Ecological responses to threats in an evolutionary context: bacterial responses to antibiotics and butterfly species' responses to climate change

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ABSTRACT

Humans are generally having a strong, widespread, and negative impact on nature. Given the many ways we are impacting nature and the many ways nature is responding, it is useful to study responses in an integrative context. My thesis is focused largely (two out of the three data chapters) on butterfly species' range shifts consistent with modern climate change in Canada. I employed a macroecological approach to my research, drawing on methods and findings from evolutionary biology, phylogenetics, conservation biology, and natural history. I answered three main research questions. First, is there a trade-off between population growth rate (r_{max}) and carrying capacity (K) at the mutation scale (Chapter 2)? I found r_{max} and K to not trade off, but in fact to positively co-vary at the mutation scale. This suggests trade-offs between these traits only emerge after selection removes mutants with low resource acquisition rates (i.e., unhealthy genotypes), revealing trade-offs between remaining genotypes with varied resource allocation strategies. Second, did butterfly species shift their northern range boundaries northward over the 1900s, consistent with climate warming (Chapter 3)? Leading a team of collaborators, we found that most butterfly species' northern range boundaries did indeed shift northward over the 1900s. But range shift rates were slower than those documented in the literature for more recent time periods, likely reflecting the weaker warming experienced in the time period of my study. Third, were species' rates of range shift related to their phylogeny (Chapter 3) or traits (Chapter 4)? I found no compelling relationships between rates of range shift and phylogeny or traits. If certain traits make some species more successful at northern boundary range expansion than others, their effect was not strong enough to emerge from the background noise inherent in the broad scale data set I used.

RÉSUMÉ

Les humains ont généralement un impact répandu, et négatif sur la nature. Compte tenu des nombreuses façons dont nous impactons la nature, et des nombreuses façons dont celle-ci répond, il est utile d'étudier ces réponses dans un contexte d'intégration. Ma thèse est axée en grande partie sur le déplacement des aires de répartition des papillons conformément aux changements climatiques contemporains au Canada. J'ai utilisé une approche macroécologique pour conduire ma recherche, en m'appuyant sur les méthodes et les conclusions de la biologie évolutive, de la phylogénie, de la biologie de conservation, et de l'histoire naturelle. J'ai répondu à trois principales questions de recherche. Tout d'abord, y a-t-il un compromis entre le taux de croissance de la population (r_{max}) et la capacité de charge (K) à l'échelle des mutations (chapitre 2)? J'ai découvert qu'il n'y a pas de compromis entre r_{max} et K, mais que ces variables covarient positivement à l'échelle des mutations. Ceci suggère que les compromis entre ces traits n'apparaissent qu'après que la sélection ait supprimé les mutants avec de faibles taux d'acquisition des ressources (p. ex. les génotypes malsains), révélant des compromis entre les génotypes restants, qui démontrent des stratégies d'allocation de ressources variées. Deuxièmement, est-ce que la limite nord des aires de répartition des espèces canadiennes de papillons s'est déplacée au cours du vingtième siècle de façon cohérente avec le réchauffement climatique (chapitre 3)? J'ai constaté que la limite nord de l'aire de répartition de la plupart des espèces de papillons s'est effectivement déplacée vers le nord au cours du vingtième siècle. Les taux de déplacement ont par contre été plus lents que ceux décrits dans la littérature pour des périodes plus récentes. Cette tendance s'explique probablement par le plus faible réchauffement connu pendant la période de mon étude. Troisièmement, est-ce que les taux de déplacement des aires de répartition des espèces étaient liés à leur phylogénie (chapitre 3) ou leurs traits (chapitre 4)? Je n'ai pas trouvé de relation convaincante entre les taux de déplacement des aires de répartition et la phylogénie ou les traits. Si certains traits favorisent la réussite d'espèces à étendre leurs aires de répartition plus que d'autres, leur effet n'était pas assez fort pour se démarquer du bruit de fond inhérent aux banques de données à grande échelle que j'ai utilisées.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION: A REVIEW OF MY RESEARCH QUESTIONS AND CONTEXT

Abstract

The effect of modern humanity on nature is generally very strong and negative. How will nature be shaped by our actions? This is the general question motivating my research. Herein I summarize the questions guiding each of my thesis chapters and their shared context of investigating biological responses to challenges at varied temporal, spatial, and taxonomic scales.

Background

Humanity's effects on nature

Humans harming nature is not new; we have been driving species to extinction for millennia (Prescott et al. 2012). What is new is the rate of our destruction. With approximately seven billion people now inhabiting almost every part of the world, we have greatly accelerated biodiversity loss (Butchart et al. 2010, Barnosky et al. 2011), and this trend is expected to continue or accelerate in the future (Millennium Ecosystem Assessment 2005, Barnosky et al. 2012). The ways in which we are driving biodiversity declines include habitat alteration, facilitating species invasions, overexploitation, pollution, and climate change (Chown and Gaston 2008). My thesis research is focused on the effects of climate change, though other threats may interact with climate change.

Earth's climate has changed rapidly in recent decades, consistent with greenhouse gas emissions produced by modern human society (Solomon et al. 2007). While warming is the most obvious and consistent manifestation of climate change (Barnett et al. 2005, Solomon et al. 2007), other environmental changes are also occurring, including:

- Intensification of heavy-precipitation events (Groisman et al. 2005, Min et al. 2011).
- Stronger winds and higher waves (Young et al. 2011).
- Less sunlight reaching Earth's surface (Stanhill and Cohen 2001) as a result of increases in fine particulates (aerosols)(Roderick and Farquhar 2002, Travis et al. 2002).
- Changing salinity of seas as a result of increased river run-off and melting glaciers (Curry and Mauritzen 2005, Wu et al. 2005) or decreased river run-off and increasing evaporation rates (Bethoux et al. 1999), depending on the region.

Climate has been constantly changing over the course of Earth's history. Evidence suggests historical climate changes had strong effects on biota including plankton losses during ocean circulation changes (Schmittner 2005) and, pertinent to my thesis topic, species' range shifts consistent with tracking suitable climates (DiMichele et al. 2004, Lyons et al. 2010, Abellán et al. 2011).

Species' range shifts with climate change

Species may have shifted their ranges with climate change in the past, but will they again under modern climate change? The answer emerging for many taxa and regions is yes (Parmesan and Yohe 2003, Hickling et al. 2006, Chen et al. 2011). Species' range shifts with modern climate change are highly variable even within regions and taxonomic groups (Pöyry et al. 2009, Angert et al. 2011). Widespread anthropogenic changes to habitat may facilitate colonization and range expansion of some species (e.g., biocontrol agents, species associated with roadsides or disturbed habitat), and may hinder range expansion for other species whose habitat we have fragmented and destroyed (Brook et al. 2008). Colonization of sites beyond a species' existing range requires not only suitable climate but also suitable habitat; since we have converted many habitats to agriculture, urban sprawl, etc., many species might be required to disperse longer distances than previously to extend range boundaries. Considering the more rapid rate of warming under modern than historic climate change, habitat corridors to range expansion have a shorter timeframe in which they are climatically-suitable (Early and Sax 2011), further inhibiting range shifts. Together with challenges from invasive species and potential interactions among various modern threats (Brook et al. 2008, Forister et al. 2010, Schweiger et al. 2010), it is remarkable that so many species have successfully shifted their ranges despite these threats, and unsurprising that there is great variation in species' success at doing so.

Which types of species are the most successful at shifting their ranges with modern climate change? The answer to this question is not yet known. It has been less than a decade since Parmesan and Yohe's landmark paper (2003) documenting species' range shifts with climate change across multiple taxa and regions, so this field of research is still new. Progress is limited in large part by the absence and heterogeneous quality of historical species distribution data (Tingley and Beissinger 2009); you cannot estimate range shifts without knowing where species used to live. This historical data limitation is likely a major reason why there is a geographic and taxonomic bias for the few studies of species' range shift responses to modern climate change. Studies have generally been based in Europe, especially Britain, where long-term records of species' occurrences are relatively abundant. Studies have generally focused on birds (Devictor et al. 2012), mammals (Moritz et al. 2008), and butterflies (Hill et al. 2002), because these charismatic and fairly easy-to-identify taxa have been collected more than “unattractive” and difficult-to-identify taxa (e.g., flies, slugs, grasses).

What types of species are most likely to shift their ranges with climate change?

While we would expect species with certain characteristics to be more likely to shift their range than others, expectations can be unclear and supporting evidence is underwhelming. It is logical to expect species that can inhabit a variety of habitat types, for example, to have more available paths to range shift than habitat specialists (Hill et al. 1999). Likewise, mobile species and dietary generalists should be more likely to disperse and find suitable food resources in colonized areas than their sedentary and specialized peer species (Peters and Darling 1985). Some traits, however, could be predicted to be associated with facilitating range shifts in opposite directions. The overwintering stage of butterflies provides one example. Butterflies overwintering as adults might be prone to frost-induced mortality (and thus less likely to colonize new sites) given the increasing variability in late Winter and early Spring temperatures; butterflies might emerge from diapause during a late Winter warm snap only to die when cold temperatures return. Butterflies overwintering as caterpillars might also be more likely to die from cold stress, predation, and starvation (hence not colonize new sites) if their diapause termination is dictated by photoperiod (a common feature in insects, Bradshaw and Holzapfel 2001) while overhead plants’ leaf-out phenology is dictated by temperature (WallisDe Vries and Van Swaay 2006). With warmer springs leaves might grow before caterpillars’ diapause terminates, which could delay larval development due to shade-induced cold, increasing caterpillars’ time vulnerable to predation and parasitism, and missing the window of young leaf palatability important to some butterfly species’ larvae (Cizek et al. 2006). Thus prior expectations of what types of species might be most likely to shift ranges with climate change can be unclear.

Two fields of trait-based ecological research can inform predictions of what types of species might be most likely to shift ranges with climate change. First, studies of the correlates of species’ endangerment and extinction risk have revealed certain phylogenetic and trait-based patterns (Fritz and Purvis 2010, Lee and Jetz 2011); it is logical to expect the types of species that tend to be declining to be less likely to expand their range in any direction including at the cool edge. Second, studies of the correlates of invasion success have revealed some traits associated with invasiveness (van Kleunen et al. 2010, Sol et al. 2012); since range expansion is akin to invading a new (albeit nearby) site, it is logical to expect the types of species most successful at invasion to also be successful at range expansion during climate change. This expectation of similarity in patterns between systems, however, may not be valid. For example, the logical assumption that the traits associated with conservation threat and invasiveness would be opposite to each other is not strongly supported (Jeschke and Strayer 2008). Existing studies of species' range shifts with modern climate change have found mixed evidence of traits associated with shift rates (e.g., Perry et al. 2005, Kharouba et al. 2009, Pöyry et al. 2009, Angert et al. 2011). Overall, we still do not know if there are any traits that consistently relate to range shift rates with modern climate change, or the conditions under which they would be influential. Continued research in this young field may improve the precision of predictions of biodiversity responses to climate change.

Choice of study taxa

I chose two study taxa that were suitable models for the questions in which I was interested. For my chapter investigating the effects of mutations I chose a microbial model taxon, *Pseudomonas fluorescens*, whose growth and mutation rates have been well-characterized. For example, the distribution of fitness effects among mutations conferring antibiotic resistance is well-characterized in this system (Kassen and Bataillon 2006). Many microbes are well suited to studies of population growth because they do not require extensive equipment or care, and their rapid generation times allow carrying capacity to be reached very quickly.

For my two chapters on range shift responses to climate change, I chose to study butterflies for several reasons. Insects are numerous, diverse, and integral to many economically important ecosystem functions (Losey and Vaughan 2006). Many insect species are at conservation risk (Dunn 2005), but are under-represented in conservation research (Clark and May 2002) and management programs (Seddon et al. 2005). This underrepresentation likely stems from the public's general disdain for insects other than butterflies, bees, and ladybeetles (Kellert 1993); taxonomic bias in ecological research generally reflects public values of taxa (Wilson et al. 2007). While there are plenty of economic, conservation, and ecological justifications for studying insects, the main reason I chose to study insects is that I think they are awesome. During my PhD years I have published research spanning three orders of insects: butterflies (Burke et al. 2011, Fitzsimmons 2012), crickets (Bertram et al. 2011), and assassin bugs (Fitzsimmons and Fitzsimmons 2010). My choice of butterflies as the main subject of my PhD research is due, indirectly, to their beauty. Being pretty, common, and relatively easy to identify, butterflies have been admired and collected for a long time, resulting in a rich knowledge of their distribution and natural history. This level of knowledge was essential to my thesis research on species' distributions, traits, and phylogenetic relationships. Butterflies are representative of other taxa for certain aspects of ecology (Parmesan 2003, Thomas 2005), but their status as indicator taxa is often over-stated (Fleishman and Murphy 2009). Without comparable studies of range shift among non-butterflies with climate change in Canada, I cannot evaluate how representative butterflies are of other taxa.

Summary of research chapters

Chapter 2: Population consequences of mutational events: effects of antibiotic resistance on the r/K *trade-off*

Trade-offs between traits are common, but at what scale do trade-offs arise? Trade-offs are a major reason why there is no single "best" genotype that outcompetes all others for all resources; there is not one strategy to rule them all. For example, Lepidoptera (moths and butterflies) species trade off the benefits of larval host plant specialization (e.g., efficient feeding, Bernays et al. 2004, toxin sequestration, Lampert and Bowers 2010) against the costs of specialization (e.g., susceptibility during mass extinction events, Labandeira et al. 2002, constraints on habitable area, Menéndez et al. 2007). During range expansion traits are expected to trade off such that the expanding frontier populations are selected for dispersal and growth rate, while core populations are selected for competitive ability (Burton et al. 2010, Llewelyn et al. 2010).

The relationship between maximum population growth rate ($r_{\max}$) and carrying capacity (K) is one of the best-studied trade-offs in ecology (MacArthur and Wilson 1967, Pianka 1970, Reznick et al. 2002). Whether these traits are positively or negatively correlated depends on the scale of study and environment. Using strains of the bacterium *P. fluorescens* that differed by a single spontaneous mutation conferring antibiotic resistance, I determined whether mutations conferring greater population growth rates also conferred greater carrying capacity, lower carrying capacity, or whether there was no relationship between these traits at the mutation scale. While r_{max} and K are population-scale traits, for asexual and parthenogenetic organisms the distinction between individual- and population-scale traits is blurred, allowing selection of population-scale attributes to operate at the scale of individual genotypes (e.g., self-sacrificing behaviour in aphids, Kurosu et al. 2003). I found a strong positive relationship between population growth rate and carrying capacity, likely reflecting mutations conferring greater magnitude of change to rates of resource acquisition (i.e., overall health) than resource allocation. This trend was consistent in four different sugar resource environments, suggesting results were not likely to be environment-specific. Together with previous studies this suggests the mutation scale is not the scale at which trade-offs between r_{max} and K become most evident; such trade-offs are more likely to appear after sufficient time for adaptation to select against low-acquisition genotypes, revealing resource allocation trade-offs among the remaining high-acquisition genotypes (Novak et al. 2006). This chapter is published in *Evolutionary Ecology* (Fitzsimmons et al. 2010).

While the links between this chapter and my subsequent chapters on range shift responses to climate change are indirect, this chapter does relate to the others in a broader context. My bacteria chapter deals with population-scale responses to individual-scale phenotypic variation. My butterfly chapters deal with species-scale responses to species-level variation in phenotypes. Thus both investigations have a common structure but differ at their scale (temporal, spatial, and taxonomic) scale of study. Further, r_{max} and K relate to the subject of range expansion with climate change because species' relative investment in r- and K-related life-history strategies may influence their likelihood of success in colonizing new environments (Sol et al. 2012). An example of research on r_{max} and K at a scale in between those of my thesis chapters is the individual-scale phenotypic responses to selection at the expanding frontier of a species' range (r-selected) and core of its range (K-selected) (Burton et al. 2010).

Chapter 3: Range shifts of Canadian butterfly species with climate change: is there a phylogenetic pattern?

