Environmental drivers of colour and size in insects

A macroecological perspective

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Contents

Summary

Climatic conditions have profound effects on the geographical distribution of species across diverse taxa and regions. However, the role of traits of species that underpin the relationship between distribution and climate is poorly understood, especially in animals. Although many physiological and local-scale studies on animals have demonstrated associations between traits and climate, to what extent such mechanistic links may impact distribution patterns of species at macroecological scales remains largely unknown. In insects, colour and size are two very important and highly multifunctional traits, which play pivotal roles in natural and sexual selection, and may hence be related to climate.

The colour of insects comprises several functions, namely crypsis, aposematism, sexual selection, species recognition, UV resistance, pathogen resistance and thermoregulation. However, the relative importance of single functions is often unclear because several functions of colouration can act simultaneously. Hitherto, thermoregulation through thermal melanism is supposed to be the dominant function of colouration in insects. Dark-coloured individuals heat up faster and attain higher steady-state body temperatures compared to light-coloured individuals of similar size under equal environmetal conditions and light-coloured individuals are supposed to have advantages in warmer climates because of a reduced risk of overheating. However, it is unknown to which extent this physical mechanism influences the colour lightness of species in response to different thermal environments at macroecological scales and across continents. Moreover, thermoregulation is not the only function of colouration of insects and it is unknown to which extent other functions of colouration dominate in particular geographical areas and taxa and thereby form macroecological patterns.

The size of insects affects almost all physiological rates (e.g. rate of oxygen consumption), which subsequently determine or constrain fertility, mortality and ecological processes such as competitive interactions between individuals or species. In this way, body size is ultimately linked to the spatiotemporal distribution and abundance of animals and has important implications for the impact of climate warming – from biomass production by single species to the structure and dynamics of communities. However, a synthesis and analysis of the major environmental driver for the large-scale geographical variation of insect body size is missing so far.

In this thesis, I use lepidopteran and odonate species to address the following questions: i) Are assemblages of diurnal insects darker coloured in colder regions and lighter coloured in warmer regions? ii) Is crypsis, pathogen resistance or protection from UV radiation associated with large-scale geographical variation in the colouration of insects? iii) What are the major environmental drivers for the large-scale geographical variation of body size in insects?

I show that dark-coloured diurnal insect species predominately occur in colder climates and light-coloured diurnal insect species in warmer climates. Thus, thermal melanism seems to be an important and general mechanism for the distribution of insects. Thereby, I provide a mechanistic link between climate, colour lightness of species and their distribution, which may be a foundation for better forecasting the effect of climate warming on many insect groups. I furthermore show that also assemblages of predominantly nocturnal geometrid moths are darker coloured in colder regions and lighter coloured in warmer regions. Unexpectedly, the best environmental predictor of this geographical pattern was not canopy cover – which would indicate the importance of crypsis depending on the light environment – but solar radiation. This result indicates a link of adult colouration to physiological processes during earlier life stages, such as thermoregulation or immune responses of larvae, and points to fundamental benefits of dark colouration in cold environments for insects.

I also show that insect voltinism (number of generations per year) is strongly determined by environmental temperature and constrains insect body size at macroecological scales. Voltinism consistently decreases with latitude, with species having on average fewer generations per year in northern Europe and more generations per year in southern Europe. Insects with the ability to extend their generation time over multiple years can overcome this constraint, which allows for a relatively large body size in cold areas. In addition, direct effects of temperature, productivity and season length on body size contrast in sign between lepidopterans (positive) and odonates (negative), with temperature having the strongest effect in both groups. These results support the idea that body sizes of terrestrial and aquatic insects show contrasting geographical patterns because they are differently affected by temperature, resources and time constraints.

In conclusion, colour and size of insects are related to their geographical distribution even at macroecological scales. The underlying mechanisms are especially driven by environmental temperatures: for colour because of its effect on thermoregulation and immune function, and for size because of the temperature dependency of metabolic rates and voltinism. With global warming, I would thus expect that especially dark-coloured and/or large aquatic insects might shift their distribution and retreat from warmer areas. Furthermore, insect species with the ability to increase their annual number of generations should benefit and extend their distribution to higher latitudes.

Zusammenfassung

Das Klima hat grundlegende Auswirkungen auf die Verbreitung von Arten. Ein mechanistisches Verständnis des Zusammenhangs zwischen Merkmalen von Arten, der räumlichen Verbreitung von Arten und klimatischen Bedingungen fehlt jedoch weitestgehend, vor allem bei Tieren. Obwohl viele physiologische und kleinräumige Studien Zusammenhänge zwischen Merkmalen von Arten und klimatischen Bedingungen gezeigt haben, ist weitgehend unbekannt, inwieweit solche mechanistischen Zusammenhänge die Verbreitung von Arten auf makroökologischer Skala beeinflussen. Bei Insekten sind Farbe und Körpergröße zwei wichtige multifunktionale Merkmale, die zentrale Rollen bei natürlicher und sexueller Selektion einnehmen und deswegen mit klimatischen Bedingungen in Beziehung stehen könnten.

Die Farbe von Insekten wird mit Tarnung, Aposematismus, sexueller Selektion, Arterkennung, Resistenz gegen UV-Strahlung und Pathogene sowie Thermoregulation assoziiert. Die relative Wichtigkeit einzelner Funktionen ist jedoch oft unklar, weil mehrere Funktionen von Farbe simultan wirken können. Bisher wird davon ausgegangen, dass Thermoregulation durch thermalen Melanismus die dominante Funktion der Farbe bei Insekten ist. Dunkle Individuen erwärmen sich schneller und erreichen höhere Körpertemperaturen verglichen mit hellen Individuen unter gleichen Umweltbedingungen. Helle Individuen hingegen sollten durch ein geringeres Risiko zu Überhitzen einen Vorteil in warmen Klimaten besitzen. Es ist jedoch unbekannt, inwiefern dieser physikalische Mechanismus die Helligkeit von Arten in Abhängigkeit der thermalen Umgebung auf makroökologischer Skala und über Kontinente hinweg beeinflusst. Darüber hinaus ist Thermoregulation nicht die einzige Funktion von Farbe und es ist unbekannt, inwiefern andere Funktionen von Farbe in bestimmten Gebieten und Taxa dominieren und dadurch makroökologische Muster generieren.

Die Körpergröße von Insekten beeinflusst fast alle physiologischen Prozesse (z.B. den Sauerstoffverbrauch), die wiederum Fertilität, Mortalität und ökologische Prozesse wie z.B. die Konkurrenz zwischen Individuen oder Arten bestimmen. Somit ist die Körpergröße letztendlich mit der raumzeitlichen Verbreitung und Abundanz von Arten verbunden, was wichtige Implikationen für Auswirkungen des Klimawandels hat – von der Biomasseproduktion einzelner Arten hin zur Struktur und Dynamik von Artgemeinschaften. Bisher gibt es jedoch noch keine Synthese und Analyse der wichtigsten Umwelteinflüsse auf die großräumige Verbreitung der Körpergröße von Insekten.

In dieser Arbeit verwendete ich Lepidopteren (Tag- und Nachtfalter) und Odonaten (Groß- und Kleinlibellen), um die folgenden Fragen zu bearbeiten: i) Sind Gemeinschaften tagaktiver Insekten im Mittel dunkler in kälteren Regionen und heller in wärmeren Regionen? ii) Ist Tarnung, Resistenz gegen Pathogene oder Schutz vor UV-Strahlung assoziiert mit großräumiger Variation in der Farbe von Insekten? iii) Was sind die wichtigsten Umwelteinflüsse auf die großräumige Variation der Körpergröße von Insekten?

Ich zeige, dass dunkle tagaktive Insektenarten vorherrschend in kühleren Klimaten vorkommen und helle tagaktive Insektenarten in wärmeren Klimaten. Von daher scheint thermaler Melanismus ein wichtiger Mechanismus für die Verbreitung von Insekten zu sein. Ich zeige somit einen mechanistischen Zusammenhang zwischen Klima, Helligkeit von Arten und der Verbreitung von Arten auf, welcher eine Grundlage sein könnte, Auswirkungen der Klimaerwärmung auf viele Gruppen von Insekten besser vorhersagen zu können. Ich zeige weiterhin, dass auch Gemeinschaften von vornehmlich nachtaktiven Motten (Geometriden) im Mittel in kühleren Regionen dunkler und in wärmeren Regionen heller sind. Unerwarteterweise war die beste erklärende Umweltvariable für dieses geographische Muster nicht die Waldbedeckung – was auf die Bedeutung von Tarnung in Abhängigkeit der vorherrschenden Lichtbedingungen hinweisen würde – sondern die solare Einstrahlung. Dieses Ergebnis gibt einen Hinweis darauf, dass die Farbe von adulten Insekten durch physiologische Prozesse in früheren Lebenstadien bedingt sein könnte, z.B. Thermoregulation oder Immunfunktion bei den Raupen, und weist auf fundamentale Vorteile einer dunklen Farbe in kühleren Regionen hin.

Ich zeige außerdem, dass der Voltinismus bei Insekten (Anzahl der Generationen pro Jahr) stark von der Umgebungstemperatur abhängt und die Körpergröße von Insekten auf makroökologischer Skala limitiert. Der Voltinismus nimmt konsistent mit der geographischen Breite ab und Arten haben im Mittel weniger Generationen pro Jahr in Nordeuropa und mehr Generationen pro Jahr in Südeuropa. Insekten mit der Fähigkeit, ihre Generationszeit über mehrere Jahre auszudehnen, können diese Beschränkung umgehen, was eine relativ große Körpergröße in kühleren Gebieten ermöglicht. Zudem sind die direkten Effekte von Umgebungstemperatur, Produktivität und Saisonlänge auf die Körpergröße gegenläufig zwischen Lepidopteren (positiver Zusammenhang) und Odonaten (negativer Zusammanhang) und die Umgebungstemperatur hat den stärksten Effekt in beiden Gruppen. Diese Ergebnisse unterstützen die Idee, dass die Körpergröße von terrestrischen und aquatischen Insekten gegensätzliche geographische Muster zeigt, weil diese beiden Gruppen unterschiedlich auf Temperatur sowie Ressourcen- und Zeitbeschränkungen reagieren.

Diese Arbeit demonstriert, dass Farbe und Körpergröße selbst auf makroökologischer Skala in Beziehung zur geographischen Verbreitung von Insekten stehen. Die zugrunde liegenden Mechanismen sind vor allem durch die Umgebungstemperatur gesteuert: Bei der Farbe durch deren Effekt auf Thermoregulation sowie Immunfunktion und bei der Körpergröße durch die Temperaturabhängigkeit metabolischer Raten und des Voltinismus. Mit der Klimaerwärmung erwarte ich von daher, dass vor allem dunkle und/oder große aquatische Insekten ihre Verbreitungsgebiete verändern und sich von wärmeren Regionen zurückziehen. Insektenarten mit der Fähigkeit, ihre jährliche Anzahl von Generationen zu erhöhen, sollten hingegen von steigenden Umgebungstemperaturen profitieren und ihre Verbreitungsgebiete in höhere Breiten ausdehnen.

Part I

Introduction

1 General background

Climatic conditions determine the occurrence of animals and climate warming has fundamental consequences for their distribution (Brown *et al.* 1996, Parmesan 2006, Lomolino *et al.* 2010). However, the roles of traits of animals that offer a mechanistic understanding of the large-scale relationships between distribution and climate are poorly understood. For endotherms, it is known that environmental temperature is related to morphological traits which are directly linked to the physiology and ecology of organisms. The most prominent geographical patterns of traits of animals were described by Gloger (1833), Bergmann (1848), and Allen (1877) and are known as ecogeographical rules.

According to *Gloger's rule*, the colouration of related forms of endotherms is often correlated with humidity of their environments, with darker colours occurring in warm and humid regions (Gloger 1833). Several mechanisms explaining this geographical pattern of colouration have been proposed, including crypsis, protection from UV radiation, thermoregulation and pathogen resistance (Burtt & Ichida 2004). According to *Bergmann's rule* the body size of endotherms increases with decreasing environmental temperature due to thermoregulatory advantages of a low surface-to-volume ratio of large body sizes which supports heat retention (Bergmann 1848). *Allen's rule* predicts reduced appendage size among endotherms with decreasing environmental temperatures, in order to conserve body heat in cold environments and to facilitate the dissipation of heat in warmer regions (Allen 1877, see also Scholander 1955).

These rules focus on the colour and size of animals and were originally postulated for endothermic vertebrates. In insects, however, which are the most diverse group of animals on Earth and which play pivotal roles for ecosystem services and functioning (e.g. pollination, decomposition, pests and diseases and control thereof; Diniz-Filho *et al.* 2010), mechanisms might be fundamentally different. Although many physiological and local-scale studies on insects have demonstrated associations between colour, size and climate, to what extent such mechanistic links impact distribution patterns at macroecological scales remains largely unknown.

The aim of this thesis is to improve our understanding of environmental drivers of colour and size in insects – two key traits potentially related to the distribution of animals. Thereby, I apply a macroecological perspective to fill important knowledge gaps and to identify potential general trends in the geographical variation of colour and size in insects.

2 Colour

Colour is probably the most prominent natural phenomenon and has attracted the attention of scientists since the early beginnings of natural history (Humboldt & Bonpland 1806, Darwin 1859, Wallace 1870, Poulton 1890). The first recognition that there might be regular variation in the colouration of animals was provided by C. L. Gloger almost 200 years ago, who qualitatively described colour variation in birds (Gloger 1833). Only recently, however, advances in the quantification of digital color data allow scientists to objectively analyse coloration, which is important for understanding the various functions of coloration as well as their geographical variation.

The colouration of animals confers a variety of important ecological and physiological functions. It influences and is controlled by various biotic and abiotic factors that operate on ecological and evolutionary scales, namely crypsis, aposematism, sexual selection, species recognition, thermoregulation, UV resistance and pathogen resistance and might be a major component involved in the adaptation to climate warming (Kettlewell 1973, True 2003, Roulin 2014). However, the relative importance of single factors affecting colouration is often unclear because several functions of colouration can act simultaneously (Mckinnon & Pierotti 2010). Furthermore, most previous studies on the colouration of animals focused on thermoregulation, and the extent to which other functions of colouration contribute to large-scale geographical patterns in species assemblages remains poorly understood (but see Dale *et al.* 2015).

In ectotherms, the appearance of the body surface and in particular its colour lightness are involved in thermoregulation (Kalmus 1941). Dark-coloured ectotherms are able to increase their body temperature above ambient air temperature more effectively than light-coloured ectotherms, and are therefore supposed to have an advantage in cool climates (*thermal melanism hypothesis*; Kingsolver 1995, Clusella Trullas *et al.* 2007). Most insects – by far the most species-rich lineage of ectotherms (Speight *et al.* 2008) – need to reach body temperatures above ambient temperature for flying, foraging or mating (Huey & Kingsolver 1989). However, being dark is only advantageous in cool climates. In areas with high temperature and insolation, insects need to protect themselves against overheating (Gibert *et al.* 1998). At high temperatures, ectothermic species with light colouration can be active for a longer period than species with dark colouration, and may be able to use a broader thermal range of habitats. Overall, heat avoidance and heat gain constraints lead to the prediction that insect assemblages should be dominated by dark-coloured species in cool climates and by light-coloured species in warm climates.

The thermal melanism hypothesis was originally postulated for terrestrial heliothermic species, whose main activity time is during the day. In non-heliothermic species, however, other mechanisms than thermoregulation are expected to be more relevant for colour variation, especially crypsis and pathogen resistance. Species that rely on crypsis should have

a colouration similar to their environment (*crypsis hypothesis*; Endler 1984). Furthermore, melanin pigments are associated with increased protection against viral, bacterial and fungal pathogens, and against abiotic stressors such as heavy metals (Wilson *et al.* 2001, True 2003, Mikkola & Rantala 2010). Hence, dark-coloured species are supposed to be more resistant to pathogens and accordingly, areas with many biotic as well as abiotic stressors should be characterized by more dark-coloured species (*pathogen resistance hypothesis*; Wilson *et al.* 2001).

In this thesis, I test whether the colour lightness of heliothermic insects is consistently correlated to the thermal environment at macroecological scales as predicted by the thermal melanism hypothesis. Therefore, I use butterfly and dragonfly species across Europe and North America (sections 6.1.1, 6.1.2 and 6.1.3). Furthermore, I test whether the colour lightness of predominantly nocturnal moths is correlated to the light and climatic conditions of their environments as predicted from considerations of crypsis and pathogen resistance (section 6.1.4).

3 Size

Body size is another key trait which influences physiological and ecological processes of an animal. Body size affects almost all physiological rates (e.g. rate of oxygen consumption; Woods 1999, Atkinson *et al.* 2006), which subsequently determine or constrain fertility, mortality and ecological processes such as competitive interactions between individuals or species. In this way, body size is ultimately linked to the spatiotemporal distribution and abundance of animals (Blackburn & Gaston 2001) and has important implications for the impact of climate warming – from biomass production by single species to the structure and dynamics of communities (Sheridan & Bickford 2011, Forster *et al.* 2012). This link between physiology and distribution was first recognized by C. Bergmann, who noted that body size increases with increasing latitude within and/or between closely related species of endothermic animals (Bergmann 1848) and was subsequently extended to also include appendage size by J. A. Allen (Allen 1877). Since publication of Bergmann's seminal work, considerable debates have flourished, especially with regard to whether Bergmann's rule constitutes a pattern, a process or both, whether it applies to intraspecific, interspecific or assemblage levels, and whether it also applies to insects (e.g. Watt *et al.* 2010).

Although the body size of endotherms appears to increase consistently with decreasing environmental temperature (e.g. Ashton *et al.* 2000, Meiri & Dayan 2003, Olson *et al.* 2009), for insects, general patterns in the geographical variation of body size are still debated. The number of studies that show an increase in insect body size with latitude or elevation is nearly equal to those that show a decrease (Shelomi 2012). It is important to note that physiological processes influencing geographical patterns of body size of insects may fundamentally differ from those of endotherms. Three major hypotheses relating body size of insects to environmental conditions have been proposed.

The first hypothesis, the *temperature-size rule* (Atkinson 1994), is based on the widely observed phenomenon that development and growth rates respond differently to variations in environmental temperature, specifically that increasing temperature accelerates the development rate more than the growth rate; hence, the adult stage is reached at a smaller body size with increasing temperature. From a geographical perspective, the temperature-size rule predicts a decrease in body size with increasing environmental temperature. The second hypothesis deals with the *availability of resources*, which is essential for growth and thus for an animal's body size. Animals adjust their body size to the potential food supply of the environment where they occur (Atkinson & Sibly 1997). Therefore, animals can grow larger in a resource-rich environment but must stay small when resources are limited. Productivity and latitude are usually negatively correlated; hence, this resource effect should, in contrast to the expectation from the temperature-size rule, lead to a decrease in body size with increasing latitude. The third hypothesis concerns the *length of the season*, which affects the time available for growth and development. A longer season means a longer growing period and hence a larger final body

size (Mousseau 1997, Chown & Gaston 2010). At high latitudes, the growing season is short, which, like the expectation from a consideration of resource availability, leads to the prediction that body size decreases with increasing latitude.

These three hypotheses, however, ignore one important factor that might constrain body size, namely voltinism, i.e. the number of generations per year. In a given area, a species with multiple generations per year has less time per generation available for growth than a species with only one generation per year. Since total growing time and body size are positively correlated, multivoltine species or populations should be smaller than univoltine species or populations (Roff 1980). Furthermore, an increase in temperature allows an increase in the number of generations per year, because it accelerates development rates so that more than one generation can be completed during the growing season (Gillooly *et al.* 2001, Altermatt 2010). Finally, the maximum number of generations per year is also constrained by resource availability and season length (e.g. Mousseau & Roff 1989). These possible indirect effects on body size via the number of generations per year stand in contrast to the possible direct effects of resource availability and season length on insect body size.

In this thesis, I use lepidopterans and odonates across Europe to analyse the relationships of body size and voltinism to temperature, productivity and season length and to test the hypothesis that voltinism constrains the body size of insects at macroecological scales (section 6.2.1). Furthermore, I test for geographical patterns in body size of European stick insects as predicted by Bergmann's and Allen's rule (section 6.2.2) and analyse the variation in body size of moths along a tropial elevational gradient in Costa Rica (section 6.2.3).

Part II

Manuscripts of this thesis

4 Outline

The manuscripts of this thesis are presented in two main sections: one on the colour and one on the size of insects (sections 6.1 & 6.2).

COLOUR

Manuscript 6.1.1 is the continuation and large extension of my bachelor and diploma theses (Zeuss 2010, 2011) and was after several revisions finally published in Nature Communications (Zeuss *et al.* 2014). This publication opened up the field for follow-up research on the colour lightness of insects (e.g Bishop *et al.* 2016, Xing *et al.* 2016, Schweiger & Beierkuhnlein 2016) and received a lot of attention in the public (see `http://www.nature.com/articles/ncomms4874/metrics`). In this manuscript, I show for the first time that the colour lightness of insects is consistently correlated to the thermal environment at a continental scale, using butterflies and dragonflies across Europe. I furthermore show that climate warming is likely to lead to an increase in the average colour lightness of insects. The other manuscripts in this section are the outcome of subsequent work on the colouration of insects with students of mine. **Manuscripts 6.1.2 and 6.1.3** extend the research to the colour lightness of dragonflies and butterflies in North America. These two manuscripts demonstrate that the geographical patterns of colour lightness and their environmental drivers are very similar in North America compared to Europe. Thus, thermal melanism might be an important and general mechanism that shapes the distribution of insects in non-tropical regions. **Manuscript 6.1.4** studies the colour lightness of predominantly nocturnal moths, for which other functions of colouration than thermal melanism were expected to contribute to large-scale geographical patterns, in particular crypsis and pathogen resistance. This manuscript also shows a distinct geographical gradient with predominantly dark-coloured species in northern regions and light-coloured species in southern regions across the Western Palearctic. Hence, the colour lightness of insects is likely to be a climate-driven trait and dark colouration seems to impose fundamental benefits in cold and moist environments.

SIZE

Manuscript 6.2.1 (Zeuss *et al.* 2017) is the first paper which analyses macroecological patterns of size and voltinism (number of annual generations) in insects. I demonstrate that voltinism in insect assemblages is strongly driven by environmental temperature, and trade-offs between voltinism and body size influence the occurrence of species at macroecological scales. This paper was featured on the front cover of Global Ecology and Biogeography (February 2017, `http://onlinelibrary.wiley.com/doi/10.1111/geb.12572/full`). I was subsequently invited by two well-known experts to participate in their research projects on the size of European stick insects and tropical moths. The outcome is presented in **manuscripts 6.2.2 and 6.2.3**. Only abstracts are presented in the latter two manuscripts due to reserved copyrights.

Furthermore, I was involved in several other manuscripts during my PhD studies which broadly deal with geographical patterns of biodiversity and which are shortly presented in section IV.

Please enjoy reading and feel free to contact me in case of questions, criticisms and ideas for collaborations.

5 List of manuscripts

Colour (section 6.1)

I	Title:	Climate warming favours light-coloured insects in Europe.
	Authors:	**Zeuss D**, Brandl R, Brändle M, Rahbek C & Brunzel S.
	Status:	*Nature Communications* 5, 3874 (2014).
	Own contribution:	Data acquisition: 100%, data analysis and figures: 100%, writing and revising: 90%, concept: 80%.
II	Title:	Colour lightness of dragonfly assemblages across North America and Europe.
	Authors:	Pinkert S, Brandl R & **Zeuss D**.
	Status:	*Ecography* 39, 1–8 (2016).
	Own contribution:	Data acquisition: 50%, writing and discussion: 50%, concept and supervision: 90%.
III	Title:	Colour lightness of butterfly assemblages across North America and Europe.
	Authors:	Stelbrink P, Brunzel S, Brandl R & **Zeuss D**.
	Status:	*In preparation*.
	Own contribution:	Data acquisition: 50%, data analysis and figures: 20%, writing and discussion: 40%, concept and supervision: 90%.
IV	Title:	The dark side of Lepidoptera: A continental gradient in the colour lightness of assemblages of geometrid moths.
	Authors:	Heidrich L, Brandl R, Fiedler K, Brändle M & **Zeuss D**.
	Status:	*In revision. Global Ecology and Biogeography.*
	Own contribution:	Data acquisition: 20%, writing and discussion: 50%, concept and supervision: 90%.

