex in this study may simply be because there is no consistent sexual bias to dispersal in the noctuid moths.

4.5.2 Variation in flight performance due to the effect of morphology, age and resource–related variables a cross species analysis

Including variables related to age and food resources into models of flight performance increased the amount of variation that was explained, compared to morphology alone, albeit by a small amount. This was most evident for total distance flown overnight for which the model including the additional variables explained 35% of the variation in the data set, compared to 28.5% in the model with morphology alone. For maximum speed the addition of age and resource related variables only increased the variation explained from 35.1 to 35.6%. This may indicate that fuel resources are important to the stamina of the individual, but maximum speed is less influenced by resources and more ‘hardwired’ in to the individual due to morphology or genetics.

The resource based variables ‘proportional food consumed’ and ‘proportional body mass change’ were significant predictors of flight performance with effect sizes in opposite directions. It is unsurprising that moths that consumed more honey solution flew both further and faster, as the food may have provided fructose and glucose to power flight muscles. Similarly, the negative relationship between the flight variables and body mass change (which would have been increasingly negative as more body mass was lost) showed that moths that lost most mass flew longer distances and faster. The moths may have used energy from lipid stores or metabolised other internal resources to power this longer, faster flight, thus losing mass.

Wing wear did not remain a significant predictor of flight performance in the cross species analyses. This may indicate that age is not an important predictor of flight performance in this range of species, or alternatively, that by selecting only quite fresh individuals the range of ages was successfully constrained and not much variation in age was present in the individuals.

4.5.3 Variation in flight performance due to the effect of morphology, age, sex and resource–related variables in a within species analysis

There were generally no interactions between species and the morphological variables in predicting the flight performance. This indicates that the overall trends, such as total distance flown and maximum speed increasing as wing length increases, all hold true within species as well as across species. The only variable where there was a significant interaction was wing wear, used as a proxy for age. This may indicate that different species vary in patterns of change in flight performance as they age, but the interaction was only borderline significant ($F_{28,561} = 1.5002$, $P = 0.049$) and thus should be treated with caution (Zurr et al. 2009).

Five species were selected to model the relationship between flight performance and morphological/physiological variables in more detail. Of these species all but one were classified as 'high mobility' in the expert survey carried out in chapter 2 (except for *Xestia xanthographa* which was 'medium') and thus variation in flight performance was reduced in this analysis compared with the full multi-species analyses. It would be interesting to see if trends were different between these species and some 'low mobility' species, but insufficient sample sizes were available for any of the 'low' species. Their small sample sizes are most likely a by-product of their low mobility, as they are less likely to fly into the trapping range of the light trap.

The modelling of relationships between morphological/physiological variables and flight performance for individual species showed that at the intra-specific level, flight morphology variables were not as important predictors of flight performance as in the multi-species analyses. This comparative unimportance of morphological variables is unsurprising as there is relatively little variation in morphology within a species compared to the difference between species. The two resource related variables proved to be consistently significant – in four of the five species for total distance and three of the five for maximum speed. For *Xestia xanthographa* the two resource related variables together accounted for 54.2% of the variation in total distance flown. Most of the individual species models, however, did not have such high R^2 values: the mean was 17.1% across the ten models. This implies that these morphological variables and the limited age and resource related variables obtained here are not enough on their own to explain the very high levels of variation within these species. As outlined in Chapter 3 this is probably largely due to fact that the individuals in this study were caught from the wild so their developmental history is unknown. The larval food resources are unknown and uncontrolled and this may have had a large effect on their lipid reserves and therefore flight activity (Gunn and Gatehouse 1993, Itoyama et al. 1999, Sakamoto et al. 2004, Boggs and Freeman 2005). Similarly their age and amount of flight prior to capture was not controlled and this may also have influenced their flight performance.

A number of other physiological features that are known to affect flight performance were not measured in this study. Metabolic rate in the flight muscles can be quantified by measuring CO_2 production as the insect is flown in a sealed container (Haag et al. 2005, Niitepold et al. 2009). Similarly the ATP/ADP ratio can be measured, with a higher ratio indicating greater ability to sustain activity (Hanski et al. 2004). The aerobic capacity of the flight muscles could be more important than simply their mass, and this can be measured by Cytochrome-c Oxidase concentration and mitochondria size and amount (Rauhamäki et al. 2014). Similarly the presence of certain alleles of the gene encoding phosphoglucose isomerase (*pgi*) have been linked to elevated flight metabolic rate and longer flight distance (Haag et al. 2005, Niitepold et al. 2009). These metabolic variables would form an ideal part of future work attempting to explain more of the intra-specific variation in flight performance

In conclusion, the inclusion of resource-related variables can explain a small additional amount of variation in flight performance in multi-species models compared to models with morphological variables alone. In single species analyses there is some indication that physiological features may be more helpful than morphological features in predicting flight performance. However the amount of variation explained by all variables in this study here was variable and often low. Controlling for factors such as age and developmental history, and measuring a greater variety of physiology variables might explain more of the high levels of variation in flight performance that we see within species.

5 Chapter 5 – The role of dispersal capability in the long term population dynamics of British noctuid moths

5.1 Abstract

Globally, many species are declining and previous analysis of abundance trends collected by the Rothamsted Insect Survey showed that two thirds of common and widespread British macro-moths have declined over the past four decades. Habitat and climate change have been shown to be two of the main drivers of these declines, and in this chapter I examined whether the dispersal ability of noctuid moth species plays a role in how species have been affected by environmental change.

Tethered flight mill data ('total distance flown overnight' and 'maximum speed attained'; data for 30 species) and adult forewing length (a good predictor of dispersal ability; data for 74 species) were compared with species trends for abundance, distribution size and northern range margin shift, using historical data from the Rothamsted Insect Survey and National Moth Recording Scheme. I tested the hypothesis that species with poorest dispersal ability would have declined most.

Wing length was a significant predictor of population trends, and explained ~14% of the variation in abundance trends and distribution size. The analysis indicated that species with intermediate dispersal ability may be declining more than those of either high or low dispersal ability. However, tethered flight measures did not predict population or distribution trends. These results give indication that dispersal has some effect on moth distribution and abundance changes but other factors such as habitat availability and responses to changing climate are likely to play a role.

5.2 Introduction

The Earth is currently undergoing biodiversity declines that may detrimentally affect ecosystem services (Butchart et al. 2010, Millennium Ecosystem Assessment 2005). Insects dominate terrestrial biodiversity in terms of species and individuals, and are vital to ecosystems, but despite being the most speciose taxonomic group globally, insect declines are understudied (McKinney 1999, Dunn 2005). One of the largest orders of insects is the Lepidoptera with around 160,000 described species worldwide (Kristensen et al. 2007). In the UK there are ~60 species of butterfly and ~2500 species of moth, of which around 900 of the latter are macro-moths (Waring et al. 2009, Thomas and Lewington 2010). Macro-moths and their caterpillars are an important food resource for bats, birds, small mammals and other invertebrates (Merckx et al. 2009), while some moth species are important pollinators and others are pests (Pettersson 1991, Scoble 1995, Devoto et al. 2011). The ecological diversity and species richness of macro-moths makes them a good indicator group for insect diversity and environmental change (Luff and Woiwod 1995, New 2004, Conrad et al. 2006). The greater diversity of moths compared with butterflies may make them a better indicator species of the ecological impacts of environmental change than the more heavily studied butterflies (Fox 2012).

Population trends of macro-moths are well studied due to the work of monitoring schemes such as the Rothamsted Insect Survey (RIS) (Woiwood and Gould 2008) and the National Moth Recording Scheme (NMRS) (Fox et al. 2011). Analysis of RIS light trap data found that of 337 common and widespread British macro-moth species only one third were stable or increased in abundance during the last 40 years; the other two thirds experienced declines, many of them at a rate of greater than 30% per decade (Conrad et al. 2006, Fox et al. 2013). Similarly the NMRS found that species which had abundance declines tended to also have reductions in distribution size, and species with abundance increases all increased in range size (Fox et al. 2011), which reflects changes in abundance and distribution of UK butterflies (Mair et al. 2014). The availability of population trend information provides the opportunity to explore which mechanisms may be influencing macro-moth declines in the UK. The pattern of increases or declines has thus far been attributed to a range of factors including habitat loss and fragmentation, climate change, agricultural intensification, urbanisation and light pollution (Conrad et al. 2004, Fox 2012, Bates et al. 2014). Of these, habitat loss and climate change are thought to be the greatest drivers, and to act synergistically in some cases (Fox 2012, Fox et al. 2014). Species traits have also been suggested as predictors of abundance trends, although their importance is unclear and more studies are required (Angert et al. 2011).

In a landscape of widespread habitat loss, dispersal ability is a trait hypothesised to be important to species' population dynamics because it enables individuals to find new suitable habitat; high dispersal ability may evolve in meta-populations and during range expansion (Hanski 1999, Berg et al. 2010, Hughes et al. 2007). Species with high levels of mobility have been shown to have lower levels of regional decline and extinction in fragmented landscapes, whereas species with lower mobility are generally more sensitive to the effects of habitat fragmentation. However, in some cases intermediate mobility may be worse than low dispersal ability if it leads to emigration from patches but failure to reach new patches of suitable habitat (Thomas 2000, Tscharntke et al. 2002). Similarly under climate change species are expected to shift their distributions towards the poles, in order to track their climate envelope (Hickling et al. 2006, Hughes et al. 2007). For species with their northern range margin in the UK this means expanding their distribution and shifting their range margin northwards (Hill et al. 1999b, Hill et al. 2011). Poor dispersal ability and barriers to mobility could prevent species from shifting their distributions (Hill et al. 1999a, Warren et al. 2001, Pearson and Dawson 2003, Berg et al. 2010, Hargreaves and Eckert 2014), and the shifting has been shown to lag behind the changing climate (Menendez et al. 2006). It also has been shown that the mobility of different moth and butterfly species can change the effectiveness of biodiversity conservation schemes, such as the benefits of wide field margins to improve local abundance of species, and the successful use of protected 'corridors' and 'stepping stones'(Merckx et al. 2009, Leidner and Haddad 2011). Thus, dispersal ability could be expected to play an important role in the population dynamics of moth species, as in butterflies (Warren et al. 2001, Pöyry et al. 2009, Burke et al. 2011, Mattila et al. 2011).

There has been a lack of knowledge about the dispersal ability of different moth species due to the difficulty in quantifying dispersal directly in nocturnal flying insects. However as outlined in Chapters 2 and 3 it has been possible to quantify the dispersal ability of a number of species of noctuid moths from flight performance on tethered flight mills, and reveal that forewing length is a good predictor of flight performance. In a multi-species analysis of 32 species, forewing length explained nearly 80% of variation in flight performance, thus justifying the use of this morphological measure as a proxy for dispersal ability (Chapter 3).

In this study I test the role of dispersal capability on inter-specific variation in the population and distribution trends of British noctuid moths. Specifically I examine: (1) population abundance trends between 1968 and 2007, estimated from Rothamsted Insect Survey (RIS) light-trap data (Fox et al. 2013); (2) distribution size change between two time periods (1970-1985 and 1995-2010), estimated by National Moth Recording Scheme (NMRS) data as the change in number of hectads (10km squares) occupied between the two periods; and (3) northern range margin shift (in km/decade) between the two time periods, estimated from the change in geographical location of the 10 most northerly occupied hectads for each species (also from NMRS data). This study aims to test the hypothesis that species with greater dispersal ability are less likely to have declining population trends and more likely to have expanding distributions than more sedentary species by:

- Examining how well flight mill performance predicts population changes in 30 intensively studied noctuid species (measures of total distance flown overnight and maximum speed from Chapters 2 – 4).
- Extending this analysis to a wider range of noctuid species using wing length as a proxy for dispersal ability, in order to determine the role of dispersal capability in the population dynamics of a wider sample (N = 74 species) of British macro-moths.

5.3 Methods

5.3.1 Measures of dispersal ability

Dispersal ability was quantified for 30 species of noctuid moth using tethered flight mills (Chapters 2 & 3). Species' mean values were calculated from individual measures of 'total distance flown overnight' and 'maximum speed attained' (Table 5.1). Forewing length was also measured from flown individuals and mean values calculated for each species. In addition, there was a strong relationship between forewing length and tethered flight performance (Chapter 3) and so forewing length was used as a measure of dispersal ability in all species with population/distribution trend information (n = 74 species; see below for species selection). Forewing length for all 74 study species was obtained by measuring life-size photographs in Skinner (1984) using callipers (accurate to 0.1 mm; 1-8 individuals measured per species). In order to check that measures from photographs were good estimates of measurements from moth specimens, wing lengths measured from the 30 study species were compared with measurements from photographs in Skinner (1984) and compared using a paired t-test and Spearman's rank correlation.

The photographic measurements of wing length were significantly slightly smaller than the specimen measurements (mean difference = 0.36 ± 0.32 mm; paired $t_{31} = -2.2889$, $p = 0.02905$) but there was very strong correlation between the two types of measures ($R_{30}= 0.95$, $p < 0.001$). Therefore, subsequent analyses of the total set of 74 species were carried out using only measures from photographs (Skinner 1984). Smaller measurements from photographs were probably because the base of the wing was obscured by the thoracic setae ("hairs") in the photographs, but specimen measures were taken from detached wings.

5.3.2 Measures of population trends, distribution and range margin shifts

Population and distribution trend data were obtained for the 30 species with flight performance data from Chapters 2 & 3 and an extra 44 species for which data on distribution trends was available from Palmer et al. (in prep) (Table 5.1). Abundance trend data were obtained from the Rothamsted Insect Survey (RIS) a national light trapping network of ~100 traps distributed across the UK, and data were available from the period 1968 to 2007. Published abundance trends, calculated for each species using data from all traps that operated for at least one complete year (Conrad et al 2006), were used in the current analyses. These abundance trends were taken from the 'State of Britain's Larger Moths 2013' report (Fox et al. 2013), and had been computed using the TRIM (TRends and Indices for Monitoring data) software package (Pannekoek and van Strien 2001). TRIM estimates the annual abundance of each species by fitting a generalised linear model with Poisson errors and a logarithmic link to the RIS data. The estimated abundance for the first year (1968) is set to 1, so each subsequent annual index is the change relative to the first year of sampling for that species. In this study, the slope of this trend was used as the measure of abundance change for each species. The value of the slope is rescaled, so that values greater than 1 indicated increasing abundance while values less than one indicated decreasing abundance.