While many species are shifting their ranges poleward with modern climate change, there is lots of variation around this general trend with some species shifting rapidly and others contracting their poleward range edge toward the equator (Parmesan and Yohe 2003, Pöyry et al. 2009, Angert et al. 2011). Can between-species variation in range shift responses to climate change be predicted? Researchers have thus far focused their attention on the influence of species' traits on range shift rates, revealing generally weak patterns (Angert et al. 2011). This approach warrants further study (including my own in Chapter Four), but could also benefit from a different perspective. Given our lack of knowledge about the traits possessed by even well-studied species (Tyler et al. 2012), trait-based predictions will only be informative for a small minority of the world's species. Phylogeny offers a crude but widely-available proxy for traits. Most traits carry a phylogenetic signal (i.e., more similar among closely- than distantly-related species) (Freckleton et al. 2002, Blomberg et al. 2003). Components of species' distributions also carry a phylogenetic signal, including signal for range size (Waldron 2007, Martin and Husband 2009) and poleward range boundary latitude (Roy et al. 2009). If traits influence species' range shift rates, and traits carry a phylogenetic signal, then we would thus expect to observe a phylogenetic signal in range shift rates. While a phylogenetic signal in range shift rates would not reveal mechanisms (i.e., which traits are influential and how), it would allow phylogeny-based predictions of species' likelihood of range shifts, just as phylogenetic signal in extinction risk (Fritz and Purvis 2010) and flowering timing responses of plants to temperature changes (Willis et al. 2008) allow phylogeny-based predictions in those systems.

I led a team of collaborators that estimated range shift rates for 137 butterfly species in Canada over the 1900s, and tested shift rates for phylogenetic signal. Northern range boundaries were estimated within each of three geo-climatic regions for the historical period (1900-1930) and recent period (1960-1990) based on collection locations of butterfly specimens held in museums (Layberry et al. 1998). Northern range boundaries were considered the average latitude of the three northern-most collection locations for a species in a region and time period, after controlling for differences in sampling effort and geographic bias between time periods. A phylogeny was constructed based on DNA sequences of three loci obtained from public databases. We found no phylogenetic signal for range shift rates; species were no more likely to shift their northern boundary at similar rates if they were closely- than distantly-related. While other studies have found individual species shifting northward in Canada consistent with modern climate change (e.g., Bowman et al. 2005, McAlpine et al. 2008, Mamet and Kershaw 2012), ours is the first study to demonstrate the generality of northward range shifts in Canada within a large taxonomic group. Ours is also the first test of a phylogenetic signal for range shifts with modern climate change in any taxonomic group or region. Our results suggest there is no phylogenetic pattern to range shift responses to climate change, but knowing how general this (lack of) pattern is requires study of additional taxa and regions.

Chapter 4: No evidence of a relationship between butterfly species' traits and northward range shift rates in Canada over the 1900s

Following up on my research from Chapter Three, the question posed in Chapter Four is: were butterflies' northward range shift rates related to species' traits? As explained earlier in this introductory chapter, it is logical to expect certain traits to facilitate poleward range expansion (e.g., habitat breadth, mobility). Several studies have investigated the relationship between range shift rates with modern climate change and species' traits, revealing a generally weak effect of traits on shift rates (Angert et al. 2011). With so few studies on the subject, and so much variation between the few taxa and regions investigated, it is prudent to conduct additional studies to determine which traits (if any) influence range shift rates and under what circumstances.

Given the limited knowledge of the influences on species' range shift rates with climate change, I felt it was appropriate to employ an exploratory approach to answer this chapter's question. Hypothesis-driven "top-down" approaches to determining the relationship between species' traits and range shift rates might omit traits that seem unrelated to range shift rates but are actually influential. Examples of non-intuitive biological relationships that might be ignored in hypothesis-driven research includes the relationship between species' genome size and flight strategy among Odonata (dragonflies and damselflies)(Ardila-Garcia and Gregory 2009), and the relationship between butterfly species' adult feeding behaviour and lifespan which was revealed through exploratory analyses (Beck and Fiedler 2009). I chose to explore the relationship between range shift rates and as many biological traits as I could find with sufficient data.

I determined the relationship between butterfly species' northern boundary shifts obtained in Chapter Three with 20 traits obtained from several sources. I found no compelling relationships between northern boundary shift rates and traits. Either shift rates were unrelated to traits, or their relationship was weak enough to be overwhelmed by the noise inherent in the data set. My results contribute to our growing understanding of species' range shifts with climate change, and the challenge of predicting such shifts with precision.

CHAPTER 2. POPULATION CONSEQUENCES OF MUTATIONAL EVENTS: EFFECTS OF ANTIBIOTIC RESISTANCE ON THE *r*/*K* TRADE-OFF

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Abstract

What are the effects of a mutational event on population dynamics? This eco-evolutionary question has relevance not only to basic biological theories but also to conservation applications. We evaluated the relationship between maximum population growth rate (r_{max}) and carrying capacity (K) among strains of the bacterium *Pseudomonas fluorescens*. Each of 65 strains differed from their common ancestor by one naturally acquired phenotypic change conferring antibiotic resistance, brought about by a single mutational event, and each was grown in isolation in four environments. We found no evidence of a trade-off between r_{max} and K. Rather, strains with rapid growth rates also had high carrying capacity, with little interaction between strain and environment. We conclude that the extensive variation in overall fitness resulting from single mutational events likely masks whatever population trade-offs may exist.

Introduction

Although the feedback between ecology and evolution has been considered for many decades, eco-evolutionary research has only recently gained widespread attention (Carroll et al. 2007). This attention is due in part to improved techniques that allow unprecedented genetic investigation (Hoffmann and Willi 2008), and in part to a growing realization that an eco-evolutionary approach can be applied to modern conservation management (Mace and Purvis 2008). At issue is the connection between genetic variation and key ecological parameters governing the long-term stability of a population such as growth rate and carrying capacity. Here we explore this question by investigating empirically the effect of mutations that arise naturally and independently during population expansion on population dynamics across a range of environments. Our main goals were to quantify the amount of genetic variation generated by mutation on two key parameters governing population growth, r_{max} (maximum population growth rate) and K (population carrying capacity; Figure 2-1), and to investigate the contribution of mutation to generating trade-offs between these parameters.

The notion of a negative association between r_{max} and K was popularized in the 1960's and 70's (MacArthur and Wilson 1967, Pianka 1970) and has been the focus of much research since (reviewed in Reznick et al. 2002, Bell 2008). Fast-growing 'weedy' strategies (*r*-selected strategies) are hypothesized to come at the expense of competitive ability in dense populations (*K*-selected strategies). This hypothesis is appealing for its common sense simplicity, but has been criticized on the grounds that it is difficult to test empirically because r_{max} and K cannot usually be directly measured, and proxy measures used to infer r_{max} and K are often inappropriate (Stearns 1977, Reznick et al. 2002). It is possible to avoid some of these criticisms by turning to microbial populations where r_{max} and K can be estimated directly from batch culture measures of population density, as resources are initially abundant but are steadily depleted as the population grows.

According to the resource acquisition-allocation model, or Y-model, r_{max} and K could be considered characters that functionally interfere with each other such that a given amount of resources must be allocated to one trait or the other. Provided there is no variance in total resources available to individuals, genetic variation in allocation leads to a negative relationship between the two traits at the population level (de Jong and van Noordwijk 1992, reviewed in Zera and Harshman 2001, Roff and Fairbairn 2007).

Experimental evolution studies have demonstrated that populations' growth rates are density-dependent and can respond to selection (reviewed in Mueller 1997). For example, *Drosophila melanogaster* populations grown at artificially-imposed high densities for several generations evolved higher population growth rates at high than low densities, while those selected at low densities evolved higher growth rates at low densities (Mueller and Ayala 1981). Our study differs from those focused on *r*- and *K*-selection or density-dependent selection in that ours focuses on indirect effects upon r_{max} and K instead of direct selection upon either trait. The timescale of our study is also novel in that we investigate the effect of single mutational events instead of consistent selective pressure, thus contributing to our understanding of the scaling of trade-offs (Rueffler et al. 2006).

Our study makes use of clonal populations of bacteria that each differ from a common ancestral strain by a single mutational event. There was thus no pre-existing genetic variation for r_{max} or K in our experiment. Any variation among mutant strains for r_{max} and K is the result of mutational events conferring antibiotic resistance. Although it is difficult to make conclusions about the mechanisms underlying trait relationships without detailed physiological and genetic investigation (Zera and Harshman 2001), by combining previous knowledge of the genetics and physiology of antibiotic resistance with the results of our population ecology experiment we can make stronger conclusions about the effect of mutational events on the relationship between traits than we could if we relied only on physiological or ecological information.

The relationship between traits can differ in magnitude and even sign between environments (Sgrò and Hoffmann 2004, Gutteling et al. 2007). In order to draw conclusions about trait relationships and evolutionary trajectories, therefore, more than two environments should be used to test trait relationships (Sgrò and Hoffmann 2004). We tested the relationship between r_{max} and K in four environments, differing in the type of sugar available as a resource. Our goal was to determine the effects of mutational events on the relationship between traits and whether these relationships are environment-dependent. This knowledge will improve our understanding of evolutionary trajectories and the nature of novel mutational events.

Methods

Bacteria strains

The strains used in our experiment come from the collection of nalidixic acid resistant mutants of the soil bacterium *Pseudomonas fluorescens* reported by Kassen and Bataillon (2006), who provide details on the protocol for mutant isolation. Briefly, a collection of independently-derived strains containing a single phenotypic change conferring resistance to the antibiotic nalidixic acid was obtained by screening over 2000 populations of the strain SBW25 (Rainey and Bailey 1996) in a conventional fluctuation assay (Luria and Delbrück 1943). All mutant strains were thus derived from independent mutational events occurring naturally during population expansion without regard to their pleiotropic fitness effects in the assay environment.

We chose 66 strains from this collection of 665 for analysis. Eighteen of these strains were non-randomly selected from the few that had been classified by Kassen and Bataillon (2006) as 'beneficial' because they produced higher population densities than their common ancestor after 24 hours of growth in permissive Luria Bertrani (LB) medium, the same medium in which antibiotic selection was performed. The remaining 48 strains were chosen at random from the larger collection, and had been classified as 'non-beneficial' because they produced lower population densities than their common ancestor in LB medium (Kassen and Bataillon 2006). Strains were stored at -80°C in solutions of 60% culture : 40% glycerol by volume.

Without sequencing the entire genome of each strain we cannot be certain that each strain diverged by a single mutation. A 500 base pair segment of the quinolone resistance determining region (QRDR) of *gyrA* was sequenced for the 18 beneficial strains (E. Ouellet and R. Kassen, unpublished data). Four different point mutations were found (never more than one per strain) among the 13 strains that had a mutation at this locus, with the remaining five strains potentially having mutations at one of several other loci known to confer resistance to nalidixic acid (Jacoby 2005). Five of the eighteen strains sequenced shared the same point mutation (T for G at position 259), suggesting approximately 28% of our strains may share identical resistance-conferring mutations, assuming non-beneficial strains have similar rates of mutation similarity as beneficial strains. It is thus possible for some strains to have independently acquired identical mutations (estimated probability of 28% calculated above) resulting in pseudoreplication. We remain confident in our results due to the strength of the relationships that emerged from our analyses; even if there were pseudoreplication of up to 28% our relationships would remain strong. Some strains may have accumulated more than one fitness-altering mutation (probability estimated by Kassen and Bataillon 2006 to be approximately one in a million), or epigenetic changes may have conferred antibiotic resistance (unlikely since strains' resistance was heritable; see Adam et al. 2008 for an empirical example of antibiotic resistance caused by epigenetic effects). These possibilities are all included along with typical single resistance-conferring mutations in our scale of study: single phenotypic changes on the timescale of single ‘mutational events.’

Experimental setup

We first grew all 66 mutant strains at 28°C on 1.2% agar LB plates with nalidixic acid at the same antibiotic concentration (1 mg / L) as used by Kassen and Bataillon (2006). The common ancestor was also grown at 28°C on agar LB plates, but without nalidixic acid. Single colonies were selected from plates (to ensure only one genotype was used) and grown in 30 mL glass vials with 5 mL LB liquid media without antibiotic for 48 hours at 28°C. Racks of vials were constantly shaken at 0.35 *g* to maintain culture homogeneity and adequate aeration. Two microliters of this culture were transferred into microwells of media to initiate our experiment.

We inoculated all 67 strains (66 mutants + 1 common ancestor) into 24-well microwell plates (Cellstar, Greiner Bio-One) containing 2 mL of one of four media environments that differed only in the source of carbon: glucose, mannitol, mannose, or sorbitol (4.78 mM Na_2HPO_4, 2.20 mM KH_2PO_4, 0.86 mM NaCl, 1.87 mM NH_4Cl, 0.20 mM $MgSO_4 \cdot 7H_2O$, 2.21 mM sugar). Microwell plates were shaken at 0.35 *g* at 28°C. Enough plates were used at once to ensure two replicate wells of each strain × environment combination (2 replicates × 67 strains × 4 environments = 536 wells). Each plate included one well with the common ancestor, and one well without any bacteria added (negative control) to detect contamination and measure spectrophotometric optical density of media without bacteria at each reading point. All negative controls were free of contamination, and all produced similar optical density (OD) readings regardless of media type or time of measurement (mean ± SD = 0.030 ± 0.001).

Optical density of wells was read at 630 nm on an ELX800 Microplate Reader (Bio-Tek Instruments Inc., Winooski, Vermont, USA) using KC Junior 1.41 software (Bio-Tek Instruments Inc.) every hour following inoculation until the end of exponential growth phase and every two hours thereafter until 27 hours of growth, and once again 19 hours later to verify that carrying capacity had been reached. Optical density is commonly used as an indicator of cell density, with OD and cell number being positively related in a sub-sample of wells tested ($r^2_{87}= 0.564$, $P < 0.001$).

Data analysis

We fitted both logistic and Gompertz equations (Zwietering et al. 1990) to ln-transformed OD data for each well using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA) to obtain estimates of growth parameters. Both equations gave comparable results so we report only those obtained from the logistic equation. The growth parameters were maximum linear population growth rate (r_{max}), population carrying capacity (K), and the intersection of the slope r_{max} with the initial ln(OD) value which represents the time lag before the growth phase (*tlag*)(Zwietering et al. 1990) for each replicate of each strain × environment combination (Marquardt iteration method used, other iteration methods produced similar results, data not shown). We excluded from data analyses ten wells that had so little growth that the program could not converge upon parameter estimates. Among these were all eight wells containing the strain 3-1A10. All analyses were thus performed on the remaining 65 mutant strains.

We evaluated the relationship between r_{max} and K by calculating the Pearson correlation coefficient among mutant strains within each environment. Because we were concerned explicitly with the variance contributed by mutation, we excluded the common ancestor from these analyses. Average values of replicates were used. All analyses were conducted using JMP 5.1 or 7.0.2 (SAS Institute Inc.).

We tested for the significance of the strain × environment interaction term in standard two-fixed-factor ANOVAs for each of r_{max} and K to detect variability among strains in growth across environments. We then estimated the variance components of strain, environment, their interaction, and residual error upon r_{max} and K (one model for each of r_{max} and K). We used sum of squares for each effect using the restricted maximum likelihood (REML) method with all effects considered random. The resulting variance components were then used to calculate the fraction of total variance of r_{max} and K attributable to each term in the model. We also calculated the Pearson correlation coefficient for each of r_{max} and K between pairwise environments to further determine whether strains with high r_{max} or K in one environment also had high values in the other environments.

Results

Relationship between r_{max} and K

r_{max} and K were strongly positively correlated among all strains in each environment (glucose r^2_{64}= 0.466, $P < 0.0001$; mannitol r^2_{64}= 0.764, $P < 0.0001$; mannose r^2_{63}= 0.773, $P < 0.0001$; sorbitol r^2_{64}= 0.763, $P < 0.0001$; Figure 2-2).

Strain × environment interaction

The strain × environment interaction term, along with each term independently, explained significant variation in both r_{max} and K in ANOVA tests (Table 2-1). The main effects of strain and environment together accounted for more than 85% of the variance in both r_{max} and K, with their interaction accounting for 11.2% and 2.8% of the variance in r_{max} and K, respectively (Table 2-1). Strains' values for r_{max} and K were positively correlated between environments (adjusted r^2 always greater than 0.447, $P < 0.0001$; Table 2-2).

Discussion

There was a positive relationship between growth rate and carrying capacity among strains in each environment. Strains that had high r_{max} and K in one environment also had high values in the other environments, and the strain × environment interaction explained little variation in r_{max} and K. This suggests that our results are not environment-specific. Our results are supported by research on *Escherichia coli* bacteria, in which mutational events conferring a fitness advantage in one environment also conferred a fitness advantage in most other environments (Ostrowski et al. 2005). *Pseudomonas fluorescens* readily specializes on different sugar medium environments following selection over a few hundred generations (Jasmin and Kassen 2007). Any environment-specificity contributed by our mutational events was masked by overall variation in fitness.