Size (section 6.2)

V	Title:	Environmental drivers of voltinism and body size in insect assemblages across Europe.
	Authors:	**Zeuss D**, Brunzel S & Brandl R.
	Status:	*Global Ecology and Biogeography* 26, 154–165 (2017).
	Own contribution:	Data acquisition: 80%, data analysis and figures: 100%, writing and revising: 95%, concept: 100%.
VI	Title:	Bergmann's and Allen's rule in native European and Mediterranean Phasmatodea.
	Authors:	Shelomi M & **Zeuss D**.
	Status:	*In revision. Frontiers in Ecology and Evolution*.
	Own contribution:	Data analysis 50%, writing and discussion: 25%, concept: 50%.
VII	Title:	Body size of species rich moths increases along a large tropical elevational gradient.
	Authors:	Brehm G & **Zeuss D**.
	Status:	*In preparation*.
	Own contribution:	Writing and revising: 50%.

Other manuscripts (section IV)

VIII	Title:	Chromosome numbers in three species groups of freshwater flatworms increase with increasing latitude.
	Authors:	Lorch S, **Zeuss D**, Brandl R & Brändle M.
	Status:	*Ecology and Evolution* 6, 1420–1429 (2016).
	Own contribution:	Data acquisition: 20%, data analysis: 20%, writing and revising: 20%.
IX	Title:	Evolutionary processes, dispersal limitations and climatic history shape current diversity patterns of European dragonflies.
	Authors:	Pinkert S, Dijkstra KD, **Zeuss D**, Reudenbach C, Brandl R & Hof C.
	Status:	*In review. Ecography*.
	Own contribution:	Writing and discussion: 20%, concept: 10%.
X	Title:	Understanding the drivers of cross-taxon congruence.
	Authors:	Pinkert S, **Zeuss D**, Dijkstra KD, Kipping J, Clausnitzer V, Bannar-Martin K & Brandl R.
	Status:	*In preparation*.
	Own contribution:	Writing and discussion: 20%.
XI	Title:	Inferring biotic interactions from macroecological data: a review.
	Authors:	Dormann C, Bobrowski M, Dehling M, Hartig F, Lischke H, Moretti M, Pagel J, Pinkert S, Schleuning M, Schmidt SI, Sheppard CS, Steinbauer MJ, **Zeuss D** & Kraan C.
	Status:	*In preparation*.
	Own contribution:	Writing and discussion: 1%.

6 Manuscripts

6.1 Colour

Global warming favours light-coloured insects in Europe

with
Roland Brandl, Martin Brändle, Carsten Rahbek & Stefan Brunzel
published in Nature Communications

Global warming favours light-coloured insects in Europe

ABSTRACT

Associations between biological traits of animals and climate are well documented by physiological and local-scale studies. However, whether an ecophysiological phenomenon can affect large-scale biogeographical patterns of insects is largely unknown. Insects absorb energy from the sun to become mobile, and their colouration varies depending on the prevailing climate where they live. Here we show, using data of 473 European butterfly and dragonfly species, that dark-coloured insect species are favoured in cooler climates and light-coloured species in warmer climates. By comparing distribution maps of dragonflies from 1988 and 2006, we provide support for a mechanistic link between climate, functional traits, and species that affects geographical distributions even at continental scales. Our results constitute a foundation for better forecasting the effect of climate change on many insect groups.

INTRODUCTION

During the last decades, considerable interest has arisen in predicting the distribution of species, assemblages of species, and characteristics of assemblages under various scenarios of climate change (Hill *et al.* 2002, Walther *et al.* 2002, Elith *et al.* 2006, Lenoir *et al.* 2008). Most of these predictions, however, are based on phenomenological models (Settele *et al.* 2008) and provide little mechanistic understanding of the underlying processes (Franklin 2010). A first step towards understanding the impact of climate change is to consider traits of species that underpin the relationship between species and climate (Millien *et al.* 2006). This approach has been very successful for plants. For instance, leaf size as well as leaf shape have been used to reconstruct temperature and precipitation of paleoclimates (Wing *et al.* 2005). However, the relationships between traits of animals and climate are more difficult to identify. One trait – the body size of endotherms – may be related to climate. Bergmann's rule predicts a decrease in their body size within species and across closely related species with increasing temperature (Ashton *et al.* 2000, Blackburn & Hawkins 2004). In line with this ecogeographic rule, a recent study found that the body size of some mammals decreases with increasing temperature (Diniz-Filho *et al.* 2009). Although many physiological and local-scale studies on insects have demonstrated associations between traits and climate, to what extent such mechanistic links may impact

distribution patterns of insect species at a biogeographical scale remains largely unknown (but see Chown & Gaston 2010).

In ectotherms, the appearance of the body surface and in particular its colour value are involved in thermoregulation (Kalmus 1941). Dark-coloured ectotherms are able to increase their body temperatures above ambient air temperature more effectively than light-coloured ectotherms and therefore have an advantage in cool climates (thermal melanism hypothesis: Clusella Trullas *et al.* 2007, Kingsolver 1995). Most insects – by far the most species-rich lineage of ectotherms (Speight *et al.* 2008) – need to reach body temperatures above ambient temperature for flying, foraging, or mating (Huey & Kingsolver 1989). However, being dark is only advantageous in cool climates. In areas with high temperatures and insolation, insects need to protect themselves against overheating (Gibert *et al.* 1998). At high temperatures, ectothermic species with light colouration can be active for a longer period than species with dark colouration, and may be able to use a broader thermal range of habitats. Overall, heat avoidance and heat gain constraints lead to the prediction that insect assemblages should be dominated by dark-coloured species in cool climates and by light-coloured species in warm climates. The first hint that this holds even at a biogeographical scale was provided by E. H. Rapoport (Rapoport 1969) almost 50 years ago, when he used a rough estimate of springtail colour value to show a positive correlation of the percentage of dark-coloured species in species assemblages with latitude and altitude.

In this study, we combine recent digital image analysis and phylogenetic statistics to demonstrate that colour lightness of insects is consistently correlated to the thermal environment across Europe. We furthermore show that assemblages of dragonflies became on average lighter-coloured during the last century, which we attribute to global warming.

MATERIAL AND METHODS

Distribution data

We used distribution maps of 434 European butterfly species (Kudrna 2002). After matching these with the species illustrated in Tolman & Lewington (2009), we were able to consider 366 species across 1,825 grid cells of 50 km × 50 km. We created a database of 107 dragonfly species across 1,845 grid cells (Dijkstra & Lewington 2006; Appendix 1 Tables S1–S3). The butterfly distribution maps were presence/absence maps; the dragonfly distribution maps were outline maps.

Computer-assisted digital image analysis

Computer programs for the analysis of digital images provide possibilities to measure the colour value from scanned published illustrations (Oberholzer *et al.* 1996, Motoyoshi *et al.* 2007). We scanned ventral and dorsal butterfly wings and dragonfly bodies and converted the scanned RGB-colour illustrations into 8 bit grey values. We assigned each pixel a value between 0 (complete black) and 255 (complete white) and averaged these values across all pixels of the considered area to obtain a single colour value for each

species. Usually, software like PhotoShop weights the three RGB channels according to the perception of human eyes when converting to grey values (e.g. $0.21 \times R + 0.72 \times G + 0.07 \times B$). This is because some colours are subjectively perceived darker than others. For example, blue looks darker than green for humans at the same value of colour lightness. We decided to calculate the unweighted mean across the three RGB channels. Note that dark-coloured species have a low value and light-coloured species have a high value.

In particular, we scanned the ventral and dorsal part of the wings of female butterflies illustrated in Tolman & Lewington (2009) with an EPSON Perfection 4490 Photo Scanner with 1,200 dpi and 24 bit in the RGB colour spectrum. All further steps to estimate the colour value were done with Adobe Photoshop CS2, Adobe Photoshop Elements 2, Epson Scan Version 2.75G and ImageMagick 6.5.5-2. In our study, we predominantly considered females because their parental investment is higher and colouration of males may be biased by sexual selection (Clutton-Brock & Parker 1992). Therefore, females can be expected to be closer to a "thermal optimum". Only if an illustration of a female was not available, we used an illustration of the male (butterflies: 28 male, 338 female). Note that dorsal and ventral colour lightness was closely and significantly correlated between the sexes (t-test, $p < 0.001$ dorsal, ventral; $r^2 = 0.62$ dorsal, 0.9 ventral; $n = 387$ dorsal, 391 ventral, all available images). For butterflies, the most relevant part of the wing for thermoregulation is the wing area near the body (Wasserthal 1975). Therefore, we decided to measure the body and 1/3 of the wing area closest to the body.

We scanned the thorax and abdomen of dragonflies illustrated in Askew (1988). For some species illustrations of both sexes were not available (74 of 114). To make use of as many species as possible, we solely processed dorsal drawings of male dragonflies. In doing so, we were able to use 107 of 114 dragonfly species presented in Askew (1988). Note that in Askew (1988) female drawings are often depicted from lateral, making comparisons difficult. Note also that beside these limitations colour lightness of male and female species was significantly correlated (t-test, $p < 0.001$, $r^2 = 0.23$, $n = 40$ due to the limited availability of female drawings). For some species the thorax was not drawn because they differ from each other only in shape and colour of the abdomen. In such cases, we composed the images of the abdomen with corresponding drawings (*Lestes viridis* with the female image; *Cordulegaster heros*, *C. picta* and *C. principes* with *Cordulegaster boltoni*). All further calculations followed the procedure for butterflies.

The colour value used in our study was derived from a three-channel evaluation of wing patterns in the visible spectrum and is therefore only a proxy for the overall properties. However, a major advantage of our method is the acquisition of standardized and hence comparable colour values. But it only gives information on pigment colouration, not structural colouration or iridescence (angel-dependent colour; e.g. wings of some male Lycaenids) and values represent mean phenotypes without intra-specific variation.

We tested the robustness of our procedure to estimate the colour value and confirmed that the extracted values represent the physical ability of the species to absorb and reflect radiation energy (Appendix 1 Figs. S3–S5).

Phylogenetic analysis

We constructed phylogenies for butterflies and dragonflies (see Appendix 1 Supplementary methods). Lynch's comparative method (Lynch 1991) was used to partition the colour value of each species into a phylogenetic and a specific component. The phylogenetic component describes the part inherited from the ancestor; the specific component measures the deviation from the relatives. We decided to apply Lynch's comparative method because it not simply "corrects" for phylogeny but also partitions the trait phenotype into a phylogenetic and a non-phylogenetic part that can be used for further analyses. Furthermore, it has the advantage to allow to incorporate the phylogenetic signal (Revell 2010) and different modes of evolution (Freckleton *et al.* 2011) in its correlation structure (for details, see refs. Lynch 1991, Housworth *et al.* 2004, Hansen & Orzack 2005). Lynch's model describes the observed trait phenotype (y_i for the i_{th} taxon) as the sum of a grand phylogenetic mean (μ), a heritable component (a_i), and a residual deviation (e_i): $y_i = \mu + a_i + e_i$. The grand phylogenetic mean μ is a scaling term that can be interpreted as the state of the ancestor at the root of the phylogeny. The quantity $\mu + a_i$ is the predicted value by the phylogeny and can be interpreted as the phylogenetically heritable component of the observed phenotype of the i_{th} taxon. It is termed "phylogenetic component" in this article. Hence, the residual deviation e_i from the phylogenetic component is termed "specific component". Subsequently, we calculated for each grid cell in Europe the mean values of the phylogenetic and specific components of the subset of the occurring species. In addition, we employed phylogenetic eigenvector regression (Diniz-Filho *et al.* 1998) to disentangle the phylogenetic and the specific components of colour lightness and found similar results (not shown).

Using Lynch's comparative method, we addressed three fundamental evolutionary assumptions of phylogenetic approaches, namely that the phylogeny is constructed without error (phylogenetic uncertainty), that more closely related species tend to show more similar characteristics than expected by chance (phylogenetic signal), and that the evolutionary model used is appropriate (evolutionary model uncertainty, Hernández *et al.* 2013).

Phylogenetic uncertainty

We resolved the multifurcations in our trees within a Bayesian framework using R and BEAST (Drummond *et al.* 2012) as described in Kuhn *et al.* (2011) (see also Ronquist 2004, Avaria-Llautureo *et al.* 2012, de Villemereuil *et al.* 2012, Pennell & Harmon 2013 for recent developments in the field). We calculated two maximum clade credibility trees with mean node heights for butterflies and dragonflies and used these trees in subsequent phylogenetic analyses. To give more probabilistic support to our results, we randomly draw 1,000 trees from the BEAST output, repeated the analyses and checked for the robustness of the results (see inserted histograms in Figs. 6.1.1.1 and 6.1.1.3). In

particular, we calculated the phylogenetic signal lambda (Pagel 1999, 2002) and used this value to scale the phylogenetic correlation matrix (see Revell 2010, Capellini *et al.* 2010, 2011 for examples within a PGLS framework) in Lynch's comparative method for each tree (see Lynch 1991 and functions `corPagel` and `compar.lynch` in the R package *ape*, Paradis *et al.* 2004). Here, the correlation structure followed a Brownian motion model with the off-diagonal elements multiplied by lambda.

Phylogenetic signal

We calculated the phylogenetic signal (lambda) for every tree with `fitContinuous` in the R package *geiger* (Harmon *et al.* 2008) version 1.99-3 and found high values of lambda, with higher values for butterflies than for dragonflies (median and mean > 0.80, Appendix 1 Fig. S6). This indicates that related species have more similar colour lightness than expected by chance and hence the phylogenetic variance-covariance matrix needs to be scaled by lambda (Revell 2010). When compared to the phylogenetic signal in body size – which we assume to be a key niche trait – there was even no significant difference to the ventral colour lightness of butterflies. However, potential micro-evolutionary causes of this pattern, e.g. gene flow, pleiotropy, or lack of genetic variability cannot be distinguished within the framework of this study.

Evolutionary model uncertainty

The resulting correlations of the trait values of species in the phylogeny can be seen as a function of an evolutionary model-specific transformation of the branch lengths of the original phylogeny. Following Hernández *et al.* (2013), a fundamental assumption of phylogenetic analyses is that the model of character evolution effectively recapitulates their history, and this implies to compare several evolutionary models. Hence, to support our main conclusions that i) colour lightness is consistently correlated to the thermal environment and that ii) dragonfly assemblages became on average lighter-coloured during the last century, we simultaneously accounted for evolutionary model and phylogenetic uncertainty. However, we would like to highlight that it is not our intention to make statements about the most adequate model for the evolution of colour lightness in butterflies and dragonflies, so we arbitrarily chose four evolutionary models to underline the robustness of our conclusions: i) The model 'lambda' (Pagel 1999, 2002) fits the extent to which the phylogeny predicts covariance among trait values for species by multiplying the off-diagonal elements in the correlation structure by the value of lambda. Interpreted as a tree transformation, values of lambda near 0 cause the phylogeny to become more star-like, and a lambda value of 1 recovers the Brownian motion model. Bounds for the estimation of lambda were set to 0 and 1. This model was used for the results presented in the main text. ii) In the model 'kappa' (Pagel 1999), character divergence is related to the number of speciation events between two species. Interpreted as a tree transformation, the model raises all branch lengths to the power kappa. Bounds for the estimation of kappa were set to 0 and 1. iii) The Early-burst (EB, Harmon *et al.* 2010) or also called ACDC (Blomberg *et al.* 2003) model is a model where the rate of evolution increases or decreases exponentially through time. Bounds for the estimation of the scaling parameter

a were set to -10 and 10. iv) The Ornstein-Uhlenbeck model (OU, Butler & King 2004) fits a random walk with a central tendency and an attraction strength proportional to the parameter alpha. Bounds for the estimation of alpha were set to 0 and 105.

Model parameters were estimated for 1,000 randomly selected phylogenetic trees in each model with the function `fitContinuous` in the R package *geiger* (Harmon *et al.* 2008) version 1.99-3. All trees were transformed with their estimated parameters (function `transform.phylo`) and used to partition colour lightness into a phylogenetic and a specific component with Lynch's comparative method (Lynch 1991). For each tree, we calculated for each grid cell in Europe the mean values of the phylogenetic and specific components of the subset of the occurring species and regressed these values against our thermal component 1. Regarding our main conclusion i) that colour lightness is consistently correlated to the thermal environment, we found that all 24,000 regressions were highly significant (t-test, $p < 0.001$) with positive slopes throughout the models, indicating that different evolutionary models do not alter the positive relationship between colour lightness and the thermal environment (Appendix 1 Fig. S7).

To give more probabilistic support to our main conclusion ii) that dragonfly assemblages became on average lighter-coloured during the last century, we followed the procedure described above and calculated for each grid cell in Europe the mean value of the phylogenetic and specific components according to the dragonfly distributional information of 1988 and 2006. We calculated the change in colour lightness for each grid cell and averaged these changes across Europe for each tree and evolutionary model. We found the majority of values to be positive, indicating that different evolutionary models do not alter the overall shift towards lighter-coloured dragonfly assemblages (Appendix 1 Fig. S8).

Environmental variables

For each grid cell across Europe, 25 environmental variables were extracted from public resources using Grass GIS version 6.4.0RC6. Since data were not available for all grid cells, the dataset was reduced to grid cells for which information was available for all environmental variables (1,825 for butterflies, 1,845 for dragonflies). i) Yearly sum of solar irradiation on horizontal surface (INS): In the data source used (Šúri *et al.* 2007, `http://re.jrc.ec.europa.eu/pvgis/download/download.htm`), atmospheric corrections were already applied to account for spatial differences in e.g. average cloud cover, atmospheric turbidity and shadowing by the local terrain. ii) Temperature-related variables: We used annual mean temperature (AMT), mean diurnal temperature range (MDTR), isothermality (IT), temperature seasonality (TS), maximum temperature of warmest month (MTWM), minimum temperature of coldest month (MTCM), temperature annual range (TAR), mean temperature of wettest quarter (MTWeQ), mean temperature of driest quarter (MTDQ), mean temperature of warmest quarter (MTWaQ) and mean temperature of coldest quarter (MTCQ) to characterize the thermal environment. Data were taken from BioClim (Hijmans *et al.* 2005), available at `http://www.worldclim.org/current`. iii) Precipitation-related variables: Since actual

evapotranspiration is reported to be a strong predictor of species richness of European butterflies (Hawkins *et al.* 2003), we considered annual precipitation (AP), precipitation of wettest month (PWM), precipitation of driest month (PDM), precipitation seasonality (PS), precipitation of wettest quarter (PWeQ), precipitation of driest quarter (PDQ), precipitation of warmest quarter (PWaQ) and precipitation of coldest quarter (PCQ). Data were taken from BioClim (Hijmans *et al.* 2005), available at `http://www.worldclim.org/current`. iv) Topography-related variables: Since precipitation and ambient temperature are related to topography, we used five measures describing the topography within each grid during subsequent analyses: average elevation (AE), lowest elevation (LE), highest elevation (HE), elevation range (ER) and standard deviation of elevation (SDE). Data are available at `http://www.biochange-lab.eu/files/europe50km.zip` (Nogues-Bravo & Araujo 2006).

Statistical analysis

Our primary interest was to identify the strongest predictors of the mean colour value of butterfly or dragonfly species co-occurring within grids across Europe. Therefore, we applied a principal component analysis (PCA) based on the correlation matrix to reduce the dimensionality in the environmental variables (Wold *et al.* 1987). We applied separate PCAs for the categories thermal environment, precipitation, and topography. Of course this leads to correlated components across the separate PCAs. The idea was, however, to characterize the different aspects of the climate and naturally the different aspects are correlated (e.g. topography and thermal environment). The PCAs extracted two components with eigenvalues > 1 from the categories temperature and insolation, which characterize the thermal environment of the respective grid cell (Thermal 1 and 2), as well as two components for precipitation (Prec 1 and Prec 2) (Appendix 1 Table S5). The PCA extracted one eigenvector (Topo) for the topography (Appendix 1 Table S5). For temperature and precipitation, the first axis (called Thermal 1 and Prec 1) characterized the overall mean, and the second axis (Thermal 2, Prec 2) characterized the variability of temperature or precipitation across the year. Since the directions of ecological associations between response and predictors in models with PCs are difficult to interpret because the sign of axis scores is arbitrary, we plotted selected variables of each category for illustration (Appendix 1 Fig. S9).

We calculated multiple regression models using the phylogenetic and specific components of the mean colour value within grids and the principal components of the environmental variables. Assumptions of an ordinary least-squares regression are that the standard deviation of the error term is constant over all values of the response, and that explanatory variables and all estimates provide equally precise information. Because the standard error of the calculated mean colour value of assemblages within grids differed in relation to the number of species recorded within grids, using the number of species per grid cell as weights to the least-squares regression improves parameter estimation (see also Olalla-Tárraga *et al.* 2010). To further improve the information quality of the data, we included only grid cells in the analysis of butterflies with more than 5 recorded species.

To account for spatial autocorrelation in the model residuals after fitting the environmental components to the data, we build spatial simultaneous autoregressive models (SAR), where the error term is predefined from a spatial neighbourhood matrix and autocorrelation in the dependent variable is modelled within a generalized least-squares framework using maximum likelihood (Kissling & Carl 2008). Calculations were performed with the function `errorsarlm` in the R package *spdep* version 0.5-65 for neighbours within 1,000 kilometres distance.

We applied hierarchical partitioning (HP) to assess whether multicollinearity between the PCs of the different categories might affect the results. HP decomposes the variation contained in a response variable into independent parts, which reveal the absolute importance of each predictor by considering all possible variable combinations in a hierarchical multivariate regression setting (Chevan & Sutherland 1991). Calculations were performed in R with the package hier.part version 1.0-3 using R^2 as the goodness-of-fit measure. We also included phylogenetic and specific parts of body size (digitally measured fore-wing length of butterflies and body length of dragonflies) in the analysis. We found qualitatively similar results compared to the findings of Table 6.1.1.1, indicating that multicollinearity and body size does not change our main results (Appendix 1 Fig. S10).

All statistical analyses were performed with the software R (`www.r-project.org`). Intensive calculations were conducted using the MaRC2 High Performance Computing cluster in Marburg.

Change in dragonfly colour lightness

To analyse changes in the colour value of dragonfly assemblages, we compared the data used in our statistical analysis (distribution of 2006, Dijkstra & Lewington 2006) to maps of the distributions prior to 198837 and plotted the distribution of the shift in colour lightness within grids and the average shift across all grids for multiple phylogenetic trees (Fig. 6.1.1.3). In addition, we extracted climatic data corresponding to the two distribution data sets of dragonflies in Europe from public resources (CRU TS3.2, Harris *et al.* 2014). We calculated the mean annual temperature for the period 1900–1988 and for 1988–2006, determined the change in annual mean temperature between these two periods, and correlated this change to the change in colour lightness of dragonfly assemblages (Fig. 6.1.1.4). We found positive correlations (t-test, $p < 0.001$) for the raw data, the phylogenetic component and the specific component of colour lightness. Correlations using SAR models to incorporate spatial autocorrelation were also all significant (t-test, $p < 0.05$) and positive. This indicates that dragonfly assemblages became lighter-coloured in regions where temperature increased during the last century, giving further support to our conclusion that climate warming favours light-coloured insects in Europe.