Change in the distribution size and northern range margin were obtained from data from the National Moth Recording Scheme dataset (Palmer et al., in prep). The NMRS assembles records from amateur and professional moth recorders throughout the UK and also collates historical macro-moth records (Fox et al. 2011, Fox et al. 2013). A 'record' for a hectad (10 by 10 km grid square) comprises a species of moth trapped at any location within the hectad in a year. In order to account for variability in recording effort only 'well recorded' locations were included in the analysis. A well recorded hectad was one which had records from at least 10% of the regional moth species (which was defined as 10% of the total number of moth species recorded from the nearest 100 hectads with moth records). The distribution changes were calculated between two time periods, 1970-1985 and 1995-2010. These time periods were chosen to provide a sufficiently long interval for change to occur, and to account for yearly variability in recording effort. Distribution size change per decade was computed as the change in the total number of hectads occupied between the two time periods divided by number of decades (2.5 decades). Northern range margin change was computed as the change in the mean latitude of the ten northernmost hectads (in km) between the two time periods, divided by number of decades. Positive values indicate a range margin that has shifted northwards and negative values those that have shifted southwards/retracted.

Table 5.1. *Summary table of 74 moth species included in analysis exploring how measures of dispersal ability predict population and distribution trends. 74 species are included of which the first 30 were flown on tethered flight mills and mean values of total distance flown overnight and maximum speed computed. Wing length is included as a morphological proxy for dispersal ability, measured from individuals flown on flight mills for the 30 study species and from life-size photographs in Skinner (1984) for the full 74 species. Abundance trends were obtained from Fox et al. (2013) analysis of Rothamsted Insect Survey light trapping data and represent the slope of abundance over the period 1968-2007. They are rescaled so abundance trend values of one indicate stable abundance, >1 increasing and <1 decreasing abundance. Northern range margin shift and distribution size change were obtained from Palmer et al. (in prep) and are calculated between two time periods (1970-1985 and 1995-2010), from National Moth Recording Scheme data. Distribution size change is the change in number of hectads (10km squares) occupied between the two periods divided by number of decades; and northern range margin shift is change in mean latitude of the 10 most northerly occupied hectads for each species between the two time periods.*

Species	**Sample size**	**Wing length (mm)**	**Total Distance (m)**	**Max Speed (m/s)**	**Wing length from photographs (mm)**	**Abundance slope**	**Northern range margin shift (km/decade)**	**Distribution size change (hectads/decade)**
Agrotis exclamationis	24	17.4	6487.1	1.46	16.36	0.9653	0.4	14.4
Agrotis puta	13	13.5	831.7	0.68	13.15	1.0139	45.2	79.2
Amphipoea oculea	11	13.6	1580.2	0.96	13.65	0.9994	0.4	38.8
Amphipyra pyramidea	21	22.8	12770.3	1.75	22.53	1.0229	92	453
Apamea anceps	9	17.7	5302.7	1.55	19.8	0.9353	-2	42.4
Apamea monoglypha	41	23.0	8930.1	2.08	22.85	0.9939	0	18
Apamea sordens	9	17.6	8725.1	1.73	17.8	0.9871	-1.6	31.6
Autographa gamma	27	18.9	5155.7	1.47	15.78	0.9846	1.6	34.8
Axylia putris	17	15.2	3172.2	1.05	15.15	0.9911	6.8	62.8
Charanyca trigrammica	8	15.9	3876.3	1.20	15.5	1.009	-7.2	98

Cosmia trapezina	6	14.8	2884.2	0.93	14.31	0.9979	0	73.2
Hoplodrina alsines	16	14.6	2364.4	1.14	13.45	1.0078	-12.8	88
Hoplodrina ambigua	14	13.3	1124.7	0.97	14.55	1.0427	73.6	132.4
Hydraecia micacea	24	18.1	2842.3	1.18	18.03	0.9522	-1.2	77.6
Lacanobia oleracea	20	17.3	5041.9	1.38	16.6	0.9927	4.8	48
Mesapamea spp	33	14.3	4136.6	1.08	14.15	1.0043	2	154
Mythimna impura	15	15.6	1460.3	0.82	14.675	0.9997	2.4	33.2
Mythimna pallens	24	15.1	2459.7	0.86	15.525	0.9778	11.6	36.4
Noctua comes	42	19.8	7024.4	1.52	18.34	1.0157	4	46.8
Noctua janthe	21	17.6	4846.8	1.15	17.75	1.007	-2	67.6
Noctua pronuba	49	25.4	11614.6	1.62	24.68	1.0266	0	15.6
Ochropleura plecta	24	13.4	606.3	0.69	13.6	1.0052	2.8	42
Oligia fasciuncula	2	10.7	1463.9	0.84	10.4	0.9973	-2	72.8
Oligia sp	12	11.8	1550.7	0.84	11.78	0.9757	-8.8	36
Omphaloscelis lunosa	18	16.1	1535.2	1.26	15.24	1.0218	6	99.6
Phlogophora meticulosa	16	22.9	9109.5	1.67	21.7	1.0072	9.6	55.2
Thalpophila matura	4	18.4	6797.1	1.17	17.2	0.9858	-12.8	19.6
Xestia c-nigrum	64	17.1	5875.5	1.18	16.4	1.0068	-7.6	57.6
Xestia triangulum	14	18.5	5326.5	1.45	18.6	0.9886	8.8	73.6
Xestia xanthographa	30	16.0	4136.9	0.96	15.5	1.0148	-2.4	60.8
Abrostola tripartita	NA	NA	NA	NA	15.2	1.0185	6.8	76
Acronicta psi	NA	NA	NA	NA	18.25	0.9681	6.4	18.8
Agrochola circellaris	NA	NA	NA	NA	16.15	0.9842	2.4	71.6
Agrochola litura	NA	NA	NA	NA	15.6	0.9576	6.8	16
Agrochola lota	NA	NA	NA	NA	15.3	1.0031	16	102
Agrochola lychnidis	NA	NA	NA	NA	15.58	0.9365	-19.2	36.8
Agrochola macilenta	NA	NA	NA	NA	15.85	1.0139	20.4	116.4

Agrotis clavis	NA	NA	NA	NA	15.58	0.9996	-39.2	81.6
Agrotis segetum	NA	NA	NA	NA	17.51	0.973	9.2	52.8
Allophyes oxyacanthae	NA	NA	NA	NA	18.55	0.9588	1.2	59.2
Amphipoea lucens	NA	NA	NA	NA	16.05	0.9765	10.4	26
Amphipyra tragopoginis	NA	NA	NA	NA	15.85	0.9544	-3.6	-4
Anaplectoides prasina	NA	NA	NA	NA	21.65	1.0236	16	111.2
Asteroscopus sphinx	NA	NA	NA	NA	18.78	0.9508	-20.8	35.2
Atethmia centrago	NA	NA	NA	NA	13.85	0.9701	20	142.8
Caradrina morpheus	NA	NA	NA	NA	14.75	0.9552	4.8	37.2
Conistra ligula	NA	NA	NA	NA	13.65	0.9875	8.4	78
Conistra vaccinii	NA	NA	NA	NA	13.53	1.0105	19.2	107.2
Cryphia domestica	NA	NA	NA	NA	11.75	1.0351	20.4	94
Dryobotodes eremita	NA	NA	NA	NA	14.9	1.0344	23.2	113.2
Eremobia ochroleuca	NA	NA	NA	NA	14.9	1.0098	15.6	58.4
Eupsilia transversa	NA	NA	NA	NA	17.38	1.0194	16.4	114
Gortyna flavago	NA	NA	NA	NA	17.6	0.9974	14.4	113.2
Hadena bicruris	NA	NA	NA	NA	15.35	0.9683	13.2	75.6
Herminia grisealis	NA	NA	NA	NA	12.35	0.9915	8	92.8
Hoplodrina blanda	NA	NA	NA	NA	13.7	0.963	25.2	98
Laspeyria flexula	NA	NA	NA	NA	13.65	0.981	32.8	54.4
Luperina testacea	NA	NA	NA	NA	15.18	0.9855	-2.8	61.6
Mamestra brassicae	NA	NA	NA	NA	19.5	0.9863	-9.6	30
Mesoligia furuncula	NA	NA	NA	NA	10.53	1.017	4.4	94.8
Mythimna comma	NA	NA	NA	NA	15.5	0.9691	6.4	76
Mythimna ferrago	NA	NA	NA	NA	16.8	0.9874	-2	49.2
Orthosia cruda	NA	NA	NA	NA	12.75	0.9993	12.4	108
Orthosia gracilis	NA	NA	NA	NA	17.21	0.9667	3.6	74.4

Orthosia munda	NA	NA	NA	NA	17.41	0.9937	29.2	124
Panolis flammea	NA	NA	NA	NA	14.5	1.038	18.8	143.2
Protodeltote pygarga	NA	NA	NA	NA	11.7	1.0274	52	136.8
Rivula sericealis	NA	NA	NA	NA	9.9	1.0022	20.8	192.4
Tholera cespitis	NA	NA	NA	NA	16.9	0.9177	14.4	12
Tholera decimalis	NA	NA	NA	NA	19.5	0.9464	-20.8	10
Xanthia aurago	NA	NA	NA	NA	13.4	0.9932	3.6	117.6
Xestia ditrapezium	NA	NA	NA	NA	17.8	0.9896	8	24.4
Xylocampa areola	NA	NA	NA	NA	14.75	0.9998	14.8	99.6
Zanclognatha tarsipennalis	NA	NA	NA	NA	13.7	0.993	14.4	107.6

5.3.3 Analysing relationships between dispersal and distribution/population changes

In order to examine whether measures of flight mill performance of 30 study species were related to population and distribution changes, General Additive Models (GAM) were fitted in R (R Core Team, 2013 using the mgcv library (Wood 2011). GAM was used so that both linear and non-linear relationships could be explored. Three population and distribution trends were included separately as response variables, and the three dispersal measures were also included separately as explanatory variables, thus nine models were created. A Gaussian distribution with a log link function and REML estimation were used in all GAMs to model moth population and distribution trends. Population and distribution change variables were transformed where necessary to ensure they conformed to a normal distribution. Penalized thin plate regression splines were used to fit 'smoothers' to data to allow non-linear relationships to be computed (Zuur et al. 2009). GAMs provide outputs in the form of the significance of the fixed effects in the model, an adjusted R^2 value for the model, and the estimated degrees of freedom (edf) which is an indication of the degree of non-linearity where, edf = 1 is linear and edf values greater than 1 indicate increasing non-linearity. Model checking for homogeneity of variances and normality of residuals was carried out with gam.check.

Nine models were computed (each pairwise combination of the three population and distribution trends and the three measures of dispersal ability), the P values were adjusted using the 'False Discovery Rate (FDR) method in R's p.adjust function. This applies a similar method to bonferroni corrections, but decreases the chance of type I errors without increasing the number of type II errors to unacceptable levels (Nakagawa 2004).

For the larger sample of 74 species, Generalised Additive Mixed Models (GAMMs) were the fitted using a response (separate models for each of the three population and distribution trends responses) and a single explanatory variable (wing length measured from photographs). The greater sample size also allowed the inclusion of Subfamily and Genus as random effects, to account for the effect of phylogeny in the absence of a detailed noctuid phylogeny. This was not possible in the 30 species analysis as the lower sample size did not permit the inclusion of these random effects.

5.4 Results

5.4.1 Measures of population trends, distribution and range margin shifts

Of the 74 noctuid study species two thirds (47) had declined in abundance in the 1968-2007 period, which reflects the fact that two thirds of common and widespread British macro-moths are known to have declined in this period. Between the periods 1970-1985 and 1995-2010 the northern range margin had shifted northwards for 52 of the species, stayed stable for three and retracted southwards for 19 species (Table 5.1). All but one of the 74 species had expanded their distribution size (increased the number of hectads occupied).

5.4.2 Relationship between dispersal and population/distribution changes

The measures of tethered flight performance were generally not significant predictors of moth population and distribution changes (Table 5.2). The only significant relationship in the analysis of 30 study species was a non-linear positive relationship between total distance flown on the flight mill and population abundance trend (edf=7.0, F=4.32, P=0.003). However inspection of the non-linear relationship shown in Figure 5.1 showed that this model has likely been over-fitted by the GAM, and the relationship appears to be primarily driven by a single species with a large increase in distribution size and a large flight capacity on the mills (*Amphipyra pyramidea*). Removal of this species resulted in a non-significant relationship.

By contrast, in the study of all 74 noctuid species, the generalised additive mixed effects (GAMM) models indicated that forewing length was a significant predictor of both abundance trend and distribution size change (Table 5.3). In both cases the amount of variation explained was ~14% (abundance trend: edf=3.2, F=4.235, P=0.007; change in distribution size: edf=1.9, F=5.724, P=0.005) and both relationships were non-linear (Figure 5.2). Species with intermediate forewing lengths had greater declines in their abundance trends compared with species with either short or long forewings (Figure 5.2a). All study species had positive (i.e. expanding) distribution size trends, but species with intermediate to long forewings had expanded their distributions less than species with shorter wings (Figure 5.2b).