The positive relationship between r_{max} and K is consistent with the fitness components model. However this result may also be consistent with the Y-model provided any functional trade-off between r_{max} and K is masked by mutational variation in total resource availability such that some strains invest lots of resources into both r_{max} and K while others invest little in both (van Noordwijk and de Jong 1986, Reznick et al. 2000). Either way, if our mutant strains were exposed to prolonged selection in a stable environment we might expect the variation contributed by mutation to be reduced as those strains with shallow r_{max} and low K would quickly be eliminated. Any trade-off between r_{max} and K could then be observed, and would in fact be expected. Long-term evolution experiments using *E. coli* support this prediction (Novak et al. 2006). After evolutionary time periods most populations were found to consist of strains that displayed a negative association between r_{max} and K (Novak et al. 2006), consistent with the hypothesis that selection removed the variation in overall fitness contributed by mutation over time, revealing a functional trade-off between r_{max} and K. The main conclusion we can draw is that mutation is not bound by any inherent functional or physiological constraint that generates a trade-off between r_{max} and K. Rather, such trade-offs are the product of the combination of mutation and selection.

An alternative explanation of positive covariance between r_{max} and K is that mutation affected neither resource acquisition nor fitness components, but shifted variation in resource allocation decisions to upper hierarchical levels thus masking trade-offs at subsequent levels (Worley et al. 2003). For example, if an animal's resources are first allocated between reproduction and somatic growth, and reproduction resources are subsequently allocated between offspring size and offspring number, a positive relationship between offspring size and number would result from mutation contributing far greater variation in allocation at the first hierarchical level (reproduction *vs*. growth) than the second. According to this explanation, our strains with high r_{max} and K have greater resources available for reproduction at the expense of resources for other functions, compared to their common ancestor. Thus strains with low r_{max} and K might have invested vast resources in cellular defences or motility rather than reproduction (for example), and would be expected to be competitively superior in environments favouring these traits. We believe this explanation is unlikely for the majority of our strains, as we expect based in part on our knowledge of the physiology of our mutational system that strains with low r_{max} and K result from mutations affecting overall fitness.

Knowledge of the physiology and genetics of our experimental system allows us to make hypotheses regarding the mechanism underlying the relationship between r_{max} and K at our scale of study that would not be possible if we were to rely only upon ecological data. A striking feature of our results, which confirms that of earlier work (Kassen and Bataillon 2006), is that the pleiotropic costs of resistance to quinolone antibiotics are highly variable both in terms of r_{max} and K. We suspect that the source of this variance in costs of resistance stems from the specific genetic targets conferring resistance. Low costs of resistance are likely to arise from mutations that induce conformational changes in DNA gyrases that prevent quinolones from binding to the enzyme-DNA complex. Such mutations should be energetically inexpensive since they result largely from amino acid substitutions that affect the tertiary folding of proteins (Hawkey 2003). High-cost mutations, on the other hand, are expected to result from mutations in regulatory genes associated with efflux systems, as the constitutive expression of such systems is likely to be energetically expensive to the cell. Preliminary evidence in support of this comes from our observation that the majority (13/18) of mutations amongst our fittest strains stem from one of four mutations within the 500 bp quinolone-resistance-determining region of *gyrA* (Ouellet and Kassen, unpublished data), which alters the target of quinolone rather than efflux systems (Hawkey 2003, Jacoby 2005). Mutants with low-cost mutations would invest plenty of resources in both r_{max} and K, while those with mutations affecting efflux systems would have few resources to invest in either r_{max} or K. Mutations affecting efflux systems might also affect resource acquisition rates if the efflux pump is overly-general and ejects resources from the cell before they can be used.

Evidence suggests the average effect of mutations on fitness is weak and deleterious (Eyre-Walker and Keightley 2007), but it is also important to understand the pleiotropic effects of mutational events to model evolutionary predictions (Johnson and Barton 2005). We have demonstrated that, at least for our study system, experimental environments, and characters evaluated, mutational events likely contribute greater variation to overall fitness than to trade-offs between fitness components.

Human degradation of natural ecosystems is expected to continue at an unprecedented rate (Millennium Ecosystem Assessment 2005), leading to phenotypic changes in wild populations that are generally greater in magnitude than those caused by natural perturbations (Hendry et al. 2008). In this setting the influence of phenotypic changes on population dynamics is not merely an academic concern. Research should advance beyond documenting changes in life-history trait values due to environmental change, and investigate the influence of those trait changes on individual fitness, population dynamics, and community- and ecosystem-scale dynamics. We have investigated the influence of one phenotypic change (antibiotic resistance, resulting from a mutational event) on two population parameters (growth rate and carrying capacity) in a controlled system that allows population-level study but lacks the multiple interactions typical of natural systems. Future research should determine the generality of population effects of mutational events and investigate eco-evolutionary dynamics in multi-species communities (Johnson and Stinchcombe 2007, Lau 2008).

Acknowledgments

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Author contributions

J.M.F. conducted the main experiments, analyzed the data, and wrote the manuscript. S.E.S. contributed ideas, helped train J.M.F., and determined the relationship between cell density and spectrophotometer values. J.T.K. participated in discussions and provided feedback on the manuscript. R.K. came up with the project idea and assisted in experimental design, choice of statistical methods, and writing.

Tables

Table 2-1. Results of two-factor ANOVAs and variance components analyses for r_{max} and K.

		r_{max}			K		
Term	df	F ratio	*P*	Total variance (%)	F ratio	*P*	Total variance (%)
Strain	64	151.50	<0.0001	64.72	106.86	<0.0001	37.42
Environment	3	187.74	<0.0001	20.63	1111.85	<0.0001	54.46
Strain × environment	192	7.47	<0.0001	11.19	4.67	<0.0001	5.27
Error	258			3.46			2.85
Total	517			100.00			100.00

Table 2-2. Adjusted Pearson correlation coefficients of strains' r_{max} and K values between environments. Values in the bottom-left of the table are for r_{max}, and those in the top-right are for K. All values are significant ($P < 0.0001$), and degrees of freedom (df) = 64 for all comparisons except those involving mannose for which df = 63.

	Glucose	Mannitol	Mannose	Sorbitol
Glucose		0.746	0.630	0.687
Mannitol	0.621		0.770	0.834
Mannose	0.447	0.733		0.849
Sorbitol	0.660	0.924	0.817	

Figures

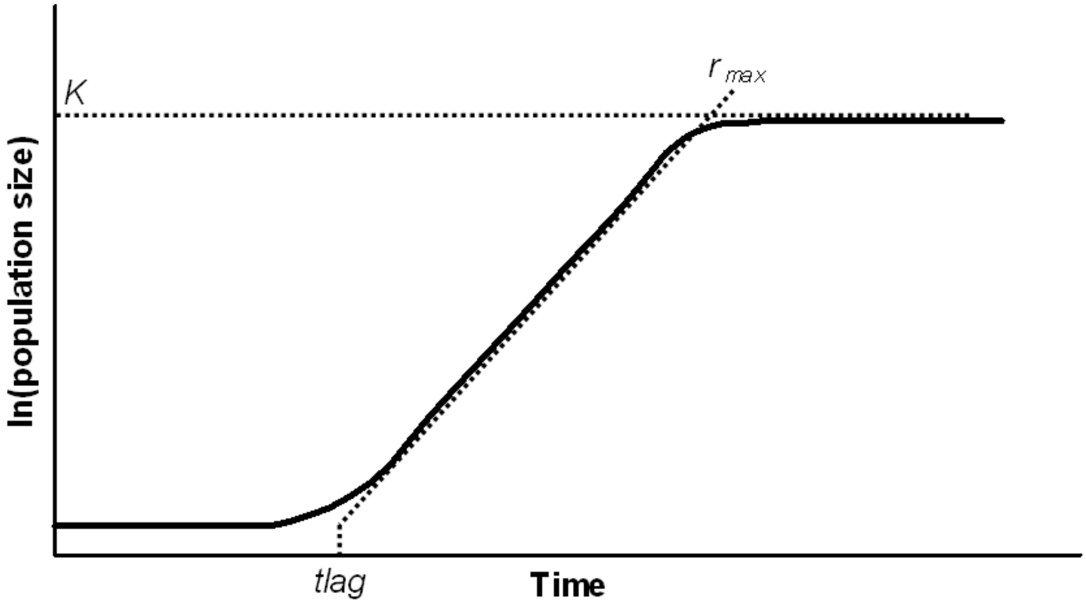

Figure 2-1. Schematic population growth curve, illustrating the population carrying capacity (*K*), maximum population growth rate (r_{max}), and the time lag (*tlag*) before exponential growth phase indicated by the intercept of r_{max} with the initial ln(population size) value.

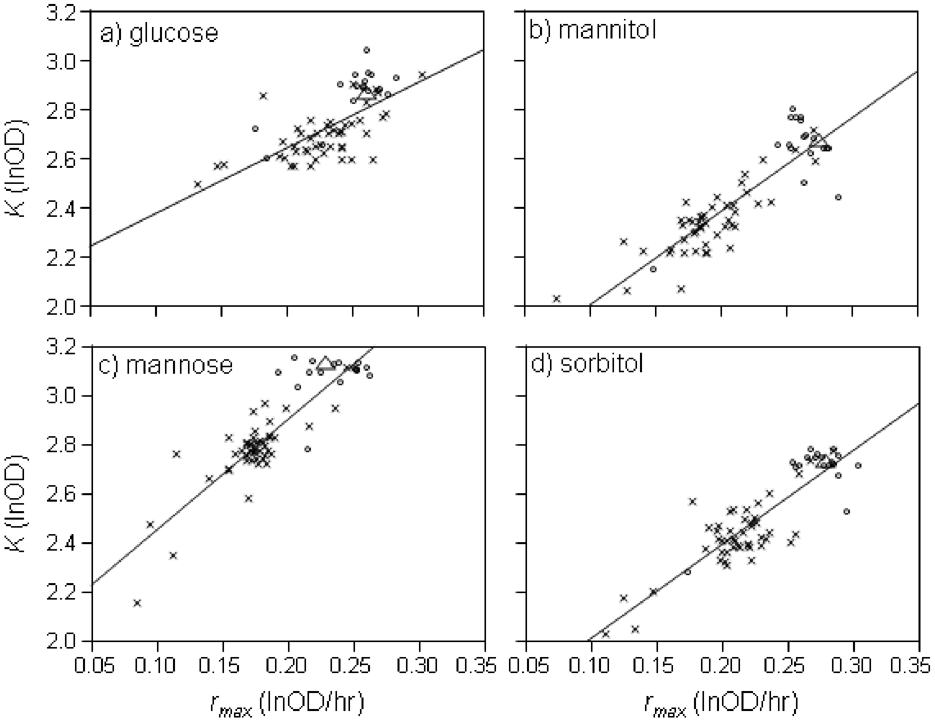

Figure 2-2. Relationship between maximum population growth rate (r_{max}) and carrying capacity (K) in ln-transformed optical density (OD) units with regression lines in each of four environments: glucose (*a*), mannitol (*b*), mannose (*c*), and sorbitol (*d*). Circles represent 'beneficial' strains, crosses represent 'non-beneficial' strains (see Methods for definitions), and the triangle represents the average of six replicates of the common ancestor (common ancestor was not included in regression analysis, and is presented for comparison purposes only).

CHAPTER 3. RANGE SHIFTS OF CANADIAN BUTTERFLY SPECIES WITH CLIMATE CHANGE: IS THERE A PHYLOGENETIC PATTERN?

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Fitzsimmons, J. M., N. D. Szabo, E. R. Young, J. Gibbs, K. E. Gibbs, J. L. Nadeau, A. C. Algar, H. M. Kharouba, A. L. MacDonald, and J. T. Kerr. Range shifts of Canadian butterfly species with climate change: is there a phylogenetic pattern?

Abstract

As the world warms with climate change many species are shifting their ranges toward the Earth's poles. There is substantial variation in species' rates, and even direction, of latitudinal range shifts which complicates conservation planning. Our goal was to determine whether there is a phylogenetic pattern to species' rates of range shift with climate change, whereby closely-related species shift at more similar rates than distantly-related species. Such a pattern would allow prediction of species' range shift responses to climate change based on related species' responses. Using collection records, we estimated 137 butterfly species' northern range boundaries in Canada for two time periods: 1900-1930 and 1960-1990. We constructed a phylogenetic tree based on publicly-available DNA sequences. We tested for a phylogenetic signal (i.e., non-random phylogenetic pattern) in northern boundary latitudinal shift distances between time periods. We found evidence of northward shifts over time, but no evidence of a phylogenetic pattern to species' boundary shift distances. At least for the taxa and time periods of our study, species' range shift responses to modern climate change cannot be predicted by phylogeny.

Introduction

Many species are shifting their ranges toward the Earth's poles with climate change (Parmesan and Yohe 2003). There is substantial variation around this general trend of poleward range shifts, with some species expanding poleward rapidly and others contracting toward the equator (Parmesan and Yohe 2003, Hickling et al. 2006, Pöyry et al. 2009). Predicting range shift responses to climate change is a core issue for conservation management. If there were a phylogenetic pattern to species' range shifts, whereby closely-related species shifted at similar rates, then it would be possible to predict range shifts of under-studied species based on known responses of closely-related well-studied species.

Range shifts with modern climate change could be expected to exhibit a phylogenetic signal (i.e., closely-related species shift at more similar rates than distantly-related species), based on two general trends. First, species' range boundaries are determined by many traits, ranging from physiological traits such as thermal tolerance to behavioural traits such as dispersal ability (Parmesan et al. 2005, Sexton et al. 2009). Second, most physiological, behavioural, and other biological traits have a phylogenetic signal (Freckleton et al. 2002, Blomberg et al. 2003). Therefore, whichever traits influence range boundaries in a phylogenetic group likely carry a phylogenetic signal, which should indirectly produce a phylogenetic signal in rates of range shift with warming over time. In agreement with this line of reasoning, there is evidence for a phylogenetic signal in species' range size (Waldron 2007, Martin and Husband 2009) and the latitude of species' northern range boundaries (Roy et al. 2009). Furthermore, there is some evidence from fossils that some groups of animals shifted their ranges more than others during Quaternary climate changes (Lyons et al. 2010, Abellán et al. 2011). To date no research has assessed phylogenetic signal in observed rates of range shift with modern climate change, but a few studies have referred to the issue. Two studies of species' range shifts (Moritz et al. 2008) and abundance changes (Breed et al. 2013) with climate change noted that there did not appear to be a pattern of similar responses among species within the same genus. Also, predictions of species' range size changes with future climate change in Europe found a weak phylogenetic signal for projected range size contractions (Thuiller et al. 2011).

Some responses to anthropogenic change do carry a phylogenetic signal, allowing phylogeny-based predictions for conservation management. For example, extinction risk carries a phylogenetic signal for several taxonomic groups (Purvis 2008, Fritz and Purvis 2010). Like range shift responses to climate change, extinction risk is not itself an evolving trait but is influenced by evolving traits. We calculated the phylogenetic signal for range boundary shifts of butterflies in Canada – a group for which extensive collection records and ecological information are available. We calculated the northern range boundaries of 137 butterfly species for two time periods of the 1900's, constructed a phylogenetic tree for these species, and determined whether there was a phylogenetic signal to northern boundary latitudinal shifts.

Methods

Northern boundary shift analyses

We used collection data from the Butterflies of Canada database (Layberry et al. 1998), which includes about 300,000 georeferenced (>80% of records located to within 1 km) collection records for 297 species dating from the late 19th century to present. Each collection record is accompanied by a preserved specimen stored at one of 40 Canadian natural history collections, identified by a lepidopterist. Our taxonomy follows Warren *et al*. (2009) for Hesperiidae, and Pelham (2008) for all other families.

To compare geographical distributions of butterfly species during a period of anthropogenic climate change, we extracted butterfly observations made from 1900 to 1930 (historical period) and from 1960 to 1990 (recent period). For species with more than one record at the same location in a time period, we used only one of the records. We excluded introduced species and species with fewer than 10 unique collection locations in each time period. We also excluded one species, *Colias pelidne* (Boisduval & LeConte), that had records removed in sampling area buffer zone corrections for the northward shift calculations (see below), leaving it with fewer than 10 historical collection records. Our final data set included 137 species from five families in the order Lepidoptera: Hesperiidae (skippers, n = 21), Lycaenidae (gossamer-winged butterflies, n = 29), Nymphalidae (brushfooted butterflies, n = 59), Papilionidae (swallowtail butterflies, n = 9), and Pieridae (white and sulfur butterflies, n = 19).

When calculating northern boundary shifts we accounted for four potential biases in our data set, described below.