RESULTS

Mechanistic adaptation of species to climate

Using a data set of nearly all European butterfly and dragonfly species, we show that insect species with dark and light colouration have advantages in cool and warm climates, and that this mechanistic adaptation of species to climate shapes the biogeographical patterns of species distributions. The results were obtained by measuring the colour value of the body and the dorsal and ventral basal wing areas of 366 butterfly species occurring in Europe using computer-assisted digital image analysis and the colour value (further on called colour lightness) of the body of 107 dragonfly species of Europe. The dorsal surface of butterflies was on average darker than the ventral surface, and the colour lightness strongly differed between families in both groups (Appendix 1 Figs. S1 and S2, Tables S1 and S2). All statistical approaches consistently showed that the mean colour lightness of assemblages increased with a synthetic variable that characterized the thermal environment within grids, i.e. colour lightness increased with increasing temperature (Fig. 6.1.1.1, Table 6.1.1.1). These results support a mechanistic link between climate and functional traits of species and indicate that an ecophysiological phenomenon can cause noticeable biogeographical patterns of insects (Fig. 6.1.1.2).

Phylogenetic and specific components of colour lightness

The colour lightness of each species has two components: a phylogenetic component and a species-specific component. The phylogenetic component is in part determined by the response of the ancestors to paleoclimates, while the species-specific response measures the deviation of each species from the ancestors. The latter component is therefore the more recent response of species to environmental factors. When we considered these two components, we found a clear decrease in the mean colour lightness of insect assemblages for both the mean phylogenetic components and the species-specific components across Europe from the Mediterranean to northern areas. This result was consistent for the ventral and dorsal surfaces of butterflies and for the dorsal surface of dragonflies (Figs. 6.1.1.1 and 6.1.1.2, Table 6.1.1.1).

Impact of climate change on insects

If colour lightness of assemblages across Europe is a reaction to climate, we would thus expect that climate change would lead to changes in the colour lightness of insect assemblages. To test this hypothesis, we compared recent distributional data of dragonflies with data prior to 1988 and plotted the shift of colour lightness within grids (Fig. 6.1.1.3). After correcting for phylogeny, we found a general shift towards lighter-coloured assemblages across Europe (Fig. 6.1.1.3c). In the phylogenetic component (Fig. 6.1.1.3b), we found shifts towards darker assemblages along the western margin of Europe, the Alps, and the Balkans. However, the variation in the phylogenetic component still contains a portion of "phylogenetically structured environmental variation" (Desdevises *et al.* 2003). In addition, the change in colour lightness was positively correlated to the change in annual mean temperature between the time periods corresponding to the distribution data (Fig. 6.1.1.4). However, we would like to stress that the strength of the shift depends on

the models used. Alterations in the distributions of species result in a complex spatial pattern in the colour lightness of assemblages, which is a clear indication that the response of assemblages of organisms to climate change does not lead to simple one-dimensional shifts (see e.g. the Alps and the Scandinavian Mountains).

Table 6.1.1.1: Multiple regression models between characteristics of the climate within grid cells and mean phylogenetic and specific components of butterfly and dragonfly colour lightness in Europe. For each model, the standardized coefficients of the predictors (slope) and the coefficient of determination (R^2) are shown. Results from weighted least-squares regressions with species richness in each grid cell as the weighting factor and models to account for spatial autocorrelation are also provided (spatial). Therm 1 and Therm 2: first two principal components of temperature- and insolation-related variables (Appendix 1 Table S5). Prec 1 and Prec 2: first two principal components of precipitation-related variables. Topo 1: first principal component of variables related to the terrain topography. Positive coefficients indicate an increase in the components (warmer, higher temperature seasonality, wetter, higher precipitation seasonality, higher elevation), negative values a decrease (butterflies: n = 1,825; dragonflies: n = 1,845). * = significant at $p < 0.001$ (t-test).

				Phylogenetic component					
	Model	Weight	Surface	Therm 1	Therm 2	Prec 1	Prec 2	Topo 1	R^2
Butterflies	Env	none	dorsal	0.44*	0.02	−0.13*	−0.22*	−0.07	0.34
			ventral	0.67*	0.16*	−0.08*	−0.01	0.11*	0.53
	Env	richness	dorsal	0.36*	−0.05	−0.16*	−0.20*	−0.04	0.41
			ventral	0.58*	0.09*	−0.12*	−0.03	0.10*	0.56
	Env (spatial)	none	dorsal	0.51*	0.07	−0.03	−0.05	−0.08*	0.38
			ventral	0.71*	0.07	−0.10*	0.02	0.10*	0.54
Dragonflies	Env	none	dorsal	0.73*	0.46*	0.06	0.07*	0.14*	0.71
	Env	richness	dorsal	0.64*	0.39*	0.06*	0.05*	0.12*	0.64
	Env (spatial)	none	dorsal	0.57*	0.24*	−0.11*	−0.06*	0.11	0.79

				Specific component					
	Model	Weight	Surface	Therm 1	Therm 2	Prec 1	Prec 2	Topo 1	R^2
Butterflies	Env	none	dorsal	0.20*	0.15*	−0.21*	−0.15*	0.25*	0.24
			ventral	0.60*	0.12*	−0.01	−0.02	0.15*	0.41
	Env	richness	dorsal	0.31*	0.09*	−0.18*	−0.10*	0.21*	0.35
			ventral	0.57*	0.06*	−0.05	−0.02	0.17*	0.52
	Env (spatial)	none	dorsal	0.22*	0.23*	−0.10	−0.05	0.19*	0.29
			ventral	0.58*	0.00	−0.07	−0.02	0.12*	0.43
Dragonflies	Env	none	dorsal	0.55*	0.30*	−0.14*	0.01	0.21*	0.51
	Env	richness	dorsal	0.58*	0.25*	−0.09*	0.02	0.16*	0.49
	Env (spatial)	none	dorsal	0.38*	0.16*	−0.25*	−0.10*	0.16*	0.55

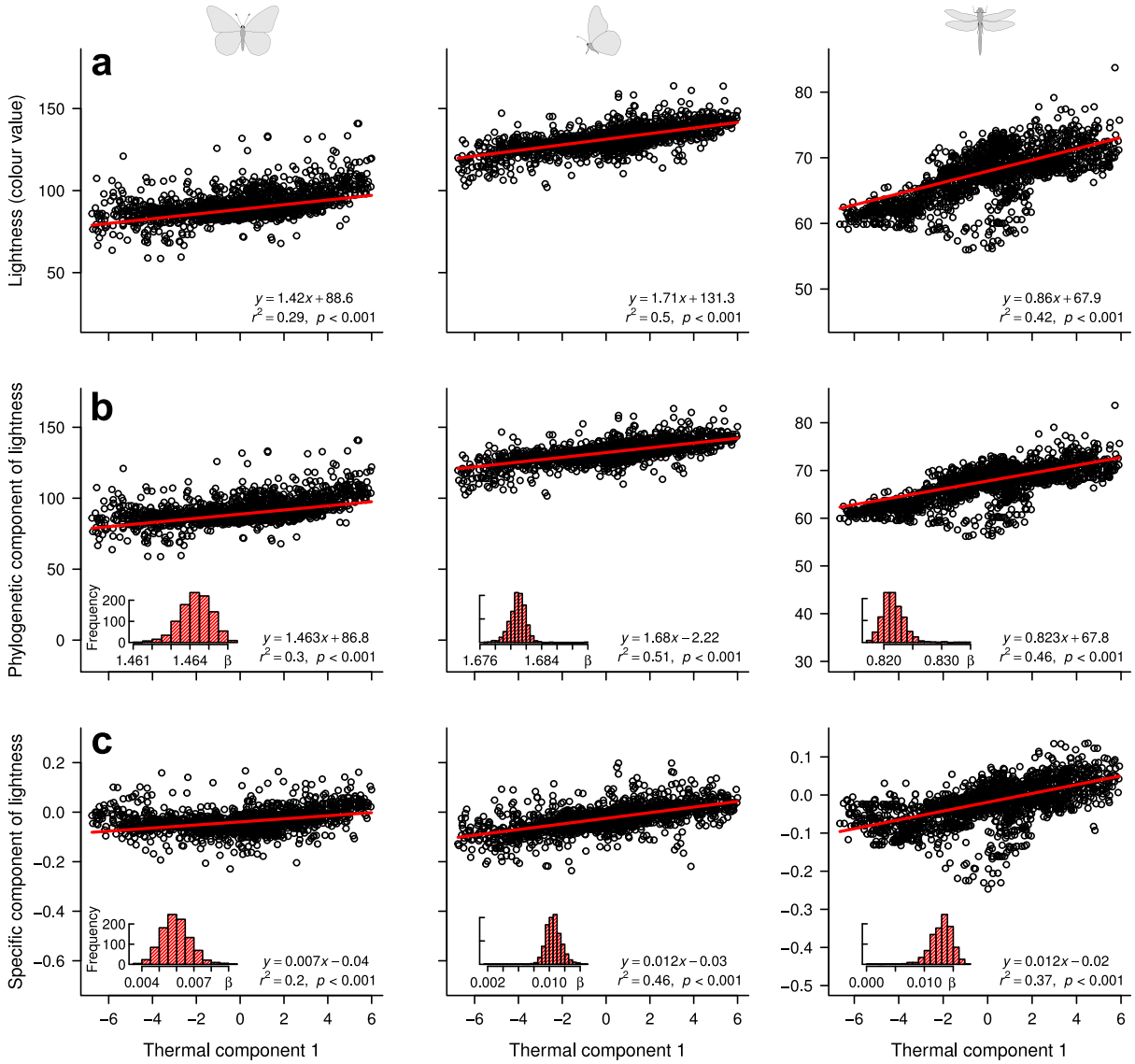

Figure 6.1.1.1: Scatterplots of the different measures of the average colour value of butterfly and dragonfly assemblages versus our thermal component 1. This component is a variable that summarizes the thermal environment within each grid from cool (low values) to warm (high values). Note that for all measures of colour value as well as for the two groups of insects, the colour lightness of assemblages is consistently correlated to the thermal environment. (**a**) Average colour values across species on a scale from 0 (black) to 255 (white). (**b**) Average phylogenetic component, i.e. the predicted colour value of each species based on the phylogeny. (**c**) Average specific component, i.e. the deviation from the colour value expected by the phylogeny. Parameters are from univariate regression models weighted with the number of species in each grid cell (butterflies: n = 1,825; dragonflies: n = 1,845). The inserted histograms in lines b and c show the distribution of regression slopes (β) calculated for 1,000 alternative phylogenetic trees. These regressions were all highly significant (t-test, $p < 0.001$) with positive slopes throughout, indicating that the positive relationship between colour lightness and the thermal environment is robust to uncertainties in the phylogenetic trees.

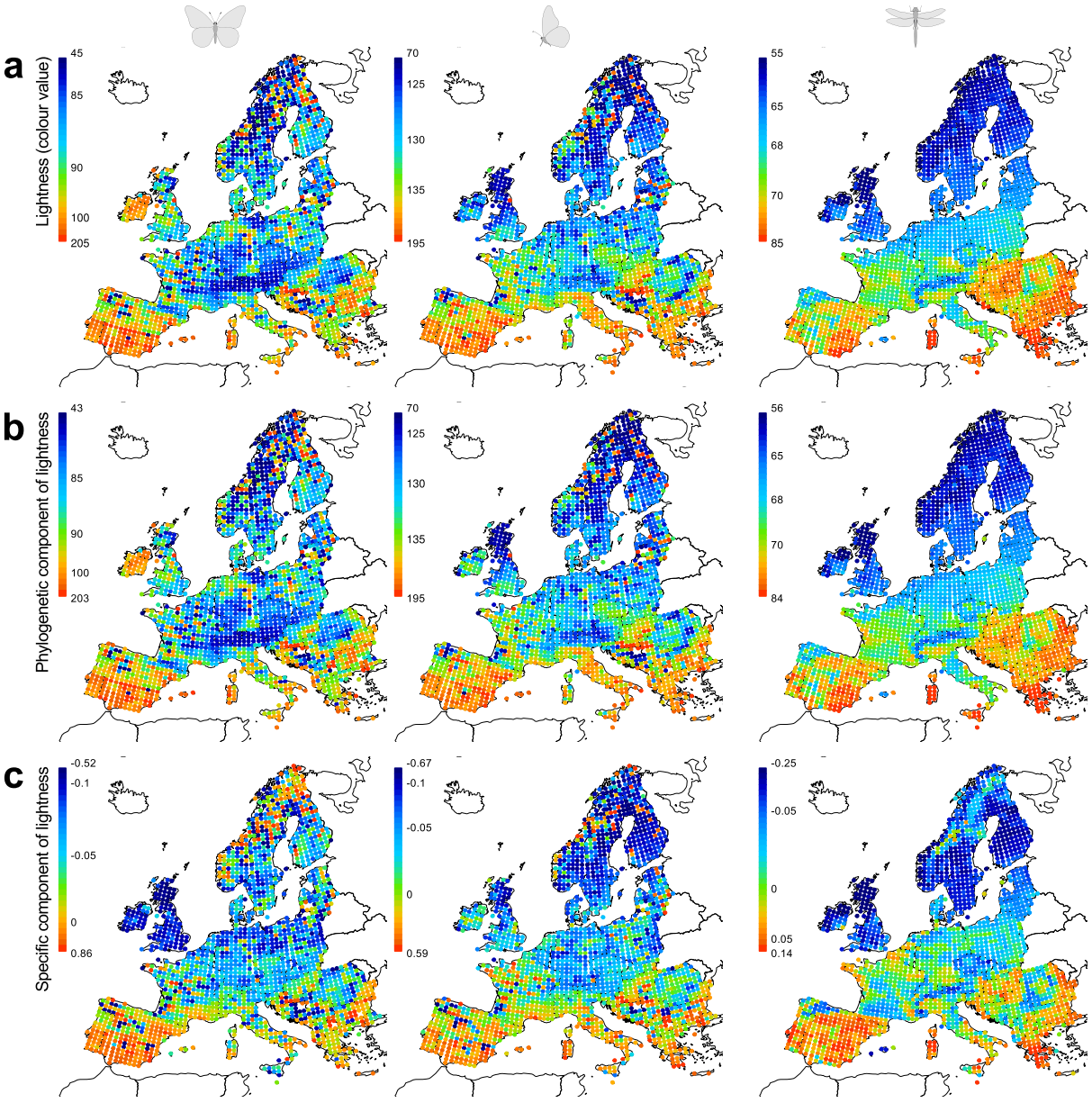

Figure 6.1.1.2: Average colour value of butterfly and dragonfly species in Europe.
(**a**) Average colour values across species on a scale from 0 (black) to 255 (white). (**b**) Average phylogenetic component, i.e. the predicted colour value of each species based on the phylogeny. (**c**) Average specific component, i.e. the deviation from the colour value expected by the phylogeny. The colour code of the individual maps is adjusted according to the specific values of each grid following equal-frequency classification; red indicates light-coloured assemblages and blue indicates dark-coloured assemblages (butterflies: n = 1,825; dragonflies: n = 1,845). For the raw colour value, the phylogenetic as well as the specific component, we found a decrease from assemblages dominated by light-coloured species in the south to assemblages dominated by dark-coloured species in the north.

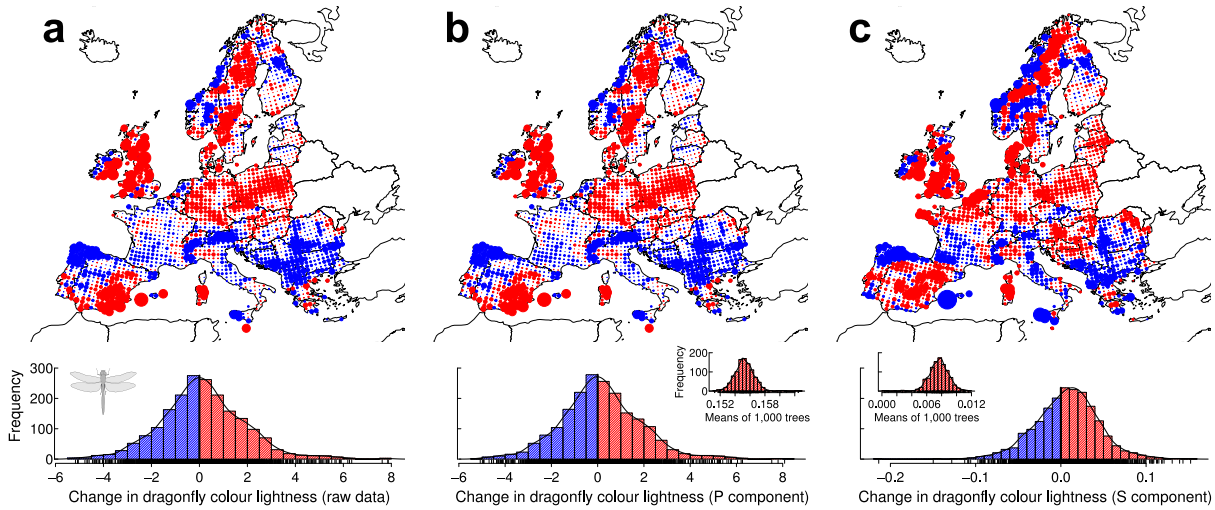

Figure 6.1.1.3: Shift in the mean colour value of dragonfly assemblages across Europe between 1988 and 2006. Shift in colour value for (**a**) the raw data; (**b**) the phylogenetic component (P); and (**c**) the specific component (S). Red indicates an increase in colour lightness; blue indicates a decrease in colour lightness. The diameter of each dot indicates the extent of the shift (n = 1,845). The distribution of the shifts shows for the specific component a clear trend towards higher (i.e. lighter-coloured) values (peak of the distribution positive; zero indicated by black line). The phylogenetic component suggests that the shifts in colour lightness have a strong phylogenetic background leading to a complex geographic mosaic in the response of assemblages to climate change. The inserted histograms show the mean change in colour lightness calculated for 1,000 alternative phylogenetic trees and are positive throughout, indicating that uncertainties in the phylogenetic hypotheses are unlikely to affect our conclusion of a general shift towards lighter assemblages. The distributional information used in the analysis is often based on a large time span, i.e., the distributional information published in 1988 summarizes data until that year using information even from the beginning of the 20th century. Rugs at the abscissa indicate observed values.

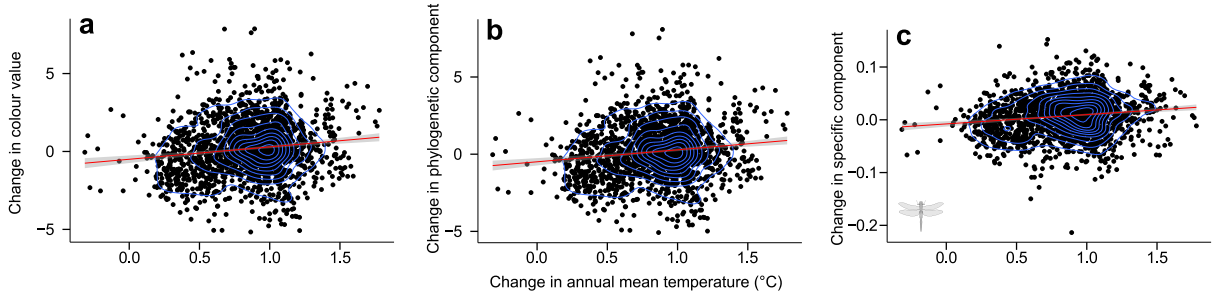

Figure 6.1.1.4: Scatterplots of the shift in colour value of European dragonfly assemblages versus the change in annual mean temperature between the periods 1900–1988 and 1988–2006. Scatterplots are presented for (**a**) the raw data; (**b**) the phylogenetic component; and (**c**) the specific component. Each dot represents a 50 km x 50 km grid cell across Europe with density estimation (blue contours, n = 1,845). Red lines are from ordinary regressions with 95% confidence intervals (grey shade). These regressions were all highly significant (t-test, $p < 0.001$, see also Methods) with positive slopes throughout, i.e. dragonfly assemblages became lighter-coloured in regions where temperature increased during the last century.

DISCUSSION

The interpretation of our results is conditional upon the assumption that thermoregulation is the predominant selective pressure for the evolution of wing or body colouration. However, colour lightness and colouration in general may be influenced by numerous other selective forces, e.g. cryptic colouration (Kettlewell 1959) or disease and parasite resistance (Wilson *et al.* 2001). Our statistical models of butterflies showed that the positive relationship between the thermal environment and colour lightness is stronger for the ventral surface than for the dorsal surface (Table 6.1.1.1), which suggests that selective pressures other than thermoregulation are more important for the colouration of the dorsal surface than of the ventral surface. A stronger relationship between colour lightness and ventral wing surfaces is not entirely surprising since several species exclusively use the ventral surfaces for thermoregulation (e.g. Colias, Gonepteryx). Furthermore, ventral wing surfaces are additionally used for heat avoidance by reflecting insolation when wings are closed (Vielmetter 1958). Despite these other potential pressures, the strong and consistent relationships between colour lightness and thermal environment across two insect groups underline the overall importance of climate for insects.

After the last glaciation, butterflies and dragonflies recolonized wide areas of Europe, but most of the butterfly and dragonfly clades evolved prior to the Pleistocene (Speight *et al.* 2008). The latitudinal gradient of the phylogenetic component suggests that exaptations (Gould & Vrba 1982) have been of considerable importance during the assembly of northern insect faunas after the last glaciation, i.e. colour lightness might have evolved for or as a by-product of other functions in a different spatio-temporal selective regime. But the trends in the species-specific component indicate that the comparatively dark-coloured species of all clades colonized northern areas.

Our finding that the assembly of insect faunas in response to the thermal environment depends on the colour lightness of certain body parts clearly demonstrates the importance of thermal energy in structuring insect assemblages — even across larger spatial scales. This has implications in forecasting the effect of climate change (Hill *et al.* 2002, Walther *et al.* 2002, Elith *et al.* 2006, Lenoir *et al.* 2008, Settele *et al.* 2008, Franklin 2010, Kerr *et al.* 2007, Deutsch *et al.* 2008, Dillon *et al.* 2010). With global warming, we would thus expect that dark-coloured insects will shift their distribution and possibly retreat from certain areas (Franco *et al.* 2006) and/or on a smaller scale will shift their habitat preference to more shady conditions (Pateman *et al.* 2012). The latter scenario has consequences for conservation strategies — conservation efforts directed exclusively toward current habitat preferences without taking into consideration the effect of ecophysiological adaptation may be futile for the future as the climate changes.

Colour lightness of dragonfly assemblages across North America and Europe

with
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ABSTRACT

Dark-coloured ectotherms absorb energy from the environment at higher rates than light-coloured ectotherms. The thermal melanism hypothesis (TMH) states that this physical mechanism links the colour lightness of the body surfaces of ectotherms to their thermal environment and hence to their geographical distribution. Studies on different insect taxa in Europe found support for this prediction of the TMH. However, whether these results hold also for other biogeographical regions remains unclear. Here, we quantify and map the colour lightness of dragonfly species in North America and directly compare our results to previously published findings for Europe. We estimated the colour lightness of 152 North American dragonfly species from published illustrations, compiled their distribution data from the literature and combined all these data with six biologically relevant environmental variables. We evaluated the importance of phylogenetic autocorrelation for the spatial variation of mean colour lightness of dragonfly assemblages (grid cells of approximately 50 km × 50 km size) by repeating all analyses also for the phylogenetically predicted component of the colour lightness of species and the species-specific deviation from this prediction. We also accounted for spatial autocorrelation with autoregressive error models. All statistical approaches showed that dragonfly assemblages from both continents consistently tended to be darker coloured in regions with cold climates and lighter coloured in regions with warm climates. Regression slopes, however, were significantly less steep, and the amount of variance explained by environmental variables was lower for North America than for Europe. Our results highlight the importance of colour lightness for the distribution of dragonfly species, but they also indicate that idiosyncrasies of the continents modify the general pattern.