Table 5.2. *Summary of generalised additive (GAM) models of measures of tethered flight performance predicting population and distribution trends in 30 noctuid moth species. Response variables were: (1) Slope of the abundance trend 1968-2007 (2) Distribution size change (hectads/decade). (3) Northern range margin shift in km/decade. Explanatory variables were total distance flown overnight (8 hours), maximum speed attained on tethered flight mills and wing length of flown individuals. These fixed effects were included in the model as smoothers (i.e. non-linear terms).*

Response variable	**Smoothed fixed effect**	**edf**	**F statistic**	**P value**	**FDR adjusted P value**	**Adjusted R^2**
Abundance	Total distance	2.0	2.002	0.143	0.417	13.3%
Abundance	Max speed	1.0	0.248	0.623	0.623	0.0%
Abundance	Wing length	3.2	1.805	0.158	0.417	17.3%
Distribution	Total distance	7.0	4.318	0.003	0.027	52.0%
Distribution	Max speed	5.2	1.013	0.441	0.567	11.8%
Distribution	Wing length	1.0	1.223	0.278	0.417	0.0%
Northern range margin	Total distance	2.1	1.629	0.206	0.417	11.7%
Northern range margin	Max speed	2.8	1.433	0.250	0.417	10.8%
Northern range margin	Wing length	1.0	0.338	0.565	0.623	0.0%

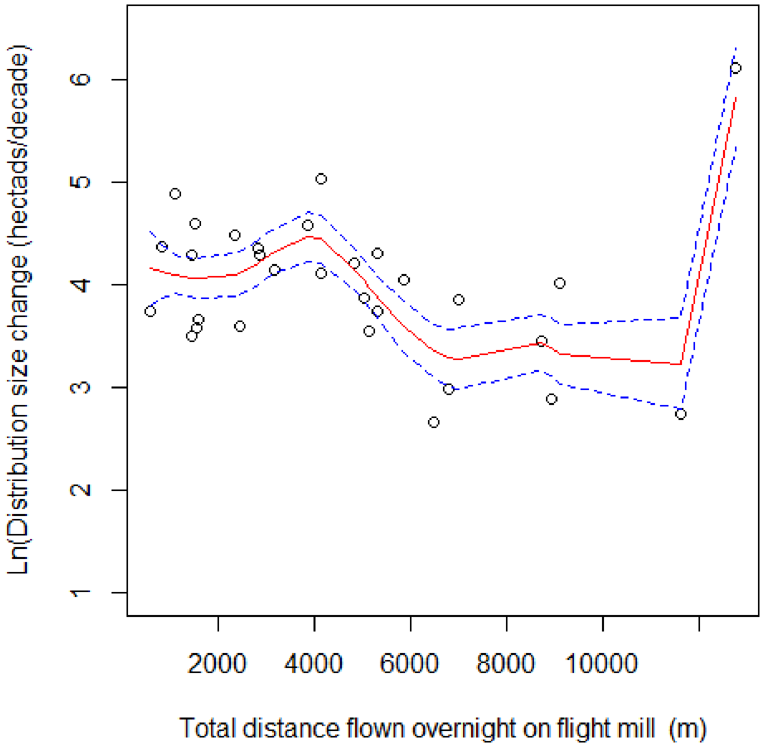

Figure 5.1. *Plot showing total distance flown overnight predicting distribution size change in 30 species of noctuid moth. Model predictions from generalised additive model (GAM) shown by solid red line with dotted blue lines indicating standard error of the model predictions. Circles denote observed values. All distribution size changes were positive.*

Table 5.3. *Summary of generalised additive mixed effects (GAMM) models of wing length as a proxy for dispersal ability predicting population and distribution trends in 74 noctuid moth species. Response variables were: (1) Slope of the abundance trend 1968-2007 (2) Distribution size change (hectads/decade). (3) Northern range margin shift in km/decade. Explanatory variable was wing length as measured from photographs in Skinner (1984). The fixed effect of wing length was included in the model as a smoother (i.e. non-linear term). Subfamily and Genus were included in the model as random effects to account for phylogeny.*

Response variable	**Smoothed fixed effect**	**Estimated df**	**F statistic**	**P value**	**Adjusted R^2**
Abundance	Wing length	3.156	4.235	0.00744	14.50%
Northern range margin	Wing length	2.287	1.206	0.303	4.26%
Distribution size	Wing length	1.963	5.724	0.00527	13.90%

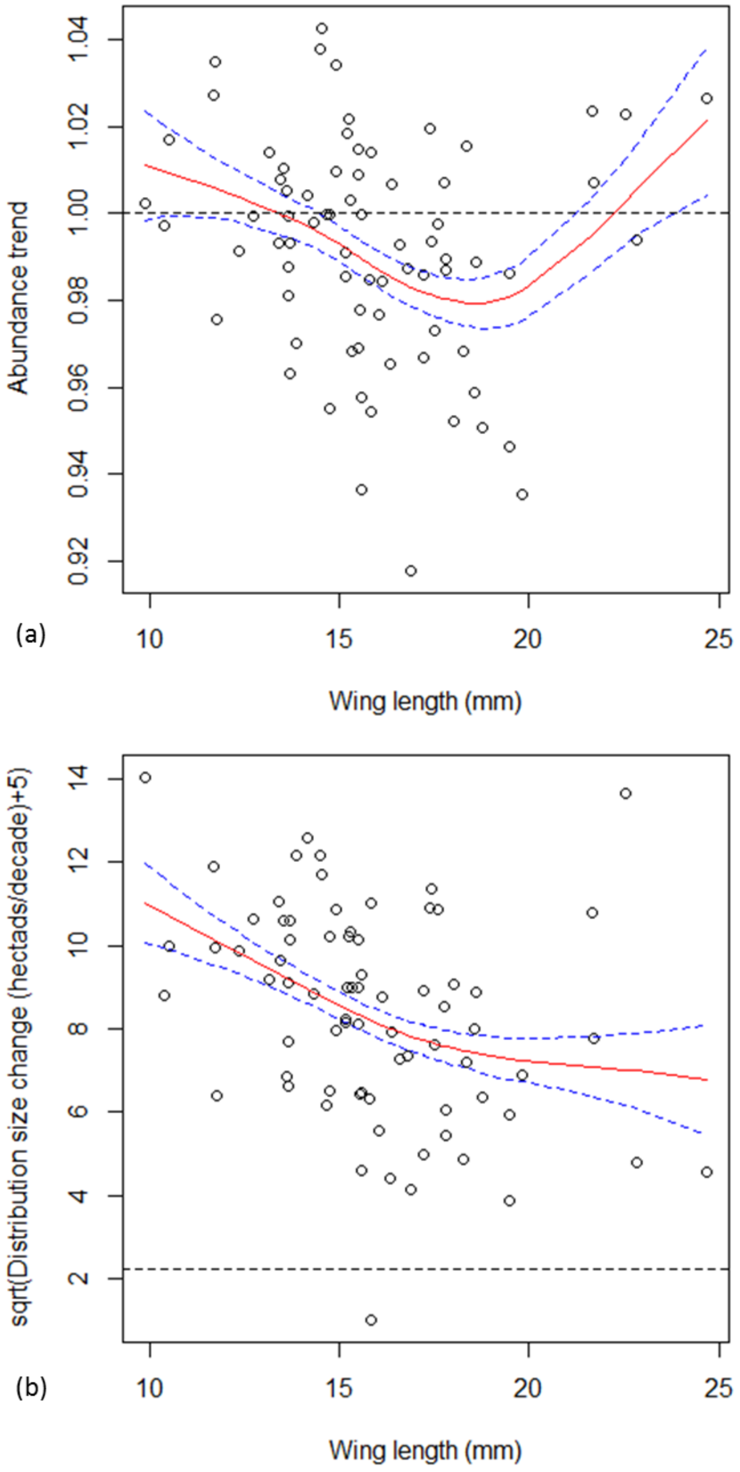

Figure 5.2. *Plots showing wing length (as a proxy for dispersal ability) predicting* ***(a)*** *abundance trend and* ***(b)*** *distribution size change in 74 species of noctuid moth. Model predictions from generalised additive mixed effects models (GAMMs) is shown by solid red lines with dotted blue lines showing standard error of the model predictions. Circles denote observed values. Dotted black line indicates zero change in abundance or distribution size. Abundance trend (a) is a measure of the slope of abundance change 1968-2007, with values >1 indicating abundance increases, <1 decreases and =1 stable populations.*

5.5 Discussion

Analysis using the subset of 30 study species found that neither total distance flown overnight on tethered flight mills, maximum speed attained on flight mills or wing length were useful predictors of the population and distribution trends. However the larger analysis of 74 species suggested that dispersal ability, through the proxy of forewing length, may explain some of the variation in moth abundance and distribution trends.

Analysis of all 74 species found that forewing length could predict ~ 14% of the variation in abundance trend and distribution size change. Thus I conclude that any effects of species dispersal traits in predicting abundance and distribution trends are relatively minor. Figure 5.2a provides some indication that species of intermediate wing length (approx. 15-20mm) may be more likely to be declining in abundance compared with species <15mm and larger species with wing lengths >20mm. This pattern may support the idea that intermediate dispersal ability is less beneficial than either high or low dispersal. Within populations, dispersal evolution models have found bimodal selection on dispersal in a fragmented landscape, due to selection against dispersal at meta-population edges, where dispersal mortality is high, and selection for high dispersal more centrally within the meta-population (Travis and Dytham 1999). Empirically a similar trend has been found by Thomas (2000) who established that intermediate dispersers declined most in a study of 56 butterfly species. The proposed mechanism for a disadvantage to intermediate dispersers was that they have higher habitat patch size requirements, but in fragmented habitat this leads to higher dispersal mortality, whereas highly mobile species tend to successfully traverse the matrix. Figure 5.2b indicates that as wing length increased species had expanded their distribution sizes less. However, only five species had wing lengths >20mm, and these 5 species had very different changes in distribution size, and so perhaps inclusion of more species within this size group might produce a more consistent relationship between size and distribution change.

In general it would be beneficial to repeat this study with a greater sample size to determine the strength of these relationships. There are around 400 noctuid species in total in the UK, and of the ten Noctuid subfamilies (Waring et al. 2009) all were represented but some only by one or two species. Although these relationships were found when controlling for phylogeny by the inclusion of subfamily and genus, ideally the analysis would account for phylogeny using a phylogenetic tree. While relationships within the Lepidoptera are starting to be elucidated, a species level phylogeny of the Noctuidae does not yet exist (Mitchell et al. 2006, Regier et al. 2013).

The species that are most rapidly-declining, which in Figure 5.2.a were the intermediate-sized species, share some life-history attributes. All bar one of the seven most declining species overwinters in the egg life-cycle stage. Conrad et al. (2004) found the greatest mean declines among species which overwintered in this life stage. Similarly the two most declining species *Tholera cespitis* and *Apamea anceps* are grassland specialists, and this larval food preference also had greater mean declines in Conrad et.al (2004). However after this the rapidly-declining species consist of a mix of specialists and generalists, indicating life-history variation alone is not enough to explain abundance trend.

5.5.1 The importance of dispersal ability

The use of wing length as a proxy for dispersal ability seems to indicate that dispersal can explain between 10 and 15% of the variation in abundance and distribution size trends of British macro-moths. This reflects other support for the importance of dispersal in shaping species responses to climate change. Distribution change, range shift and therefore extinction risk was related to dispersal in Finnish butterflies (more mobile species declined less) (Kotiaho et al. 2005, Mattila et al. 2011). Similarly, in British butterflies more sedentary species experienced more declines than mobile species, although this relationship was not always linear (Warren et al. 2001, Thomas 2000). Selection on improved dispersal ability has also been demonstrated in butterflies at expanding range edges (Hughes et al 2007, Hill et al. 1999a). The importance of sufficient dispersal ability has led to its acknowledgment as a factor in reducing extinction risk (McKinney 1997).

5.5.2 Role of species traits and extrinsic factors

By contrast, recent discussion has called into question the utility of species traits such as dispersal in predicting species abundance and distribution trends. A meta-analysis of North American birds, British Odonata, Swiss alpine plants and western North American small mammals found that species traits models had low predictive power for range shifts (Angert et al. 2011). Similarly a study of 25 butterfly species found that dispersal ability was not a significant predictor of range shifts (Mair et al. 2014). Exploring how invasive species may differ in their traits from non-invasive ones has led to a similar conclusion; a comprehensive analysis of traits of Eurasian plants which have been introduced to Argentina found that no single trait explained a large amount of variation in the data and even combining all traits measured only 21% of the differences between invasive and non-invasive (Prinzing et al. 2002). Rather than species traits being of primary importance, there is evidence that the changes in distribution of native species are likely to be dependent on extrinsic factors. These extrinsic factors include habitat availability, which has been shown to be key for butterflies (Hill et al. 2002, Mair et al. 2014) and response to climate change itself (Palmer et al. in prep). Although the importance of, and selection for, dispersal ability may seem intuitive, high dispersal ability is not helpful if the availability of habitat/host plants is too patchy or rare (Berg et al. 2010, Pelini et al. 2010).

In conclusion, this study provides evidence that dispersal ability can explain a small proportion of the variation in abundance and distribution trends of British macro-moths. Dispersal ability may not be a primary consideration when planning conservation management for macro-moths species. However, it may be worthwhile to combine information on dispersal ability with other factors in population and distribution changes, such as habitat and climate responses and life-history factors, in order to generate more accurate predictions of which species will increase or decline in the future.

6 Chapter 6 – General Discussion

6.1 Summary of Thesis Findings

The main aim of the thesis is to quantify the dispersal ability of noctuid moths, deduce which morphological features are good predictors of flight performance, and investigate the links between dispersal capacity and population trends. To investigate this, I studied dispersal in noctuid moths using tethered flight mills, measured a wide range of morphological features of flown individuals, and compared this dispersal information with abundance and distribution trends.

Dispersal is an important process in the ecology and evolution of organisms, affecting population dynamics, gene flow, species distributions and range size. The dispersal ability of British macro-moths may determine whether or not species persist in a changing environment, but knowledge of individual species dispersal abilities is lacking. This lack of knowledge is due at least in part to inherent difficulties in quantifying dispersal ability, especially for nocturnal species. For this study, a novel tethered flight mill technique was developed and in **Chapter 2** I used the tethered flight mills to quantify the dispersal ability of 24 species of British noctuid moth. Previously, tethered flight mills were designed for the study of single species; this new method allows a range of different species to be studied concurrently. I answered the question of whether flight performance on the mills is representative of natural flight ability by comparing flight performance with expert opinion. I also concluded that the tethered flight variables that were measured (‘total distance flown overnight’ and ‘maximum flight speed’) characterised the main differences in flight mill activity among the study species. This new technique will provide the opportunity to examine flight performance in a wider range of insects than was previously possible.

Biologists have long been interested in the link between morphological features and flight ability, in order to better understand and predict dispersal capabilities. In addition, due to the difficulty in measuring dispersal directly, morphological traits may act as useful proxies. Therefore, in **Chapter 3**, I explored the relationship between adult flight morphology and flight performance on the tethered flight mills in 32 species of noctuid moth, in order to answer the question of whether morphological features are useful predictors of tethered flight performance. I determined that both total distance flown overnight and maximum speed increased with increasing forewing length, and to a lesser extent, proportional thorax mass, and that morphology explained about 30% of the variation in flight performance among individuals across all species. Adult forewing length was an extremely good predictor of inter-specific variation in flight performance, explaining ~76% of the variation. I conclude that forewing length is a useful proxy for species dispersal ability in noctuid moths.