1. *Climate-latitude disparity*. A given latitude can have different climates across Canada – an issue less relevant to global change studies in smaller countries. We thus split Canada into three geo-political regions within which climate exhibits less longitudinal variation: the Western Region (British Columbia and Yukon Territory); the Central Region (Alberta, Saskatchewan, Manitoba, and Northwest and Nunavut Territories); and the Eastern Region (Ontario, Québec, and the provinces of Atlantic Canada). We calculated species' northern range boundary shifts within each region that had at least three collection records in each time period (see #4 below for how we estimated range boundaries). We estimated phylogenetic signal for range shifts within each region separately, as well as for all of Canada (see below).
2. *Sampling area bias*. If a butterfly species was observed in the recent time period at a northern location that had not been sampled for any species in the historical period, then the apparent northward shift could be real (i.e., the species actually shifted north over time) or an artefact of sampling (i.e., the species was at the northern location in the historical period but nobody collected butterflies at that location then). We eliminated all collection records from regions that were only sampled in one time period by eliminating all recent period records that fell outside 'buffer zones' of 100 km radius around each collection location in the historical period using ArcGIS version 9.2 (ESRI). We likewise eliminated all historical period records that fell outside recent collection location buffer zones, although this was less common. We chose 100 km as our buffer zone radius because it reduced sampling area bias without reducing our sample size of collection records to levels too small for reliable analyses. The number of collection locations in the recent time period fell from 6273 to 5241 after buffer zone exclusions, a reduction of 16%. The average latitude of remaining collection locations was similar between historical and recent time periods, though slightly farther south in the recent period making our tests for northward shifts conservative (historical: 49.7° N, recent: 48.8°

N). A total of 4525 and 29,344 collection records were used in final analyses for the historical and recent time periods, respectively (note that sampling intensity was equalized in analyses; see #4 below).

3. *Far-North species*. For those few species collected at the northern-most collection location of any butterfly in the historical period in a region, it is impossible to distinguish the species' northern range limit from the northern limit of where people collected butterflies. These species could have lived farther north in the historical period, but nobody collected butterflies there. Thus we excluded species collected at the northernmost collection location in the historical time period from boundary shift estimates within each region, reducing our likelihood of over-predicting shift distances. For example, *Colias nastes* Boisduval was collected at the northern-most collection location in the Eastern Region in the historical period, so even though it was collected at a farther-north location in that region in the recent period its boundary shift was not estimated for that region because we could not distinguish true boundary shift from collection effort. Because no species was collected at the northern-most collection location in all three regions, no species was completely eliminated from Canada-wide shift analyses.
4. *Sampling intensity bias*. As sampling intensity increases, estimates of range boundaries expand outward toward species' real boundaries (Shoo et al. 2006). Previous studies have used several methods to address sampling intensity bias (reviewed in Hassall and Thompson 2010). Because our data set had 6.5 times more records in the recent than historical time period, we subsampled the same number of collection records used in the historical period from the larger recent period data set. We repeated this procedure 50 times in each Canadian region, for each species, each time determining the three collection records with the highest latitude. The average northern range boundary among all 50 subsample results was used as the northern range boundary of a species in the recent time period in the given region. We used the same procedure in reverse for the few species with more collections in the historical than recent time period.

Northward shift distances (recent minus historical northern range boundary latitude) were determined for species in each region. Canada-wide shift estimates per species were calculated as the average shift distance among the three (or fewer) regions' shift estimates, weighted by historical period sample size in each region. Shift rates are equivalent to shift distances (measured in kilometers), since all species' shift distances were calculated for the same time period of 60 years.

To determine whether more species shifted north than the 50% expected by chance, we calculated two-tailed exact binomial probability tests for each region using VassarStats (vassarstats.net accessed January 3 2012).

Phylogenetic analyses

We constructed a phylogenetic tree for our species using GenBank DNA sequences (see Table 3-1 for accession numbers). We used two nuclear genes (Elongation Factor – 1α, *EF-1α*; and Wingless, *WNG*) and one mitochondrial gene (Cytochrome *c* oxidase subunit 1, *COX1*). These three genes have been advocated for use in butterfly phylogenetic construction because *EF-1α* and *WNG* resolve higher level nodes and *COX1* resolves lower level nodes as a result of its high mutation rate (Sperling 2003). Although these three genes were the most widely available for Canadian butterfly species, sequences for all three genes were not available for every species and many Canadian species had no sequence data at all. We also included sequences from 'substitute' species in the same genus as species in our data set that lacked sequence data to improve the phylogenetic resolution of the tree. Sequence length varied across taxa for each of the three genes (*EF-1α*, 1247bp; *WNG*, 417bp; *COX1*, 1524bp), and were truncated when necessary to include only the gene of interest. Sequences were aligned using CLUSTALX (Thompson et al. 1997). Aligned sequences were concatenated and entered into MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) to generate a consensus phylogenetic tree using Bayesian methods with default settings (Nruns = 2, Nchains = 4, Samplefreq = 100, Realburnin = yes, and Burninfrac = 0.25). We applied the GTR+G+I model of nucleotide substitution, following Wahlberg *et al*. (2005) who used the same three genes in their construction of a butterfly phylogeny. Each gene and each codon position within a gene was treated as a separate partition with parameters unlinked and different rates among partitions. We ran the analysis until the average standard deviation of split frequencies fell consistently below 0.005 (indicating good fit of the tree to the sequence data), which took 30,000,000 generations. For outgroups we used GenBank sequences for two moth species: *Archiearis parthenias* (family Geometridae) and *Urania leilia* (family Uraniidae).

The final consensus tree produced by MrBayes was largely congruent with recent studies of family level relationships of Lepidoptera (Wahlberg et al. 2005, Regier et al. 2009, Mutanen et al. 2010). Consistent with these studies, and with a recent taxonomic classification (van Nieukerken et al. 2011), the traditional classification of the family Hesperiidae in its own superfamily Hesperioidea (Pogue 2009) is not supported. Hesperiidae segregated among most other butterfly families, while the most recent common ancestor of the Papilionidae is suggested to be the first split in our tree. Our placement of Hesperiidae cannot be considered independent of those by Wahlberg *et al*. (2005) since we used many of their gene sequences. Our tree differed from accepted phylogenetic relationships in two aspects. First, *Danaus plexippus* (Linnaeus) is generally considered a member of the tribe Danainae, which arose from a basal node within the family Nymphalidae (Wahlberg and Wheat 2008), but resolved in our tree as an evolutionarily distinct taxon more closely related to the lycaenids than the nymphalids. Second, *Speyeria mormonia* (Boisduval) did not cluster with congeneric species.

Because genetic sequences were only available for a subset of our species, we constructed two phylogenetic trees to test phylogenetic signal: a DNA-based tree and an all-species tree. The DNA-based tree only included species for which any of the three genes' sequences were available (72 species). The all-species tree used the DNA-based tree as a foundation, with all other species added onto the tree following a set of rules based on taxonomic affiliations (Figure 3-1) using Mesquite version 2.72 (Maddison and Maddison 2009). Previous research has found that tree variants constructed in different ways produce very similar results (Corey and Waite 2008, Cadotte et al. 2009, Helmus et al. 2010). Likewise, our phylogenetic signal results were very similar with the DNA-based tree and the all-species tree (Table 3-2).

To test for phylogenetic signal in range shifts we used the REGRESSIONv2.m program (Lavin et al. 2008) in Matlab version 7.0.4.365 (MathWorks Inc.). The northern boundary shift response variable was continuous and included both southward and northward shifts. Shift rates were transformed using the Box-Cox procedure in JMP version 8.0.2 (SAS Institute Inc.) to improve normality prior to signal estimation.

REGRESSIONv2.m estimates the likelihood of several evolutionary models, including the model that phylogenetic relatedness does not relate to shift rates, given the tree and shift rate data. The evolutionary models tested were: ordinary least squares (OLS, a star-shaped phylogeny corresponding to no phylogenetic signal), generalized least squares (GLS) with Brownian motion, GLS with Ornstein-Uhlenbeck (OU) transformation, GLS with Accelerating-Decelerating (ACDC) transformation, GLS with Pagel's λ transformation, and GLS with Grafen's ρ transformation. Each of the GLS transformations makes different assumptions about evolutionary constraints and processes, and each should be considered to determine which evolutionary model fits the data best (Garland et al. 2005). Shift rates themselves are not an evolving trait so any model of evolutionary process may seem odd, but the traits influencing shift rates may have evolved via various processes thus it is appropriate to consider multiple evolutionary models in signal tests. Phylogenetic distance data were obtained from trees using PDAP module version 1.14 (Midford et al. 2003) within the Mesquite environment. The fit of each model to shift data was measured with Akaike's Information Criterion (AIC) scores, from which evidence ratios (based on Akaike weights) were calculated to produce results with intuitive interpretation (e.g., model A is three times more likely than model B given the data; Garamszegi et al. 2009).

Results

The final consensus tree produced by MrBayes was largely congruent with recent studies of family level relationships of Lepidoptera (Wahlberg et al. 2005, Regier et al. 2009, Mutanen et al. 2010). Consistent with these studies, and with a recent taxonomic classification (van Nieukerken et al. 2011), the traditional classification of the family Hesperiidae in its own superfamily Hesperioidea (Pogue 2009) is not supported. Hesperiidae segregated among most other butterfly families, while the most recent common ancestor of the Papilionidae is suggested to be the first split in our tree. Our placement of Hesperiidae cannot be considered independent of those by Wahlberg *et al*. (2005) since we used many of their study's gene sequences. Our tree differed from accepted phylogenetic relationships in two aspects. First, *Danaus plexippus* (Linnaeus) is generally considered a member of the tribe Danainae, which arose from a basal node within the family Nymphalidae (Wahlberg and Wheat 2008), but resolved in our tree as an evolutionarily distinct taxon more closely related to the lycaenids than the nymphalids. Second, *Speyeria mormonia* (Boisduval) did not cluster with congeneric species.

In each Canadian region, although not significantly so in the Eastern region, most species shifted north between 1900-1930 and 1960-1990 (Table 3-3). Canada-wide, twice as many species shifted their northern boundary north than south (68% of species; significantly more species shifted north than expected by chance, $P < 0.0001$). The median Canada-wide shift distance was 25.5 km northward (Figure 3-2), for a rate of 4.3 km north per decade, with considerable variation around this central tendency.

We found no evidence of a phylogenetic signal for Canada-wide boundary shifts. Likewise, tests for signal within each of Canada's three regions revealed that the model of no phylogenetic pattern to shifts was either the most likely model given the data or almost as likely as evolutionary models with weak rates of evolution (Table 3-2). Figure 3-3 illustrates the lack of phylogenetic pattern to northern boundary shifts.

Discussion

Northward shifts were somewhat common

Most species' northern range boundaries extended northward over time, consistent with expectations from climate change. Observed northward range shift estimates were lower than those from other studies, but the reasons for this difference are difficult to isolate. Previous multi-species studies have found northern range boundary northward shifts of 6.1 km/decade for a variety of terrestrial taxa and regions (Parmesan and Yohe 2003), 17.6 km/decade for British butterflies (Hickling et al. 2006), and 75 km/decade for Finnish butterflies (Pöyry et al. 2009). Hickling *et al*. (2006) used data from 1970-1999, and Pöyry *et al*. used data from 1992-2004, both of which were periods of more rapid and consistent warming than the time period used in our study (IPCC 2007), which may have contributed to the slower shift rates found in our study (Chen et al. 2011). Canada warmed by approximately 1.1 °C over the time period of our study with variation in warming trends between Canada's 11 climatic regions (Skinner and Gullett 1993). Our methods excluded recent collection locations that were not within 100 km of historical collection locations. This buffer zone exclusion was necessary to detect real boundary shifts as opposed to increased sampling coverage over time, but likely rendered estimates of shift rates conservative. Determination of whether differences in shift rates among studies arise from differences in time periods, regions, taxa, or methods requires additional studies of latitudinal range shifts.

Our finding of northward range shifts for Canadian butterflies builds upon previous research that documented shifts in potential habitat for these species (Kharouba et al. 2009). Canadian butterfly species' abiotic niches (climate and habitat requirements) have generally remained similar between the time periods considered in our study (Kharouba et al. 2009), and potential habitat for some species has expanded as Canada has warmed (White and Kerr 2006). The approach used in the present study does not use any information regarding species' niches, only their observation locations. Both lines of evidence are complementary, suggesting that species' potential habitat is shifting according to climatic changes (White and Kerr 2006, Kharouba et al. 2009), and species are expanding their northern range boundaries farther north into such newly-suitable habitat (our study).

Lack of phylogenetic signal

Below we discuss five potential reasons why we did not detect a phylogenetic signal to northern boundary shift distances: 1) the spatial extent of our study may have been too large and heterogeneous for consistent patterns to emerge; 2) Canada may not have warmed enough over the time period of our study to allow species with certain traits to consistently shift farther north than other species; 3) our phylogenetic tree may not be accurate; 4) phylogenetic signal of multiple influential traits may have cancelled each other out; and 5) the factors responsible for shift rates may not have a phylogenetic signal themselves.

First, the spatial extent of our study may have been too large and heterogeneous for a clear phylogenetic pattern to emerge. By splitting Canada into three regions we reduced the spatial heterogeneity in our data set, but each region was still very large and heterogeneous. Many factors are known to influence butterfly survival and dispersal and to vary among landscapes including urbanization, biotic interactions, and degree of warming. This heterogeneity may influence butterfly species' site occupancy so much that shift rates between populations within a species differ as much as shift rates between species. This would require that studies of shift rate variation between species be conducted at local scales. For example, Helmus *et al*. (2010) found a phylogenetic signal for zooplankton species' resilience to lake disturbance within lakes but no consistent pattern to which species were resilient between lakes, suggesting considerable site-specific effects. We echo the sentiments of Melles *et al*. (2011) that more research is required to understand the mechanisms determining range shift rates for various species at multiple spatial scales.

Second, the magnitude of warming that took place between the time periods in our study may not have been sufficient to give an advantage to species with traits associated with range shift capacity. While Canada warmed between the two time periods considered in our study (Skinner and Gullett 1993), it has warmed much more rapidly over the past 20 years (Whitewood and Phillips 2010). Perhaps northward shifts during a period of slight warming were driven less by species' intrinsic capacity for range expansion and more by stochasticity, but with the challenge of more rapid warming those species with certain traits would have consistently shifted farther than species without those traits. If this reasoning is correct, and if the traits underlying shift responses to climate change carry a phylogenetic signal, then more recent rapid warming would reveal a phylogenetic signal in range shift rates.

Third, a failure to detect true phylogenetic signal in range shifts may arise from an inaccurate phylogenetic tree. This explanation seems unlikely for three reasons. First, our phylogenetic tree is largely congruent with previously published butterfly trees (Wahlberg et al. 2005, Regier et al. 2009, Mutanen et al. 2010). This suggests that, while errors in branch lengths and branching patterns are possible, they are likely to be minor compared to the major restructuring of the tree that would be required to produce a phylogenetic pattern to range expansion (Figure 3-3). Second, tests of phylogenetic signal are generally robust to errors in branch lengths (Münkemüller et al. 2012), suggesting that our test for phylogenetic signal should be robust to the types of errors that may exist in our tree. Third, because our tree includes a large number of species, tests of phylogenetic signal should not be affected by small tree size, to which tests of phylogenetic signal are very sensitive (Münkemüller et al. 2012).

Fourth, range shift rates may be unrelated to phylogeny if the signal patterns of multiple traits cancel each other out. For example, if both mobility and larval host plant breadth facilitate range shifts and carry a phylogenetic signal, but the taxa that are highly mobile are not the same as those that are host plant generalists, then phylogenetic signal in range shifts would be less evident. Given the large number of traits that may influence rates of range shift, their conflicting phylogenetic patterns may muddy the signal in range shifts considerably.

Fifth, range shift rates may be the result of traits that are not phylogenetically conserved. Although in the minority, many traits do not carry a phylogenetic signal (Losos 2008), and these traits may influence range shifts. An example of a mechanism by which range shifts could be unrelated to phylogeny is if range shifts are largely the product of long-distance dispersal events and long-distance dispersal does not itself carry a phylogenetic signal. It has been suggested that long-distance dispersal may influence species' range expansion more than the central tendency of dispersal distance (Lester et al. 2007). Whether long-distance dispersal rates are related to species' local dispersal rates is an open question (Ronce 2007), but it is possible that long-distance dispersal is responsible for the majority of range expansions and is largely a stochastic process unrelated to phylogenetically conserved traits. Some traits are related to range shift rates with modern climate change, but their influence is generally weak and differs among the few studies yet conducted on this topic, leaving open the question of what traits influence range shift rates and under what circumstances (Angert et al. 2011).