INTRODUCTION

Climate, particularly temperature and rainfall, is the most important driver of the distribution and abundance of organisms (Currie & Paquin 1987, Hawkins *et al.* 2003, Willig *et al.* 2003, Davies *et al.* 2007, Dunn *et al.* 2009). However, the sensitivity to climatic conditions differs fundamentally between endothermic and ectothermic organisms (Buckley *et al.* 2012). In contrast to endotherms, ectotherms need to absorb energy from their abiotic environment to reach their thermal optimum (Huey & Kingsolver 1989). Consequently, given the importance of ectotherm body temperature for physiological rates (e.g. metabolic and development rate; Gillooly *et al.* 2001, 2002) and for locomotion (Huey & Kingsolver 1989), ectotherms are sensitive to the temperature regime in which they live. Heat gain and loss depends on morphological traits (e.g. body size, skin reflectance; Gates 1980 as well as physiological and behavioural traits (e.g. posture, movement between shaded and sunny microhabitats; May 1976, Chown *et al.* 2002). Therefore, those traits involved in the regulation of body temperature of ectotherms should vary along with the temperature regime of the habitat of the species.

Associations between biological traits of animals and temperature are well documented (Clusella Trullas *et al.* 2007, Chown & Gaston 2008). For example, recently we have shown that the mean lightness of wing or body colour of assemblages of butterflies and dragonflies decreases with decreasing environmental temperature across Europe (Zeuss *et al.* 2014, see also Schweiger & Beierkuhnlein 2016). This was interpreted to be consistent with the thermal melanism hypothesis (TMH, sensu Clusella Trullas *et al.* 2007; also known as the Bogert's rule, Bogert 1949), which states that in regions with low temperatures, darker coloured ectotherms have an advantage over lighter coloured ectotherms because they absorb more solar irradiance (Gates 1980), which leads to higher heating rates and equilibrium temperatures (Kalmus 1941, Watt 1968). By contrast, species inhabiting hot climates profit from enhanced reflectance facilitated by light colours that reduce heat load and avoid overheating (Gates 1980). So far, a number of studies have documented support for the TMH within and between ectothermic species (summarised in Clusella Trullas *et al.* 2007). The association of colour lightness of body surfaces with temperature might, however, depend also on other functions of colouration, such as camouflage, pathogen resistance, UV protection and sexual selection (Roulin 2014) as well as other mechanisms of thermoregulation (e.g. wing whirring, obelisk posture; May 1976). Even if the underlying ecological processes are universal, these other functions might offset the importance of thermal melanism for single species or localities. Therefore, broad-scale and interspecific analyses of functional traits are important supplementary approaches for understanding the generality of the mechanisms that shape species distributions (Spicer & Gaston 2009).

Our previous analysis found the first broad-scale support for thermal melanism in assemblages of odonates across Europe (Zeuss *et al.* 2014). However, other biogeographic regions differ from Europe in the geographical setting as well as in the processes influencing the assembly of local or regional faunas, e.g. during the post-glacial recolonisation

(Hewitt 2000, Swenson & Howard 2005). Consequently, further macro-scale and interspecific studies are a necessary next step to test the generality of the association between colour lightness and the distribution of odonates as proposed by the TMH.

Here, we test the prediction derived from our previous findings for Europe (Zeuss *et al.* 2014) that the geographic variation in the average colour lightness of dragonfly assemblages also in North America is positively correlated with temperature. Specifically, we predict that assemblages in colder regions are composed of more dark-coloured species, while assemblages in warmer regions are composed of more light-coloured species, which would result in a gradient of increasing colour lightness from north to south. If the mechanistic link between climate, colour lightness and physiology as proposed by the TMH is of general importance for the geographical distributions of odonates, we expected the patterns of colour lightness of the two continents to be similar despite differences in their biogeographic history and geographic setting.

MATERIAL AND METHODS

Damselflies and dragonflies are an ideal system to study the evolution and ecology of functional traits like colour lightness because they are highly dependent on ambient conditions for various activities, e.g. flight, foraging and mating (Corbet 1980). These prominent and colourful insects are globally popular among entomologists and scientists, and therefore represent one of the best-investigated insect orders (Kalkman *et al.* 2008). The long history of natural science resulted in comprehensive literature on their distribution, life histories and functional traits (Corbet 1980, Dijkstra & Lewington 2006).

Colour lightness data

To achieve a maximum of comparability, we generated the colour data exactly according to the protocol of Zeuss *et al.* (2014). We used the R package *png* to extract data on the colour lightness of the body of 152 North American dragonfly species (suborder Anisoptera, hereafter termed "dragonflies") using scans of lateral drawings (24 bit sRGB colour space, 1200 dpi resolution) published by Needham *et al.* (2000). The colour lightness of 74 European dragonfly species was taken from Zeuss *et al.* (2014). We considered only drawings of male individuals because of their availability, which is reasonable for the study as the colour lightness of the two sexes is highly correlated (Zeuss *et al.* 2014 and Appendix 2 Fig. A1). We extracted the body image from the scans and averaged the red, green and blue channels of the body colour of each species to obtain 8 bit lightness values on a scale from 0 (absolute black) to 255 (pure white; referred to as 'colour lightness'). For subspecies with different colour patterns, we averaged colour lightness estimates if no further information regarding their distribution was available (five species: *Cordulegaster dorsalis* ssp. *dorsalis/deserticola*, *Cordulegaster obliqua* ssp. *obliqua/fasciata*, *Erpetogomphus lampropeltis* ssp. *lampropeltis/natrix*, *Ophiogomphus severus* ssp. *severus/montanus*). Colour lightness estimates of both sexes of the same species (44 species in North America and 19 in Europe) as well as between drawings of

the same European species from two different data sources were significantly correlated (Appendix 2 Fig. A2; for the frequency distribution of the lightness values, Appendix 2 Fig. A3).

Distribution data

Distribution ranges of the 152 North American dragonfly species were digitalised from contour maps published in Paulson (2012) using QGIS. The polygons obtained were resampled to halfdegree grid cells with functions provided in the R package *RSAGA*. For the analysis of European dragonflies, we used the distribution data of approximately half-degree resolution for 74 European dragonfly species (Anisoptera) also used by Zeuss *et al.* (2014) (original contour maps are from Dijkstra & Lewington 2006). For both continents, grid cells with less than five species were excluded from the analysis to avoid the effect of very low sampling sizes for analyses at the assemblage level. However, this decision does not change our main results (Appendix 2 Fig. A4). Assemblage data was imported and converted to a spatial pixel data frame for the analysis with the R packages *rgdal* and *sp*. Classifications of the average colour lightness for cartographic illustrations were generated with the R package *classInt*.

Different types of distribution data have certain advantages and disadvantages for macroecological analysis. For instance, whereas spatially explicit occurrence records are likely to underestimate the true distributions of species, contour maps are interpolations of occurrences of species across potential habitats and may thus overestimate their true distributions. We therefore also processed a second dataset of 48470 occurrence records of dragonfly species of North America provided by `www.OdonataCentral.org` (Abbott 2006) in addition to the distribution data mentioned above. We harmonised these data to one-degree grid cells and repeated all analyses. The coarse resolution, large areas with no data available and overall lower geographical coverage of this second dataset, however, led us to decide not to discuss it in the main text; instead, we present parts of the results in Appendix 2 Figs. A5 and A6.

Environmental data

According to the predictions of the TMH, we decided to use three variables associated with annual and seasonal trends in ambient temperature, namely annual mean temperature (AMT), temperature seasonality (TS; i.e. the standard deviation of annual temperature multiplied by 100) and mean temperature of warmest quarter (TWaQ). We considered temperature seasonality because it captures temporal variability in temperature conditions that might be relevant for the TMH.We also included annual precipitation (AP) and precipitation of warmest quarter (PWaQ). It has been hypothesized that at least in endothermic ('warm-blooded') species precipitation is negatively related to colour lightness because melanin pigments might improve pathogen resistance under moist conditions (Gloger's rule; Roulin *et al.* 2011). However, a number of studies have also found support for the assumption that high degrees of melanisation are associated with increased desiccation tolerance, resulting in the opposite pattern (Kalmus 1941). Furthermore, we considered altitude (A) as a topographical variable that, in addition to

its negative correlation with temperature, might explain an independent proportion of the spatial variation in colour lightness because mountainous regions harbour a multitude of microhabitats (e.g. different exposures to sunlight). These variables were downloaded from `www.worldClim.org` at a resolution of 2.5 arcminutes (Hijmans *et al.* 2005; BIOCLIM ver.1.4; current condition records). We calculated mean values of these variables for polygon mask layers containing 8127 half-degree grid cells in North America (EPSG: 4326) and 1839 half-degree grid cells in Europe (EPSG: 3575). Note that small differences in grids used for North America (rectangular latitude and longitude grid) and Europe (equal areas; CGRS) influence the cartographic illustrations (see the small gaps in Fig. 6.1.2.1 that compensate for this effect in Europe) but not the results of our analyses.

Statistical analyses

Phylogenetic autocorrelation

For analysing the influence of the phylogenetic relationship of species on the geographical patterns in colour lightness, we first constructed a phylogeny for North American dragonfly species (Appendix 2 Fig. A7). Since no comprehensive phylogeny is currently available, we combined phylogenetic and taxonomic information published by Misof *et al.* (2001), Ware *et al.* (2007), Fleck *et al.* (2008), Dumont *et al.* (2010), van Tol (2013). The phylogeny of European dragonfly species was taken from Zeuss *et al.* (2014) (Appendix 2 Fig. A6). However, phylogenetic information for dragonflies is incomplete, which forced us to introduce a number of multifurcations. To evaluate the effect of this uncertainty on our results, we resolved these multifurcations of the original trees randomly 10 000 times using the function `multi2di` of the R package *ape* (Paradis *et al.* 2004). Branch lengths were estimated as the number of leaves of each subtree minus one. All of the following phylogenetic analyses were repeated for these 10 000 alternative phylogenies to assess the robustness of our results to phylogenetic uncertainty. We found a significant phylogenetic signal (Pagel's lambda; Pagel 1999) in the colour lightness of North American and European dragonfly species using functions provided in the R package *geiger* (Harmon *et al.* 2008; Appendix 2 Fig. A8). Therefore, we partitioned the total variance of the average colour lightness into a phylogenetic (P) and a specific (S) component using Lynch's comparative method (Lynch 1991), as implemented in the R package *ape* (Paradis *et al.* 2004). The P component represents the variation in colour lightness that is predicted by the phylogenetic relationships of the species, whereas the S component, added to the estimated root value μ, represents the model residuals and hence the species-specific deviation from this phylogenetic prediction. The P component can be interpreted as the outcome of macroevolutionary processes (Lynch 1991). In addition, we calculated P and S components with phylogenetic eigenvector regression (Diniz-Filho *et al.* 2011) provided in the R package *adephylo*. The results of the two approaches were highly similar (Appendix 2 Fig. A9), and therefore we present only the former.

Spatial autocorrelation

We evaluated spatial autocorrelation for colour lightness in North America and Europe with spatial correlograms generated with the R package *ncf*. We calculated Moran's I for

the residuals of the regression models (see below) and found that spatial independence, i.e. the distance beyond which the effect of spatial similarity was no longer significant, was reached at about 1400 km in North America and at about 1500 km in Europe (Appendix 2 Fig. A10).

Regression models

We analysed the importance of environmental factors for the spatial variation in colour lightness of dragonfly assemblages using several linear regression models. In the simplest analysis, we considered the average colour lightness of each assemblage as the dependent variable and environmental variables as predictors in ordinary least-squares regressions. However, residuals from these models were spatially autocorrelated (Appendix 2 Fig. A10), which can affect parameter estimates and measures characterizing the fit (Kissling & Carl 2008). Therefore, we used spatial simultaneous autoregressive error models (SAR), as implemented in the R package *spdep*, to fit a spatial dependency weight using the distances at which spatial independence was reached as upper boundaries in an Euclidean distance classification. To compare regression slopes between the two continents, we pooled the data for North America and the data for Europe into a single dataset and analysed whether the interaction between the treatment variable – continent – and the predictor variables was significant in ordinary least-squares regressions. In this analysis, we corrected for spatial autocorrelation structures using generalized additive models that include a trend surface instead of SAR because region-specific differences in spatial autocorrelation of broad-scale patterns in colour lightness across North America and Europe may be confounded when data for both continents are pooled. SAR would use a single distance matrix for both continents as a spatial weight, whereas generalized additive models enable to fit a smoothed term for the coordinates as a predictor variable. Effective degrees of freedom of the smooth terms were estimated (i.e. not fixed) to obtain best fit. Slopes of single regression models between continents were compared for all environmental variables. For comparison of slopes of multiple regression models between continents, however, only the variables mean temperature of the warm- est quarter, mean altitude and annual mean precipitation were selected to minimize multicollinearity. We chose these three out of the six considered environmental variables because each highly contributed to one of three principal components (Appendix 2 Table A1). Principal components were calculated based on a correlation matrix without rotations using functions of the R package *psych*.

Hierarchical partitioning

Although each of the selected environmental variables is informative, some are closely correlated with another variables (e.g. AMT and TWaQ, Appendix 2 Table A1). Therefore, we decomposed the conjoined, i.e. multicollinear, contribution from the independent contribution of each predictor variable using a hierarchical partitioning analysis with functions of the R package *hier.part*. This method allows us to identify the environmental variables with the highest independent effects on the spatial variation in average colour lightness of dragonfly assemblages (Chevan & Sutherland 1991).

RESULTS

Dragonfly assemblages in both North America and Europe were composed of more light-coloured species in the south and more dark-coloured species in the north (Fig. 6.1.2.1; for a map of the phylogenetic (P) and specific (S) components, Appendix 2 Fig. A11). Nevertheless, the geographical patterns of colour lightness differed in detail between the two continents. In Europe, the cline was almost perpendicular to latitude, whereas in North America, colour lightness changed roughly from southwest to northeast (Fig. 6.1.2.1; Appendix 2 Fig. A11). The results for North America were consistent across the two different types of distribution data (occurrence records and contour maps of distribution ranges) and resolutions (Appendix 2 Figs. A5 and A6). In both North America and Europe, colour lightness and environmental variables were significantly correlated (Table 6.1.2.1, Fig. 6.1.2.2 and Appendix 2 Fig. A12) and the temperature variables AMT and TWaQ were the most important predictors in all statistical models. These results were also supported by hierarchical partitioning analysis (Fig. 6.1.2.3). The tendencies of the correlation trends of the two continents were very similar (Table 6.1.2.1), but the explained variance was slightly higher for models of Europe ($0.69 \leq R^2 \leq 0.84$) than for models of North America ($0.58 \leq R^2 \leq 0.77$). Furthermore, except for annual mean precipitation, the slopes of multiple and single regressions between colour lightness and environmental variables were significantly less steep across North America than across Europe (Table 6.1.2.2 and Appendix 2 Table A2).

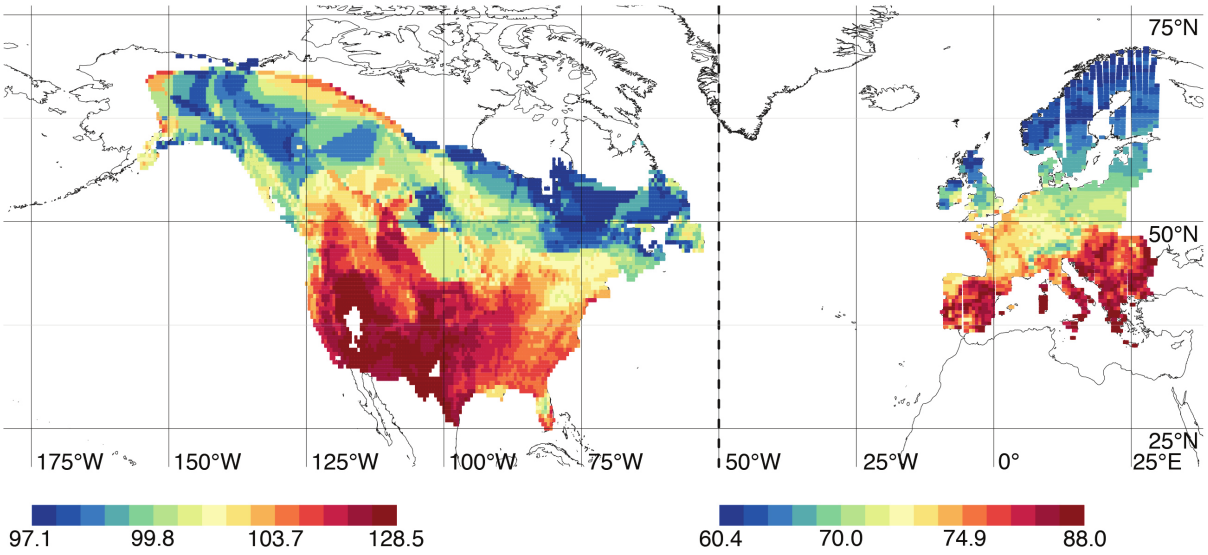

Figure 6.1.2.1: Map of the spatial variation in average colour lightness of North American and European dragonfly assemblages. Colour lightness ranges from 0 (absolute black) to 255 (pure white). Colour scale intervals follow an equal-frequency classification, ranging from blue (darkest) to red (lightest). Because of different data sources for North America and Europe, only the classes but not the values can be directly compared. The datasets comprise 8127 half-degree grid cells in North America and 1839 approximately half-degree grid cells in Europe (EPSG: 4326 and EPSG: 3537; rectangular latitude and longitude grid).

Table 6.1.2.1: Regression models between different measures of colour lightness of 8127 North American and 1839 European dragonfly assemblages and environmental variables. The proportion of explained variance from single (r^2) and multiple regression (R^2) models with all six environmental variables for average colour lightness and the P and S components are given. The P component represents the phylogenetically predicted part of colour lightness averaged across 10 000 alternative phylogenetic trees; the S component represents the respective deviation from the P component. In addition, models calculated with a spatial dependency weight (SAR) are given. Variables: AMT, annual mean temperature; TS, temperature seasonality; TWaQ, mean temperature of the warmest quarter; A, altitude; AP, mean annual precipitation; and PWaQ, mean precipitation of the warmest quarter. The highest and second highest r^2 values are in boldface. All results were significant at $p < 0.001$. +/–, tendency of the relationship (positive/negative); [1] = Nagelkerke pseudo-r^2/R^2 based on maximum likelihood.

Model characteristics			r^2						
Colour lightness	Weight	Continent	AMT	TS	TWaQ	A	AP	PWaQ	R^2
Average	None	N. America	**+0.48**	–0.26	**+0.45**	+0.13	–0.01	–0.06	0.77
		Europe	**+0.67**	–0.10	**+0.64**	+0.01	–0.01	–0.17	0.82
Average	SAR[1]	N. America	**+0.68**	–0.62	**+0.81**	–0.62	+0.62	–0.63	0.77
		Europe	**+0.77**	–0.55	**+0.76**	–0.54	–0.56	–0.68	0.84
P component	None	N. America	**+0.33**	–0.23	**+0.28**	+0.12	–0.00	–0.06	0.58
		Europe	**+0.69**	–0.11	**+0.64**	+0.01	–0.01	–0.17	0.82
P component	SAR[1]	N. America	**+0.56**	–0.52	**+0.55**	–0.50	–0.50	–0.50	0.61
		Europe	**+0.78**	–0.56	**+0.76**	–0.54	–0.55	–0.66	0.84
S component	None	N. America	**+0.48**	–0.23	**+0.47**	+0.10	–0.01	–0.05	0.74
		Europe	**+0.53**	–0.01	**+0.55**	+0.01	–0.03	–0.17	0.69
S component	SAR[1]	N. America	**+0.63**	–0.58	**+0.65**	+0.58	–0.60	–0.60	0.74
		Europe	**+0.63**	–0.45	**+0.65**	–0.45	–0.50	–0.61	0.72

Table 6.1.2.2: Individual slopes and intercept from single regression models between colour lightness of 8127 North American and 1839 European dragonfly assemblages and environmental variables. Models were controlled for spatial autocorrelation using trend surface generalized additive models, i.e. a smoothing term for longitude and latitudes (effective degrees of freedom = 29). Differences in slopes between the two continents that were significant at $p < 0.001$ are shaded grey. Variables: AMT, annual mean temperature; TS, temperature seasonality; TWaQ, mean temperature of the warmest quarter; A, mean altitude; AP, mean annual precipitation; and PWaQ, mean precipitation of the warmest quarter. The steepest and second steepest slopes are in boldface. All results, except for the relationship of colour lightness of North American dragonflies and PWaQ that was corrected for spatial autocorrelation, were significant at $p < 0.001$.