Variation in dispersal ability among individuals of a species can have important effects on the fitness of those individuals and the viability of their populations. In **Chapter 4**, I explored variation among individuals, and asked whether additional variation in tethered flight performance could be explained by moth sex, age and resource use. In a multi-species analysis an additional 0.5-6.5% of variation could be explained by the inclusion of resource –related variables, but wing wear (a proxy for age) did not explain any additional variation, and there were no detectable differences between males and females in flight performance. I also conducted separate analyses for a sub-set of five species where a large number of individuals had been studied, and these analyses indicated that the effects of flight morphology were generally less important and varied considerably among the five study species. In contrast to the multi-species analyses, resource-related variables sometimes explained high levels of variation (up to 54%) in some species. I conclude that additional physiological and behavioural features to those measured in this study must be responsible for the high levels of intra-specific variation in flight performance.

Around two thirds of common and widespread UK macro-moths have experienced declines in the last 40 years. Habitat and climate change have been shown to be two of the main drivers of these declines, and in **Chapter 5** I examined whether the dispersal ability of noctuid moth species also plays a role in how species have been affected by recent environmental change. I tested the hypothesis that over the past four decades, moths with greater dispersal ability had fared better than less mobile species. I compared three measures of species' dispersal ability with changes in abundance, distribution size and northern range margin shift. The dispersal measures I examined were 'total distance flown overnight', 'maximum speed attained' and adult forewing length (as a proxy for dispersal ability). I showed that forewing length explained ~14% of the variation in abundance trends and distribution size. Species with intermediate wing lengths were more likely to decline in abundance and expand their distribution less compared with species with either short or long wings. I concluded that dispersal may play a role in how species are faring under climate change, but other factors such as habitat availability and climate may be more influential.

6.2 Tethered flight as a tool to study dispersal ability

The tethered flight mill system used in this thesis is a great improvement on previous systems available, and the lightweight, low friction arm has enabled me to compare the flight performance of moths across a wide range of species of different sizes. Criticism can be levelled at the technique however, primarily because it is not clear how 'natural' dispersal behaviours recorded on the flight mills really are. As highlighted in Chapter 3, the maximum speeds attained by moths in this study (0.7-2 m/s) were lower than those estimated in the wild from radar studies (speeds of 3 - 4 m/s; e.g. Chapman et al 2008). These slower speeds are likely to be due to the added effort of pushing the flight mill arm. It is important to understand how distances flown on the flight mill might relate to dispersal distances in the wild. It is difficult to simulate all the cues that an insect may require to fly, which is especially important if flight propensity is a point of interest (Colvin and Gatehouse 1993c), and so insects may not behaviour appropriately when tethered. For example, moths may not receive appropriate cues to take off, or once in flight, this lack of cues may prolong the insect's flight unnaturally if they don't receive cues influencing them to land. In addition, the lack of tarsal contact with the ground and the inability to land may make insects fly for an extended period of time (Gatehouse and Hackett 1980). Conversely, the added physical effort of pushing the flight mill while flying may cause the insect to tire and cease flight more quickly than in the wild.

Despite these criticisms, tethered flight mills are an invaluable tool in studying the flight performance of nocturnal and/or high flying insects for which no observation of natural flight duration and movement pathways may be possible. Although tethered flight may not provide direct information on natural flight speeds and distances, it has been demonstrated to represent differences in dispersal ability between different groups (Blackmer et al. 2004, Taylor et al. 2010). In my study, I showed that performance on the flight mill reflected expert opinion on dispersal ability of different species, supporting the notion that flight mills are a useful technique for comparing flight ability of different groups. In the future work section below, I outline ways in which it might be possible to develop these techniques to provide a better understanding of relationships between moth performance on tethered flight mills and natural flight.

6.3 Forewing length as a proxy for dispersal ability

In Chapter 3, I determined that forewing length was a good predictor of dispersal ability, and I therefore used it as a proxy for dispersal ability in analyses of the role of dispersal ability in abundance and distribution trends in Chapter 5. The selection of a measure of body size as the best morphological predictor of dispersal ability was not surprising, because body size is one of the most widely supported morphological predictors of dispersal ability in animals. Allometry (biological scaling of variables with body size) of dispersal has been demonstrated across a wide range of taxonomic groups, including mammals, birds and fish (Sutherland et al. 2000, Bradbury et al. 2008), and there is also some evidence in bees, butterflies and moths (Greenleaf et al. 2007, Sekar 2012, Nieminen et al. 1999). There is some evidence that positive relationships between size and dispersal distance are only evident in active dispersers, such as the animals outlined above, and not passive dispersers such as plant seeds and planktonic larvae, where propagule size is generally not a useful predictor of dispersal distance (Jenkins et al. 2007). This supports the conclusion that size is important in animals such as moths, for which self-powered locomotion permits dispersal.

The mechanism determining why dispersal increases with size in active dispersers is likely to be due to relative costs of locomotion, including species' metabolic rates and energy consumption (Hein et al. 2012). Energy usage per unit distance travelled increases as body size increases, but the energy usage per gram of mass decreases with increasing body size, across running, swimming and flying mammals (Schmidt-Nielsen 1972, Peters 1983). In addition, the muscles of running, flying and swimming animals work more efficiently as muscles get larger (Alexander 2005). Larger animals also have longer maximum movement distances because they can carry more fuel and they utilise that fuel at a slower rate per gram than a smaller animal (Peters 1983). Similarly, larger animals can gather and store fuel more easily than smaller animals (Brown and Maurer 1989), and due to the biomechanics of movement, larger animals can achieve higher speeds which leads to greater distances (Peters 1983). Larger wings specifically, rather than just larger size, are more likely to lead to greater dispersal ability as the longer the wings are in relation to the weight of the animal the less induced power is required for flight (the induced power is the power required to maintain enough lift to overcome the force of gravity; Pennycuick 1972).

However while this information demonstrates why a measure of moth body size, such as forewing length, might be a good predictor of dispersal ability, the use of body size *per se* as a proxy to explore the role of dispersal ability in population trends could be called into question. Other life history traits such as lifespan, range size, local population abundances and fecundity vary allometrically (Calder 1984) and these traits may affect population trends. Species with greater dispersal ability might be expected to fare better under environmental change, but larger body size has also been related to increased extinction risk (McKinney 1997, Purvis et al. 2000). For example, larger species of moths had greater distribution declines in a study of Finnish noctuid moths (Mattila et al. 2008). The use of wing length as a proxy for dispersal ability should therefore be used with caution and an awareness of the complex interactions of species traits in their role in population dynamics. However as noted by Sekar (2012) for butterflies, wing length is a ‘satisfactory proxy’ that may be essential for analyses of large numbers of species for which no direct information on dispersal exists.

6.4 Association between dispersal and other life-history traits

There has been recent interest in identifying ‘dispersal syndromes’ which are patterns of covariation of morphological, behavioural and other life-history traits that are associated with dispersal (Ronce and Clobert 2012, Stevens et al. 2014). The existence of dispersal syndromes has been detected across a wide range of taxa including mammals, birds, amphibians, spiders and insects, including butterflies (Stevens et al. 2014).

In Chapter 3 I demonstrated that morphological features such as wing length covary with dispersal ability in noctuid moths, but I have not looked at the relationship of any other species traits with dispersal ability, which could lead to further insights into dispersal syndromes and provide more detailed understanding of dispersal processes in macro-moths. In butterflies, Stevens et al (2012)found a very strong association between demography (such as fecundity, number of generations per year and growth rate) and dispersal ability. It was concluded that high dispersal ability was part of an r-strategy, whereby some species evolve to reproduce rapidly, with many offspring in order to make the most of variable and ephemeral resources (Reznick et al. 2002). Therefore the challenges posed by habitat fragmentation and climate change should disproportionately affect those species at the other end of the spectrum, with long generation times and low dispersal rates. (K selected) There is already some indication that combinations of traits are important for moth responses to a changing environment; species with low mobility, narrow feeding niche and low reproduction showed the greatest effect of habitat loss (Ockinger et al. 2010).

The increasing universality of dispersal syndromes, and the evidence of them in butterflies, strongly suggests that patterns of covariation of dispersal with other life-history traits are very likely to be present in macro-moths. Apart from their close phylogenetic relatedness, macro-moths occupy the same trophic level, are ectothermic and use the same locomotory mode (flying) which is indication that they are likely to have similar dispersal syndromes (Stevens et al. 2013). There is evidence that high dispersal ability can be accompanied by fast generation time and high fecundity in a well-studied moth – the Silver Y *Autographa gamma* (Spitzer et al. 1984), and some information on life-histories of different moth species is widely available in field guides (e.g. Waring et al. 2009). This existing information on macro-moth life-histories means that investigation of associations between life-history traits and dispersal ability should therefore become easier provided information on dispersal abilities of more species becomes available (such as with further flight mill studies).

Dispersal ability alone may not be a very good predictor of species abundance and distribution trends, but focus could be on detecting species or populations that exhibit dispersal syndromes such as high fecundity and rapid maturation alongside high dispersal ability. This would enable us to investigate if these suites of traits are good predictors of population trends, which may help us to know which species are of most or least conservation concern.

6.5 Future work

The results presented in this thesis add to our knowledge of dispersal ability of macro-moths, and how dispersal is related to flight morphology and species abundance and distribution trends. There are still many gaps in our knowledge, so in this section I will outline some ways of furthering the work that I have presented.

6.5.1 Extending the scope beyond noctuid moths

In Chapter 2, I showed that the newly-developed tethered flight mill system could quantify dispersal ability in flying insects, providing measures of flight performance that corresponded with expert opinion. In this thesis, I studied noctuid moths, but a much wider range of macro-moths has been shown to be in decline and so it is important to quantify the dispersal ability of species from other macro-moth families, and to test if the relationships with morphology and abundance and distribution trends that I have found hold true for other families. Noctuid moths, while a large and diverse family, are known to be highly mobile, powerful fliers (Waring et al. 2009) and so species from other moth families that are less mobile may be more threatened. As part of the expert survey in Chapter 2, the experts suggested that all of my study species could be classified as 'very mobile' when compared to macro-moths in general (P. Waring pers. comm.). Using the flight mills for studying species in families that have a similar body plan and flying style to the noctuids, such as the Hepialidae and Arctiidae, would require the same methodology I used in the thesis, but with more trapping effort to get appropriate sample sizes of non-noctuid moths, which are not as frequently trapped as noctuid.

New tethered flight methods may be required for species with very different body plans and flying styles. As part of my studies, preliminary testing of six individuals from five geometrid species on the flight mills led to my conclusion that the tethered flight mills were not suited to the flying style of geometrids. It seemed that the attachment 'handle' obstructed their wings, and their wings were often tattered or stuck on the handle on inspection in the morning, after flying on the mills. In addition, extremely short flight distances (all <400m) were recorded, implying the moths were failing to fly properly on the mills. A variety of aerodynamic mechanisms have been demonstrated in butterflies that may make species using these flight mechanisms unsuitable for flight mills (Srygley and Thomas 2002), including the 'clap and fling' flying motion which involves 'clapping' the wings together at the end of the upstroke of a wing beat (Weis-Fogh 1973). If this flying style is employed by moths such as the geometrids, it might be obstructed by the attachment handle. However an alternative attachment has been developed for mosquitoes, and could be appropriate for geometrids. The handle consists of a curved, slightly flexible wire that attaches horizontally to the insects thorax and leads back behind the wings before curving up to attach to the flight mill, thereby preventing obstruction with flapping wings. The geometrids should be a priority for such a tethered flight study because they are the second most speciose macro-moth family, with 300+ species in Britain.

For larger species such as Sphingidae (hawkmoths), and Lasiocampidae (Eggar moths), their flight performance could be quantified using a larger version of the tethered flight mills that I used for noctuids, but with a 1 m circular trajectory. Similarly, smaller moths from any family could be flown on the 25 cm circular trajectory flight mills. The main problem that remains to be overcome with the current apparatus is calibrating the different arm-lengths of flight mill so that speeds obtained with different arm types can be compared. If this can be achieved it would not only enable the comprehensive and comparable quantification of flight ability for all families of macro-moths, but also other insect species. As discussed above, wing length may not be an ideal proxy for dispersal ability, so more direct quantification of dispersal ability for a wider range of species should be a priority.

6.5.2 Relating flight mill performance to natural dispersal ability

In Chapter 2 I discovered that measures of 'total distance flown overnight' and 'maximum flight speed' characterised the main differences in flight mill activity among the study species. Although this was shown to represent differences in mobility as estimated by experts, it would be useful to understand how these flight mill variables represent distances and speeds in the field.

A potential method to calibrate flight mill performance relative to *in situ* flight performance would be to use harmonic radar. Harmonic radar can be used to track the flights of tagged insects within an area of a few hundred metres (Chapman et al. 2011), and this method could be used to obtain natural flight speeds (both average and maximum) of a range of species, which could then be compared with flight speeds on the tethered flight mills. The speeds of moths I recorded from the flight mill were lower than speeds reported from *in situ* observation, implying some sort of correction factor may be needed if the fraction by which the moths are 'slowed' by the flight mill can be computed. Such information could be used to parameterise metapopulation dynamic models, and models used in pest management and disease transmission, and population viability analyses (Dwyer and Elkinton 1995, Brook et al. 2000, Hanski et al. 2004). As outlined above, it is unclear whether the flight mills may shorten flight distances because of the added effort of pushing the flight mills arm, or extend them due to lack of habitat cues to promote landing. A possible method to calibrate flight mill distances and natural measures would be to use a study group for which mobility and dispersal distances are well-studied, such as the butterflies (Stevens et al. 2010). If a range of butterfly species of different dispersal abilities and known *in situ* dispersal distances could be flown on the flight mills, this might provide a means of calibrating the relationships between flight mill performance and natural dispersal. This relationship could then be extended to the macro-moths and other insect species for which information on dispersal abilities is lacking.

This refinement and extension of the tethered flight mill technique would enable not only quantification of dispersal ability across a wide range of flying insects, but ideal conditions in which to explore the relationship of dispersal ability with other species traits and the potential to detect dispersal syndromes as outlined above. The technique provides the means to explore the effect of developmental history, disease status, population source, and myriad other factors on dispersal ability. As it is a laboratory technique it would also permit investigation of the effect of abiotic environmental factors on flight performance by manipulation of laboratory conditions.