Understanding species' responses to climate change in the recent past is essential to predicting future responses. The integration of phylogenetic and macroecological approaches provides a potentially powerful approach to investigating large-scale responses to climate change (Cavender-Bares et al. 2009, Wiens et al. 2010). According to our results, phylogenetic relatedness cannot be used to predict species' latitudinal range shifts with modern climate change. Future research should explore whether the lack of phylogenetic pattern to range shifts depends on the spatial, temporal, and taxonomic scale considered, or whether the lack of pattern we detected is more general.

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Author contributions

J.M.F. came up with the project idea, organized the project, conducted statistical analyses, and wrote the manuscript. N.D.S., E.R.Y., K.E.G., and J.L.N. calculated species' northern boundary range shift rates. J.G. compiled DNA sequences and worked with J.M.F. to construct the DNA-based tree. A.C.A., H.M.K., A.L.M., and J.T.K. participated in discussions and provided feedback on the manuscript.

Tables

Table 3-1. GenBank accession numbers for sequences used to construct the DNA-based tree. Includes ‘substitute’ species used in tree construction but not included in shift analysis.

Species	Name in GenBank (if different)	*COX1*	*EF-1α*	*WNG*
Amblyscirtes exoteria		EU364479	EU364274	EU364072
Ancyloxypha numitor		EU364463	EU364258	
Anthocharis belia		AY954574	AY870560	AY954604
Archiearis parthenias		DQ018928	DQ018899	DQ018869
Boloria bellona				AF246530
Boloria epithore		AF170862	AF173402	
Boloria eunomia		DQ922869	DQ922901	DQ922837
Boloria selene		AY090201	AY090167	AY090134
Carterocephalus palaemon		EU364388	EU364183	EU363990
Celastrina neglecta		EU141355	EU136662	EU141236
Cercyonis pegala		AY218239	AY218259	AY218277
Chlosyne gorgone		AF187772	AY788728	AY788489
Chlosyne harrisii		AF187773	AY788729	AY788490
Chlosyne nycteis		AF187788	AY788732	AY788493
Chlosyne palla		AF187791	AY788733	AY788494
Coenonympha tullia		AF170860	AF173399	DQ351126
Colias alexandra		AF044872		
Colias eurytheme		AF044024	AF173400	AY569040
Colias hecla	*Colias hecla sulitelma*	AF073940		
Colias nastes	*Colias nastes werdandi*	AF073941		
Colias palaeno	*Colias palaeno aias*	AB107925		
Colias philodice		AY954570	AY870565	AY954600
Danaus plexippus		DQ018954	DQ018921	DQ018891
Erebia palarica		AY090212	AY090178	AY090145
Erebia vidleri		AB324843		
Erynnis afranius		EU364378	EU364173	EU363980
Erynnis horatius		EU364377	EU364172	EU363979
Euchloe ausonides		AF044881	AY870558	
Euphydryas chalcedona		AF187752	AY788744	AY788505
Euphydryas editha		AF187765	AY788745	AY788506
Euphydryas phaeton		AF187797	AY788747	AY788508
Euphyes vestris		EU364477	EU364272	
Euptoieta claudia		DQ922864	DQ922896	DQ922832
Glaucopsyche lygdamus		AY675411	AY675364	
Hesperia comma				AY700706
Hesperia leonardus		EU364470	EU364265	EU364065
Lethe eurydice	*Satyrodes eurydice*	DQ338772	DQ338914	DQ338621
Lethe portlandia	*Enodia portlandia*	AY508536	AY509062	
Limenitis archippus		DQ205128	EF643262	EU433935

Limenitis arthemis	*Limenitis arthemis astyanax*	DQ205113	EF643315	EU433941
Limenitis lorquini		DQ205132	EF643335	EU433944
Lycaena helloides		AY954562	AY954622	DQ018886
Megisto cymela		AY508558	AY509084	AF246547
Neominois ridingsii		DQ338870	DQ339026	DQ338735
Neophasia menapia		DQ082765	AY870536	DQ082814
Notamblyscirtes simius	*Amblyscirtes simius*	EU364480	EU364275	EU364073
Nymphalis antiopa		AY218246	AY218266	AY218284
Oarisma garita		EU364464	EU364259	EU364059
Ochlodes sylvanoides		DQ018931	DQ018902	DQ018872
Oeneis jutta		DQ018958	DQ018925	DQ018896
Papilio canadensis		AF044014	AF044816	AY569125
Papilio glaucus		EU141368	EU136675	EU141249
Papilio machaon		EF473834	EF485106	AY569124
Papilio polyxenes		AF044010	AF044823	
Papilio rutulus		AY954560	AY954620	
Papilio zelicaon		AF044008	AF044827	
Parnassius clodius		EF473795	EF485078	DQ351134
Parnassius smintheus		EF473777	EF485052	AY569045
Phyciodes batesii		AF187747	EF494005	EF493898
Phyciodes cocyta		AY156606	EF494008	EF493901
Phyciodes pallida		AF187792	AY788794	AY788554
Phyciodes tharos		EF493955	EF494010	EF493903
Pieris rapae		AY954581	AY870550	AY954611
Plebejus argus		AY350459	AY496828	
Plebejus idas	*Lycaeides idas anna*	AY496806		
Plebejus melissa	*Lycaeides melissa*	DQ234691		
Plebejus pheretiades	*Agriades pheretiades*	AY496709	AY496849	
Plebejus pyrenaicus	*Agriades pyrenaicus*	AY557030		
Poanes taxiles		EU364474	EU364269	EU364069
Polites themistocles		EU364471	EU364266	EU364066
Polygonia comma		AY248794	AY248819	AF412781
Polygonia faunus		AY248798	AY248823	AY248837
Polygonia gracilis		AY248797	AY248822	AY248836
Polygonia interrogationis		AY248793	AY248818	AY248834
Polygonia progne		AY248795	AY248820	AF412765
Polygonia satyrus		AY248796	AY248821	AY248835
Pontia callidice		EF584870	AY870548	
Pontia occidentalis		DQ148933		
Pontia protodice		AF044886		
Pontia sisymbrii		AF044890		
Pyrgus communis		EU364384	EU364179	EU363986
Pyrgus ruralis		EU364382	EU364177	EU363984
Satyrium esculi		AY556950		
Satyrium hyrcanicum		AY557057		
Speyeria idalia		AF295040		
Speyeria mormonia		AF044891		
Thorybes pylades		EU364331	EU364126	EU363933
Urania leilia		DQ018927	DQ018898	DQ018868
Vanessa atalanta		AY090221	AY090187	AF246542

Vanessa cardui	AY248782	AY248807	AF412770
Vanessa virginiensis	AY248783	AY248808	AY248827

Table 3-2. Results of tests for phylogenetic signal in species' northern boundary shifts using both the DNA-based and the all-species tree (see Methods for the distinction between the two trees). Models are sorted from most- to least-likely given the shift data with the DNA-based tree. OLS represents the "no phylogenetic signal" model, and GLS models represent phylogenetic models with various evolutionary processes (see Methods).

		DNA-based tree			All-species tree		
Region	Model	Transform estimate[a]	AIC	ER[b]	Transform estimate	AIC	ER
West	OLS	-	219.7	1	-	431.1	1
	GLS (OU)	<0.01	221.6	2.6	<0.01	433.1	2.7
	GLS (λ)	<0.01	222.9	5.0	<0.01	434.6	5.8
	GLS (ACDC)	0.48	311.6	$> 10^3$	1.1	569.7	$> 10^3$
	GLS (Brownian)	-	316.7	$> 10^3$	-	567.7	$> 10^3$
	GLS (ρ)	1.4	318.5	$> 10^3$	1.9	564.3	$> 10^3$
Central	OLS	-	221.2	1	-	522.4	1.1
	GLS (OU)	<0.01	223.3	2.7	<0.01	524.4	2.9
	GLS (λ)	0.25	224.5	5.0	0.13	522.3	1
	GLS (ACDC)	0.65	335.6	$> 10^3$	0.95	657.7	$> 10^3$
	GLS (Brownian)	-	337.2	$> 10^3$	-	656.3	$> 10^3$
	GLS (ρ)	1.2	339.0	$> 10^3$	1.3	657.6	$> 10^3$
East	GLS (λ)	0.31	134.6	1	<0.01	238.1	1
	OLS	-	135.2	1.4	-	238.4	1.2
	GLS (OU)	0.72	136.7	3.0	<0.01	238.6	1.3
	GLS (ACDC)	0.58	162.5	$> 10^3$	0.68	283.6	$> 10^3$
	GLS (ρ)	0.40	165.7	$> 10^3$	0.42	286.8	$> 10^3$
	GLS (Brownian)	-	175.8	$> 10^3$	-	298.0	$> 10^3$
Canada-wide	OLS	-	235.9	1	-	527.8	1
	GLS (OU)	<0.01	237.8	2.5	<0.01	528.6	1.5
	GLS (λ)	<0.01	238.3	3.2	<0.01	529.3	2.1
	GLS (ACDC)	0.59	346.2	$> 10^3$	0.95	669.9	$> 10^3$
	GLS (Brownian)	-	349.3	$> 10^3$	-	668.4	$> 10^3$
	GLS (ρ)	1.0	351.3	$> 10^3$	-[c]		

[a] Transformation values can generally be considered the effect size of the evolutionary rate transformation, with zero representing no influence of evolutionary history and one the amount of niche conservativism expected from Brownian motion (Lavin et al. 2008).

[b] The evidence ratio (ER) can be interpreted as how much more likely the best-ranked model is than a given model (Garamszegi et al. 2009).

[c] GLS (ρ) could not be calculated for Canada-wide shifts with the all-species tree.

Table 3-3. Median distance (in km) of northward shift among species' northern range boundaries between 1900-1930 and 1960-1990 in the three regions of Canada and the weighted Canada-wide average shift distances. Numbers (and percentages) of species shifting their northern boundary north and south are also presented, with the number shifting north appearing bold when its binomial probability is significantly greater than 50%. Species were often present in more than one region.

Measure	West	Central	East	Canada-wide
Median shift (km)	73	13	8	25
Species shifting north	**68 (75%)**	**71 (60%)**	42 (58%)	**93 (68%)**
Species shifting south	23 (25%)	48 (40%)	31 (42%)	44 (32%)
Total species	91	119	73	137

Figures

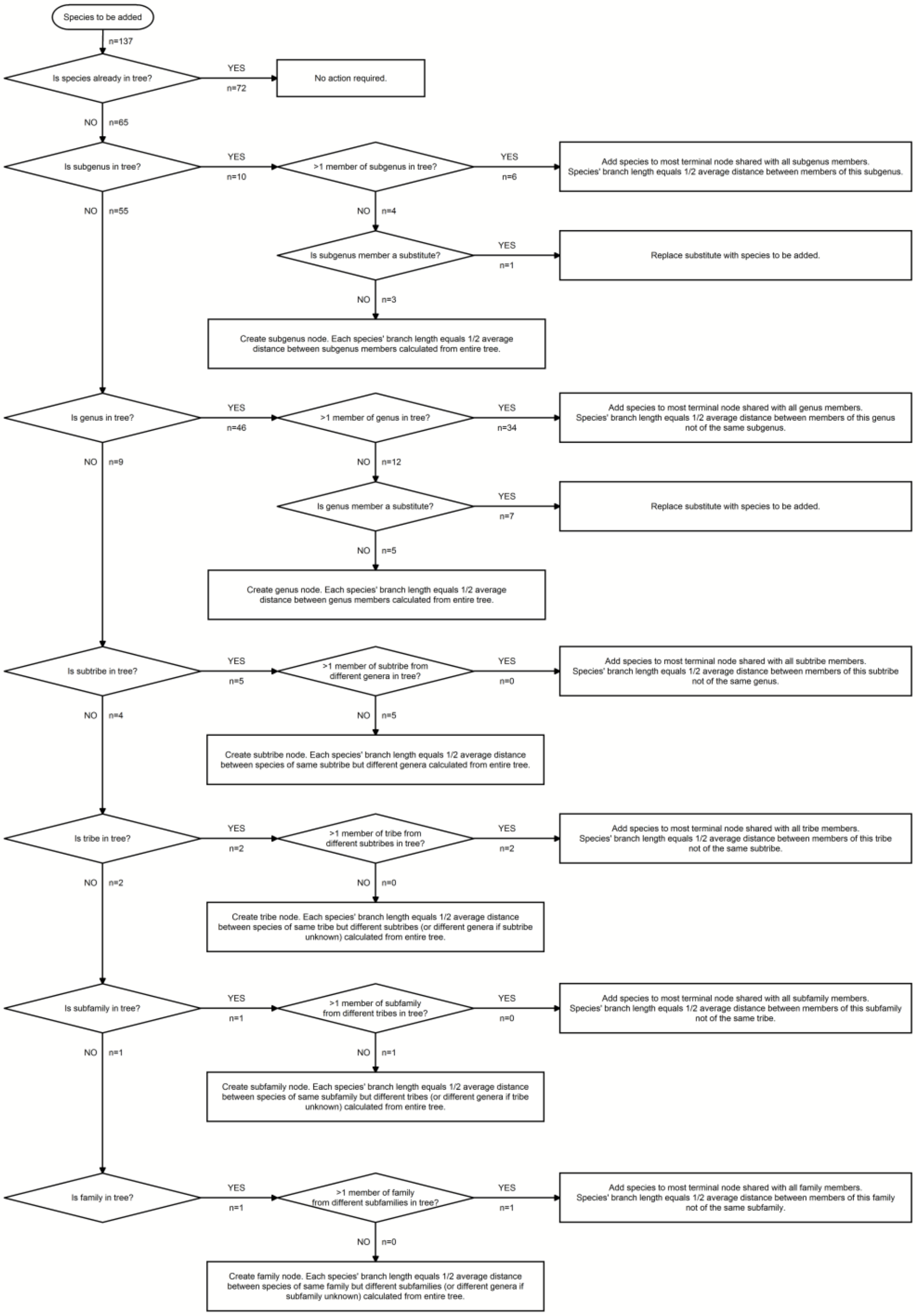

Figure 3-1. Decision tree used to construct the all-species phylogenetic tree. The only exception we made to this tree was ignoring the presence of *Speyeria mormonia* and proceeding as though *S. idalia* was the only species of its genus in the tree when adding congeneric species, because *S. mormonia* resolved at an improbable position in our tree.

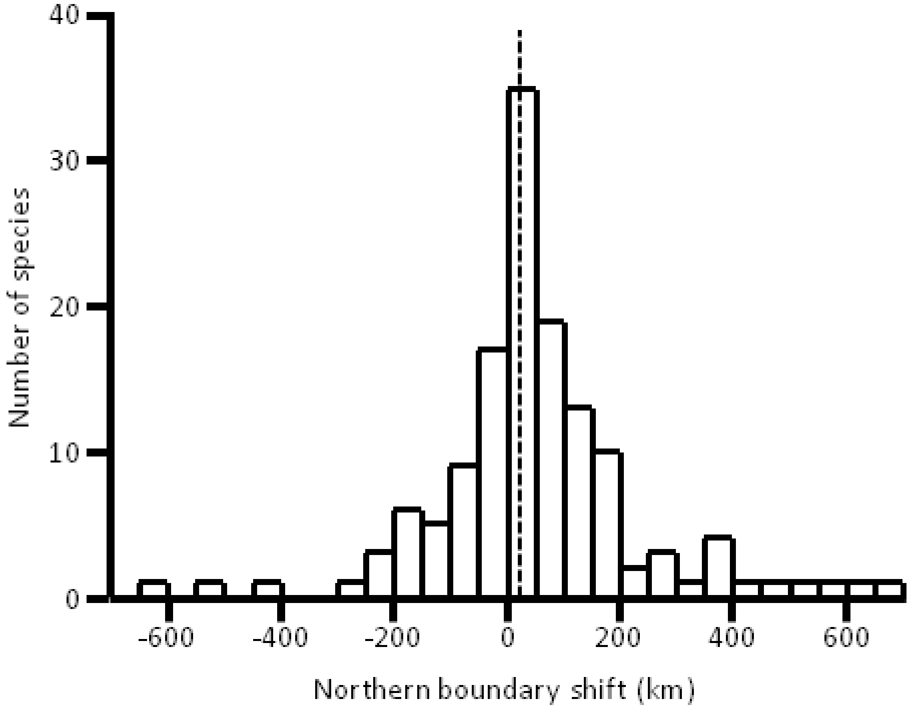

Figure 3-2. Histogram of species' Canada-wide northward range shifts between time periods (1900-1930 to 1960-1990). The dashed line represents the median shift distance of 25.5 km.