		Individual slopes ± SE		
Model	Variable	North America	Europe	Intercept ± SE
None	AMT	$\mathbf{4.6 \times 10^{-1}}$	$\mathbf{1.1 \times 10^{0}}$	1.0×10^{2}
		$\pm \mathbf{5.2 \times 10^{-3}}$	$\pm \mathbf{1.9 \times 10^{-2}}$	$\pm 4.5 \times 10^{2}$
	TS	-9.0×10^{-4}	-1.4×10^{-3}	1.1×10^{2}
		$\pm 1.8 \times 10^{-5}$	$\pm 7.9 \times 10^{-5}$	$\pm 1.9 \times 10^{-1}$
	TWaQ	$\mathbf{6.3 \times 10^{-1}}$	$\mathbf{1.3 \times 10^{0}}$	9.2×10^{1}
		$\pm \mathbf{7.7 \times 10^{-3}}$	$\pm \mathbf{2.4 \times 10^{-2}}$	$\pm 1.3 \times 10^{-2}$
	A	3.2×10^{-3}	1.9×10^{-3}	1.0×10^{2}
		$\pm 9.9 \times 10^{-5}$	$\pm 3.3 \times 10^{-4}$	$\pm 8.4 \times 10^{2}$
	AP	-1.1×10^{-3}	-2.7×10^{-3}	1.0×10^{2}
		$\pm 1.3 \times 10^{-4}$	$\pm 4.7 \times 10^{-4}$	$\pm 1.1 \times 10^{-1}$
	PWaQ	-1.4×10^{-2}	-3.2×10^{-2}	1.1×10^{2}
		$\pm 5.9 \times 10^{-4}$	$\pm 1.5 \times 10^{-3}$	$\pm 1.4 \times 10^{-1}$
Corrected	AMT	$\mathbf{2.4 \times 10^{-1}}$	$\mathbf{2.8 \times 10^{-1}}$	9.5×10^{1}
		$\pm \mathbf{1.5 \times 10^{-2}}$	$\pm \mathbf{2.4 \times 10^{-2}}$	$\pm 6.2 \times 10^{-2}$
	TS	3.3×10^{-4}	-2.0×10^{-5}	9.4×10^{2}
		$\pm 2.9 \times 10^{-5}$	$\pm 9.1 \times 10^{-5}$	$\pm 2.8 \times 10^{-1}$
	TWaQ	$\mathbf{2.9 \times 10^{-1}}$	$\mathbf{2.6 \times 10^{0}}$	9.2×10^{1}
		$\pm \mathbf{1.3 \times 10^{-2}}$	$\pm \mathbf{2.3 \times 10^{-2}}$	$\pm 2.0 \times 10^{-1}$
	A	-3.7×10^{-4}	-2.3×10^{-3}	1.0×10^{2}
		$\pm 7.9 \times 10^{-5}$	$\pm 1.6 \times 10^{-4}$	$\pm 8.4 \times 10^{2}$
	AP	-1.1×10^{-3}	-3.0×10^{-3}	9.8×10^{1}
		$\pm 9.7 \times 10^{-4}$	$\pm 2.3 \times 10^{-4}$	$\pm 6.8 \times 10^{-2}$
	PWaQ	-1.4×10^{-2}	-1.3×10^{-2}	9.7×10^{1}
		$\pm 5.9 \times 10^{-4}$	$\pm 9.0 \times 10^{-4}$	$\pm 1.0 \times 10^{-1}$

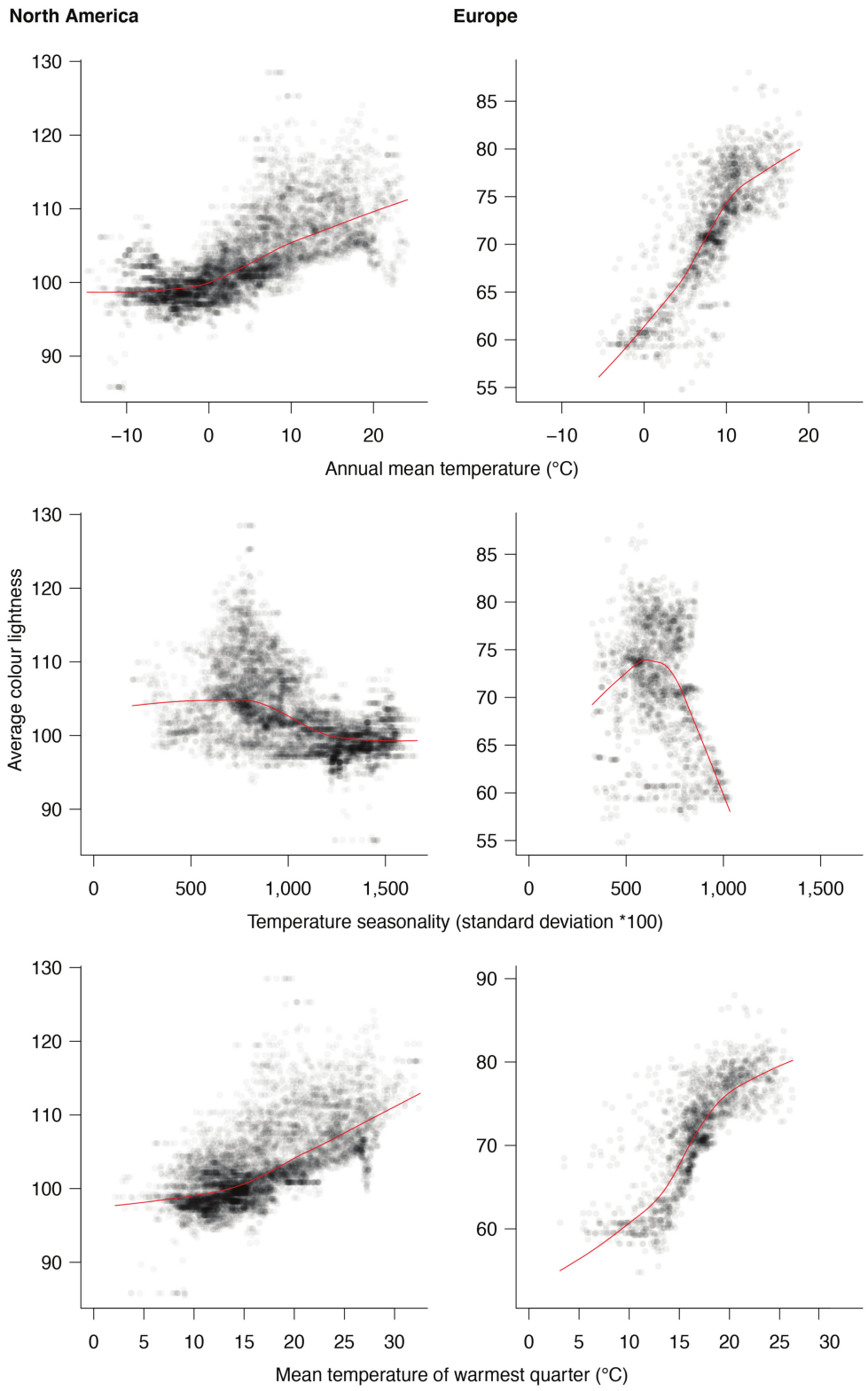

Figure 6.1.2.2: Scatterplots of the average colour lightness of 8127 North American and 1839 European dragonfly assemblages and variables that characterise the thermal environment. Partly transparent dots indicate data density. Red lines were fitted with spline-based smoothed regressions.

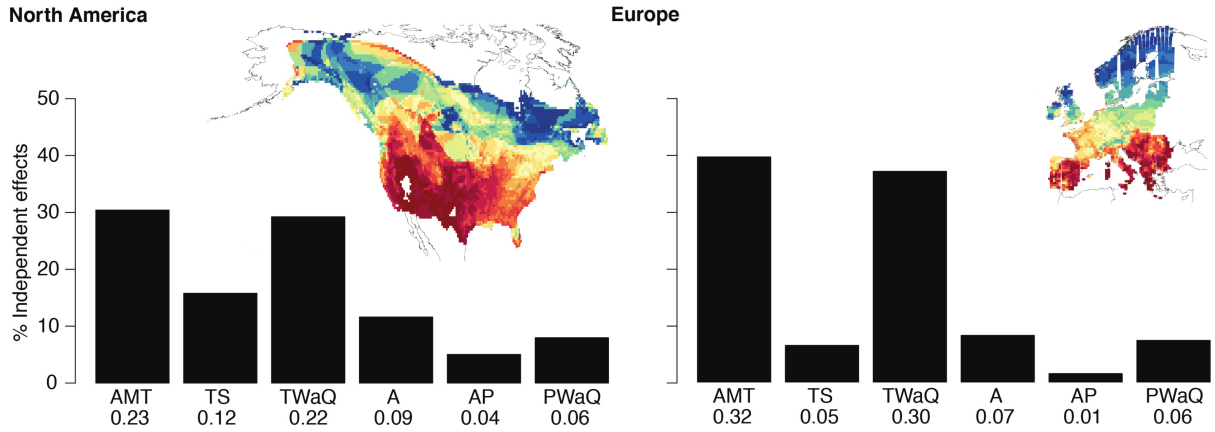

Figure 6.1.2.3: Hierarchical partitioning showing independent contributions of six environmental variables on the spatial variation in average colour lightness of 8127 North American and 1839 European dragonfly assemblages. Variables: AMT, annual mean temperature; TS, temperature seasonality; TWaQ, mean temperature of the warmest quarter; A, altitude; AP, mean annual precipitation; and PWaQ, mean precipitation of the warmest quarter. The independent contribution (r^2) of each environmental variable is indicated underneath the respective abbreviation.

DISCUSSION

Our results demonstrated that the spatial variation in colour lightness of regional dragonfly assemblages across North America is basically consistent with the spatial variation across Europe – with on average more dark-coloured species occurring in colder regions and more light-coloured species occurring in warmer regions. In both North America and Europe, the thermal environment explained the largest proportion of this geographical pattern. Hence, for both continents, the results are consistent with the predictions of the thermal melanism hypothesis. Therefore, our results as well as physiological studies and studies on smaller spatial scales (for ectotherm species: Clusella Trullas *et al.* 2007 and particularly for dragonflies: May 1991, Samejima & Tsubaki 2010) support the conclusion that surface colour lightness is an important trait that influences the distribution of insects across continents.

We found also differences between the two continents. First, the slopes of the relationships between environmental variables and average colour lightness across assemblages were significantly less steep for North America than for Europe. Second, the amount of explained variance was slightly lower for North America than for Europe. The finding that the variation of a functional trait is more complex and patchier across North America than across Europe is not unique to our study. For example, Olalla-Tárraga *et al.* (2006) also found that the correlation between body size of reptile assemblages and environmental variables was less steep and explained less of the spatial variation across North America than across Europe. Thus in North America, factors and processes not captured by environmental variables potentially influence the spatial variation of functional traits. We are aware of two such factors, namely geographical setting and biogeographical history, and the factors are intertwined.

Geographical setting

Mountain ranges in North America are oriented in a north–south direction, whereas in Europe, the mountain ranges are oriented mostly in an east–west direction (Hewitt 2000). In Europe, the mountain ranges are barriers, and re-colonisation of northern areas might therefore have been slower in Europe than in North America. Thus, northern assemblages in Europe might not have reached equilibrium since the last glaciation, with only a comparatively small subset of dark-coloured species being able to colonise northern areas. Indeed, several studies have shown that quaternary climate still influences current macrocecological patterns (Araújo *et al.* 2008, Hortal *et al.* 2011). In addition, phylogeographic studies suggest that North America and Europe experienced different histories during climate oscillations (Hewitt 2000). For example, the ice sheets of North America spread more widely across the relatively plain landscape down to about 40°N in the south-east. With the absence of effective geophysical barriers, large areas have been subjected to repeated waves of extinction and repopulation (Hewitt 2000). Unfortunately, knowledge of the phylogeography of dragonflies is still poor. However, a recent study of the dragonfly genus Cordulegaster in Europe found an array of unique evolutionary histories that can be interpreted as a diversification of this group in a refuge in eastern

Europe (Froufe *et al.* 2014). Similar to species of the genus Cordulegaster, which breed today almost exclusively in small streams and permanent springs at higher elevations (Dijkstra & Lewington 2006), some species could have survived a glacial maximum in the Balkan region, and then when isolated could have experienced an enhanced selection pressure towards thermal melanism.

Biogeographical history

The biogeographical history of North American and European dragonflies could have been very different. Of the 20 dragonfly genera of North America, 10 do not occur in Europe, and of the 223 species used in this analysis, only three species are shared between the two continents. This highlights the independence of the species pools of North America and Europe. North America is directly connected to tropical regions, and nine genera of North American dragonflies have a tropical origin. By contrast, in Europe, the arid belt forms a barrier for most lineages with tropical origin. In tropical lineages, however, temperature might have been less important for the evolution of thermal melanism. This idea is supported by our finding that temperature explained a larger proportion of the variation in the phylogenetic component of colour lightness in Europe than in North America.

In conclusion, our comparison of dragonfly assemblages of North America and Europe demonstrates that the geographic patterns of colour lightness and their environmental drivers on these two continents are very similar. Thus, thermal melanism might be an important and general mechanism that shapes the distribution of dragonflies in non-tropical regions. Our results furthermore underline the importance of differences between continents for the geographical variation in the average colour lightness of assemblages and clearly indicate that more comparisons across large geographic regions are needed to understand the evolutionary and ecological significance of colour lightness for community assembly of insects.

Colour lightness of butterfly assemblages across North America and Europe

with
Pablo Stelbrink, Stefan Brunzel & Roland Brandl
in preparation

Colour lightness of butterfly assemblages across North America and Europe

ABSTRACT

The vast majority of animals on earth are ectotherms and rely on external energy to reach the body temperature necessary for activity. Thereby their colouration is supposed to determine the rate of energy absorption from the environment. We test the prediction that dark-coloured butterfly species are favoured in cooler climates and light-coloured butterfly species in warmer climates by comparing the link between colour lightness, distribution of species, and climate across butterfly assemblages of North America and Europe. We furthermore compare strength and direction of this link for butterfly families of both continents to elucidate the taxonomic composition of the overall pattern. We combined data on the distribution and colouration of 367 butterfly species of North America with data on the distribution and colouration of 326 butterfly species of Europe. Distribution data for butterflies of North America were provided by the BAMONA project and data for Europe was taken from Zeuss *et al.* (2014). We used an assemblage-based approach and calculated the average colour lightness of all species occurring within each grid cell as dependent variables in our analyses. We calculated average climatic conditions within each grid cell from global datasets and used them as explanatory variables in single and multiple regressions. The colour lightness of each butterfly species was calculated using computer-assisted digital image analysis. Dorsal and ventral butterfly colour lightness were positively correlated with insolation and temperature and negatively correlated with humidity in both continents. In North America, insolation was stronger correlated to dorsal colour lightness than to ventral colour lightness. By contrast, in European butterflies, insolation explained less of the variance in dorsal colour lightness compared to ventral colour lightness. Butterflies in both study areas were on average darker at the dorsal side than at the ventral side. This study revealed that the conjunction of distribution ranges, colour lightness, and annual temperature and insolation already found for Europe is also valid for North American butterfly assemblages. Our study hence further supports the hypothesis that colour lightness is of considerable importance for the large-scale distribution of insects.

INTRODUCTION

The vast majority of animals on earth are ectotherms and rely on external energy to reach the body temperature necessary for foraging or mating. Thereby their colouration is supposed to determine the rate of energy absorption from the environment, leading to the hypothesis that darker coloured ectotherms should have an advantage in colder climates because of higher absorption rates (Watt 1968, Gates 1980, Clusella Trullas *et al.* 2007), and lighter coloured ectotherms in warmer climates because of higher reflection rates to protect against overheating (Watt 1968, Gibert *et al.* 1998). The link between colour lightness, distribution of species, and climate takes effect on single individuals but as a long term evolutionary process should also lead to large-scale distribution patterns across taxonomic groups and regions.

This hypothesis has been tested with a variety of organisms at the intraspecific level (e.g. beetles: Lusis 1961, Brakefield 1984a,b,c, Brakefield & Willmer 1985, DeJong *et al.* 1996, butterflies: Guppy 1986a,b, Davis *et al.* 2005, Karl *et al.* 2009, flies: Munjal *et al.* 1997, grashoppers: Forsman *et al.* 2002, snakes: Gibson & Falls 1979, Andren & Nilson 1981, Capula & Luiselli 1994, Tanaka 2007, spittlebugs: Stewart & Lees 1996 and at the interspecific level (butterflies: Watt 1968, Roland 1982, Kingsolver & Wiernasz 1991, Ellers & Boggs 2004, chameleons: Walton & Bennett 1993, lizards: Norris 1967, Clusella Trullas *et al.* 2009). Although most of these studies suggest that the hypothesis is generally supported, three characteristics of the investigations limit its generality. Firstly, the large majority of investigations covers either a single or a small set of closely related species at small spatial scales (but see Zeuss *et al.* 2014). Secondly, the investigations are limited to an either intraspecific or interspecific perspective and thus do not explicitly consider the geographic context with the data. Thirdly, a direct comparison between related taxa from different biogeographic regions is missing so far, which could demonstrate that the link between colour lightness, distribution of species, and climate is present despite different evolutionary histories and climatic regimes.

Furthermore, the existence of assemblage-based patterns is often implicitly assumed in statements about the characteristic physiological traits of species inhabiting particular kinds of environments and is thus important in the generalization of variation in physiology (Gaston *et al.* 2009). However, their documentation has been remarkably poor.

Here, we test the prediction that dark-coloured butterfly species are favoured in cooler climates and light-coloured butterfly species in warmer climates by comparing the link between colour lightness, distribution of species, and climate within butterfly assemblages of North America and Europe. We furthermore compare strength and direction of this link for butterfly families of both continents to elucidate the taxonomic composition of the overall pattern.

Butterflies are excellent study organisms because they strongly rely on elevated body temperatures for flight (Clench 1966, Kammer 1970, Kingsolver 1983, Tsuji *et al.* 1986)

and thermoregulation via colouration properties of the surface is constraint by other functions like aposematism and sexual selection. Furthermore, butterflies are a taxonomically well studied group in regard to trait and distribution data, especially for the northern Hemisphere. A comparison between butterfly assemblages of North America and Europe is promising because both continents experienced different biogeographic histories since their separation about 200 Myr ago. Today, they differ in their topography, with e.g. mountain ranges mainly oriented in north-south direction in North America in contrast to mainly east-west orientated mountain ranges in Europe, leading to different climatic gradients.

Similar findings for both continents and across families would underline the importance of colour lightness as a mechanistic adaptation of species to climate which shapes biogeographical patterns of species distributions.

MATERIAL AND METHODS

To compare the link between colour lightness, distribution of species, and climate across USA and Europe as well as between butterfly families, we combined data on the distribution and colouration of 367 butterfly species of the USA with data on the distribution and colouration of 326 butterfly species of Europe from Zeuss *et al.* (2014). We applied an assemblage-based approach and used the average colour lightness of the species occurring within each grid cell as dependent variables in our analyses. We used a half degree grid for the USA with 3593 (2535 occupied) cells (EPSG: 4326, average cell size 2,400 km^2) covering 8.6 m km^2 and a CGRS grid in Europe with 1943 cells (EPSG: 4326, average cell size 2,500 km^2). We calculated average climatic conditions within each grid cell from global datasets and used them as explanatory variables.

Species

We analysed the four most specious butterfly families of the Papilionoidea (Lycaenidae, Nymphalidae, Papilionidae, Pieridae) and in doing so included most butterfly species from the USA and Europe. Family classification followed Brock et al. (2003, USA) and Tolman & Lewington (2009, Europe). In case of species occurring in both study regions and originally classified into different families, we used the family classification of Brock et al. (2003, *Hamearis lucina* and *Danaus plexippus*, classified as Nymphalidae).

Colouration data

The colour lightness of each butterfly species was calculated using computer-assisted digital image analysis, following the protocol described in Zeuss *et al.* (2014). In particular, we scanned dorsal and ventral images of butterfly species from the USA in Brock *et al.* (2003) with a resolution of 1200 dpi and 24 bit in the RGB colour space. We only measured the body and 1/3 of the wing area closest to the body because this area is supposed to be most important for thermoregulation (Wasserthal 1975). Image processing was done using Adobe Photoshop CS2. Only images of females and phenotypes which could not be differentiated between sexes were included, because colouration of males is supposed to

be more adapted to sexual selection compared to females (Wiernasz 1989; see also Zeuss *et al.* 2014). We computed colour lightness values as the arithmetic means of the red, green and blue colour values of the considered area using the R package *EBImage*. These values range from 0 (black) to 255 (white). Data of different morphs within species in Brock *et al.* (2003) were averaged for the dorsal and ventral surface, respectively. Colour lightness of European butterfly species were computed in the same way as in Zeuss *et al.* (2014) based on images published in Tolman & Lewington (2009).

Distribution data

Distribution data for butterflies of the USA were kindly provided by the BAMONA project (`www.butterfliesandmoths.org`). We used point records as well as county records and processed these to presence data (n=103,262) for each grid cell. We cropped our study area to the mainland of the USA because the density of records beyond this boundary sharply declined. Distribution data for butterflies of Europe were extracted from contour distribution maps (Kudrna 2002) and converted to presence data for each grid cell within a CGRS grid (see Zeuss *et al.* 2014). In addition, we digitized the distribution maps provided in Scott (1997) and repeated all analyes with this data source to test for the reliability of our results. Results presented here were obtained with data from the BAMONA project. The maps and scatterplots were generated with data from Scott (1997).

Environmental variables

Zeuss *et al.* (2014) showed that colour lightness of butterfly assemblages is consistently correlated to the thermal environment across Europe using principal components. Here, we decided to use untransformed environmental variables for both USA and Europe to further differentiate the influence of the thermal environment on butterfly colour lightness and to make a direct comparison between both regions possible. Colour lightness is expected to be connected with humidity as well. Therefore, we included relative air humidity in our analysis. Data were taken from microclim, which provides global hourly data for an average day of each month of the year (Kearney *et al.* 2014). We calculated annual mean values of the variables solar radiation, air temperature, and relative humidity, which were provided in a spatial resolution of 10 arcmin. Hence, we calculated the arithmetic means of all data points located in each grid cell. Finally, we used annual mean hourly solar insolation (INS, Wm^{-2}), annual mean air temperature at 1.2 m above ground (TMP, °C) and annual mean relative humidity at 1.2 m above ground (HUM, %) as predictors in our models. Environmental data was processed in the same way for USA and Europe with the R package *raster* 2.3-24 and QGIS 2.6.1.

Statistical analysis

Due to the case that some areas of USA are not well sampled, there are grid cells with records of only few species. Expecting these low species numbers not to be representative for the real species set, in our statistical analysis we only included grid cells with at least 5 species. Due to reasons of consistency, this selection was performed for both study

areas. Thereby, numbers of grid cells in the statistical analysis were reduced to 2142 in USA and 1846 in Europe.

To test for mean colour lightness of butterfly assemblages depending on insolation, air temperature and humidity we used an ordinary least square regression, each. Tests were differentiated after study areas, family and dorsal/ventral wing and body side. Due to high number of grid cells, level of significance was set to $p < 0.001$. Especially to detect differences of adaptations in melanisation between the families and continents, it is necessary to determine the most important environmental influences. Hierarchical partitioning (HP) of the environmental variables was used to decompose the independent contributions. HP results the relative independent importance of several explanatory variables on one depending variable by computing all possible multiple regressions (Chevan & Sutherland 1991). Calculations were conducted with the R package *hier.part* using 'r^2' as goodness-of-fit measure. All data handling and analysis was performed in R (R Core Team 2016).

RESULTS

Colour lightness

Butterflies in both study areas were in mean darker at dorsal body and wing side than at ventral body and wing side (USA: dorsal: 98.81, ventral: 128.36, d.f. = 731.68, t = -11.34, $p < 0.001$; Europe: dorsal: 89.22, ventral: 130.95, d.f. = 647.38, t = -14.40, $p < 0.001$). This pattern of darker dorsal side than ventral side was found in families Lycaenidae and Nymphalidae as well, but not in Papilionidae and Pieridae, all consistently between study areas (Fig. 6.1.3.1).

General pattern

Both, dorsal and ventral butterfly colour lightness in both study areas, USA and Europe were positively correlated with insolation and temperature and negatively correlated with humidity (Figs. 6.1.3.2 & 6.1.3.3). In USA insolation explained more of variance in dorsal colour lightness ($r^2 = 0.20$, $p < 0.001$) than of variance in ventral colour lightness ($r^2 = 0.07$, $p < 0.001$). In European butterflies insolation explained less of variance in dorsal colour lightness ($r^2 = 0.25$, $p < 0.001$) than of variance in ventral colour lightness ($r^2 = 0.45$, $p < 0.001$). We found the same relation of correlation strengths in correlations with temperature even though correlation coefficients were even lower in USA data and even higher in European data.

Families

Pattern of increasing colour lightness at increasing temperature and insolation was generally found in family subset data of Lycaenidae and Nymphalidae as well. However, in study area USA Lycaenid dorsal colour lightness was strongly positively correlated with insolation and temperature (INS: $r^2 = 0.25$, $p < 0.001$, TMP: $r^2 = 0.34$, $p < 0.001$) whereas ventral colour lightness was not significantly or weakly negatively correlated. In

European data dorsal and ventral colour lightness of Lycaenidae was positively correlated with insolation and temperature. Nymphalid dorsal and ventral colour lightness was strongly correlated with insolation and temperature in USA and Europe, especially ventral data in Europe (INS: $r^2 = 0.55 < 0.001$, TMP: $r^2 = 0.54$, $p < 0.001$).

Contrasting, colour lightness of Papilionidae was strongly negatively correlated with temperature in both study areas, USA and Europe and similarly negatively correlated with insolation in Europe. Likewise, we found negative correlations in Pierid colour lightness data with insolation and temperature in USA (INS: dorsal: $r^2 = 0.17$, $p < 0.001$, ventral: $r^2 = 0.28$, $p < 0.001$). In European data, Pierid colour lightness was not significantly or weakly positively correlated with insolation and temperature.

Hierarchical partitioning

Hierarchical partitioning revealed always insolation or temperature and never humidity as strongest predictor for distribution of colour lightness, across and within the four butterfly families in USA as well as in Europe. Across all families in study area USA insolation explained higher amount of variance in dorsal and ventral colour lightness data than temperature, whereas in Europe temperature explained higher amount of variance in dorsal as well as ventral colour lightness data (Fig. 6.1.3.4).