6.6 Conclusions

Betzholtz and Franzen (2011) stated that for moths there is ‘an urgent need for an increased knowledge of mobility and associated traits’, and in this thesis I have provided more knowledge.

A novel tethered flight technique allowed me to quantify and compare the flight performances from a wide range of moths, something that was previously not possible. I showed that morphological features associated with flight, such as wing size and proportional thorax mass, and measures of resource availability play a role in flight performance both within and across species. I determined that moth forewing length was a strong predictor of flight performance among species, and I elucidated that dispersal ability played a role in the abundance and distribution trends of British noctuid moths.

Appendix A: Example of raw data generated by tethered flight mill.

Table A. *Example section of file generated by tethered flight mill software, updating every five seconds for eight separate flight mill channels (Ch.)*

Line No.	Date	Time	Ch. 1	Ch. 2	Ch. 3	Ch. 4	Ch. 5	Ch. 6	Ch. 7	Ch. 8
5261	02-Jul-13	03:18:20	0	0	0	2.7	0	0	0	3.4
5262	02-Jul-13	03:18:25	0	0	0	2.8	0	0	0	3.5
5263	02-Jul-13	03:18:30	0	0	0	2.8	0	0	0	3.8
5264	02-Jul-13	03:18:35	0.1	0	0	2.9	0	0	0	3.9
5265	02-Jul-13	03:18:40	0.7	0	0	2.9	0	0	0	3.7
5266	02-Jul-13	03:18:45	0.4	0	0	2.8	0	0	0	3.6
5267	02-Jul-13	03:18:50	0.2	0	0	2.8	0	0	0	3.6
5268	02-Jul-13	03:18:55	0	0	0	2.5	0	0	0	3.7
5269	02-Jul-13	03:19:00	0.1	0	0	2.9	0	0	0	4.1
5270	02-Jul-13	03:19:05	0.4	0.5	0	3	0	0	0	4.3
5271	02-Jul-13	03:19:10	0	0.5	0	3.2	0	0	0	4.1
5272	02-Jul-13	03:19:15	0	0.1	0	3.2	0	0	0	3.9
5273	02-Jul-13	03:19:20	0	0	0	3.1	0	0	0	3.9
5274	02-Jul-13	03:19:25	0	0	0	3.1	0	0	0	4
5275	02-Jul-13	03:19:30	0	0	0	3	0	0	0	3.7
5276	02-Jul-13	03:19:35	0	0	0	3	0	0	0	3.6
5277	02-Jul-13	03:19:40	0	0	0	2.9	0	0	0	4.2
5278	02-Jul-13	03:19:45	0	0	0	2.7	0	0	0	4.3
5279	02-Jul-13	03:19:50	0	0	0	2.5	0	0	0	4.4
5280	02-Jul-13	03:19:55	0	0	0	2.1	0	0	0	4.3
5281	02-Jul-13	03:20:00	0	0	0	1.9	0	0	0	4.4
5282	02-Jul-13	03:20:05	0	0	0	1.6	0	0	0	4.2
5283	02-Jul-13	03:20:10	0	0	0	1.8	0	0	0	3.3
5284	02-Jul-13	03:20:15	0	0	0	1.8	0	0	0	2.8
5285	02-Jul-13	03:20:20	0	0	0	1.8	0	0	0	2.4
5286	02-Jul-13	03:20:25	0	0	0	1.6	0	0	0	2.3
5287	02-Jul-13	03:20:30	0	0	0	1.4	0	0	0	2.1
5288	02-Jul-13	03:20:35	0	0	0	1.2	0	0	0	2.2
5289	02-Jul-13	03:20:40	0	0	0	0.9	0	0	0	2.7
5290	02-Jul-13	03:20:45	0	0.1	0	0.7	0	0	0	3.2
5291	02-Jul-13	03:20:50	0	0	0	0.5	0	0	0	3.1
5292	02-Jul-13	03:20:55	0	0	0	0.2	0	0	0	3.1
5293	02-Jul-13	03:21:00	0	0.9	0	0	0	0	0	3.4
5294	02-Jul-13	03:21:05	0	0	0	0	0	0	0	3.3
5295	02-Jul-13	03:21:10	0	0	0	0	0	0	0	3.5
5296	02-Jul-13	03:21:15	0	0	0	0	0	0	0	3.6

Appendix B: Selection and preparation of moths for tethered flight.

We avoided flying moths that were worn to constrain insect age– only categories 3 & 4 (Figure B.1b & B.1a) insects were used.

Figure B.1. *Wing wear categories as per Thomas (1983) demonstrated in Apamea monoglypha.* ***a.*** *Fresh(4).* ***b.*** *Good (3).* ***c.*** *Poor (2).* ***d.*** *Worn (1)*

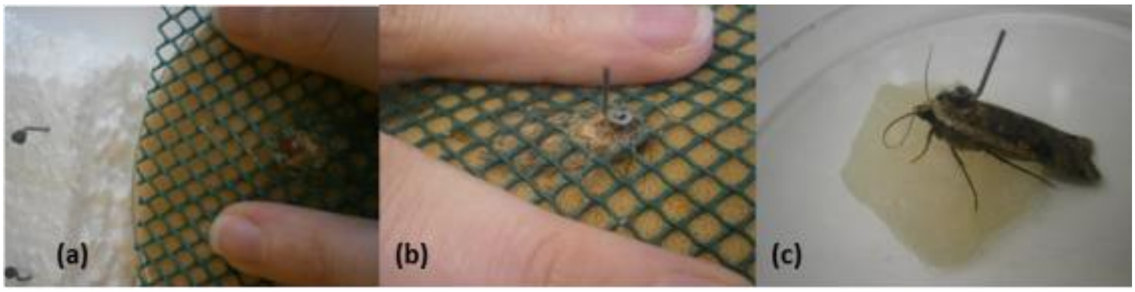

Figure B.2. *Preparing moths for tethered flight.* ***a.*** *Removal of scales from thorax.* ***b.*** *Attachment of flight handle with contact adhesive.* ***c.*** *Feeding with honey solution.*

Appendix C: Experts survey

A survey of opinions was created as the experience and opinion of lepidopterists has been shown to be a valid tool in quantifying dispersal ability (Stevens et al. 2010b, Burke et al. 2011). A list of the 24 species with ≥10 sample size was sent to a number of experts, of which five replied with suitable responses. They were asked to designate each of the noctuid species to one of three dispersal categories based on their relative dispersal ability. The categories were sedentary, mobile and very mobile and corresponded to 0, 1 or 2 mobility points in the table below.

Table C. *Mobility points assigned to noctuid species in expert survey*.

Species	**Expert 1**	**Expert 2**	**Expert 3**	**Expert 4**	**Expert 5**	**Mean points**
Agrotis exclamationis	2	1	1	1	0	**1**
Agrotis puta	2	1	1	1	1	**1.2**
Amphipoea oculea	1	1	0	1	1	**0.8**
Amphipyra pyramidea	1	1	0	1	1	**0.8**
Apamea monoglypha	2	2	1	1	1	**1.4**
Autographa gamma	2	2	2	2	2	**2**
Axylia putris	1	1	0	1	0	**0.6**
Hoplodrina alsines	2	1	0	1	0	**0.8**
Hoplodrina ambigua	2	1	1	1	2	**1.4**
Hydraecia micacea	1	1	0	1	0	**0.6**
Lacanobia oleracea	1	1	0	1	0	**0.6**
Mesapamea secalis	2	1	0	1	0	**0.8**
Mesapamea didyma	2	1	0	1	0	**0.8**
Mythimna impura	2	1	0	1	0	**0.8**
Mythimna pallens	2	1	0	1	0	**0.8**
Noctua comes	2	2	1	1	0	**1.2**
Noctua janthe	2	2	1	1	1	**1.4**
Noctua pronuba	2	2	2	2	2	**2**
Ochropleura plecta	1	1	1	1	1	**1**
Omphaloscelis lunosa	2	1	0	1	1	**1**
Phlogophora meticulosa	2	2	0	2	2	**1.6**
Xestia c-nigrum	2	1	1	1	2	**1.4**
Xestia triangulum	1	1	1	1	0	**0.8**
Xestia xanthographa	2	1	0	1	0	**0.8**

Appendix D: Investigating flight cut-off thresholds

We explored whether there was an appropriate place to draw a threshold of which flights could be classified as 'noise', by determining what impact cut-off threshold of minimum flight duration has on analysis. ANOVA analysis from *Flight mill validation* section of paper was carried out on data generated by different cut-off thresholds. Table D below shows very little difference in ANOVA, resulting in a highly significant analysis regardless of flight threshold. Figures D.1 & D.2 show that total distances flown overnight are highly correlated despite different thresholds of what is a 'true' flight.

Table D: *Numbers of moth individuals that made valid flights according to different threshold values of 'true' flight. ANOVA between dispersal categories (low, medium and high) repeated for total distance at different cut-off points.*

True flight cut-off point (secs)	Total qualifying moths	***Dispersal Categories ANOVA results***			
		effect df	**error df**	**F statistic**	**P-value**
0	457	2	21	8.7	0.002
5	456	2	21	8.69	0.002
10	452	2	21	8.47	0.002
15	450	2	21	8.49	0.002
20	449	2	21	8.4	0.002
25	448	2	21	8.34	0.002
30	447	2	21	8.26	0.002
45	444	2	21	8.7	0.002
60	442	2	21	8.48	0.002
120	427	2	21	8.2	0.002
180	416	2	21	7.54	0.003
240	405	2	21	7.22	0.004
300	391	2	21	7.51	0.003

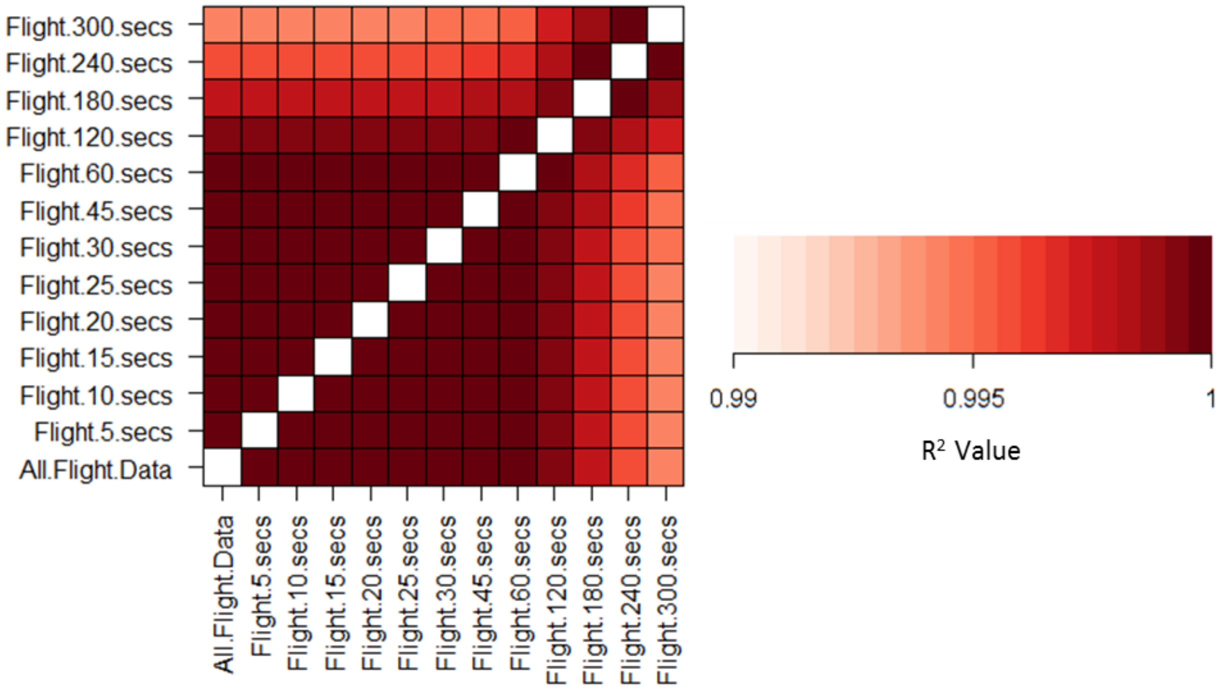

Figure D.1. *Matrix of correlations for values of total distance flown generated by different 'true' flight cut-off thresholds. Cut-off threshold means excluding all flights of duration equal to or less than the number of seconds specified. Note no correlations had R2 lower than 0.99.*

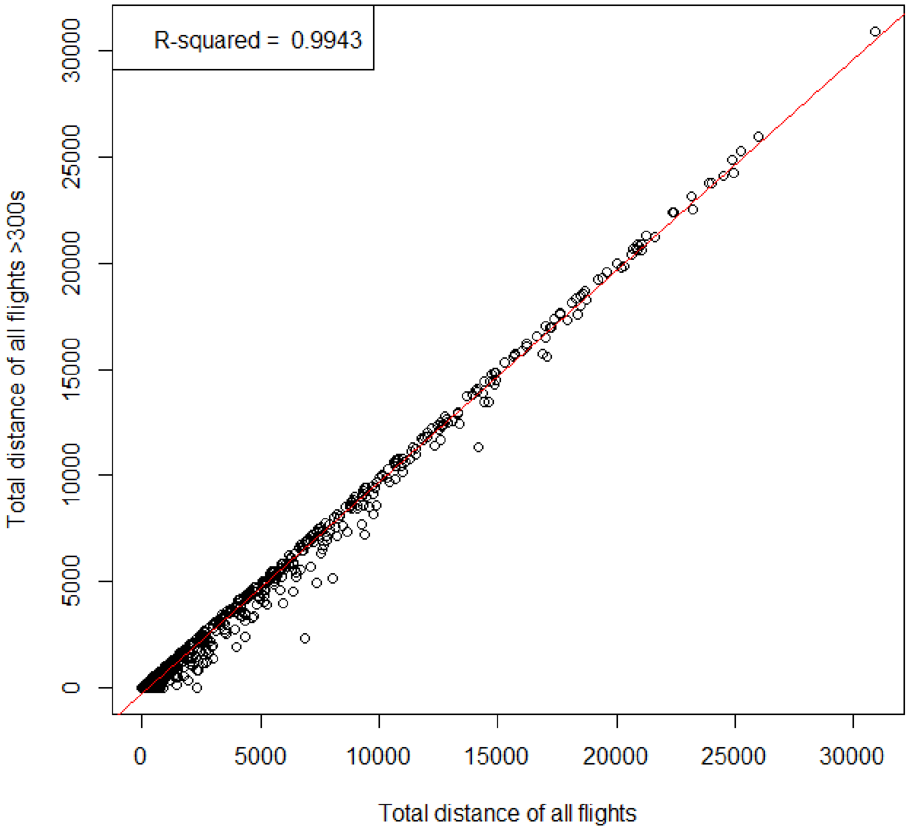

Figure D.2. *Scatterplot showing total distances for each individual moth, between the most severe true flight threshold (>300seconds) and total distance with all flights included.*

Appendix E: Separate ANOVA analysis of suction trap presence and expert opinion mobility categories

In order to test the assumption that tethered flight performance reflects natural dispersal behaviour in the wild, all study species were assigned to a mobility category based on two sources of information as outlined in Chapter 2.3.4. These two sources of information (presence in top 25% of species caught in suction traps and expert opinion on mobility) were combined to create one mobility score in Chapter 2. In this Appendix the analysis is shown for the two mobility measures separately (Categories shown in Table E.1). ANOVA was used to compare tethered flight variables among moth species assigned to mobility categories.