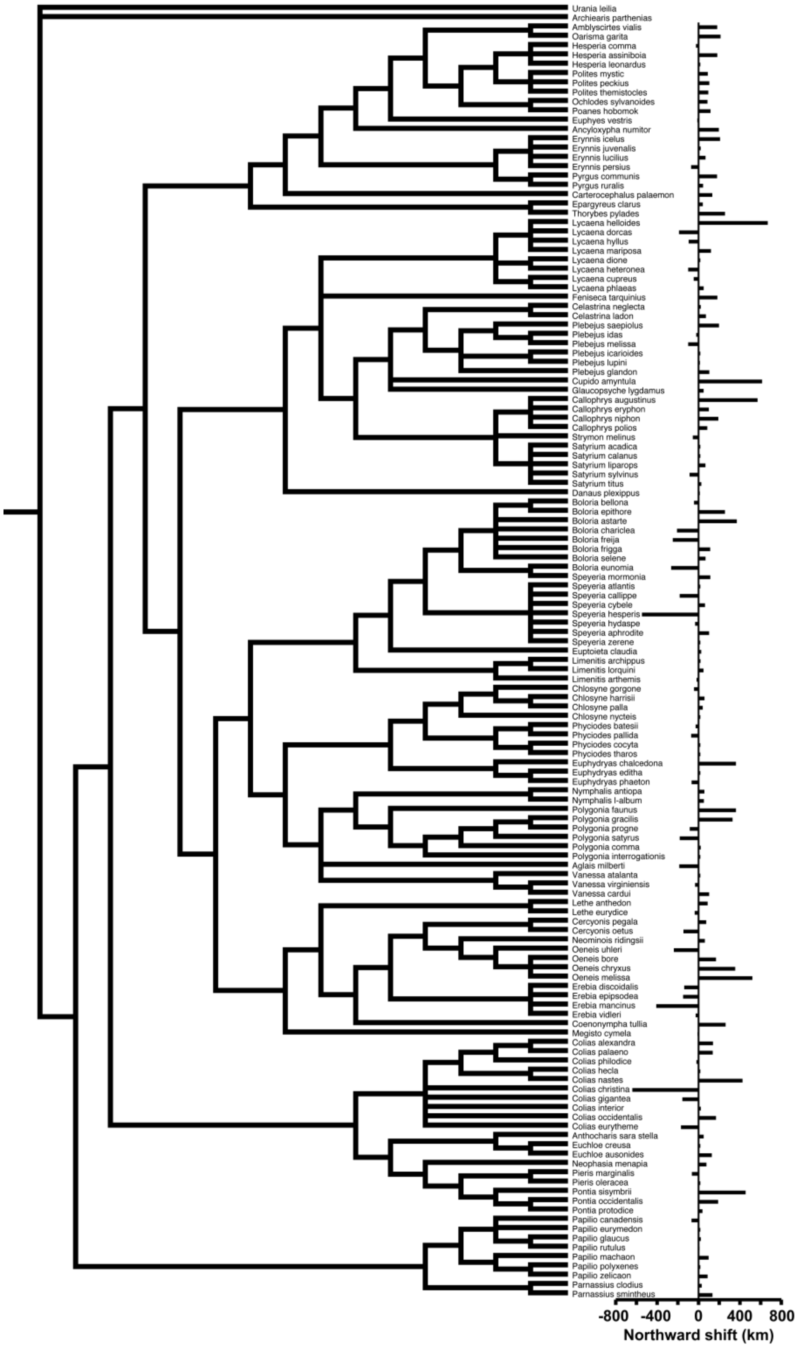

Figure 3-3. All-species phylogenetic tree with Canada-wide northward range shift distances for each species (km). Positive shift values (bars extending to the right) indicate northward shifts of the northern range boundary, negative values (bars extending to the left) indicate southward shifts. Branch lengths have been ultrametricized so tree tips are flush for illustrative purposes. Moth outgroup species at the top were not included in range shift analyses.

CHAPTER 4. NO EVIDENCE OF A RELATIONSHIP BETWEEN BUTTERFLY SPECIES’ TRAITS AND NORTHWARD RANGE SHIFT RATES IN CANADA OVER THE 1900s

Citation of unpublished manuscript:
Fitzsimmons, J. M. No evidence of a relationship between butterfly species’ traits and northward range shift rates in Canada over the 1900s.

Abstract

As the world warms, species are frequently shifting ranges poleward. Around this general poleward shift trend there is considerable variation among species’ rates of range shift. Logical predictions and some evidence suggest rates of poleward range shift could be related to species’ traits (e.g., mobile habitat generalists should shift more rapidly than less-mobile habitat specialists). The relationship between traits and poleward range shifts has been little-studied, especially outside Europe. I investigated whether butterfly species’ northern boundary shifts over the 1900s were related to traits. I analyzed northern boundary latitudinal shifts for 137 butterfly species across Canada between the periods 1900-1930 and 1960-1990. I related shift distances to 20 traits (anatomical, life-history, behavioural, and ecological) in a phylogenetic context. I found no compelling relationship between species’ traits and northern boundary shift rates. This lack of relationship is likely the result of a low signal-to-noise ratio. Range shifts were evident but less frequent and distant than findings from time periods with greater warming (i.e., low signal). The broad-scale approach to studying shifts across a country as large as Canada brings with it inherent noise arising from the nature of the data. While species’ traits may influence rates of range shift with climate change, their effects likely vary among taxa, regions, and timescales. There is no evidence that traits were related to range shifts at the scale of my study.

Introduction

Many species are shifting their ranges poleward and upward in elevation, consistent with warming trends over recent decades (Parmesan and Yohe 2003, Thomas 2010, Chen et al. 2011). Within this general pattern of range shifts is a great deal of variation in rate, and even direction, of range shifts among time periods, regions, and species (Lenoir et al. 2010, Chen et al. 2011). Unless we can account for this substantial variation, predictions of species' responses to climate change will remain imprecise rules of thumb largely unsuitable to conservation management.

It is logical to expect species with certain traits to be more likely to shift ranges than species without those traits. For example, mobile dietary generalists should be more likely to successfully expand range margins than sedentary dietary specialists because the former are more likely to: 1) disperse into new areas and 2) find suitable food in new areas (Peters and Darling 1985). While there is some support for species' traits correlating with poleward range shifts (e.g., Pöyry et al. 2009), the strength of traits' relationships with range shift rates is variable across studies and overall not very strong (Angert et al. 2011). What traits affect poleward range shift rates, and under what circumstances, is still largely an open question.

Given the recency of research on range shifts with modern climate change, and our lack of knowledge about what affects shift rates, it is appropriate to pursue an exploratory approach to discover trends. Exploratory methods are data-driven rather than hypothesis-driven, and thus permit analyses of variables and relationships that would not be allowed under a strictly hypothesis-driven framework (Kelling et al. 2009). Luck et al. (2012) recommend that trait researchers should follow a top-down framework, only including traits with sufficient justification in their analyses. I have instead chosen a bottom-up approach, searching for relationships with range shift rates among as many biologically-reasonable traits as data permitted. A combination of approaches is likely required for progress in ecological understanding (Aarssen 1997), such as exploratory results informing subsequent hypothesis testing (Gorelick 2011).

I investigated the relationship between species' traits and northern range boundary latitudinal shifts for 137 Canadian butterfly species. Northern boundary shifts were calculated between 1900-1930 and 1960-1990, a period of slight warming during which most of these species shifted their northern boundaries northward (Chapter 3). Given closely-related species' similarity for most traits (Freckleton et al. 2002, Blomberg et al. 2003), I previously tested whether closely-related species had similar rates of northern boundary shifts (i.e., phylogenetic signal for northern boundary shifts; Chapter 3). I found no such phylogenetic signal. This could be the result of northern boundary shift rates not being related to species' traits, or shift rates being related to traits that do not display a phylogenetic signal themselves. Thus herein I test directly the relationships between species' traits and northern boundary shift rates while accounting for phylogenetic autocorrelation. I use 20 traits including behavioural (e.g., mate search strategy), life history (e.g., overwintering stage), anatomical (e.g., wingspan), and ecological traits (e.g., habitat breadth), thus providing the most comprehensive analysis of the traits associated with species' range boundary shifts in North America.

Methods

Species used, occurrence data

I used collection data from the Butterflies of Canada database (Layberry et al. 1998), which includes about 300,000 georeferenced (>80% of records located to within 1 km) collection records for 297 species dating from the late 19th century to present. Each collection record is accompanied by a preserved specimen stored at one of 40 Canadian natural history collections, identified by a lepidopterist. I updated nomenclature when necessary following Pelham (2008).

Northern boundary shift estimates

Estimates of species' northern boundary latitudinal shifts were obtained from Chapter 3, and described in detail therein. Briefly, each species' northern range boundary was estimated in each of two time periods: 1900 to 1930 (historical period) and 1960 to 1990 (recent period). Boundaries were calculated as the average latitude of the three northern-most occurrence records for a species in a time period. A species' northern boundary range shift is simply its recent northern boundary minus its historical northern boundary.

Several sources of potential bias in estimates of northern boundary shifts were accounted for. Introduced species were excluded because their historical ranges were not likely at equilibrium. Differences in climate among Canadian regions were accounted for by splitting analyses into three geographic regions: the Western Region (British Columbia and Yukon Territory); the Central Region (Alberta, Saskatchewan, Manitoba, and Northwest and Nunavut Territories); and the Eastern Region (Ontario, Québec, and the provinces of Atlantic Canada). Species' northern range boundary shifts were calculated within each region, and a weighted average shift estimate in which regions' influence on the average was weighted by their sample size for a species. Sampling area bias was accounted for by excluding sampling locations that were only sampled in one time period. Sampling intensity bias (generally more occurrence records in the recent than historical period) was accounted for by using the same number of occurrence records in both time periods for a species in a region. Northern boundary shift estimates for 137 species were thus obtained.

Species' traits

I obtained species' data on a multitude of traits from five authoritative sources: two atlases (Bird et al. 1995, Layberry et al. 1998), two field guides (Brock and Kaufman 2003, Wagner 2005), and one online resource (Opler et al. 2011). While some traits' data were easily-transcribed (e.g., wingspan in mm), others required a conversion from verbal descriptions to numerical values (e.g., caterpillar host plant breadth). The rules I followed for such classifications, and further description of the traits, are available in Appendix A.

I adopted an exploratory approach to trait selection, including all biologically-reasonable traits meeting certain criteria. Some traits were excluded due to inadequate sample sizes or collinearity with other traits. I excluded mudpuddling and ant association traits because neither had data for more than 40 species in any source; all remaining traits had records for at least 99 of the 137 species in at least one of the sources. Minimum forewing span (in mm) was excluded because it was highly correlated with maximum forewing span. Twenty traits met these criteria and were used in analyses.

Because trait data were sometimes inconsistent between sources (Fitzsimmons, unpublished results), I analyzed relationships between range boundary shifts and traits using two sets of sources. If results are consistent between analyses using different sets of sources then we can be more confident in their reliability. For each trait the primary source was the one with the greatest species coverage, and the secondary source was that with the second-greatest coverage. I preferentially designated Layberry et al. (1998) the primary source when its species coverage was only slightly less than another source (fewer than 10 species difference), because this source is most relevant to Canadian butterflies (which is beneficial for traits that may vary within a species across geographic areas). Traits, sources, and sample sizes are listed in Table 4-1.

Phylogenetic autocorrelation

I accounted for phylogenetic relationships in analyses because closely-related species are not independent data. It is especially important to account for phylogenetic autocorrelation when the effect being analyzed has a strong phylogenetic signal (Chamberlain et al. 2012). It is still important to account for phylogenetic autocorrelation when there is no phylogenetic signal in the response variable, as is the case for butterfly boundary shifts (Chapter 3), because phylogenetic autocorrelation pertains to phylogenetic patterns in model residuals, not in variables themselves (Revell 2010).

I used the phylogenetic tree described in Chapter 3, which was based on DNA sequences and taxonomic affiliations. I modified this tree using R 2.15.0 (R Development Core Team 2012) to satisfy common assumptions of phylogenetic analyses. Using the *Ape* package ver 3.0-3 (Paradis et al. 2004) I made the tree ultrametric (i.e., all tips flush) with the chronopl function. I also removed polytomies with *Ape*'s multi2di function, which adds branches of zero length to polytomies (tree nodes with more than two branches) to produce a dichotomous branching pattern. Many phylogenetic analyses require trees to be ultrametric and dichotomous to satisfy assumptions, but phylogenetic generalized least squares (pGLS), which is the type of analysis I used, is quite robust to these challenges (Stone 2011).

All pGLS analyses were performed in R with the *caper* package version 0.5 (Orme et al. 2012). This version of *caper* has a known "bug," to be fixed in an upcoming version, whereby it includes models' y intercepts in calculations of F statistics' numerator degrees of freedom, thus inflating the numerator degrees of freedom by one (David Orme, pers. comm. Jan 28 2013). This often results in models having lower P values than they should, leading to inflated numbers of significant models. I accounted for this bug by re-calculating P-values for models based on a numerator degrees of freedom reduced by one, using VassarStats (vassarstats.net accessed January 28 2012). Because *caper* calculates predictor variable coefficients using correct degrees of freedom, re-calculated P-values of models with a single predictor term were identical to the single term's coefficient P-value.

Single-predictor analyses

To evaluate what traits were related to species' northern boundary shifts I used a combination of exploratory single-predictor analyses and null hypothesis testing for optimal multiple-predictor models.

Each predictor variable in the primary source set (Table 4-1) was analyzed in a pGLS model (i.e., accounting for phylogenetic autocorrelation) with northern boundary shift as the response. Separate models were analyzed for each of the 20 predictor variables and each of the four shift responses (West, Central, East, and Weighted Average) for a total of 80 models. Because these were exploratory analyses I did not account for multiple testing, but readers should interpret P values with caution since 5% (i.e., four) of these 80 models would be expected to be significant by random chance under null conditions. I thus compared the number of significant models observed against the number of models expected to be significant by chance with a two-tailed exact binomial probability test in VassarStats (vassarstats.net accessed January 29 2012).

Multiple-predictor analyses

I performed stepwise pGLS to find the optimal set of traits explaining variance in each of the four northern boundary shift response variables. Stepwise methods have been the subject of some criticism (Whittingham et al. 2006). These criticisms do not justify abandoning stepwise methods; instead stepwise analyses should follow certain protocols to avoid known vulnerabilities. One criticism of stepwise procedures is that they inflate Type

I error by effectively running many tests under the banner of a single stepwise test (Mundry and Nunn 2009). To avoid Type I error inflation, researchers should first test the full model (i.e., with all non-collinear predictors); only if that model is significant should researchers proceed to stepwise selection of the optimal set of predictors (Blanchet et al. 2008). A second criticism of stepwise procedures is that it can include "nuisance" predictor variables that are significant but barely influential (Whittingham et al. 2006). This criticism is not unique to stepwise procedures – in fact stepwise procedures tend to include fewer "nuisance" variables than Akaike's Information Criterion (AIC) methods (Raffalovich et al. 2008, Murtaugh 2009) – but removing non-influential predictor variables is nonetheless desirable. Forward model selection should thus be based not only on predictor variables' P values being less than 0.05, but also require the whole-model R^2_{adj} to increase as a result of the predictor variable's addition (Blanchet et al. 2008). Thus for each of my four pGLS models (West, Central, East, and Weighted Average) I first tested the full model with all non-collinear predictors. Only if that model was significant (which none were; see Results) would I have proceeded with forward model selection starting with the predictor trait with the lowest P value, adding trait variables according to the combined P and R^2_{adj} criteria outlined above.

Given the high level of noise in the data set, I tested an additional model with a subset of high-quality data (hereafter the southeastern subset). Southern Ontario, along with southern parts of Québec and the Maritime provinces, has been inhabited and sampled by people at higher densities than elsewhere in Canada, reflecting human population's westward migration tendency across Canada over the 1900s. I thus restricted a subset analysis to species whose most northern collection location in the East region was south of 48°N in both time periods, and which had at least ten geographically-unique collections in each time period in this region. Eighteen species met these criteria.

Prior to testing each full model I removed predictor variables that were multicollinear with each other, to minimize error surrounding parameters' coefficient estimates. Because the sample size of the dummy level for categorical variables can bias tests of autocorrelation (Wissmann et al. 2007), I re-levelled categorical predictor variables using the relevel function in R when necessary to ensure all dummy levels were categories with at least 20% of species. This did not alter values for species, but rather changed which category was classified as the null/dummy in analyses to ensure only common categories were dummies. I then evaluated Generalized Variance Inflation Factors (GVIF) for all predictors in each model using the *car* package ver 2.0-12 in R (Fox and Weisberg 2011), based on the GVIF formula constructed by Fox and Monette (1992). Multicollinearity cannot yet be evaluated within a phylogenetic context, so GVIF was calculated in ordinary least squares models. I sequentially eliminated predictor variables with $\mathrm{GVIF}^{1/2\mathrm{df}} \geq 3$, indicating the standard error of the predictor was ≥ 3 times greater than it would have been without multicollinearity. The resulting pared set of predictor variables was fed into pGLS models for full-model significance testing. Thus the overall sequence of multiple-trait model testing was: 1) Eliminate multicollinear predictor variables; 2) Test significance of (non-collinear) full models in a phylogenetic context; and 3) If the full model was significant then I would have proceeded with forward stepwise regression adding predictors following P- and R^2_{adj}-criteria.