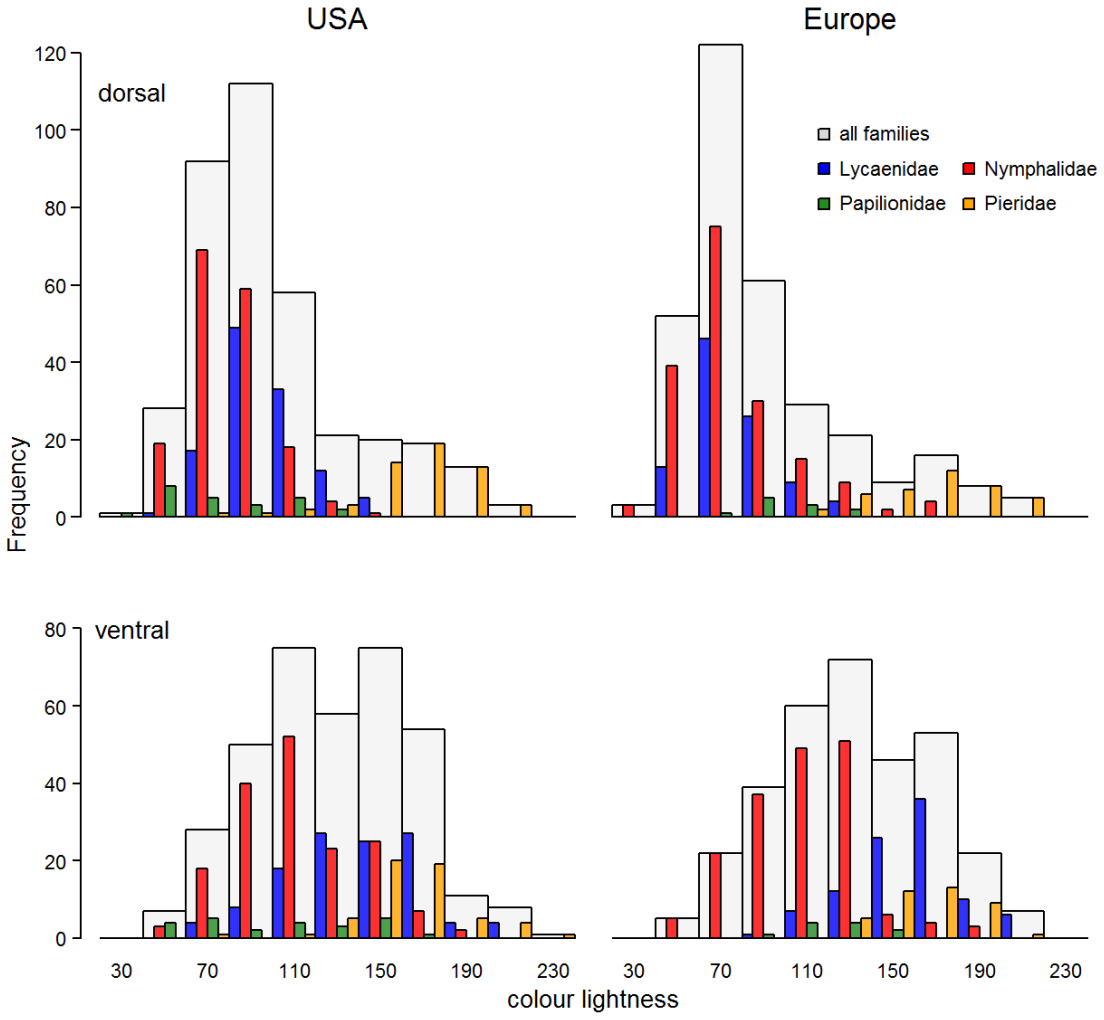

Figure 6.1.3.1: Histograms of dorsal (top) and ventral (bottom) colour lightness (grey value) of 435 North American butterfly species separated after the four most speciose families. Note the overall and within each family lighter coloured (high grey value) ventral wing and body side.

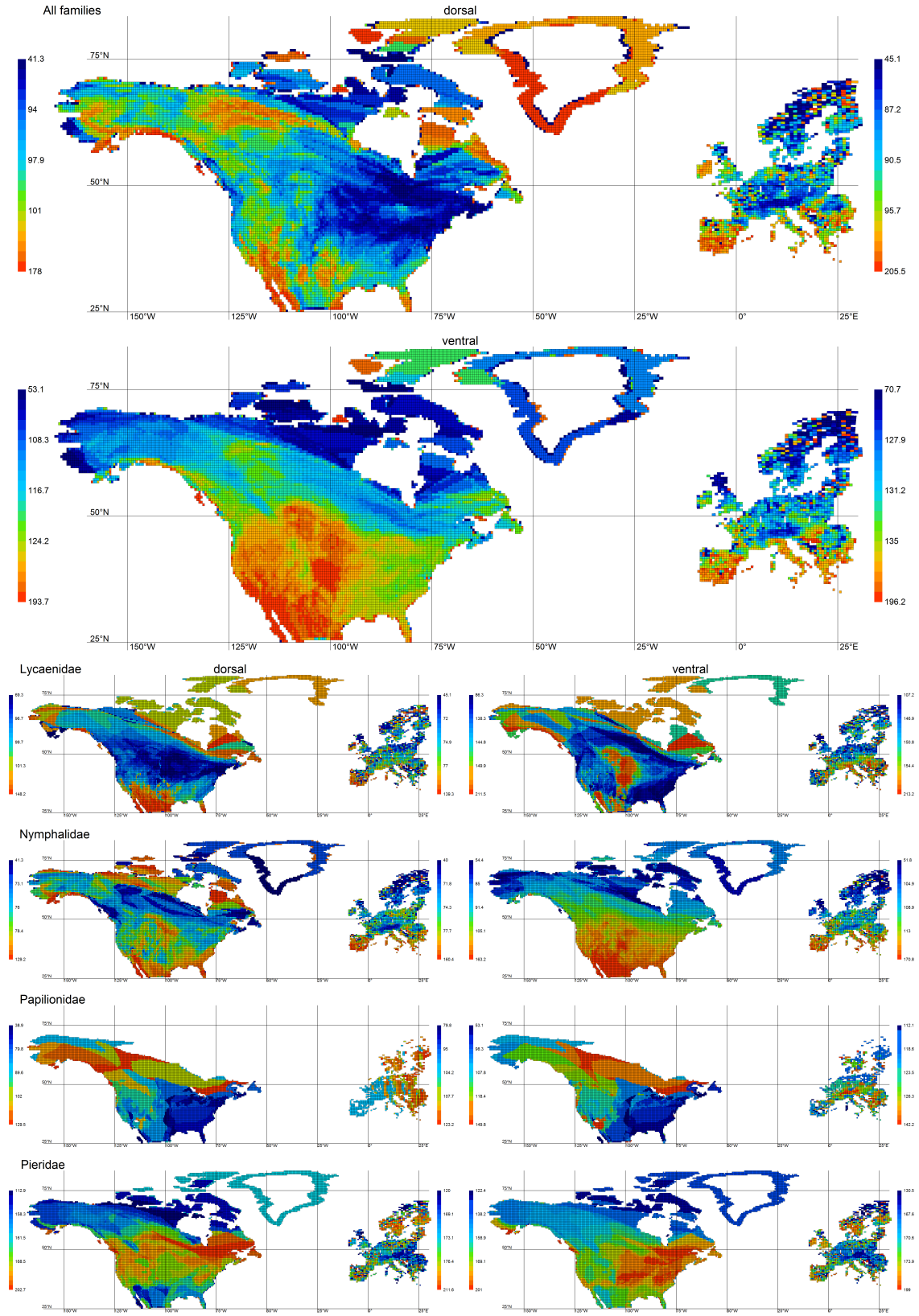

Figure 6.1.3.2: Map of the spatial variation in average colour lightness of North American and European butterfly assemblages. First and second row: colour lightness of the dorsal and ventral surface of all species combined. Bottom rows: colour lightness according to butterfly families. Colour lightness ranges from 0 (absolute black) to 255 (pure white). Colour scale intervals follow an equal-frequency classification, ranging from blue (darkest) to red (lightest).

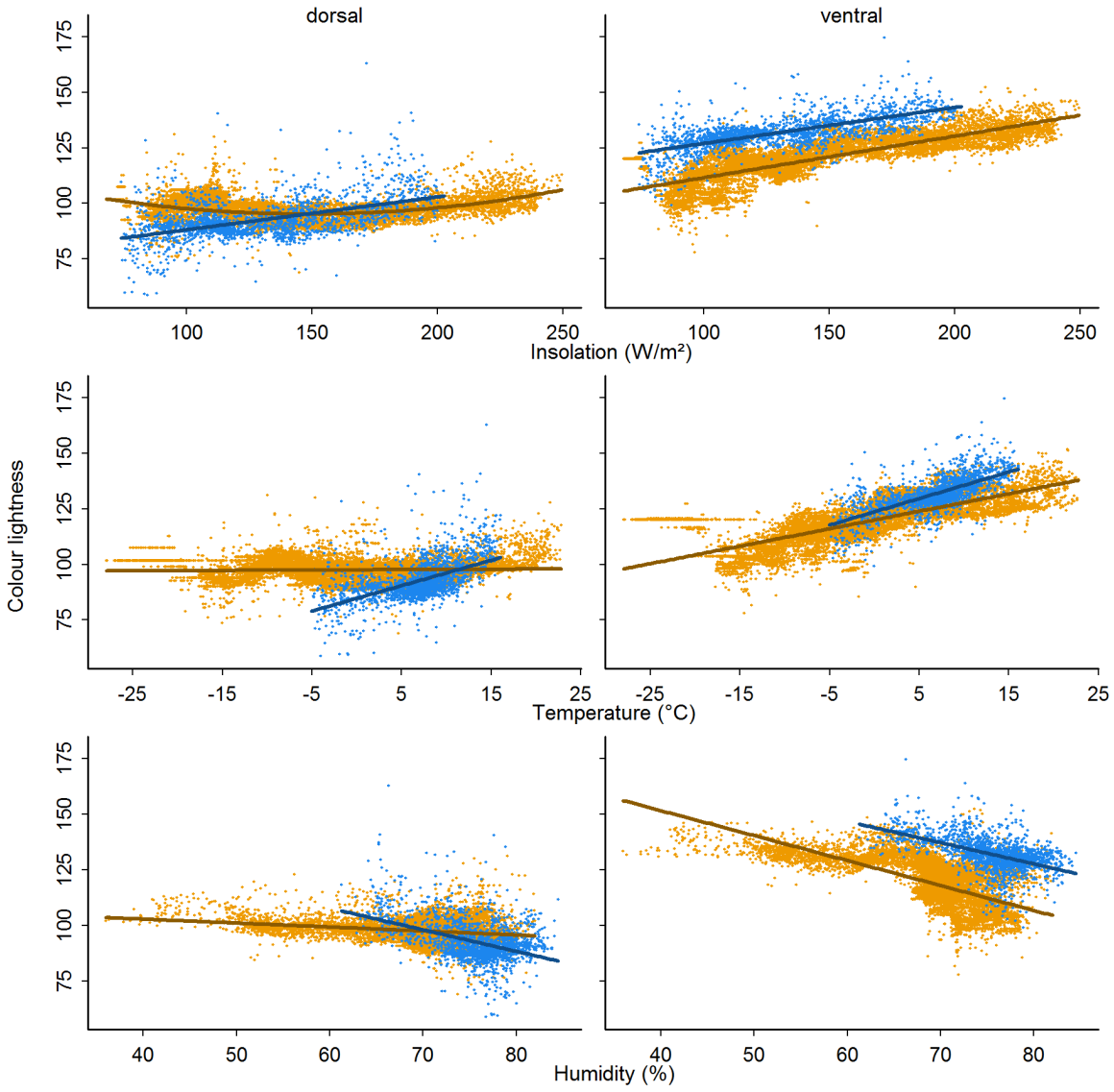

Figure 6.1.3.3: Scatterplots between the average colour lightness in assemblages of North American butterflies and insolation, temperature and humidity. Note that regression trends are similar between North America and Europe, especially for the ventral surface.

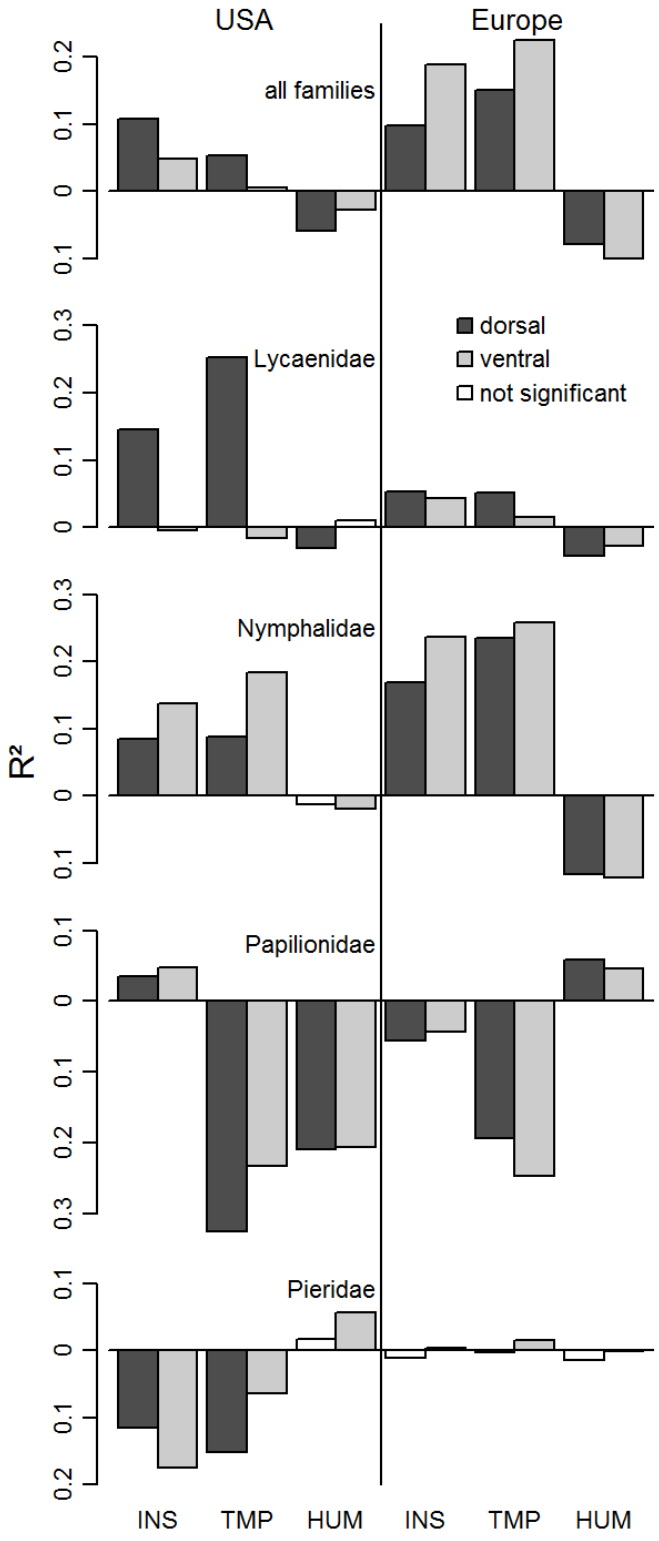

Figure 6.1.3.4: Independent contributions (R^2-values) of insolation (INS), temperature (TMP) and humidity (HUM) for explaining the average colour lightness of North American and European butterfly assemblages. Analyses were separated after dorsal (left bars) and ventral (right bars) colour lightness and the four most species-rich families. According to ordinary least square regressions, positive correlations are drawn upwards, negative correlations are drawn downwards and not significant ($p > 0.001$) correlations are coloured white.

DISCUSSION

As supposed by the thermal melanism hypothesis (TMH) our analyses revealed that the thermal environment in general is a strong predictor for melanism in North American as well as in European butterfly assemblages and mean colour lightness of all assemblages increased with temperature and insolation. This result is in line with previous large scale studies on European butterflies and dragonflies by Zeuss *et al.* (2014). Furthermore, mean colour lightness generally decreased with increasing humidity in both North America and Europe. In addition to this general tendency, our analyses show another corresponding finding of colouration of North American and European butterfly assemblages: in contrast to the general pattern, the papilionids and the pierids exhibit deviant patterns. In North America, pierids showed a negative and in Europe no correlation between insolation and mean colour lightness of assemblages. In North America and Europe, the mean colour lightness of papilionid assemblages is even negatively correlated to temperature: the higher the mean annual temperature, the darker the assemblages.

Despite this congruences between North America and Europe – which support the general relationships between colour lightness and the thermal environment in ectotherms already stated by Zeuss *et al.* (2014) and the THM (Clusella Trullas *et al.* 2007) – there are some striking differences between patterns of colour lightness of butterfly assemblages in North America and Europe. Firstly, least square regression models showed that the environmental variables insolation, mean annual temperature and humidity generally explain a higher amount of variance of mean colour lightness in Europe than in North America. This genereal difference may partly be caused by the mere fact that the species distribution data and thus the assemblages in Europe base on a $50 \times 50\,\mathrm{km}$ grid, in North America the distrubution data base on point records or county records.

Secondly, across all families in North America insolation explained a higher amount of variance in dorsal and ventral colour lightness of assemblages than annual temperature did, whereas in Europe temperature explained a higher amount of variance in dorsal as well as in ventral colour lightness. This finding may be generated by the most prominent difference in the geography of North America and Europe: Whereas the distribution of sea and mountain ranges structures Europe predominantly according the latitudes, North America is structured longitudinal by the south-north stretched Rocky Mountains. Furthermore, North America thus has a continuous connection to the tropics and this geographical characteristics offer an easier northward access for migrating tropical and sub-tropical species. Regarding the difference in the importance of the predictors insolation and temperature between North America and Europe we assume that the north-south stretched mountain ranges disrupt an originally latitudinal structured continuous gradient of temperatures between South and North stronger than a continuous insolation gradient. In a mountain range grid insolation between low and high altitudes does not vary as much as annual temperatures should do. Therefore, the part of mean colour lightness of the butterfly assemblage of this grid explained by annual temperature should be weakened thus leading to less explained variance in the respective regression models.

Taking into account this hypotheses to explain the differences between North America and Europe regarding the amount of explained variance, the north-south structured geography and thus the correspondingly north-south-structured meteorological patterns may shed further light on the generally weaker correlations in North America than in Europe. The rather longitudinal structured geograpyh of North America is particularly mirrored in the colour lightness patterns of the papilionid family: Mean dorsal as well as ventral colour lightness of papilionid assemblages was distinctly split in two halfs. Apparently, western north-american papilionids were on average lighter coloured than papilionids in the eastern study region. Beside from few exceptions in border areas of the study region, single papilionid species occurring only in one of the two halfs, either in eastern or western half of North America (see Scott 1997, Brock *et al.* 2003). These north to south stretched and east to west restricted distribution ranges were found in several North American species and can be attributed to north-south orientated distribution limits such as mountain ranges. The exceptional spatial pattern of papilionid colour lightness probably determine the disparate results of WLSR and HP. Furthermore, Papilionidae are the largest butterflies in the study region (Brock *et al.* 2003). As it is hypothesized to exist a trait-off between melanism and body size adaptation (see Clusella Trullas *et al.* 2007), there might be deviation in papilionid melanism evolution forces. Additionally, the Papilionids are the species poorest family in this study with assemblages with on average less than six species. Colouration adaptations characteristics of single species such as cryptic colouration or aposematism, which overlay thermal colouration adaptation (see e.g. Forsman 2011) will affect mean colour lightness of species-poor assemblages much more than mean trait of species-rich assemblages. A multispecies approach, as applied in this study, cannot consider and is even not intended to account for characteristics of each species. Suchlike top-down investigations shall detect major patterns and coherences. Hence, this study supports general assumptions of the TMH as assemblages of the both specious families Nymphalidae and Lycaenidae follow the TMH.

The longitudinal structured geographical pattern of North America may generate another possible cause of the generally lower explained variance of colour lightness by the predictors insolation, annual temperature and humidity. The temperate and boreal zone in North America should be much easier accessible for species predominantly occurring in the tropics. In North America much more species which originated in the tropics spread occasionally or frequently far to the north and thus built a larger proportion of the assemblages of the temperate zone than in Europe, where only 6 species occur in tropical regions and simultaneously in Europe (The Canaries and Madeira excluded). A discrimination between species which evolutionary origin was in the tropics or in the temperate zone would have been more appropriate to analyse the impact of tropical taxa but this was not possible due to a lack of data. Interestingly, the two families with the deviant pattern, papilionids and pierids, exhibit a larger proportion of species occurring in the tropical and temperate zone than the other families, but these difference were not significant. In dividing species distributed exclusively in the temperate zone and species occurring in both zones, for papilionids and pierids we would have expected a change of

the regression slopes toward the "right" direction (like in the other families) when omitting the species distributed also in the tropics. This analyses revealed no change towards the expected positive correlation between thermal environment (insolation and mean annual temperature) and colour lightness of papilionids and pierids. A further test of the hypothesis that the high proportion of tropical species in papilionids and pierids revealed no significant differences between colour lightness of species occurring in the tropics and the temperate zone and species inhabiting exclusively the temperate zone. This falsification of our hypotheses to explain the differences between the families may be caused by the fact that we are currently not able to correctly determine the classification "tropic" versus "non-tropic" back to the evolutionary origin. The study of Condamine *et al.* (2012) shows a clear tropic evolutionary origin for the papilionids but major differences exist between genera and subfamilies: The Parnassiinae have evolved in the temperate zone whereas the Papilioninae for example have evolved in the tropical climates. In North American taxa the main discrimination between temperate species and species occurring in both climate zones seem to be on the level of subfamilies or even on genera level. But currently, however, we found no hint that the origin or at least the distributional preferences of climate zones of species explain family differences.

This study revealed that the conjunction of distribution ranges, colour lightness, and annual temperature and insolation found for Europe (Zeuss *et al.* 2014) is also valid for North American butterfly assemblages. This is important for the estimation of possible effects of global warming. Species may shift their distribution ranges to the environmental conditions they are adapted to, which needs particular considerations in conservation efforts (see e.g. Parmesan *et al.* 1999, Franco *et al.* 2006, Gonzalez-Megias *et al.* 2008). A possible shift of distribution ranges due to global warming should be easier for North American butterfly species compared to European species due to the lack of latitudinal geographic dispersal barriers like the Alps or the Mediterranean Sea. As colour lightness differ between the families, and especially the differences between western end eastern parts of North America emphasizes that conservation tactics have to be adjusted not only to general trends, but distinct spatial responses have to be regarded.

The dark side of Lepidoptera: A continental gradient in the colour lightness of assemblages of geometrid moths

with
Lea Heidrich, Konrad Fiedler, Axel Hausmann, Martin Brändle & Roland Brandl
in review in Global Ecology and Biogeography

The dark side of Lepidoptera: A continental gradient in the colour lightness of assemblages of geometrid moths

ABSTRACT

The colouration of animals confers a variety of important ecological and physiological functions. Recent studies showed that thermoregulation shapes macroecological patterns of colour lightness in heliothermic insects. However, we expect that other functions than thermoregulation determine the colour lightness of nocturnal insects. Here, we use assemblages of predominantly nocturnal geometrid moths to test for geographical clines in colour lightness potentially associated with crypsis, pathogen resistance and protection from UV radiation. We used computer-assisted digital image analysis to assess the wing colour lightness of 637 species of geometrids and compiled their distribution across 3777 grid cells of 50 km × 50 km. We calculated the average colour lightness of all species within each grid cell and tested for relationships to forest cover, dew point temperature and solar radiation as proxies for crypsis, pathogen resistance and protection from radiation, respectively. We addressed effects of spatial autocorrelation and species richness with autoregressive error models and randomization tests. Structural equation models were used to differentiate the thermal aspects of solar radiation and dew point temperature. We found a distinct geographical gradient with predominantly dark-coloured species in northern regions and light-coloured species in southern regions ($r^2 = 0.63$). Average colour lightness within grid cells increased with increasing solar radiation and dew point temperature, but was not correlated with forest cover. The colour lightness of insects is a climate-driven multifunctional trait. The clear geographical gradient in colour lightness of nocturnal moths coincides with the geographical pattern predicted by thermoregulatory functions of heliothermic insects. This unexpected result indicates linkages of adult colouration to physiological processes during earlier life stages, such as thermoregulation or immune responses of larvae, and point to fundamental benefits of dark colouration in cold and moist environments.