Suction trap presence

Presence in top 25% of species caught in suction traps had a significant effect on flight distance but not speed (total distance: $F_{1,22} = 8.85$, $P = 0.007$; maximum speed: $F_{1,22} = 2.76$, $P = 0.111$; Figure D.1).

Expert opinion survey

Although there were three categories in the expert survey, the reluctance of three of the five experts to assign any species to the ‘sedentary’ category meant that after taking means scores no moths were in the low category, thus this analysis has moths assigned only to the medium and high categories as outlined in table D.1.

Mobility category assigned by experts had a significant effect on flight distance but not speed (total distance: $F_{1,22} = 86.22$, $P = 0.021$; maximum speed: $F_{1,22} = 3.20$, $P = 0.088$; Figure D.2).

Table E.1. *Summary table of 24 individual moth species flown on tethered flight mills. All individuals are males. Suction trap score was assigned if species were in the top 25% of species caught in Rothamsted Insect Survey (RIS) suction traps (mean yearly catch over period 2000-2009). Expert mean score is the mean value of responses where 5 experts were asked to assign species to categories of low (0), medium (1) and high (2) mobility (see Appendix C).*

Species	N flown	Suction trap score	Expert mean score	Expert category
Agrotis exclamationis	18	1	1	Medium
Agrotis puta	8	1	1.2	Medium
Amphipoea oculea	11		0.8	Medium
Amphipyra pyramidea	14		0.8	Medium
Apamea monoglypha	39	1	1.4	Medium
Autographa gamma	13	1	2	High
Axylia putris	14		0.6	Medium
Hoplodrina alsines	13		0.8	Medium
Hoplodrina ambigua	13		1.4	Medium
Hydraecia micacea	23		0.6	Medium
Lacanobia oleracea	16		0.6	Medium
Mesapamea didyma	10	1	0.8	Medium
Mesapamea secalis	16	1	0.8	Medium
Mythimna impura	11		0.8	Medium
Mythimna pallens	19		0.8	Medium
Noctua comes	26		1.2	Medium
Noctua janthe	13		1.4	Medium
Noctua pronuba	37	1	2	High
Ochropleura plecta	20		1	Medium
Omphaloscelis lunosa	16		1	Medium
Phlogophora meticulosa	10	1	1.6	High
Xestia c-nigrum	59	1	1.4	Medium
Xestia triangulum	12		0.8	Medium
Xestia xanthographa	25	1	0.8	Medium

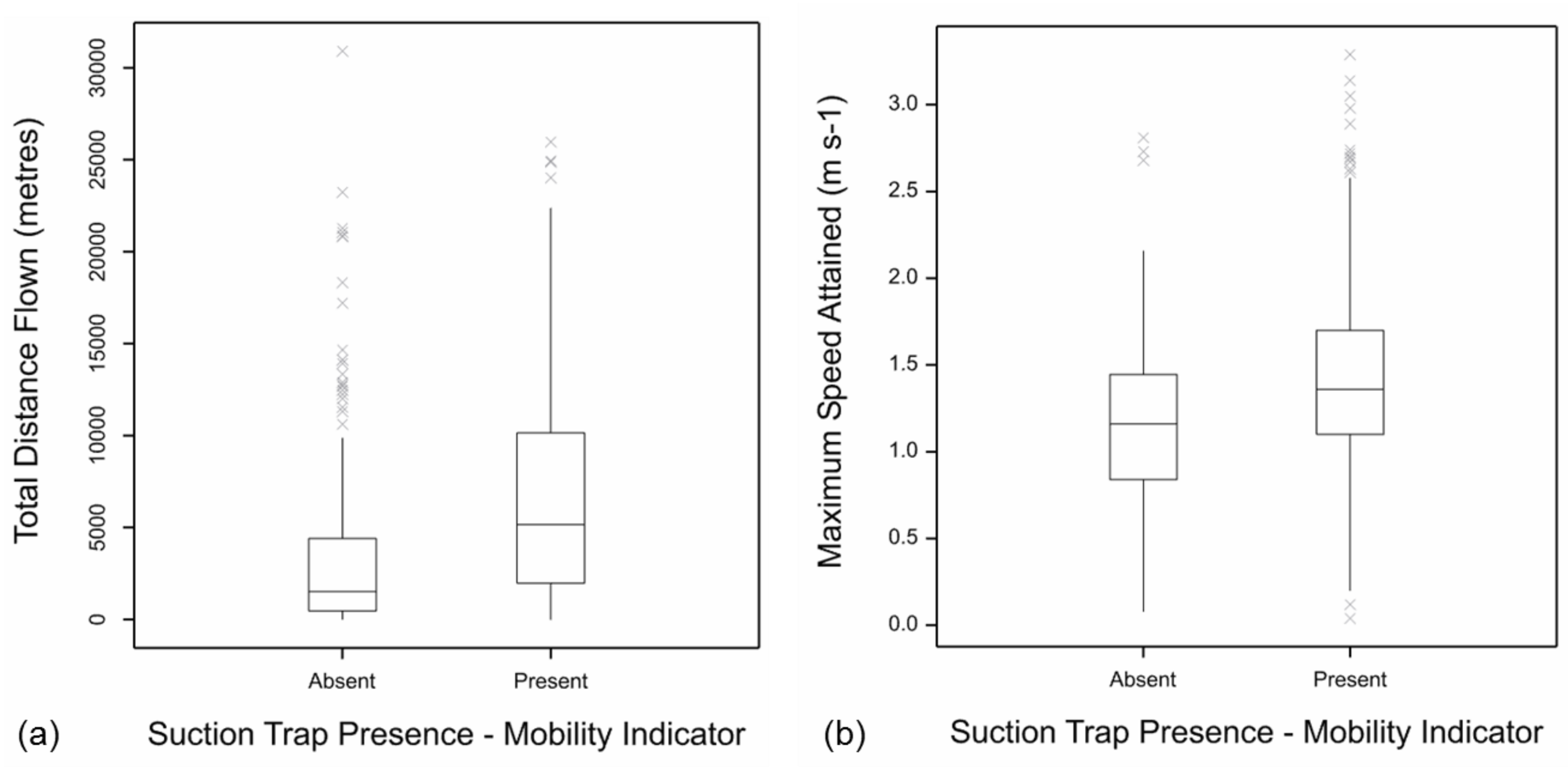

Figure E.1. *Boxplots showing* ***(a)*** *total distance flown and* ***(b)*** *maximum speed attained on tethered flight mills of 456 individuals in two mobility categories; present or absent from top 25% of species caught in suction traps (Table E.1). Boxes span the interquartile range of values, with the line dissecting the box indicating the median. Whiskers extend to 1.5 times the interquartile range beyond the quartiles. Beyond this outliers are plotted as a cross.*

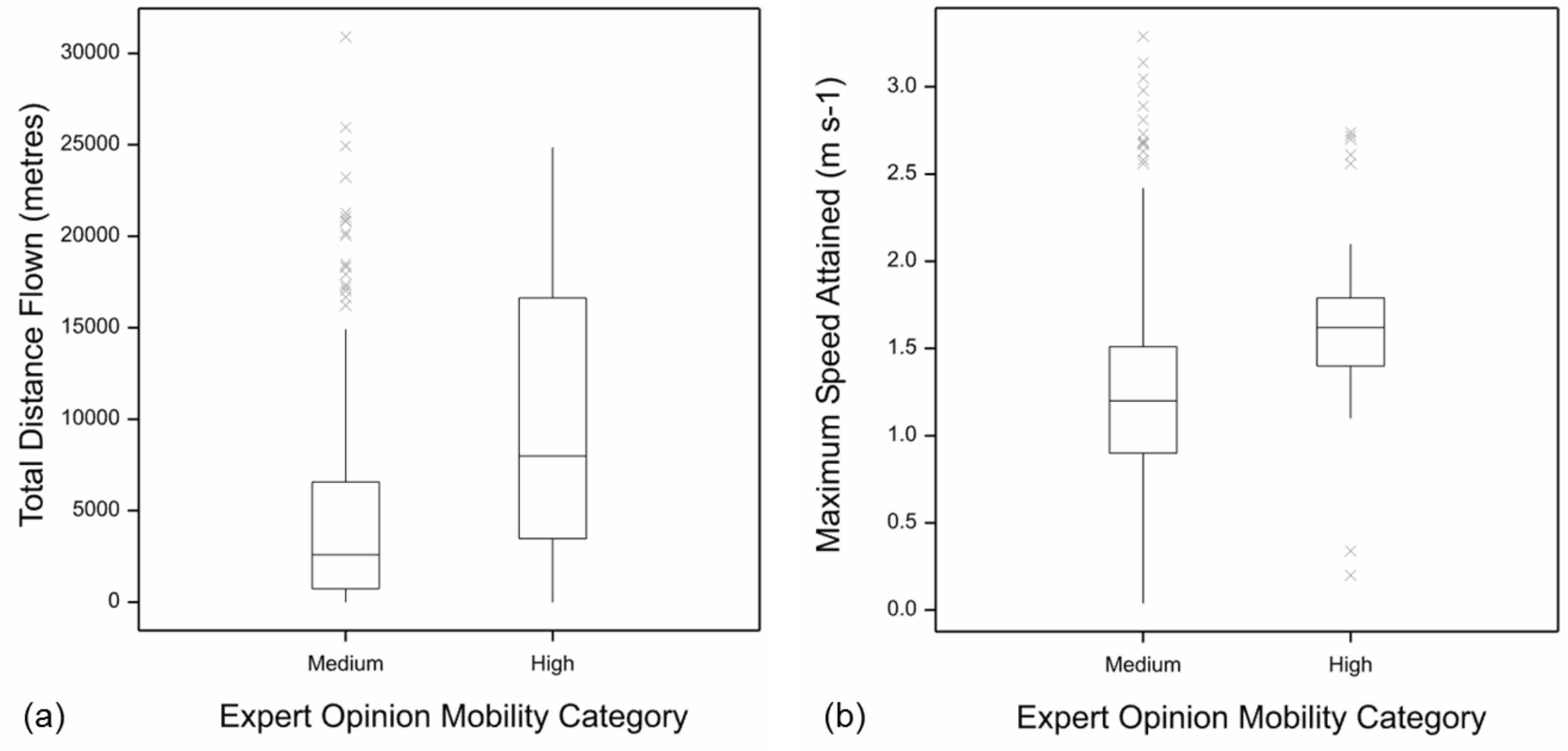

Figure E.2. *Boxplots showing (a) total distance flown and (b) maximum speed attained on tethered flight mills of 456 individuals assigned to two mobility categories according to their species by experts (Table E.1). Boxes span the interquartile range of values, with the line dissecting the box indicating the median. Whiskers extend to 1.5 times the interquartile range beyond the quartiles. Beyond this outliers are plotted as a cross.*

Appendix F: Exploratory analyses of tethered flight variables

Sixteen flight variables were computed from tethered flight mill data (as outlined in Table 2.1), a number of exploratory analyses were carried out to see how these variables were related to each other and to select a minimum number of them. A matrix of pairwise correlations (Figure F.1) and a Principal Components Analysis (Figure 2.3) show that measures of distance and duration are correlated with each other especially within different flight types such as first or longest flight. Measures of maximum and mean speed are also correlated in the same way. Features of first, longest and furthest flights are correlated with the equivalent measures over the whole night, indicating this level of detail is not necessary. Number of flights is not correlated with any other variable.