Results

Exploratory single-predictor analyses

Exploratory pGLS analyses of each trait with each of the four boundary shift responses (West, Central, East, and weighted average) revealed few compelling relationships (Table 4-2). Only two of the 80 possible relationships had $R^2_{\mathrm{adj}} \geq 0.10$. Only six of the 80 possible relationships was significant at $P < 0.05$, which is not significantly different from the proportion of models expected to be significant by chance (6 observed significant models *vs*. 4 expected by chance; exact binomial probability test $P = 0.422$).

Multiple predictor analyses

Of the five boundary shift full models (West, Central, East, weighted average, and southeastern subset), only the weighted average and southeastern subset models were significant. I thus proceeded to forward stepwise regression only with the weighted average and southeastern subset boundary shift responses. Forward stepwise regression could not commence, however, because no predictor's coefficient was significant for either model. Therefore, none of the multiple trait models produced significant results.

Discussion

I found no compelling relationships between species' traits and northern boundary shift rates. This lack of relationship was the general pattern regardless of which Canadian region, or the intensively sampled southeastern subset, was the focus of analysis. The few relationships that were found between boundary shift rates and individual traits were weak, and inconsistent in magnitude and even direction among regions of Canada (Table 4-2).

Why were species' traits unrelated to shift rates?

There are several potential reasons why I did not detect a compelling relationship between species' traits and northern boundary shift rates, which I have grouped into four categories below.

First, the time period of my study (1900-1930 to 1960-1990) may not have experienced sufficient warming for traits to reliably facilitate boundary shifts. Canada warmed by approximately 1.1 °C over this time period, with warming being heterogeneous across regions and decades (Skinner and Gullett 1993). My range shift estimates were based on less warming because they were generally based on 60 years (median years of both time periods) rather than the full 90 years, and generally located south of the rapidly-warming Arctic and sub-Arctic regions. A meta-analysis found that the rates of species' poleward range shifts are positively correlated with the degree of warming during a study's time period (Chen et al. 2011), consistent with the relatively small average northward range shifts among the species in this data set (Chapter 3). Canada has warmed much more rapidly since 1990 and is expected to continue warming rapidly in the future (Christensen et al. 2007), so species' traits may be more likely to relate to range shift rates in the recent past and future if they are indeed influential.

Second, the traits used in my analyses may not be the appropriate ones to use. An example of a potentially influential trait I did not consider is relative brain size, which is related to invasion success among bird species likely by conferring an ability to use novel habitats and resources (Sol et al. 2012). Any trait-based study is vulnerable to the criticism that they ignored potentially important traits. Having used more traits than any previous study of trait influences on range shift rates, I minimized the risk of ignoring influential traits. In my opinion a more likely vulnerability in my (and others') trait-based approach is the magnitude of intraspecific variation in species' traits. Assigning single trait values to species assumes intraspecific variation is minimal compared to between-species variation, but this assumption is not always valid. Populations within butterfly species can vary in traits including host plant preference (Pelini et al. 2010, Downey and Nice 2011), wing morphology (Cassel-Lundhagen et al. 2009), and dispersal (Stevens et al. 2010). Theory predicts populations at the expanding frontier of species' ranges to experience selection in favour of increased dispersal (Burton et al. 2010), consistent with evidence from species rapidly expanding their ranges such as Australia's invasive cane toad (Llewelyn et al. 2010). If selection can result in intraspecific variation that rivals between-species variation in traits facilitating range expansion then species-specific trait values would lose precision. This could result in less-precise or even biased trait coefficients (Hansen and Bartoszek 2012), although in practice the effect of intraspecific variation on comparative analyses tends to be small (Garamszegi and Møller 2010). I have accounted for intraspecific variation to some degree by using alternative data sources for species' traits and finding broadly consistent results, but this approach underestimates true intraspecific variation. Thus while I may have missed influential traits and underestimated intraspecific variation in species' traits, I have attempted to minimize these vulnerabilities by employing an exhaustive list of traits and multiple sources for each when available.

Third, the spatial scale of my study may be so broad that local phenomena produce sufficient noise to overwhelm broad-scale trends. Range expansions are inherently local events, occurring through the actions of individual butterflies colonizing and reproducing in new habitats. Local abiotic (e.g., extreme weather events, Diez et al. 2012) and biotic (e.g., host preference of local parasitoids, Menéndez et al. 2008) factors can facilitate or impede expansion into new areas. One example relevant to the region and time period of my study is the regional heterogeneity in treeline expansion rates in northern Canada over the 1900s; treeline dynamics varied among sites experiencing similar climate change, perhaps as a result of local humidity and biotic interaction factors (Mamet and Kershaw 2012). Local-scale influences on range expansion rates are undoubtedly important, and can be accounted for with two opposite approaches: local studies and macroecological studies. Confining studies to local regions will produce more precise results because local features and selective pressures can be accounted for, but this approach is research-intensive and has unknown applicability to areas beyond the location of study. A macroecological approach gains general applicability of findings (Kerr et al. 2007) at the cost of reduced precision. Including traits such as larval host plant breadth and habitat breadth allow general patterns to emerge despite heterogeneity in shift rates across local sites. A bog specialist species, for instance, could be more likely to expand north in a boggy area than other species, but it will be less likely to expand in non-boggy areas. While expansion rates will vary across sites, the general macroecological trend should be for habitat generalists to expand northward more readily than habitat specialists. Thus local scale influences on range expansion rates are expected and do not compromise the broad-scale approach I used in my study unless general trends are not sufficiently powerful to emerge beyond location-specific noise.

Fourth, the 30 year timescale used to estimate northern range boundaries in the historical period and recent period may be too coarse for reliable boundary estimates. Range boundaries can shift rapidly over short timescales, introducing noise of northward and southward boundary shifts within each 30 year window. For example, the flying squirrel *Glaucomys volans* expanded its range northward in Ontario by approximately 200 km over nine years of warming (1994-2003) before the range contracted southward by approximately 240 km after a food-shortage Autumn and cold Winter in 2004 (Bowman et al. 2005). Different sites may have experienced different opportunities for range expansion during 30 year windows if the path between occupied and uncolonized habitat experienced a narrow "climate path," or time in which that corridor was climatically suitable (Early and Sax 2011). These factors contribute noise to the data set, resulting in less-precise boundary estimates than studies conducted at finer temporal scales. I reduced the influence of single-year anomalies by relying on the average of three northern-most collection locations, rather than the single northern-most location, to estimate range boundaries in each time period. A macroecological approach will miss year-to-year fluctuations in boundaries but should reveal general boundary shift trends over decades.

The lack of relationships between species' traits and boundary shift rates likely results from a combination of a weak signal and powerful noise in the data set. The weak signal represents the short northward shifts observed during this period of inconsistent and weak warming trends, relative to recent rapid northward range shifts and warming. The powerful noise results from a variety of sources inherent in broad-scale macroecology. These include threats other than climate change affecting species in trait-specific ways (Fréville et al. 2007), intraspecific variation in traits, stochasticity in range boundaries resulting from meta-population dynamics, biotic interactions, habitat variability, and the nature of presence-only opportunistic data (Tingley and Beissinger 2009).

Accurately predicting species' future distributions requires knowledge of reliable range shift patterns past and present. As I and others have found, there has been great variation in species' poleward range shifts over the past century. Evidence is accumulating that some of this inter-species variation is related to species' traits (Angert et al. 2011), but knowing how much and under what circumstances traits influence range shifts requires studies like this one. Macroecological approaches result in strong inference for general trends, but lack the precision necessary for understanding events for any one species, year, or location as a result of the noisy data upon which most studies rely. Regional studies, such as analyses of butterfly species' abundance trends in Massachusetts (Breed et al. 2013) and Britain (González-Megías et al. 2008), provide greater precision than macroecological studies but have unknown applicability to other regions. At a finer scale, natural history research on individuals and populations can reveal mechanisms underlying population trends, such as how snow melt dates affect population dynamics in the butterfly *Speyeria mormonia* (Boggs and Inouye 2012). Through a combination of these approaches we can advance our understanding of species' responses to climate change.

Acknowledgements

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Tables

Table 4-1. Traits used in analyses, with their sources and sample sizes.

Trait	Primary source	Primary source n	Secondary source	Secondary source n	Data type
Disturbed habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Dry habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Open habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Rocky habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Shrub habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Wet habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Woody habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no

Woods' opening habitat association	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	yes / no
Habitat breadth	(Layberry et al. 1998)	127	(Opler et al. 2011)	131	Scale 1-8 (number of habitat types associated with species)
Hilltopping	(Opler et al. 2011)	116	N/A		yes / no
Male mate searching behaviour	(Opler et al. 2011)	99	N/A		percher / patroler
Overwinter stage	(Opler et al. 2011)	121	(Bird et al. 1995)	89	egg / larva / pupa / adult
Maximum wingspan (between forewing tips)	(Layberry et al. 1998)	133	(Opler et al. 2011)	132	Continuous (in mm)
Flight period length	(Layberry et al. 1998)	131	(Brock and Kaufman 2003)	128	Number of months between start and end of flight period
Flight period season	(Layberry et al. 1998)	131	(Brock and Kaufman 2003)	128	early (start month Jan-May) / late (start month June-Dec)
Larval host plant	(Layberry et al.	124	(Opler et al.	132	Taxonomic diversity of larval host

breadth	1998)		2011)		plants on scale of 1-5. 1 = one species; 2 = one genus; 3 = one family; 4 = one order; 5 = host plants in more than one order
Mobility	(Burke et al. 2011)	133	N/A		Continuous scale of 0-10, with higher numbers representing greater mobility. Log-transformed and centred.
Species range size (North America, north of Mexico)	(Burke et al. 2011)	114	N/A		Continuous scale in km^2. Log-transformed and centred.
Conservation status	(NatureServe 2009, as described in Burke et al. 2011)	133	N/A		Scale 1-5 (higher numbers represent less risk)
Generations per year	(Brock and Kaufman 2003)	128	(Layberry et al. 1998)	66	univoltine (maximum one generation per year) / multivoltine (maximum more than one generation per year)

Table 4-2. Exploratory results of individual predictors of northern boundary latitudinal shifts in each Canadian region. Only models with $P < 0.10$ are shown; all blank relationships had $P \geq 0.10$. Bolded coefficient (Coeff) and R^2_{adj} values indicate $P < 0.05$.

	West		Central		East		Weighted average	
Term	Coeff	R^2_{adj} (df)	Coeff	R^2_{adj} (df)	Coeff	R^2_{adj} (df)	Coeff	R^2_{adj} (df)
Conservation status								
Flight period length							-23.91	0.02 (128)
Flight period season (early *vs* late)								
Generations per year								
Hilltopping								
Larval host plant breadth					-94.83	0.04 (64)		
Maximum wingspan			-3.16	0.02 (112)				
Mobility	-3.13	0.02 (86)						
Overwinter stage								
Range size					**-0.00022**	**0.19 (60)**		
Perching (rather than patrolling) mate search strategy					**296.85**	**0.07 (55)**		
Habitat breadth					**-135.20**	**0.09 (60)**		
Disturbed habitat								

association						
Dry habitat association						
Open habitat association			-224.28	0.04 (60)		
Rocky habitat association			544.55	0.05 (60)	91.25	0.02 (124)
Shrub habitat association	**177.27**	**0.04 (107)**	-321.79	0.03 (60)		
Wet habitat association			**-284.68**	**0.06 (60)**		
Wood edges habitat association						
Woody habitat association	**-230.50**	**0.10 (107)**				

CHAPTER 5. CONCLUSION: THE SCALING OF BIODIVERSITY THREATS AND RESPONSES IN A HUMAN-DOMINATED WORLD

Abstract

I evaluate the influence of spatial and temporal scales on biodiversity responses to threats. Focusing on any one scale of threat or response can only reveal partial information about biological responses to modern threats. I discuss the influence of temporal scale on trade-offs in microbial evolution. I then turn to ecological responses to climate change and evaluate the different scales at which "successful" responses can occur. I then critically evaluate the potential contribution macroecological studies can make to conservation management. Finally, I assess the state of knowledge of species range shifts with climate change in Canada and beyond.

Answers to research chapters' questions

In Chapter Two I asked whether there was a trade-off between r_{max} and K at the mutation scale. The answer is no; there was a strong positive correlation between r_{max} and K at this scale. Trade-offs likely require time for selection to remove unfit mutant strains. In Chapter Three I asked whether butterfly species had shifted their northern range boundaries northward over the 1900s. The answer is a qualified yes; most species shifted northward, but not by much. In Chapters Three and Four I asked whether species' rates of range shift were related to their phylogeny or traits. The answer, at least for the regions, taxa, time periods, and traits I considered, is no. Given the slow rate of range shift accompanying the weak warming trend over the period of study, if any characteristics do facilitate northward shifts their influence was likely overwhelmed by the noise inherent in the data set.

Microbial evolution: the importance of scale

Whether traits co-vary positively or negatively is largely dependent on the evolutionary scale of the investigation. In Chapter Two I investigated the relationship between r_{max} and K at the scale of single-clone populations differing by approximately one mutation. At this scale of short-term evolution, the two traits co-varied positively. At the scale of longer term experimental evolution studies (Novak et al. 2006) and comparison of yeast strains artificially selected for industrial uses (Spor et al. 2008), there is a negative relationship between r_{max} and K such that strains that grow relatively quickly have relatively low carrying capacity, and *vice versa*. Together, this suggests that when mutations first arise, they bestow greater variation to cells' overall "health" than to resource allocation strategies. This is consistent with our understanding of the highly variable effects of mutations on fitness (Eyre-Walker and Keightley 2007). At longer timescales, once selection has had the opportunity to remove strains with low r and K and improve remaining straits' fitness through additional mutations, what remains is a variety of strains with successful resource acquisition strategies. Only then are strains' differences in resource allocation strategies evident. This illustrates the importance of temporal scale on evolutionary strategies and trade-offs; only through investigations at several timescales can we fully understand the nature of these relationships.

Climate change research and the importance of scale

Species and population scale responses to climate change

What is best for the individual, population, and species do not always coincide, reflecting the different scales at which "success" is assessed by natural selection (Dawkins 1989). In the case of climate change, what might be considered an optimal response can differ substantially across scales. For an individual, the optimal response to climate change (experienced as weather at short timescales) is, as usual, to survive and reproduce by any means necessary. Individuals are not capable of the clairvoyance required to predict what will be best for their gene pool generations hence. Preferentially mating with immigrants from southern populations, for example, might be beneficial to an individual's descendents because it might integrate alleles selected for warmer conditions. But preferential mating with such immigrants could only arise through the trial-and-error process of natural selection. If immigrants from southern populations were distinct, visually, acoustically, or otherwise, and mating with individuals possessing the southern phenotype produced offspring with greater fitness than offspring from non-directional matings, then selection could eventually result in the evolution of mate choice preference.

At the population (gene pool) scale, selection may favour a variety of changes that bestow greater fitness for individuals at a fixed location. Shifts in phenology (Fitter and Fitter 2002, Forister and Shapiro 2003, Parmesan 2007), host plants (Pateman et al. 2012), and body size (Daufresne et al. 2009, Gardner et al. 2009, Winder et al. 2009) may be selected as fitness-enhancing responses to climate change. These responses may benefit the long-term persistence of the population, but the long-term persistence of the species likely requires additional responses.

At the species scale, shifting a range to match the climate to which the species is adapted would be beneficial to species' continued existence. If a species does not expand its cold-edge borders and instead remains restricted to its current range, the area with habitable climate available (i.e., the species' potential Grinnellian niche as per Soberón and

Nakamura 2009) will shrink over time with climate change (Peterson et al. 2004). Warm-adapted species to the south may colonize southern edges of the non-shifting species' range, outcompeting it and further contributing to warm-edge range decay (Ackerly 2003). Because species with small ranges are often at greater risk of extinction than widely distributed species (Jablonski 2008, Lee and Jetz 2011), species that do not expand their ranges poleward and/or upward will be at greater risk of extinction as their habitable ranges shrink.