INTRODUCTION

The colouration of animals confers a variety of important ecological and physiological functions. It influences and is controlled by various biotic and abiotic factors that operate on ecological and evolutionary scales, namely crypsis, aposematism, sexual selection, species recognition, thermoregulation, UV resistance and pathogen resistance and might be a major component involved in the adaptation to climate warming (True 2003, Roulin 2014).

However, the relative importance of single factors is often unclear because several factors of colouration can act simultaneously (Mckinnon & Pierotti 2010). Furthermore, it is unknown to which extent the importance of particular functions and determining factors of colouration vary across geographical regions. To date, geographically explicit large-scale studies have revealed colouration gradients in several taxa (e.g. Clusella Trullas *et al.* 2008, Zeuss *et al.* 2014, Bishop *et al.* 2016, Pinkert *et al.* 2016, Schweiger & Beierkuhnlein 2016. However, most of these previous studies focused on thermoregulation, and the extent to which other functions of colouration contribute to large-scale geographical patterns in species assemblages remains poorly understood (but see Bastide *et al.* 2014).

The thermoregulatory function of colouration forms the heart of the thermal melanism hypothesis. Melanization, i.e. dark colouration, increases absorption of solar energy and hence allows dark-coloured animals to reach their optimum body temperature faster than light-coloured animals (Gates 2003, Clusella Trullas *et al.* 2007, Xing *et al.* 2016). It is thought that this ecophysiological phenomenon results in better thermoregulatory performance and thus longer activity times, which in turn leads to a positive effect for evolutionary fitness of dark-coloured ectotherms in cold climates. By contrast, it is believed that light colouration is advantageous under warm conditions because it prevents overheating (Clusella Trullas *et al.* 2007). Therefore, melanin-based thermoregulatory constraints should lead to more light-coloured ectotherms in warm climates and more dark-coloured ectotherms in cold climates. However, this functional relationship should primarily apply to diurnal ectotherms that regulate their body temperature by absorbing solar energy.

Thermoregulation is not the only function that is ascribed to the colouration of animals. Three other main functions of colouration have been proposed. One other function of animal colouration is the influence of both intraspecific and interspecific interactions. Predator avoidance strategies, such as crypsis and aposematism, have generated famous textbook examples of natural selection, e.g. *Biston betularia* (Kettlewell 1973, Majerus 2009, van't Hof *et al.* 2016) and Heliconius butterflies (Merrill *et al.* 2014). Species that rely on crypsis should have a colouration similar to their environment (crypsis hypothesis; Endler 1984).

Another function of colouration relates to the immune response of insects, which is partly based on melanin and the melanin-producing enzyme phenol oxidase (Armitage & Siva-

Jothy 2005). Melanin pigments are associated with increased protection against viral, bacterial and fungal pathogens, and against abiotic stressors, such as heavy metals (Wilson *et al.* 2001, True 2003, Mikkola & Rantala 2010). Hence, more darkly coloured species could be more resistant to pathogens and accordingly, areas with many biotic as well as abiotic stressors should be characterized by more dark-coloured species (pathogen resistance hypothesis; Wilson *et al.* 2001).

The third additional function of colouration is protection against harmful UV radiation by means of melanin (UV-resistance hypothesis; Ortonne 2002). It is believed that the concentration of melanin in the cuticle of species increases towards the equator and at high elevations, where UV radiation is high and more UV protection is needed. In this study, we address these possible functions of colouration, namely thermoregulation, crypsis, pathogen resistance and UV resistance in geometrid moths across the Western Palearctic. We chose geometrid moths because they are a species-rich and well-studied family of lepidopterans that primarily rely on olfactory signals for intraspecific interactions and on crypsis for predator avoidance while resting (Majerus 2009, Kang *et al.* 2015). Hence, sexual selection, aposematism, mimicry and species recognition by visual cues are of minor importance. Geometrid moths are mostly nocturnal and therefore do not bask in the sun like heliothermic butterflies; yet, compared to other nocturnal moths, geometrids are able to fly when the temperature of the thorax is relatively low (Casey & Joos 1983, Heinrich 1993). Hence, thermal melanism should only play a minor role, at least for adults.

Each of these major potential functions of the colouration of geometrid moths can be related to one distinct environmental factor. i) The effectiveness of crypsis depends on the light habitat (Endler 1993), which describes the average light conditions of the environment. If crypsis is of evolutionary importance, the colour lightness of geometrid moths should match their predominant light environment. In this large-scale study, we used forest cover as a proxy for the light habitat of moth assemblages, which becomes darker with increasing forest cover (Endler 1993). ii) Pathogen pressure on lepidopterans mostly affects their herbivorous larval stages and depends on the temperature and humidity of the environment. Pathogens thrive particularly well under moist and warm conditions (Elderd & Reilly 2014). If pathogen resistance is an important function of colouration of geometrid moths, assemblages in humid and warm regions should be composed of predominantly dark-coloured species. Under warm and humid conditions, the dew point temperature is high, and under cool and arid conditions, the dew point temperature is low. We therefore used dew point temperature, i.e. the temperature at which the water saturation of air is reached, as a proxy for pathogen pressure. iii) We used total solar radiation to address the potential thermoregulatory function of melanin as well as its protective function against UV radiation. Solar radiation is not only highly correlated to UV radiation (Appendix 3-S1) but has also been shown to be a better predictor variable for testing the thermal melanism hypothesis than annual temperature (Clusella Trullas *et al.* 2008). A decrease in colour lightness of assemblages of geometrid moths with in-

creasing solar radiation would support a UV-protective function of colouration, whereas an increase in colour lightness with increasing solar radiation would indicate a thermoregulatory function of colouration.

Here, we tested for correlations between the average wing colour lightness in assemblages of geometrid moths and forest cover, dew point temperature and solar radiation across the Western Palearctic. We expected that i) crypsis is the dominant selection mechanism for colour lightness in assemblages of geometrid moths and hence that geometrids are more darkly coloured in highly forested areas, ii) pathogen resistance and UV protection is of secondary importance and iii) thermal melanism is of no relevance for these predominantly nocturnal moths.

MATERIAL AND METHODS

Digital image analysis

We calculated the colour lightness of geometrid moths as one of the most basic characteristics of colouration. We scanned the standardized photographs of museum specimens published in the monograph book series "The Geometrid Moths of Europe Vols. I–IV" (Hausmann 2001, 2004, Hausmann & Viidalepp 2012, Mironov 2003), which include the subfamilies Archiearinae, Orthostixinae, Desmobathrinae, Alsophilinae, Geometrinae, Sterrhinae and Larentiinae, with an EPSON Perfection 4490 Photo Scanner (2400 dpi, 24 bit, RGB colour space).

We then removed the background and converted the images to 8 bit grey values by calculating the unweighted means of the RGB channels per pixel (called "colour lightness" in this study; see also Zeuss *et al.* 2014). Colour lightness was scaled to range from 0 (completely black) to 1 (completely white, R package *png*) and averaged across all pixels of each image. If several images of one species were available, they were also averaged. We used the full surface area of the body and wings to calculate the colour lightness of species.

We processed a total of 3443 images of 681 species. Subspecies were excluded. As there was no relevant sexual dimorphism in colour lightness (Appendix 3-S2), we used all available images per species without taking into account the sex of the specimens. We considered only those species for which data on the distribution were available and excluded images of brachypterous females, leading to a dataset of 3367 images of 646 species.

Distributional data

We digitized contour distribution maps of the selected geometrid species, thereby dividing the study area into 50 km × 50 km grid cells of a polygon mask layer (Common European Chorological Grid Reference System) and obtained presence/absence lists per grid cell. Our study covered the Western Palearctic, ranging from the Mediterranean area and Black Sea in the south to the Ural Mountains in the east, the British Isles in the west

and northernmost Fennoscandia in the north. Coastal grids (defined as $< 50\%$ land mass) were excluded. This resulted in a total of 3821 grid cells (assemblages) with 637 species.

Environmental data

We used the percentage of forest cover in each grid cell as a proxy for the light environment in a particular area. Compared to blue sky, forest canopy has a lower radiance, and thus dims the light environment Endler (1993). The higher the percentage of canopy cover, the lower the lightness of the light environment should be. The Vegetation Continuous Field collection (DiMiceli *et al.* 2011) from MODIS provides estimates of canopy cover with a spatial resolution of 250 m (data of the year 2000). In this dataset, canopy cover was defined as the percentage of horizontal ground in each 30 m pixel covered by woody vegetation greater than 5 m in height, including leaves, stems and branches.

The Microclim dataset (Kearney *et al.* 2014) provides estimates of clear sky solar radiation, temperature and humidity in hourly intervals and at a spatial resolution of 15 km. For each grid cell, the environmental data were condensed to annual values and averaged over a 25 km radius from the centroid (R package *raster*). The dew point temperature was calculated using the formula:

$$\tau = \Phi^{\frac{1}{8.02}} \times (109.8 + t) - 109.8$$

(Berber *et al.* 2014), with τ being the dew point temperature, Φ being the annual relative humidity and t being the annual mean temperature.

Statistical analysis

We used the total data set (hereafter referred to as the Geometridae data set) as well as subsets to account both for possible phylogenetic effects and for differences in the activity of the species. In each data set, only grids with at least five species were included in the analyses to improve the information quality of the estimated mean value of colour lightness. The final complete Geometridae data set included 637 species over a total of 3777 grid cells, which covers approximately 65% of the total geometrid fauna of Europe. Most of the missing geometrid moth species belong to the subfamily Ennominae, which has not yet been completely treated in the monograph series. The subsets of the Geometridae data set comprised a) the subfamily Larentiinae (401 species, 3776 grid cells), b) the subfamily Sterrhinae (197 species, 3275 grid cells), c) strictly nocturnal geometrid moths (160 species, 3,614 grid cells) and d) at least partially diurnal geometrid moths (102 species, 3775 grid cells). Classification as nocturnal or at least partially diurnal geometrid moths is based on species accounts and our own field observations. Note that many species could not be classified according to a specific time of activity. In addition, geometrid moths that fly at dusk or dawn or that can be startled during daylight were not classified.

For each data set, the colour- lightness values of all species occurring within each grid cell were averaged. Thereby, one value was obtained for each cell, and this value was used as a dependent variable in the analysis. First, single ordinary least-square regressions (OLS) were applied. Generalized least squares (GLS) models were used to correct for effects of species richness and spatial autocorrelation in a multivariate analysis (R package *nlme*). Thereby, our models were weighted by the number of species per grid cell and the spatial autocorrelation structure was included using a Gaussian variogram model, which estimates how much of the similarity of two points can be accounted for by their spatial proximity. Environmental variables were z-transformed prior to analyses to alleviate scale effects. We used model averaging of all possible combinations of the explanatory variables from the GLS model (R package *MuMIn*) to obtain z-values of the averaged model as estimates for the relative importance of single predictor variables.

A null model analysis was run to test whether the observed geographical variation in the colour lightness of assemblages is a random effect of species composition or driven by environmental gradients. For this, we randomly resampled the colour-lightness values across the data sets, thus breaking phylogenetic relationships of species and colour lightness, and subsequently calculated the average colour lightness for each grid cell using the randomized data. Thereby, the species distribution ranges and number of species per grid cell remained unchanged. This procedure was repeated 1000 times, and the results were averaged for each grid cell. To quantify the deviation of the observed colour lightness per grid (CL_{obs}) from random colour- lightness values (CL_{null}), standardized effect sizes (SES) were calculated. The SES per grid cell was calculated as (CL_{obs} - CL_{null})/sd(CL_{null}). SES values indicate significant divergence from random colour lightness when the SES is larger than 1.96 or smaller than -1.96; this corresponds to the 95% confidence interval of a Gaussian distribution. Subsequently, the statistical analyses described above were repeated with SES as a dependent variable.

In an additional step, we used structural equation models (SEM, R package *lavaan*) to disentangle potential inter-correlating effects of the climatic variables. Solar radiation comprises UV-B radiation as well as temperature, and temperature is also incorporated in the calculation of the dew point temperature. Hence, we constructed an a priori SEM that accounts for the correlation between the predictor variables. All statistical analysis and calculations were conducted in R Version 3.1.3.

RESULTS

Colour-lightness patterns

In the full Geometridae data set, the average colour lightness of assemblages decreased with increasing latitude (OLS: $r^2 = 0.63$, $P < 0.01$), thus showing a clear geographical gradient, with predominantly dark-coloured species occurring in northern Europe and in alpine regions and predominantly light-coloured species occurring in southern Europe (Fig. 6.1.4.1). This pattern was also found for Larentiinae (OLS: $r^2 = 0.37$, $P < 0.01$; Fig.

6.1.4.2a), nocturnal species assemblages (OLS: $r^2 = 0.64$, $P < 0.01$; Fig. 6.1.4.2c) and diurnal species assemblages (OLS: $r^2 = 0.45$, $P < 0.01$; Fig. 6.1.4.2d), whereas Sterrhinae assemblages showed a more random colour- lightness pattern across Europe (OLS: $r^2 < 0.01$, $P = 0.04$; Fig. 6.1.4.2b).

Colour lightness of individual species ranged from 0.30 (Baptria tibiale) to 0.97 (Scopula subpunctaria), with a mean of 0.70 and a standard deviation of 0.13 (Appendix 3-S3). Species belonging to the subfamily Larentiinae were on average more darkly coloured than species belonging to the subfamily Sterrhinae (t-test, $P < 0.01$, Appendix 3-S4), whereas nocturnal and diurnal species did not differ in their colour lightness (t-test, $P = 0.17$, Appendix 3-S4)

The average colour lightness of Geometridae assemblages ranged from 0.59 to 0.86 (mean 0.68 ± 0.03 SD). The average colour lightness of Larentiinae assemblages ranged from 0.59 to 0.70 (mean 0.64 ± 0.01 SD) and that of Sterrhinae assemblages ranged from 0.75 to 0.88 (mean 0.8 ± 0.02 SD). The average colour lightness of nocturnal assemblages ranged from 0.59 to 0.80 (mean 0.68 ± 0.03 SD), and that of diurnal assemblages ranged from 0.59 to 0.81 (mean 0.67 ± 0.03 SD).

Regression models

Colour lightness of moth assemblages increased with increasing solar radiation in the Geometridae data set (OLS: $P < 0.01$, $r^2 = 0.67$; GLS: $P < 0.001$), Larentiinae subset (OLS: $P < 0.01$, $r^2 = 0.41$; GLS: $P < 0.01$), nocturnal subset (OLS: $P < 0.01$, $r^2 = 0.60$; GLS: $P < 0.001$) and diurnal subset (OLS: $P < 0.01$, $r^2 = 0.45$; GLS: $P < 0.01$). Solar radiation was the most important single predictor in the GLS of the Geometridae data set (z-value = 18.8), nocturnal subset (z-value = 12.9) and diurnal subset (z-value = 10.1) and the second most important predictor in the Larentiinae subset (z-value = 3.06; Table 6.1.4.1). Solar radiation explained almost no variance in the colour lightness in the Sterrhinae subset (OLS: $P < 0.01$, $r^2 = 0.01$; GLS: n.s.; Table 1).

The dew point temperature was also positively correlated to colour lightness in the Geometridae data set (OLS: $P < 0.01$, $r^2 = 0.37$; GLS: $P < 0.01$), Larentiinae subset (OLS: $P < 0.01$, $r^2 = 0.23$), nocturnal subset (OLS: $P < 0.01$, $r^2 = 0.40$; GLS: $P < 0.01$) and diurnal subset (OLS: $P < 0.01$, $r^2 = 0.34$; GLS: $P < 0.01$). It was the most important predictor in the Larentiinae subset (z-value = 9.41) and the second most important predictor in the Geometridae data set (z-value = 14.8), nocturnal subset (z-value = 12.2) and diurnal subset (z-value = 8.14; Table 1). Dew point temperature explained almost no variance in the colour lightness of the Sterrhinae subset (OLS: $P < 0.01$, $r^2 = 0.01$; GLS: n.s., Table 1).

Colour lightness decreased with increasing forest cover in the OLS models in the Geometridae data set ($P < 0.01$, $r^2 = 0.28$), Larentiinae subset ($P < 0.01$, $r^2 = 0.19$), nocturnal subset ($P < 0.01$, $r^2 = 0.38$) and diurnal subset ($P < 0.01$, $r^2 = 0.09$), but was not correlated to the forest cover after correcting for species numbers and spatial autocorrelation, neither in the Geometridae data set nor in any subset (GLS models,

Table 1). The colour lightness of the Sterrhinae subset increased with increasing forest cover in the OLS model ($P < 0.01$, $r^2 = 0.12$), but showed no correlation in the GLS model (positive ΔAIC, Table 1).

GLS models with SES values as dependent variables and GLS models with colour lightness as a dependent variable showed similar correlations to the environmental variables (Table 1). Radiation had the highest z-values in the nocturnal subset (14.6) and diurnal subset (10.1), closely followed by the dew point temperature (nocturnal: 12.8, diurnal: 8.14). The dew point temperature had the highest z-value in the Geometridae data set (17.0) and the Larentiinae subset (11.1), followed by radiation (Geometridae: 11.7, Larentiinae: 3.20).

SEM analysis

The SEM of the complete Geometridae data set (Fig. 6.1.4.3) accounted for 66% of the variation in the colour lightness of the assemblages. Colour lightness was strongly positively correlated to temperature (standardized parameter estimation = 1.90) and negatively correlated to dew point temperature (-1.60). Radiation was only weakly correlated to colour lightness (0.45). The SEMs of the subsets of Larentiinae, nocturnal geometrid moths and diurnal geometrid moths resembled these correlation trends, whereas the colour lightness of the Sterrhinae assemblages was positively correlated to dew point temperature, negatively correlated to temperature and only weakly correlated to solar radiation (R^2 and standardized parameter estimations are given in the figures in Appendix 3-S5).

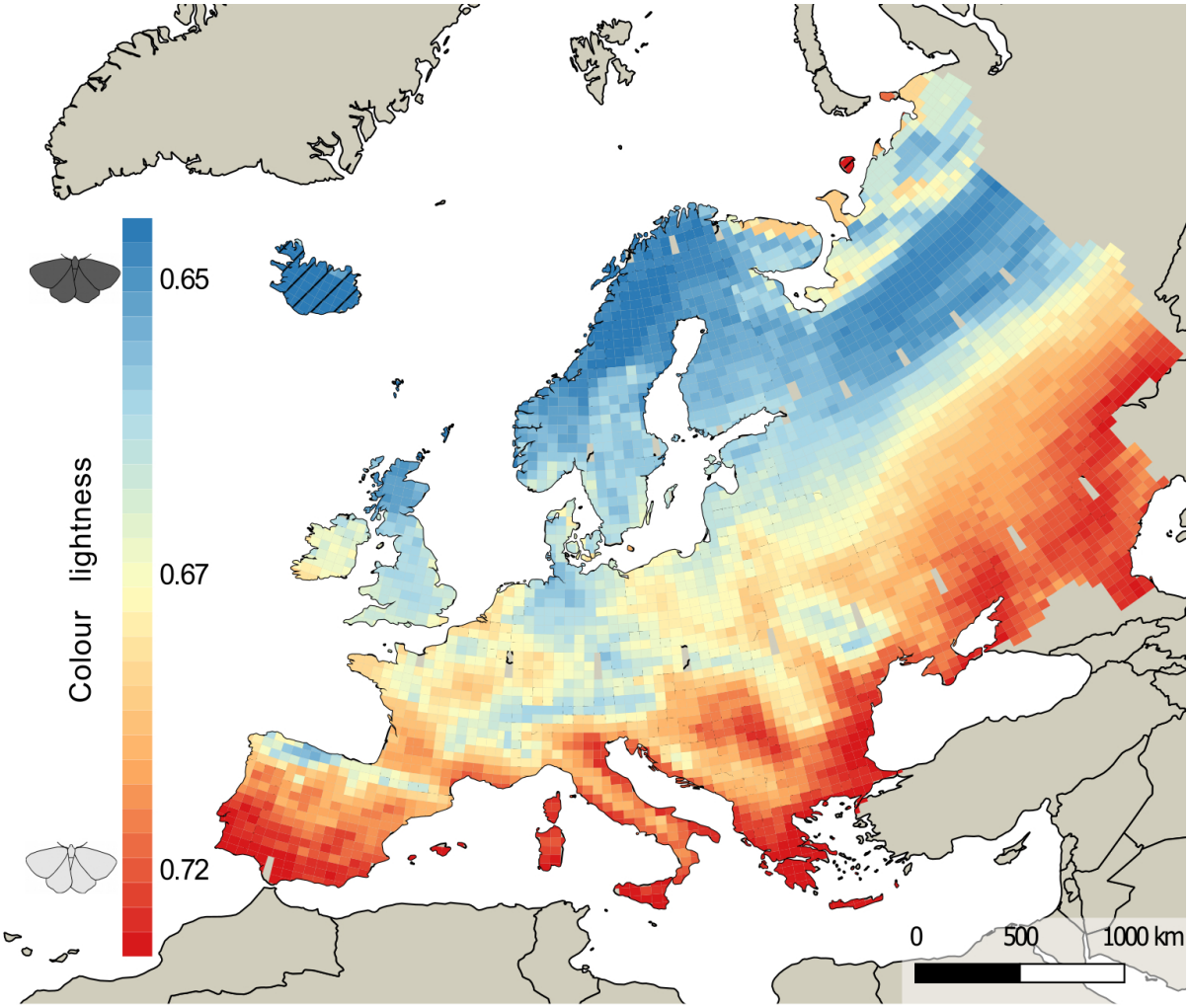

Figure 6.1.4.1: Mean colour lightness of assemblages of geometrid moths across the Western Palearctic. The colour coding represents quantiles, ranging from 0 (black) to 1 (white), with blue indicating dark colouration and red indicating light colouration. Grids with less than five species are hachured. Map created using Lambert Azimuthal Equal-Area projection. Dark-coloured species predominantly occur in northern regions and light-coloured species predominantly occur in southern regions.