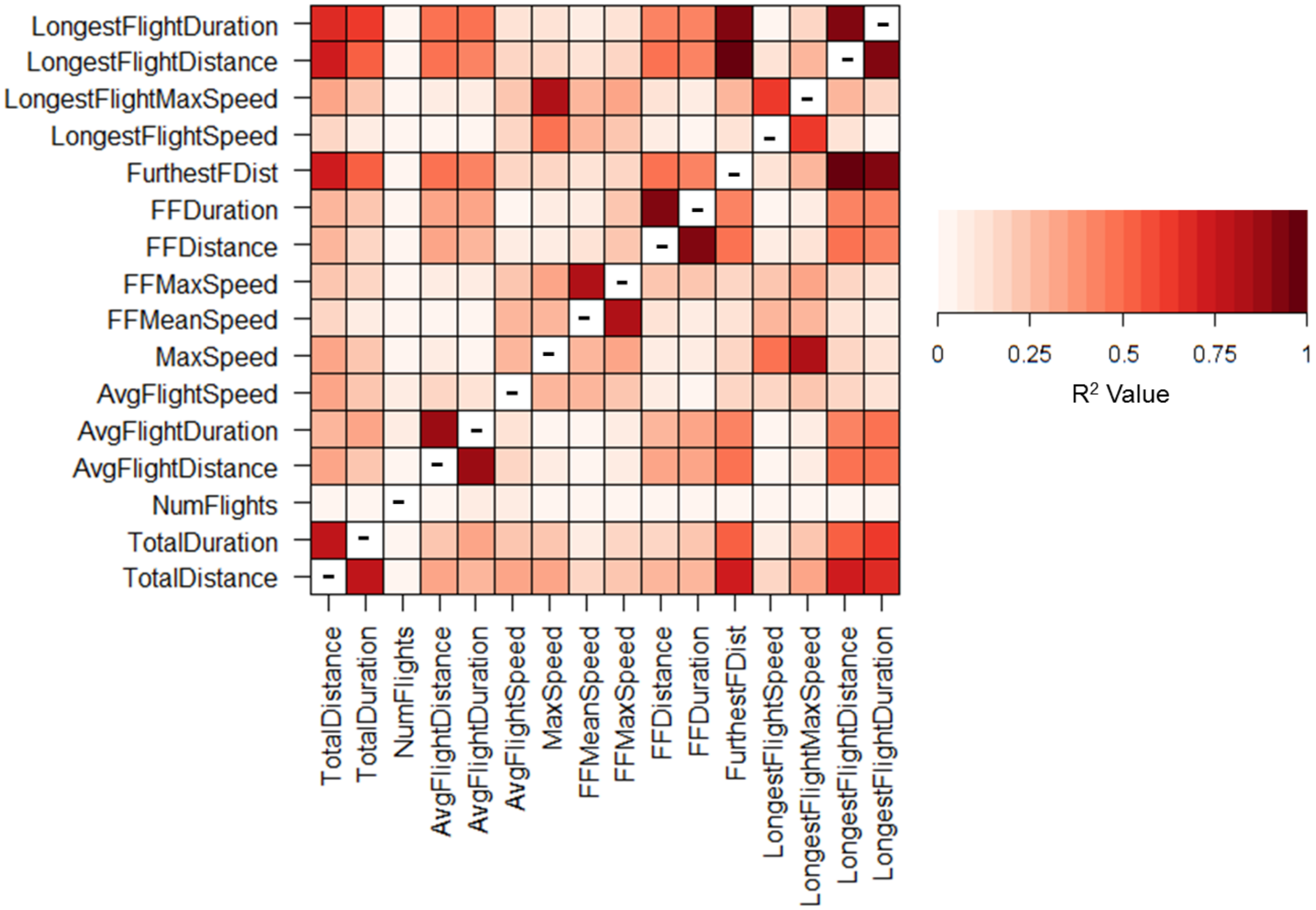

Figure F.1. *Matrix of pair-wise correlations of tethered flight variables. A dash indicates a cell where a correlation value has not been computed.*

A Canonical Variates Analysis was carried out to see which of the tethered flight variables were most useful for distinguishing between different species. The CV loadings values (Table F.2) show that measures of speed, especially maximum speed are useful to separate species.

Table F.2 *Canonical Variates Analysis was performed on sixteen tethered flight variables. Loadings values of the variables in the first five canonical variates are shown. Values in brackets next to CV number are the percentage variance in the dataset accounted for by that canonical variate.*

Tethered flight measurement	CV1 (45.46)	CV2 (14.75)	CV3 (10.57)	CV4 (7.26)	CV5 (7.1)
AvgFlightDistance	-0.0002	0.0001	0.0004	0	0.0001
AvgFlightDuration	0.0001	-0.0002	-0.0004	0	-0.0002
AvgFlightSpeed	0.8207	-3.5807	-0.5541	-2.5477	2.1785
FFDistance	0.0001	0.0003	-0.0002	-0.0004	0.0002
FFDuration	-0.0001	-0.0002	0.0002	0.0002	-0.0001
FFMaxSpeed	0.3871	1.3461	-1.1428	0.7091	-0.8464
FFMeanSpeed	-1.0561	-1.3326	0.902	0.8578	3.5797
FurthestFDist	-0.0001	-0.0001	-0.0001	0.0005	0
LongestFlightDistance	-0.0001	-0.0001	-0.0001	0.0005	0
LongestFlightDuration	0.0001	0.0002	0	-0.0007	0.0001
LongestFlightMaxSpeed	1.1193	0.4216	1.392	-1.12	1.3157
LongestFlightSpeed	0.0129	0.9717	0.4125	-1.8829	0.8313
MaxSpeed	1.302	0.5732	-1.5167	1.1944	-1.9183
NumFlights	0.0095	-0.0076	0.0325	-0.0066	0.0087
TotalDistance	0.0001	0.0001	0.0001	-0.0004	-0.0003
TotalDuration	0	-0.0002	0.0001	0.0003	0.0002

Appendix G: Species means for all tethered flight and morphological variables

Table G.1. *Definitions of all variables obtained from tethered flight mills or by morphological measurement of moth individuals.*

Variable	Unit	Definition
Wing wear		See Appendix B
Mass before feed	grams	See chpt 2.3.2
Mass before flight	grams	See chpt 2.3.2
Mass after flight	grams	See chpt 2.3.2
Food consumed	grams	Mass before flight- mass before feed
Mass change during flight	grams	Mass after flight- mass before flight
Mass change from pre feed	grams	Mass after flight - mass before feed
Total mass	grams	Dry insect mass
Thorax mass	grams	Dry thorax mass
Abdomen mass	grams	Dry abdomen mass
Thorax width	millimetres	See Figure 3.1
Thorax length	millimetres	See Figure 3.1
Wing length	millimetres	See Figure 3.1
Wing breadth	millimetres	See Figure 3.1
Forewing area	millimetres^2	Measured using MARVIN digital seed analyser
Forewing aspect ratio		(4*forewing length2)/forewing area
Wing loading		Total mass/forewing area
Proportional thorax mass		Thorax mass/total mass
Proportional abdomen mass		Abdomen mass/total mass
Thorax squareness		Thorax width/thorax length
TotalDistance	metres	Sum of distance covered by all flights
TotalDuration	seconds	Sum of duration of all flights
NumFlights		Count of flights
AvgFlightDistance	metres	Mean of distances of flights
AvgFlightDuration	seconds	Mean of duration of flights
AvgFlightSpeed	metres/sec	Mean of the speeds of individual flights (calculated as distance/duration)
MaxSpeed	metres/sec	Greatest distance attained in any 5 second interval/5 – of the whole night
FFMeanSpeed	metres/sec	Speed of first flight of the night(calculated as distance/duration)
FFMaxSpeed	metres/sec	Greatest speed attained in any 5 second interval of the first valid flight
FFDistance	metres	Distance of first flight of the night
FFDuration	seconds	Duration of first flight of the night
FurthestFDist	metres	Distance travelled in the flight of greatest distance of the whole night
LongestFlightSpeed	metres/sec	Speed of the flight with greatest duration(calculated as distance/duration)
LongestFlightMaxSpeed	metres/sec	Greatest speed attained in any 5 second interval of the flight of greatest duration
LongestFlightDistance	metres	Distance travelled in the flight of greatest duration of the whole night
LongestFlightDuration	seconds	Duration of the flight with greatest duration

Table G.2. *Summary table of 30 moth species studied with species means for all tethered flight variables and morphological variables as defined in table G.1.*

Species	**Agrotis exclamationis**	**Agrotis puta**	**Amphipoea oculea**	**Amphipyra pyramidea**	**Apamea anceps**
Sample size	24	13	11	21	9
Wing wear	3.708333333	3.923076923	3.636363636	3.738095238	3.555555556
Mass before feed	0.154945417	0.072079231	0.063087273	0.253101905	0.127221111
Mass before flight	0.222170833	0.105708462	0.095457273	0.41777619	0.161176667
Mass after flight	0.153625	0.068587692	0.067105455	0.26016	0.115758889
Food consumed	0.067225417	0.033629231	0.03237	0.164674286	0.033955556
Mass change during flight	-0.068545833	-0.037120769	-0.028351818	-0.15761619	-0.045417778
Mass change from pre feed	-0.001320417	-0.003491538	0.004018182	0.007058095	-0.011462222
Total mass	0.049947917	0.021843077	0.021824545	0.098668095	0.038022222
Thorax mass	0.017725217	0.007347692	0.008935455	0.03140381	0.01505
Abdomen mass	0.02165	0.010363846	0.008478182	0.046943333	0.015378889
Thorax width	4.722083333	3.515384615	3.627272727	6.05	4.288888889
Thorax length	4.4	3.103846154	3.204545455	5.419047619	4.144444444
Wing length	17.41041667	13.45769231	13.57727273	22.775	17.71111111
Wing breadth	7.2	5.515384615	5.877272727	10.90333333	7.366666667
Forewing area	93.62916667	55.43846154	59.36818182	180.4333333	94.90555556
Forewing aspect ratio	12.981711	13.13512153	12.43685359	11.51564622	13.2559866
Wing loading	0.000534248	0.000388047	0.000367479	0.000544053	0.000397523
Proportional thorax mass	0.363083691	0.349790092	0.410558285	0.32685052	0.403715925
Proportional abdomen mass	0.423725595	0.451766902	0.386366323	0.46146762	0.393108389
Thorax squareness	1.08246292	1.135885669	1.134466137	1.120986817	1.041634166
TotalDistance	6487.072917	831.7307692	1580.181818	12770.27857	5302.744444
TotalDuration	9572.291667	2992.307692	2893.636364	16428.09524	8151.666667
NumFlights	13.95833333	16.30769231	13.63636364	19.19047619	32.33333333
AvgFlightDistance	721.7648091	41.67085139	148.1874387	5215.524499	237.0755167
AvgFlightDuration	1109.820909	144.4114631	257.7101371	5895.554323	388.4927665
AvgFlightSpeed	0.414754602	0.154686908	0.318391414	0.520134543	0.346236165
MaxSpeed	1.4575	0.678461538	0.961818182	1.753809524	1.546666667
FFMeanSpeed	0.7325	0.223846154	0.469090909	0.656190476	0.65
FFMaxSpeed	1.243333333	0.34	0.769090909	1.26	1.268888889
FFDistance	2079.0375	227.0538462	561.1	6563.538095	1191.733333
FFDuration	2674.375	413.8461538	758.1818182	7601.190476	1630.555556
FurthestFDist	3514.210417	364.4192308	768.3545455	10650.28571	2170.488889
LongestFlightSpeed	0.79375	0.278461538	0.533636364	0.82047619	0.862222222
LongestFlightMaxSpeed	1.354166667	0.438461538	0.852727273	1.707142857	1.473333333
LongestFlightDistance	3514.210417	364.4192308	768.3545455	10650.28571	2170.488889
LongestFlightDuration	4784.791667	905.7692308	1135.454545	12871.66667	2427.777778

Species	**Apamea monoglypha**	**Apamea sordens**	**Autographa gamma**	**Axylia putris**	**Charancya trigrammica**
Sample size	41	9	27	17	8
Wing wear	3.695121951	3.277777778	3.814814815	4	3
Mass before feed	0.230775854	0.128233333	0.110662593	0.080951765	0.126525
Mass before flight	0.302693902	0.162623333	0.185152593	0.121579412	0.17165375
Mass after flight	0.219161463	0.119756667	0.119858889	0.082474118	0.132375
Food consumed	0.071918049	0.03439	0.07449	0.040627647	0.04512875
Mass change during flight	-0.083532439	-0.042866667	-0.065293704	-0.039105294	-0.03927875
Mass change from pre feed	-0.01161439	-0.008476667	0.009196296	0.001522353	0.00585
Total mass	0.07947561	0.040058889	0.043128148	0.027267647	0.0392225
Thorax mass	0.027948293	0.014756667	0.016792222	0.009747647	0.01577375
Abdomen mass	0.036060976	0.014534	0.017415556	0.012358235	0.01575125
Thorax width	5.493902439	4.222222222	4.524074074	3.826470588	4.45
Thorax length	5.198780488	3.922222222	4.424074074	3.265625	3.9
Wing length	23.03292683	17.59444444	18.90185185	15.17941176	15.9375
Wing breadth	9.284146341	7.333333333	8.237037037	5.958823529	7.2
Forewing area	150.5414634	92.77222222	108.9092593	68.56176471	86.58571429
Forewing aspect ratio	14.12458942	13.37699208	13.20027478	13.47436016	11.94557284
Wing loading	0.000526598	0.000429174	0.00038969	0.000397226	0.000448347
Proportional thorax mass	0.354891636	0.374536265	0.396312402	0.364232103	0.403367754
Proportional abdomen mass	0.44905404	0.380707941	0.393814924	0.443423151	0.398308164
Thorax squareness	1.060722779	1.079739699	1.029466554	1.183127524	1.151029418
TotalDistance	8930.057317	8725.088889	5155.696296	3172.2	3876.25625
TotalDuration	11365.4878	10979.44444	6948.888889	5261.176471	6174.375
NumFlights	31.17073171	31.88888889	10.11111111	12.70588235	16
AvgFlightDistance	570.5695792	466.7591409	1068.804076	437.1312872	359.4814236
AvgFlightDuration	725.8058639	662.2057419	1506.598318	673.14666	526.5402449
AvgFlightSpeed	0.465852691	0.498168308	0.493558103	0.436174075	0.445833915
MaxSpeed	2.075609756	1.732222222	1.471851852	1.050588235	1.2
FFMeanSpeed	0.6	0.79	0.761481481	0.573529412	0.53125
FFMaxSpeed	1.100487805	1.346666667	1.285925926	0.923529412	0.9025
FFDistance	1382.092683	1426.277778	1981.316667	612.3029412	222.5125
FFDuration	1390.243902	1608.333333	2360.37037	832.6470588	323.75
FurthestFDist	4222.49878	3934.2	3733.701852	1163.197059	1454.525
LongestFlightSpeed	0.986097561	0.88	0.832592593	0.591764706	0.7125
LongestFlightMaxSpeed	1.886341463	1.553333333	1.374074074	0.945882353	1.195
LongestFlightDistance	4222.49878	3934.2	3733.701852	1163.197059	1454.525
LongestFlightDuration	4587.926829	5011.666667	4602.592593	1806.470588	1788.75