Cool edge range expansion would generally be beneficial for species' persistence, but what conditions are required to enable it? Here we must return to the individual scale. Individuals that disperse and colonize new habitats must survive and reproduce at a rate that leads to population growth. Selection may thus favour individuals predisposed to disperse at the cool edges of species' ranges, since they should encounter climatically suitable habitats with little conspecific competition or parasitism (Menéndez et al. 2008). This individual-scale selection rewarding dispersal at species' northern frontier may lead to increasingly rapid rates of dispersal, colonization, and thus range expansion at poleward edges of species' ranges. A similar pattern has been observed in cane toads in Australia, whereby populations at the expanding frontier of this invasive species' range hop more than those at the core of its range (Llewelyn et al. 2010), resulting in faster rates of range expansion over sequential generations of selection (Phillips et al. 2006). Individual-scale selection could also, however, lead to reduced cool edge range expansion. If a population is surrounded by inhospitable habitat (e.g., agriculture), selection may act against dispersal (Cassel-Lundhagen et al. 2009), resulting in reduced likelihood of range expansion. The likelihood and rate of species' cool edge range expansion is thus intricately linked to individual-, population-, and evolutionary-scale processes. The differences in threats and optimal responses among scales illustrate the benefits of considering biological responses to climate change at multiple scales, and the perils of careless extrapolation across scales.

Macroecology: why bother?

The main goal of macroecology is to discover and investigate broad-scale trends. Depending on the topic under investigation there can be substantial error surrounding general macroecological trends, arising from several sources including the individual- and population-scale processes described earlier. Specifically considering macroecological trends related to responses to modern conservation threats (e.g., likelihood of introduced species becoming invasive), the error surrounding general trends can be substantial.

Broad-scale trends are seldom directly relevant to traditional local-scale conservation management because the error surrounding trends arises in part from local-scale processes. When conservation practitioners prioritize needs, broad-scale studies of species' general tendencies are not considered very useful (Cardillo and Meijaard 2012). This reveals a mismatch between academic biologists' and conservation practitioners' priorities. There are a variety of reasons why academic ecologists do their research, but to influence applied conservation management is surely a laudable one (Cooke 2011, Neff 2011). Given the influence of local-scale processes on broad-scale trends (e.g., Cowlishaw et al. 2009), and given the disinterested shrug conservation practitioners display toward much macroecological research, is there any conservation benefit to macroecology?

In my opinion macroecology can make useful contributions to conservation management in two main areas. First, macroecology can provide probability-based estimates of species' responses to threats when local information on the species is lacking. In the absence of local information, general trends based on species' traits, phylogenetic relationships, or other characters could provide rules of thumb that are superior to no information at all. For example, if a wildlife manager wanted to evaluate the conservation risk of a local data deficient species of bird, she could estimate its probability of extirpation risk based on the conservation status of closely-related species (Fritz and Purvis 2010) and the bird's traits (Lee and Jetz 2011). Of key importance here is the provision of uncertainty estimates to inform managers of how accurately such trends can predict individual species' risk without placing undue confidence in uncertain predictions (Blanco et al. 2012).

A second area in which macroecology can inform conservation management is when the scales of threat and ecological response are broad. For example, polar bears (*Ursus maritimus*) are under threat from habitat loss (reduced extent, thickness, and duration of annual sea ice) arising from climate change (Stirling and Derocher 2012). This is a broad-scale threat. But existing management policies for polar bear were designed to address local-scale issues, most notably over-hunting (Meek 2011). This mismatch between the scales of conservation threat and management response seems unlikely to result in conservation success. While polar bears will likely decline in abundance regardless of management approach, broad-scale management approaches have some hope of minimizing the bears' decline. Examples of broad scale research topics that could benefit conservation management include regional sea ice trends, forecast seal population dynamics under climate scenarios, and estimating productivity of formerly-perennial sea ice in the far North after it transforms into annual sea ice. As the threats to wildlife are increasingly broad-scale, conservation management may increasingly be informed by broad-scale ecological trends supplied by macroecological research.

Knowns and unknowns of species' range shift responses to modern climate change

There is a general trend of species shifting their ranges upward in latitude and elevation with climate change (Parmesan and Yohe 2003, Parmesan 2006, Lenoir et al. 2008, Angert et al. 2011), and rates of range shift positively correlate with the extent of warming experienced over the time period of study (Chen et al. 2011). Around this general trend of poleward and upward shifts, however, is a great deal of variation across species, regions, and time periods. It would be logical to expect some of this variation to be explained by differences in species' traits (e.g., generalist species having more opportunities to expand their range than specialists, Peters and Darling 1985). While some studies have indeed found rates of range shift to be related to species' traits (e.g., Pöyry et al. 2009, Betzholtz et al. in press), in general traits only slightly improve the accuracy of range shift predictions (Angert et al. 2011). The number of studies that have thus far investigated the relationship between species' traits and range shifts is small, and the quality of their underlying data sets is heterogeneous. More studies are required to determine whether traits or phylogeny can be used to predict range shifts with various taxa, regions, time periods, and methods. For now, the general pattern is that traits can predict range shifts, but only sometimes and only weakly.

Great White Northward expansions

What is known about species range shifts with climate change in Canada? While my study (Chapter 3) is the first to quantify rates of range shift consistent with climate change in a large taxonomic group in Canada, several Canadian studies have investigated range shifts in other ways. Kharouba et al. (2009) constructed ecological niche models for Canadian butterflies for the same time periods as my studies, investigating shifts in species' potential habitat over time. Kharouba et al.'s study (2009) and my own (Chapter 3) employed macroecological methods, investigating broad-scale trends using a dataset comprised of opportunistic collection records. An alternative approach is to sample a small number of sites thoroughly over several time periods to compare presence, absence, and abundance shifts over time. Three recent studies on insects employed this method in northern Canada, comparing species composition at sites over several decades (Fernandez-Triana et al. 2011, Renaud et al. 2012, Timms et al. in press). Their results illustrate the idiosyncratic nature of ecological responses to climate change, with one study finding evidence consistent with southerly species shifting north over time (Fernandez-Triana et al. 2011), and the other two studies finding little evidence of range shifts over time despite their studies being focused on the same taxonomic group (parasitoid wasps, Timms et al. in press) or location (Churchill, Manitoba, Renaud et al. 2012) as that of Fernandez-Triana et al. (2011). Substantial inter-species and inter-site variation was also found in an assessment of Canada's northern treeline shifts over time at several well-sampled sites (Mamet and Kershaw 2012). Another approach to investigating range shifts is to study a small number of species at several locations along a latitudinal gradient over time. Bowman et al. (2005) took this approach, sampling flying squirrels across a latitudinal gradient in Ontario in 2002-2004. They traced the northward expansion of the southern flying squirrel (*Glaucomys volans*) until the final year when its range collapsed 240 km south after a low-food Autumn and cold Winter, demonstrating the dynamic nature of species' range boundaries (Bowman et al. 2005). Finally, the most common approach to exploring species' range shifts in Canada is through documentation of species newly recorded in a region (e.g., McAlpine et al. 2008, Hussell et al. 2012). Usually such studies cannot verify whether the species already inhabited the region but went unnoticed (Tingley and Beissinger 2009), nor can they attribute the purported range expansion to climate change since such attribution requires a heavy burden of proof (Parmesan et al. 2011). Despite these drawbacks, the accumulation of evidence from such studies can inform local wildlife management and provide insight into broader trends (Parmesan and Yohe 2003).

Overall, species do seem to be shifting northward in Canada but at rates that vary from southward contractions to rapid northward expansions. Too few studies yet exist to estimate how much of the variation in range shift rates is attributable to species, regions, time periods, methods of sampling and analysis, or their interactions. This situation resembles that for the world at large, with abundant variation in range shift responses reflecting largely unknown factors. The diversity of life is what appeals to us as ecologists. But the diversity of ecological responses to environmental changes can be frustrating when we seek to predict and protect nature.

APPENDIX A. DESCRIPTION AND SCORING OF BUTTERFLY TRAITS

Habitat association is a family of eight traits, each a yes/no response to whether a species is associated with one of eight habitat types: disturbed, dry, open, rocky, shrub, wet, woods, and woods' clearings.

Hilltopping is the tendency for butterflies to congregate on hilltops for the purposes of finding a mate (Shields 1967). Sources seldom described a species as non-hilltopping, thus the data file included only one category (yes) and many species with no mention of hilltopping. Because an alternate (i.e., "no") state is required for analysis of inter-source agreement, I chose to classify species as non-hilltopping when a source listed information on mate search habit (indicating knowledge of adults' behaviour) without mention of hilltopping.

Mate search habit is the tendency for males to search for females by perching (i.e., waiting for females then chasing them) or patroling (i.e., scanning for females while flying) (Wiklund 2003). Occasionally a species was described as engaging in both perching and patroling strategies, in which case I coded the species as whichever strategy was described as being more prevalent, and omitted the species if neither strategy was described as being more prevalent.

Overwintering stage describes the life-history stage (egg, larva, pupa, or adult) in which butterflies overwinter. When more than one stage was listed for a species in a source (e.g., high-altitude species that require two years to develop and overwinter in different stages each winter) I recorded the earliest stage.

Maximum wingspan is the length, in mm, between distal tips of forewings. Sources provided lower and upper boundaries of wingspan for species; because minimum and maximum wingspan were highly correlated only maximum wingspan is considered here.

Flight period is the time of year when adult butterflies are flying. The start and end of the flight period were recorded as two separate ordinal variables, with numbers corresponding to months (e.g., eight represents August). Flight period length is simply the end month minus the start month, while flight period season was coded as early (start month Jan-May) or late (start month June-Dec).

Generations per year was coded as univoltine (maximum one generation per year) or multivoltine (maximum more than one generation per year).

Habitat breadth is the sum of the number of habitat types classified as suitable for a species. Thus habitat breadth could range from one to eight as an ordinal variable (but seven was the highest breadth for any species in my data set), with higher numbers representing greater breadth (habitat generalists).

Larval host plant breadth represents the phylogenetic diversity of host plants listed in a source. This trait was coded as an ordinal variable from one to five with higher numbers representing greater breadth as follows: one host plant species (1); more than one host plant species within one genus (2); more than one host plant genus within one family (3); more than one host plant family within one order (4); host plants in multiple orders (5). Plant species' taxonomic associations were obtained from Wikipedia, which has been found to be highly accurate in its taxonomic information (Page 2010). Other researchers employ measures of host plant breadth similar to the one I have used, such as Komonen et al. (2004) and Burke et al. (2011) who classified butterflies within the first three categories (i.e., ignoring variation above family level). It is logical to classify dietary breadth with taxonomic hierarchies since host plant breadth is often limited by foliage chemistry (Ehrlich and Raven 1964, Janz and Nylin 1998), and closely-related plants often have similar chemistry (Ricklefs 2008). Host plant breadth is likely confounded with sampling effort, so species with numerous host plant records (e.g., common species) are more likely to be found on non-preferred plant species leading to broader niche classification whereas butterflies with few host plant records are more likely to only be found on their preferred plant species and be classified as specialists (Beck et al. 2006).

APPENDIX B. TITLES AND ABSTRACTS OF MY NON-THESIS CHAPTER PUBLICATIONS DURING PhD YEARS

1. **Fitzsimmons, J. M.** (2012) Local species trading cards: an activity to encourage scientific creativity and ecological predictions from species' traits. Journal of Natural History Education and Experience. 6: 10-15.

Abstract

Species' traits (e.g., body size, generation time, diet breadth) are being used by biologists with increasing frequency to predict ecological responses to modern environmental threats. Given the importance of traits for ecological research, and the accessibility of traits to learners, it is important to develop effective teaching methods for the relationship between species' traits and ecological responses. I describe a short (approximately 45 minutes) activity that encourages youth to critically evaluate species' traits in the context of predicted responses to modern climate change. The activity uses trading cards for local butterfly species akin to sports trading cards, with photographs of the species on the front and their trait statistics on the back. Participants are asked to make trait-based predictions of species' responses to climate change. I describe my experience leading this activity with a youth naturalist club, and provide supplementary files allowing readers to modify this activity for other taxa, traits, and ecological responses.

2. Bertram, S. M., V. L. M. Rook, **J. M. Fitzsimmons**, and L. P. Fitzsimmons. (2011). Fine- and broad-scale approaches to understanding the evolution of aggression in crickets. Ethology 117: 1067-1080.

Abstract

Male field crickets frequently engage in agonistic contests to establish dominance in social interactions and gain access to mate attraction territories. Crickets (Orthoptera: Gryllidae) are often used as a model taxon to study aggression, but limited documentation of aggression in some cricket species hinders our understanding of its evolutionary costs and benefits. Our study investigated cricket aggression at two scales: the within-species scale for two cricket species, *Gryllus assimilis* and *G. veletis*, whose aggression had not been adequately documented; and the among-species scale to detect evolutionary patterns in species' levels of aggression. In both *G. veletis* and *G. assimilis*, winners spent more time being aggressive than losers but they were not larger or heavier. Our results reveal that *G. veletis* males are more aggressive than *G. assimilis*. Male *G. veletis* had higher aggression scores that male *G. assimilis*. The majority of *G. veletis* contests escalated to grappling (a highly aggressive behavior), while less than one quarter of *G. assimilis* contests escalated to grappling. Further, *G. veletis* males transitioned between two of the most aggressive behaviors most often while *G. assimilis* transitioned between two of the least aggressive behaviors most often. We integrate this new information on aggression for *G. assimilis* and *G. veletis* with previously documented aggression data for many cricket species to investigate aggression in a broader evolutionary context than previously possible. Within a phylogenetic context we test the hypothesis that species whose males use burrows from which to call and attract females are more aggressive than species with non-burrowing males. We found evidence consistent with this hypothesis; species with burrowing males tended to be more aggressive than species with non-burrowing males. Together our study provides fine-scale understanding of aggression in two cricket species and broad-scale evolutionary context for aggression across cricket species.

3. Burke, R. J., **J. M. Fitzsimmons**, and J. T. Kerr. (2011) A mobility index for Canadian butterfly species based on naturalists' knowledge. Biodiversity and Conservation 20: 2273-2295.

Abstract

Mobility is a key component of species' biology. Research on mobility is inherently difficult, however, resulting in studies of narrow taxonomic, spatial, and temporal scope with results that are difficult to compare between studies. We had three goals for our research: 1) construct a data set of mobility estimates for the butterfly species of Canada based on naturalists' knowledge; 2) develop methods to evaluate aspects of accuracy and precision for knowledge-based ecological research such as ours; and 3) using our data set, test mobility-related hypotheses of species-level relationships. We distributed a questionnaire to amateur and professional lepidopterists in Canada and northern USA, asking them to estimate the mobility of Canadian butterfly species based on their field experience. Based on responses from 51 lepidopterists with approximately 800 years of combined field experience, we received mobility estimates for almost all (291 out of 307) of Canada's butterfly taxa. Mobility estimates were consistent among respondents and were not affected by respondent expertise. Mobility carries a strong phylogenetic signal and is positively related to wingspan (albeit weakly), range size, and host plant breadth, and negatively related to conservation risk. Reliance upon naturalists' experience was essential to the feasibility of our project, and provides a promising method for many types of ecological research.

4. **Fitzsimmons, J. M.** (2011) Online publication: a natural progression for The Canadian Field-Naturalist. Canadian Field-Naturalist 125: 5-6. [not peer-reviewed]

Abstract

Without dismissing potential limitations, the benefits and opportunities of electronic publication are numerous. For The Canadian Field-Naturalist, these are focussed within three main areas, as summarized below [online content, online manuscript management, and supplementary files].

5. **Fitzsimmons, J. M.** and J. H. Skevington. (2010) Metrics: don't dismiss journals with a low impact factor. Nature 466: 179. [not peer-reviewed]

Main paragraph

We have debated whether we should eliminate our ‘Notes’ section, which comprises short descriptions of natural history that are often single observations of previously undocumented animal behaviour. ‘Notes’ adversely affect our impact factor because they contribute as much to the denominator of the impact-factor equation as full articles, but are cited far less frequently.

6. **Fitzsimmons, J. M.** and L. P. Fitzsimmons. (2010) Pre-copulatory behavior of the wheel bug *Arilus gallus* (Hemiptera: Reduviidae). Entomological News 121: 304-307.

Abstract

We describe the pre-copulatory behaviour of a pair of *Arilus gallus* Stål (Heteroptera: Reduviidae) observed in a tropical dry forest in Sector Santa Rosa of Area de Conservación Guanacaste, northwestern Costa Rica (10°40'N, 85°30'W). To our knowledge this is the first description of mating behaviour in the genus *Arilus* other than several notes on the time of year *A. cristatus* (Linné) mates in the USA (Barber 1920, Moul 1945, Hagerty and McPherson 2000).

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