Species	**Cosmia trapezina**	**Hoplodrina alsines**	**Hoplodrina ambigua**	**Hydraecia micacea**	**Lacanobia oleracea**
Sample size	6	16	14	24	20
Wing wear	3.333333333	3.875	3.571428571	3.458333333	3.2
Mass before feed	0.055293333	0.094818125	0.068131429	0.159040417	0.135821
Mass before flight	0.080986667	0.112003125	0.094361429	0.196754583	0.1738695
Mass after flight	0.063323333	0.089704375	0.065898571	0.15884	0.1311025
Food consumed	0.025693333	0.017185	0.02623	0.037714167	0.0380485
Mass change during flight	-0.017663333	-0.02229875	-0.028462857	-0.037914583	-0.042767
Mass change from pre feed	0.00803	-0.00511375	-0.002232857	-0.000200417	-0.0047185
Total mass	0.02024	0.030635	0.022412143	0.064462917	0.039909
Thorax mass	0.007211667	0.0109975	0.00869	0.018944167	0.0139375
Abdomen mass	0.008856667	0.013653125	0.008614786	0.035960417	0.018589
Thorax width	3.391666667	4.15	3.732142857	4.879166667	4.41
Thorax length	3.125	3.559375	3.332142857	4.239583333	3.845
Wing length	14.78333333	14.646875	13.26071429	18.1375	17.26
Wing breadth	6.966666667	6.5875	5.989285714	7.479166667	7.43
Forewing area	72.475	72.7875	58.63214286	101.4958333	93.4
Forewing aspect ratio	12.1078618	11.81458063	12.00451642	13.03363921	12.78078982
Wing loading	0.000273092	0.000420536	0.000381892	0.000623563	0.000422936
Proportional thorax mass	0.37741364	0.363974205	0.389689633	0.318736768	0.35821118
Proportional abdomen mass	0.405145973	0.438286173	0.386973595	0.519906708	0.454309421
Thorax squareness	1.086439771	1.168818743	1.124277719	1.154536555	1.154282483
TotalDistance	2884.183333	2364.359375	1124.657143	2842.3	5041.8875
TotalDuration	6060	3743.125	2356.785714	5584.166667	8812.5
NumFlights	11	18.3125	17.21428571	11.04166667	22.4
AvgFlightDistance	358.3456937	213.5451903	136.4013282	614.5805121	433.2200448
AvgFlightDuration	716.9295274	285.2064281	242.130213	1052.496169	800.0811797
AvgFlightSpeed	0.358280411	0.282710971	0.21878594	0.305830644	0.369464403
MaxSpeed	0.926666667	1.13875	0.972857143	1.18	1.3825
FFMeanSpeed	0.47	0.548125	0.33	0.449583333	0.628
FFMaxSpeed	0.71	0.95	0.645714286	0.8525	1.0575
FFDistance	595.9	1569.69375	394.8142857	239.0375	1300.2925
FFDuration	1005	1660.625	596.0714286	496.0416667	1713.25
FurthestFDist	1837.466667	1802.475	775.9071429	2223.45	2853.9075
LongestFlightSpeed	0.638333333	0.615625	0.527857143	0.521666667	0.6765
LongestFlightMaxSpeed	0.926666667	1.065	0.875714286	0.971666667	1.288
LongestFlightDistance	1837.466667	1802.475	775.9071429	2223.45	2853.9075
LongestFlightDuration	3537.5	2029.6875	1174.285714	3931.041667	4042.75

Species	Mesapamea didyma	Mesapamea secalis	Mythimna impura	Mythimna pallens	Noctua comes
Sample size	10	16	15	24	42
Wing wear	4	3.6875	3.933333333	3.958333333	3.404761905
Mass before feed	0.066376	0.06624	0.088486	0.0821725	0.199852619
Mass before flight	0.084168	0.0825725	0.112296667	0.102960833	0.233951429
Mass after flight	0.061045	0.061208125	0.08151	0.074295417	0.188222619
Food consumed	0.017792	0.0163325	0.023810667	0.020788333	0.03409881
Mass change during flight	-0.023123	-0.021364375	-0.030786667	-0.028665417	-0.04572881
Mass change from pre feed	-0.005331	-0.005031875	-0.006976	-0.007877083	-0.01163
Total mass	0.021485	0.02175875	0.027329333	0.024296667	0.06936
Thorax mass	0.009201	0.0093525	0.008872	0.007735417	0.023027143
Abdomen mass	0.007696	0.0078475	0.013255714	0.01234375	0.033024286
Thorax width	3.635	3.6875	3.796666667	3.607708333	5.364285714
Thorax length	3.31	3.303125	3.31	3.002083333	5.046341463
Wing length	14.365	14.05625	15.63333333	15.11666667	19.75642857
Wing breadth	6.505	6.3875	6.306666667	5.90625	8.635714286
Forewing area	67.815	65.89375	74.61666667	66.55833333	125.977381
Forewing aspect ratio	12.19800986	12.01818154	13.12479943	13.75895248	12.42561313
Wing loading	0.000315537	0.000329945	0.000365636	0.000363406	0.000545591
Proportional thorax mass	0.427608784	0.430511007	0.329268091	0.323529763	0.346827093
Proportional abdomen mass	0.358603897	0.360478398	0.46805045	0.496640846	0.452115904
Thorax squareness	1.102870146	1.124745755	1.149421419	1.210224572	1.062641088
TotalDistance	3598.105	3573.69375	1460.343333	2459.69375	7024.419048
TotalDuration	7483.5	6548.75	4416.333333	5973.541667	9899.52381
NumFlights	22.5	26.6875	25.8	26.45833333	10.30952381
AvgFlightDistance	283.2622415	386.4309785	54.29643279	169.7991175	1731.735017
AvgFlightDuration	522.1136822	608.9841926	163.6217908	367.2153443	2254.826487
AvgFlightSpeed	0.333735127	0.303655617	0.149818154	0.213797671	0.421861826
MaxSpeed	1.112	1.04625	0.822666667	0.863333333	1.516666667
FFMeanSpeed	0.667	0.435625	0.222666667	0.32375	0.603571429
FFMaxSpeed	1.076	0.6975	0.464	0.583333333	1.113571429
FFDistance	1673.05	1431.83125	487.5733333	404.8208333	3763.164286
FFDuration	2716	1831.875	1438	756.875	4209.166667
FurthestFDist	1966.83	2504.309375	796.2333333	1125.670833	5319.161905
LongestFlightSpeed	0.656	0.61125	0.466	0.457916667	0.722142857
LongestFlightMaxSpeed	1.022	0.94375	0.758666667	0.8175	1.422380952
LongestFlightDistance	1966.83	2504.309375	796.2333333	1125.670833	5319.161905
LongestFlightDuration	3135	3869.375	1822	2192.5	6625.357143

Species	**Noctua janthe**	**Noctua pronuba**	**Ochropleura plecta**	**Oligia fasciuncula**	**Oligia latruncula**
Sample size	21	49	24	2	1
Wing wear	3.619047619	3.836734694	3.291666667	3.25	4
Mass before feed	0.114307619	0.405471224	0.059910833	0.034275	0.03688
Mass before flight	0.133605238	0.482066939	0.08430125	0.06352	0.05612
Mass after flight	0.111734286	0.381954082	0.059181667	0.037985	0.0264
Food consumed	0.019297619	0.076595714	0.024390417	0.029245	0.01924
Mass change during flight	-0.021870952	-0.100112857	-0.025119583	-0.025535	-0.02972
Mass change from pre feed	-0.002573333	-0.023517143	-0.000729167	0.00371	-0.01048
Total mass	0.041327143	0.14356898	0.01988375	0.01231	0.01319
Thorax mass	0.014809048	0.042303469	0.0072225	0.00543	0.00426
Abdomen mass	0.018208095	0.078458163	0.008690417	0.00467	0.00644
Thorax width	4.445238095	6.616326531	3.40625	3	2.8
Thorax length	4.257142857	6.397959184	2.983333333	2.6	2.45
Wing length	17.58809524	25.36938776	13.36666667	10.725	11.3
Wing breadth	7.583333333	10.05	5.7625	4.875	5
Forewing area	96.29047619	182.4173469	58.51041667	39.4	44.8
Forewing aspect ratio	12.88541559	14.14145671	12.23315653	11.68083756	11.40089286
Wing loading	0.000427913	0.000783353	0.000339618	0.000312437	0.00029442
Proportional thorax mass	0.364951342	0.307108335	0.367333361	0.445165574	0.322971948
Proportional abdomen mass	0.428585536	0.525739614	0.431342525	0.372793884	0.488248673
Thorax squareness	1.047557035	1.037304971	1.146712543	1.155555556	1.142857143
TotalDistance	4846.761905	11614.60816	606.3416667	1463.85	1587.1
TotalDuration	7567.857143	17365.20408	2174.375	2957.5	3655
NumFlights	15.85714286	15.69387755	19.04166667	9	17
AvgFlightDistance	638.9839569	4065.687011	54.99112677	267.4735294	93.35882353
AvgFlightDuration	1013.988003	5751.179854	197.5726093	564.5588236	215
AvgFlightSpeed	0.387126393	0.605041381	0.1670018	0.388529412	0.282352941
MaxSpeed	1.146666667	1.62244898	0.69	0.84	0.9
FFMeanSpeed	0.605238095	0.628571429	0.281666667	0.56	0.56
FFMaxSpeed	0.95047619	1.149795918	0.493333333	0.84	0.9
FFDistance	1218.809524	3460.204082	145.4125	214.15	447.8
FFDuration	1364.285714	4802.244898	341.6666667	447.5	795
FurthestFDist	2698.157143	7692.236735	253.6270833	631.95	447.8
LongestFlightSpeed	0.69	0.716734694	0.37375	0.535	0.56
LongestFlightMaxSpeed	1.106666667	1.445306122	0.649166667	0.81	0.9
LongestFlightDistance	2698.157143	7692.236735	253.6270833	631.95	447.8
LongestFlightDuration	3740.47619	10914.38776	669.375	1135	795

Species	Oligia strigilis	Omphaloscelis lunosa	Phlogophora meticulosa	Thalophila matura	Xestia c-nigrum
Sample size	7	18	16	4	64
Wing wear	3.714285714	3.777777778	3.875	3.375	3.90625
Mass before feed	0.047952857	0.119327222	0.22827875	0.1125175	0.113919063
Mass before flight	0.070107143	0.142374444	0.24903375	0.171545	0.150966547
Mass after flight	0.047958571	0.11789	0.21139625	0.127235	0.104828125
Food consumed	0.022154286	0.023047222	0.020755	0.0590275	0.037047484
Mass change during flight	-0.022148571	-0.024484444	-0.0376375	-0.04431	-0.046138422
Mass change from pre feed	5.71E-06	-0.001437222	-0.0168825	0.0147175	-0.009090938
Total mass	0.016232857	0.046885	0.082141875	0.0432525	0.037173281
Thorax mass	0.006331429	0.013327778	0.0232025	0.01977	0.012317969
Abdomen mass	0.006741429	0.025836667	0.046660625	0.013565	0.018088438
Thorax width	3.328571429	4.344444444	5.2125	4.825	4.16328125
Thorax length	2.771428571	3.486111111	4.715625	4.4125	3.599765625
Wing length	12.19285714	16.13611111	22.90625	18.3875	17.0765625
Wing breadth	5.585714286	6.794444444	8.771875	8.8375	7.1515625
Forewing area	51.14285714	81.03888889	138.75625	118.0625	88.578125
Forewing aspect ratio	11.66915085	12.86281849	15.15490741	11.47327669	13.1906614
Wing loading	0.000316738	0.000577365	0.000588344	0.000369054	0.000418796
Proportional thorax mass	0.39146689	0.298646551	0.29761068	0.460379794	0.334956864
Proportional abdomen mass	0.414069337	0.528593038	0.544017772	0.308513725	0.481293103
Thorax squareness	1.209878247	1.255318368	1.114751832	1.095288864	1.163225385
TotalDistance	1912.814286	1535.244444	9109.53125	6797.075	5875.538281
TotalDuration	2778.571429	2555	13019.375	8286.25	9654.140625
NumFlights	8.714285714	20.27777778	28.1875	2.75	34.703125
AvgFlightDistance	235.1459921	231.4382203	754.6473804	5312.048333	826.2210696
AvgFlightDuration	361.6256949	356.0065101	902.5122024	6147.958333	1085.421386
AvgFlightSpeed	0.379925204	0.324904451	0.328729795	0.600916667	0.363467739
MaxSpeed	0.988571429	1.26	1.666875	1.17	1.1753125
FFMeanSpeed	0.338571429	0.479444444	0.73375	0.6175	0.483125
FFMaxSpeed	0.488571429	0.821111111	1.354375	1.145	0.8178125
FFDistance	426.5714286	462.5666667	3272.190625	6097.475	1515.8625
FFDuration	554.2857143	594.7222222	3095.9375	7287.5	2121.953125
FurthestFDist	1147.114286	887.6833333	6034.13125	6167.075	2888.020313
LongestFlightSpeed	0.712857143	0.731111111	0.80625	0.6875	0.60296875
LongestFlightMaxSpeed	0.974285714	1.246666667	1.570625	1.16	1.068125
LongestFlightDistance	1147.114286	887.6833333	6034.13125	6167.075	2888.020313
LongestFlightDuration	1411.428571	1112.222222	6180	7368.75	4058.59375

Species	**Xestia triangulum**	**Xestia xanthographa**
Sample size	14	30
Wing wear	3.785714286	3.6
Mass before feed	0.151417857	0.100761333
Mass before flight	0.201236429	0.144934
Mass after flight	0.146502857	0.100889
Food consumed	0.049818571	0.044172667
Mass change during flight	-0.054733571	-0.044045
Mass change from pre feed	-0.004915	0.000127667
Total mass	0.050453571	0.038012667
Thorax mass	0.014912143	0.012970333
Abdomen mass	0.026640714	0.017348667
Thorax width	4.428571429	4.158333333
Thorax length	3.832142857	3.788333333
Wing length	18.45	16.02166667
Wing breadth	8.114285714	7.325
Forewing area	107.5785714	87.28333333
Forewing aspect ratio	12.67095414	11.79231502
Wing loading	0.000467472	0.000433212
Proportional thorax mass	0.301505064	0.347003664
Proportional abdomen mass	0.519329262	0.447893974
Thorax squareness	1.165366803	1.104252397
TotalDistance	5326.510714	4136.893333
TotalDuration	7662.857143	8087.333333
NumFlights	24	20.53333333
AvgFlightDistance	362.3651015	551.8763127
AvgFlightDuration	579.9762187	1036.663005
AvgFlightSpeed	0.33185807	0.28527359
MaxSpeed	1.445	0.959
FFMeanSpeed	0.639285714	0.403333333
FFMaxSpeed	1.102857143	0.663666667
FFDistance	1872.835714	995.2433333
FFDuration	2507.5	1858.5
FurthestFDist	2958.010714	2528.273333
LongestFlightSpeed	0.734285714	0.530666667
LongestFlightMaxSpeed	1.28	0.905666667
LongestFlightDistance	2958.010714	2528.273333
LongestFlightDuration	3664.285714	4260

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