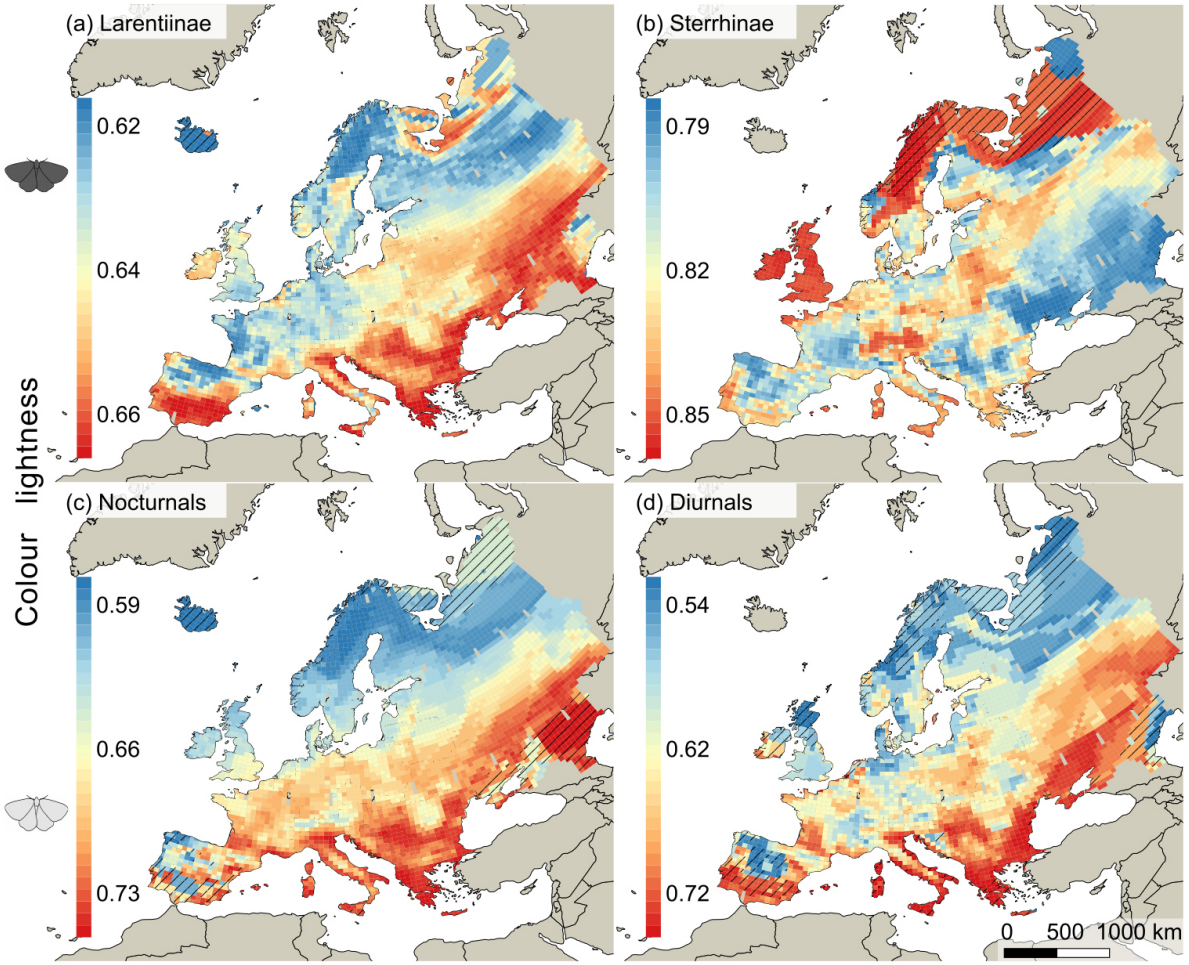

Figure 6.1.4.2: Mean colour lightness of subsets of assemblages of geometrid moths across the Western Palearctic. **a**) Subfamily Larentiinae, **b**) subfamily Sterrhinae, **c**) nocturnal species and **d**) diurnal species. The colour coding represents quantiles ranging from 0 (black) to 1 (white), with blue indicating dark colouration and red indicating light colouration. Grids with less than five species are hachured. Map created using Lambert Azimuthal Equal-Area projection. For the subfamily Larentiinae and for nocturnal and diurnal species, dark-coloured species predominantly occur in northern regions and light-coloured species predominantly occur in southern regions. The mean colour lightness of assemblages of Sterrhinae shows no clear gradient.

Table 6.1.4.1: Model results. Estimated coefficients of generalized least squares models (GLS) using average colour lightness (CL) of geometrid moths per grid cell and standardized effect sizes (SES) as dependent variables and standardized environmental variables as predictors. Models were calculated for the full data set (Geometridae) and for subsets (subfamilies: Larentiinae, Sterrhinae; activity period: nocturnal, diurnal). GLS models include a species-richness weight and a Gaussian spatial covariance matrix. ΔAIC shows the difference between the GLS model with all environmental variables and the intercept model; a negative sign represents lower AIC values in the GLS model compared to the intercept model and a positive sign represents higher AIC values (*** , $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P > 0.05$). The colour lightness of assemblages of geometrid moths increased with increasing dew point temperature and solar radiation and was not correlated to forest cover. This trend was consistent over all subsets except for Sterrhinae, for which the GLS model had a higher AIC than the intercept model.

Subset	Intercept	Forest cover		Dew point		Solar radiation		ΔAIC
Geometridae								
CL	0.68	< 0.001	n.s.	0.005	***	0.012	***	-214.4
SES	-3.16	0.001	n.s.	0.715	***	0.924	***	-290.1
Larentiinae								
CL	0.63	< -0.001	n.s.	0.003	***	0.002	**	-42.78
SES	-0.49	0.019	n.s.	0.373	***	0.186	**	-105.3
Sterrhinae								
CL	0.82	< -0.001	n.s	0.001	n.s.	< 0.001	n.s.	43.00
SES	-0.28	-0.032	*	0.048	n.s.	< 0.001	n.s.	14.33
Nocturnal								
CL	0.67	0.001	n.s.	0.005	***	0.010	***	-103.5
SES	-1.68	0.014	n.s.	0.471	***	0.999	***	-216.2
Diurnal								
CL	0.67	< 0.001	n.s.	0.006	***	0.011	***	-104.1
SES	-0.08	0.014	n.s.	0.216	***	0.407	***	-120.6

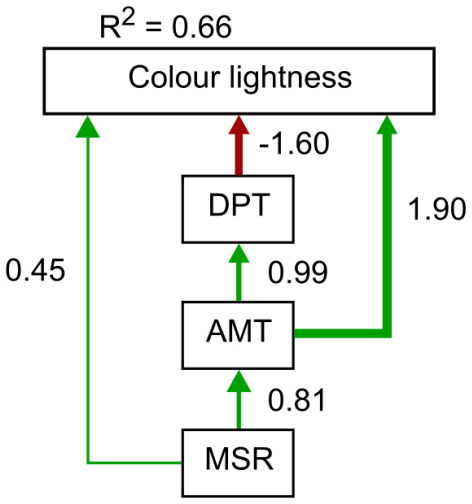

Figure 6.1.4.3: Results of structural equation modelling used to distinguish effects of annul mean temperature (AMT), mean solar radiation (MSR) and dew point temperature (DPT) on the colour lightness of assemblages of geometrid moths. This model explained 66% of the variation in colour lightness. Arrows represent causal paths, and the thickness is proportional to path coefficients. All standardized coefficients shown were significant at $P < 0.01$. The colour lightness of assemblages of geometrid moths decreases with increasing dew point temperature, and increases with increasing MSR and AMT. AMT has the strongest direct effect on colour lightness.

DISCUSSION

We found that the average colour lightness of assemblages of geometrid moths decreased clearly from south to north across the Western Palearctic, forming a very distinct gradient ranging from light-coloured assemblages in Mediterranean Europe to dark-coloured assemblages in northern Europe and north-western Russia. We also found similar trends towards more darkly coloured assemblages at high elevations in mountain chains, e.g. Alps, Pyrenees and Carpathians (Fig. 6.1.4.1). Unexpectedly, however, the best environmental predictors of this geographical pattern among these largely nocturnal insects did not include canopy cover, which would indicate the importance of crypsis and the light environment, but solar radiation and dew point temperature.

Our study was the first attempt to relate the colour lightness of species to their light environment on a macroecological scale. Although colour lightness is probably the most fundamental component of camouflage (Stevens *et al.* 2006), its effectiveness depends on multiple factors that act at smaller spatial scales, such as the specialization on resting sites (Endler 1984), effectiveness of disruptive colouration (Schaefer & Stobbe 2006) and body orientation (Kang *et al.* 2015). Furthermore, both tree species composition and seasonality affect the forest light environment (Endler 1993). By using forest cover as a proxy for the average light environment on a grid cell scale of 50 km × 50 km, we naturally missed much of this fine-scale variation, which might have blurred the results.

In contrast to forest cover, however, both dew point temperature and solar radiation

were strongly correlated to the colour lightness of assemblages of geometrid moths. At first glance, the positive correlation between dew point temperature and colour lightness, i.e. light-coloured assemblages found in warm and humid regions, stands in contrast to the pathogen resistance hypothesis. However, both radiation and dew point temperature contain a thermal aspect that seems to be the dominant factor shaping the colour lightness of assemblages of geometrid moths according to our structural equation models (Fig. 6.1.4.3, Appendix 3-S5). This thermal aspect seems to be responsible for the positive relationship between colour lightness and dew point temperature in our GLS models. This result supports the temperature-dependent immune investment hypothesis postulated by Fedorka *et al.* (2013). According to this hypothesis, temperature is the dominant factor shaping colour lightness, and it indirectly influences melanin-based immunity by altering the quantity of melanin pathway components. As a consequence, thermoregulation through light colouration to prevent overheating might act as an immune constraint for insects in warm climates, especially if environments are also humid, and therefore exert a higher pathogen burden.

Interestingly, the correlations between colour lightness and dew point temperature as well as solar radiation were observed for both diurnal and nocturnal species. Dark colouration should obviously not be advantageous from a strictly thermoregulatory perspective for species whose activity period is during the night. However, the temporal niche of nocturnal activity decreases with latitude in summer, which leads to a decreasing share of nocturnal species in northern areas (Appendix 3-S6). Thus, misclassification of the predominant activity period due to latitudinal variability in the temporal flight activity niche might have biased our results for nocturnal species. However, this does not explain the dark colouration of nocturnal geometrid moth assemblages in mountainous regions further south.

Furthermore, thermal melanism might also positively affect physiological processes during the resting phase. But because moths generally are more likely to seek shelter under branches or leaves during their resting phase to avoid direct exposure to predators (Mikkola 1984), solar radiation is unlikely to serve as a substantial direct heat source for the majority of resting nocturnal geometrid moths during daytime, at least for adults. This leads to the question why also nocturnal geometrid moths show a colouration gradient as expected by the thermal melanism hypothesis. We can think of three possible explanations. First, the observed continental gradient in the colour lightness of assemblages of geometrid moths could be an effect of a structural shift within the phylogenetic composition. Secondly, it could be related to thermal melanism of the larvae, many of which are at least partially active during the day. Thirdly, the thermal environment could affect colour lightness via developmental and physiological interdependencies.

Phylogenetic composition

The decreasing share of light-coloured Sterrhinae and the increasing share of dark-coloured Larentiinae with latitude (Appendix 3-S6) could have led to the observed overall gradient in colour lightness. However, we found essentially the same distinct colour-

lightness gradient within the Larentiinae (Fig. 6.1.4.2a). Hence, the observed gradient is more than a mere phylogenetic effect, and is rather the result of a functional relationship between the colour lightness of species and their environment. This conclusion is also supported by the results of our randomization tests (Table 6.1.4.1). The dominance of Larentiinae in northern Europe and the Alps (a phenomenon also recurring in other mountain areas of the world: Brehm & Fiedler 2003) might occur precisely because of a selective advantage that is related to their on average darker colouration.

A carry-over effect of thermal melanism in juvenile life stages?

Holometabolic insects such as lepidopterans go through fundamental transformations during metamorphosis, which frequently include changes from diurnal to nocturnal activity niches and vice versa. However, transitions in species traits from juveniles to adults are poorly understood (Pechenik 2006). In particular, it is unclear whether the colour lightness of adult insects depends on the colour lightness of their larvae. Temperature-related colouration of juvenile lepidopterans has been reported for several species (e.g. Davis *et al.* 2005, Karl *et al.* 2009). If such relationships are common amongst the Lepidoptera, the geographical pattern of colour lightness that we found might be the result of thermal melanism in the juvenile life stages being translated into adult pigmentation. However, also contrasting findings have been reported. For example, Välimäki *et al.* (2015) found that larval colouration was independent of temperature and Davis et al. (2005) found no evidence that colour lightness of the larvae was correlated to adult wing colour lightness within individuals of lepidopterans.

Interdependencies to other temperature-driven traits?

The thermal melanism hypothesis implies that under warm environmental conditions, dark colouration might ultimately lead to overheating. However, physiological and behavioural adjustments, e.g. through the choice of microhabitats, could diminish such disadvantages (Forsman 2011, Xing *et al.* 2016). Without being thermally restricted, dark colouration could per se be an overall positive trait in terms of fitness, as shown for *Biston betularia*, in which melanics reach adulthood more often than typical morphs (Creed *et al.* 1980).

In insects, melanin production is limited by the intake of nitrogen (Lee *et al.* 2008), which is especially true for holometabolous insects, which accumulate resources primarily as larvae (Karlsson *et al.* 2008). Hence, there possibly exists a trade-off between melanin production and other physiological and morphological traits that demand chemical compounds from the same limited pool (Roff & Fairbairn 2013). Co-variation of colour polymorphisms and life-history traits, including clutch size (Forsman 2001), gonad mass (Roff & Fairbairn 2013) and body size (Ahnesjö & Forsman 2003, Schweiger & Beierkuhnlein 2016, Xing *et al.* 2016), has often been reported (True 2003). These findings suggest that colour lightness might represent a trait that is involved in multiple developmental and physiological interdependencies, for example via genetic coupling (Mckinnon & Pierotti 2010) or by the regulation of juvenile hormone titres (Suzuki & Nijhout 2008).

The choice whether reproductive organs or somatic maintenance and survival are favoured by resource allocation depends on the species' life history: species with a longer expected life span must invest more resources in somatic maintenance, whereas those with a shorter expected life span instead invest in reproductive organs (Karlsson *et al.* 2008). The species life history itself is constrained by the length of the season: if the season is sufficiently long, a directly developing generation with a shorter life span might emerge next to the obligatory hibernating generation (Kivelä *et al.* 2013). An investment in melanin, and thus in immune system functioning, UV resistance and cuticle hardening, leads to higher somatic maintenance costs. Increasing voltinism (number of generations per year), and thus an increase of short living generations which favour an investment in reproductive organs rather than in melanin, should lead to an overall increase of colour lightness (lifetime melanin investment hypothesis). This hypothesis is supported by the strong positive relationship between environmental temperature and voltinism of lepidopteran assemblages (Zeuss *et al.* 2017), which coincides with our observed decrease of colour lightness with latitude.

Conclusions

Our results showed that the colour lightness of geometrid moths is a climate-driven multifunctional trait. The observed colour-lightness pattern fits to a colour-lightness pattern contingent on thermal melanism. However, a dark colouration in cold and moist environments not only seems to be advantageous in the thermal economy of basking ectotherms, but also likely provides additional fundamental fitness benefits for somatic maintenance. Increasing temperatures owing to climate warming might shift the allocation from investment in melanin towards investment in reproduction, or might affect colour lightness through other developmental and physiological interdependencies, which will lead to on average more lightly coloured assemblages of species.

6.2 Size

Environmental drivers of voltinism and body size in insect assemblages across Europe

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Environmental drivers of voltinism and body size in insect assemblages across Europe

ABSTRACT

General geographical patterns of insect body size are still a matter of considerable debate, mainly because the annual number of generations (voltinism) and its relationship with body size have largely been ignored. We present the first analyses of voltinism and body size of insect assemblages at a continental scale using lepidopteran and odonate species. We hypothesize that voltinism is strongly driven by environmental conditions and constrains body size on macroecological scales. We compiled the distribution, voltinism and body size of 943 lepidopteran and odonate species within a 50 km × 50 km grid system, thereby presenting a novel method for estimating the body volume of species from digital images. Regressions and structural equation modelling were applied to distinguish the effects of temperature, productivity and season length on mean voltinism and body size within grid cells. We accounted for spatial autocorrelation with autoregressive models and analysed the possible effect of species richness and intraspecific variability. Voltinism consistently decreased with latitude for both lepidopterans ($r^2 = 0.76$) and odonates ($r^2 = 0.86$), with species having on average fewer generations per year in northern Europe and more generations per year in southern Europe. The effects of temperature, productivity and season length on body size contrasted in sign between lepidopterans and odonates, leading to opposing geographical patterns across Europe. Voltinism in insect assemblages is strongly driven by environmental temperature, and trade-offs between voltinism and body size influence the occurrence of species at macroecological scales. Insects with the ability to extend their generation time over multiple years can overcome this constraint, allowing for a relatively large body size in cold areas. Our results furthermore support the idea that body sizes of terrestrial and aquatic insects form contrasting geographical patterns because they are differently affected by temperature and resource constraints.

INTRODUCTION

The most important trait influencing the physiological and ecological processes of an animal is its body size (LaBarbera 1989). Body size affects almost all physiological rates (e.g. the rate of oxygen consumption; Woods 1999, Atkinson *et al.* 2006), which subsequently determine or constrain fertility, mortality and ecological processes such as competitive interactions between individuals or species. In this way, body size is ultimately linked to the spatio-temporal distribution and abundance of animals (Blackburn & Gaston 2001) and has important implications for the impact of climate warming – from biomass production by single species to the structure and dynamics of communities (Sheridan & Bickford 2011, Forster *et al.* 2012). This link between physiology and distribution was first recognized by Bergmann (1848), who noted that body size increases with increasing latitude within and/or between closely related species of endothermic animals. Bergmann proposed that thermoregulatory advantages explain this pattern because of the low surface-to-volume ratio of large organisms and consequent improved heat retention in cold geographical areas. Since the publication of Bergmann's seminal work, considerable debates have flourished, especially with regard to whether Bergmann's rule constitutes a pattern, a process or both, whether it applies to intraspecific, interspecific or assemblage levels, and whether it also applies to ectothermic animals (e.g. Watt *et al.* 2010).

The body size of endotherms appears to increase consistently with decreasing environmental temperature (e.g. Ashton *et al.* 2000, Meiri & Dayan 2003, Olson *et al.* 2009), although it is clear that thermoregulation is not the only factor that influences the evolution of body size, given the many processes in which it is involved (see also Geist 1987). However, for ectotherms, especially insects, general patterns in the geographical variation of body size are still debated. The number of intraspecific and interspecific studies that show an increase in insect body size with latitude or elevation is nearly equal to those that show a decrease (Shelomi 2012). It is important to keep in mind that the physiological processes influencing geographical patterns of body size of ectotherms may fundamentally differ from those of endotherms. Three major hypotheses relating the body size of ectotherms to environmental conditions have been proposed, all of which operate at the individual level. However, if these hypotheses are also important during speciation, then their effects should be mirrored by the convergence of species traits in a particular environment (e.g. Gaston *et al.* 2008).

The first hypothesis, the temperature–size rule (TSR; Atkinson 1994), is based on the widely observed phenomenon that development and growth rates respond differently to variations in environmental temperature, specifically that increasing temperature accelerates the development rate more than the growth rate; hence, the adult stage is reached at a smaller body size with increasing temperature. This holds true for both unicellular and multicellular organisms despite differences in reproductive methods and ontogenetic timing, which suggests that there are different ultimate causes for the TSR (Forster *et al.* 2011). For example, it has been proposed that oxygen supply is an important driver of

the TSR in animals, especially in aquatic environments where oxygen uptake is more costly than in terrestrial environments (e.g. Forster *et al.* 2012, Horne *et al.* 2015; but see also Angilletta *et al.* 2004, Walters & Hassall 2006, Kingsolver & Huey 2008). From a geographical perspective, the TSR predicts a decrease in body size with increasing environmental temperature.

The second hypothesis deals with the availability of resources, which is essential for growth and thus for an animal's body size. Animals adjust their body size to the potential food supply of the environment in which they occur (Atkinson & Sibly 1997). Therefore, animals can grow larger in a resource-rich environment but must stay small when resources are limited. Productivity and latitude are usually negatively correlated; hence, this resource effect should, in contrast to the expectation from the TSR, lead to a decrease in body size with increasing latitude.

The third hypothesis concerns the length of the season, which affects the time available for growth and development. A longer season means a longer growing period and hence a larger final body size (Mousseau 1997, Chown & Gaston 2010). At high latitudes, the growing season is short, which, like the expectation from considerations of resource availability, leads to the prediction that body size should decrease with increasing latitude.

These three hypotheses, however, are influenced by one important factor that might constrain body size, namely vol- tinism, i.e. the number of generations per year. In a given area, a species with multiple generations per year has less time per generation available for growth than a species with only one generation per year. Since total growing time and body size are positively correlated, multivoltine species or populations should be smaller than univoltine species or populations (Roff 1980; but see Kivelä *et al.* 2011), as already suggested by Masaki (1967) and shown, for example, for the striped ground cricket, *Allonemobious fascia* (Mousseau & Roff 1989). In a recent intraspecific study, Horne *et al.* (2015) found that voltinism significantly affects strength and direction of latitude–size clines across different orders of terrestrial arthropods. Furthermore, an increase in temperature allows an increase in the number of generations per year, because it accelerates development rates so that more than one generation can be completed during the growing season (Gillooly *et al.* 2001, Altermatt 2010). Finally, the maximum number of generations per year is also constrained by resource availability and season length (e.g. Mousseau & Roff 1989). These possible indirect effects on body size via the number of generations per year stand in contrast to the possible direct effects of resource availability and season length on insect body size. Whether organisms in a particular environment invest in a large adult size or in several small generations is a matter of resource allocation, and of course differs within and between species (Kozlowski *et al.* 2004).

Here, we analyse direct and indirect effects of temperature, productivity and season length on geographical patterns of voltinism and body size of assemblages of lepidopterans and odonates within grid cells across Europe. So far it is largely unknown how environmental

filters influence the large-scale geographical distribution of voltinism and body size of insect species, which might lead to the convergence of traits within local assemblages. In contrast to previous studies (e.g. Altermatt 2010, Forster *et al.* 2012), our analyses hence focus on assemblages on a large spatial scale. For example, Horne *et al.* (2015), in an intraspecific study, found that body size of aquatic arthropod species generally decreases with warming and decreasing latitude in contrast to body size of terrestrial arthropod species, which shows reduced and often opposite clines. A calculation of averages across the species co-occurring within an assemblage may reveal subtle trends in the variation of traits across environmental gradients, despite considerable variation within and between species. We chose lepidopterans and odonates as study groups because reliable data on their large-scale distributions are available. Furthermore, the two groups differ in important biological aspects: butterflies and moths are terrestrial herbivores, whereas dragonflies and damselflies are carnivores with aquatic larvae. Lepidopterans have to cope with the phenology and low nitrogen content of many plants, whereas odonates continuously feed on a comparatively nitrogen-rich diet.

In particular, we: (1) analyse the relationships of voltinism and body size within assemblages to temperature, productivity and season length and (2) test the hypothesis that voltinism constrains the body size of lepidopterans as well as odonates. We predict that the body size of terrestrial lepidopterans decreases with latitude, whereas the body size of semiaquatic odonates increases with latitude. We furthermore hypothesize that voltinism is strongly determined by environmental conditions and constrains insect body size at macroecological scales.

MATERIAL AND METHODS

Body size data

Linear measurements, such as wing length or head width, are commonly used as a proxy for the body size of lepidopterans and odonates (e.g. Hawkins & Lawton 1995, Bried 2009; but see Hassall *et al.* 2008). Instead of these linear measures, we used the body volume (caput, thorax and abdomen) as a proxy for body size. The advantages of this threedimensional measure are that it also captures differences in shape that influence body mass and that it is not confounded by variations in wing morphology, which covaries with habitat or landscape structure (Van Dyck & Matthysen 1999). We scanned illustrations of adult species (imagos) from existing literature (see Appendix 4-S1 in the Supporting Information) with an Epson Perfection 4490 photo scanner and manually clipped background, wings and legs with GIMP 2.6 tools. We then calculated the total body volume as the sum of the volumes of each row of the clipped image ($\pi \times r^2 \times$ edge length pixel) using the resolution of the image to obtain metric values in mm^3. This procedure assumes that the body shapes are axially symmetric. Given the bilateral symmetry of higher organisms, this assumption seems to be acceptable. The images that we analysed were all of the same scale, and we obtained real size values by applying the scale factors given in the data sources. The obtained values are a surrogate for the average

adult body size of species (ignoring intraspecific variability). However, there might be intraspecific variability in body size due to, for example, sexual dimorphism, time of year, number of specimens used to estimate the body size of species and differences in body size within the distributional range of species, and such variability could bias the results. Therefore, we accounted for the possible effect of intraspecific variability on the results by running simulation tests (see the section 'Statistical analyses'). This seemed to be the most appropriate solution to this problem as sufficient spatially and temporally explicit data on within-species body size variation are not available for the species studied here.

We tested the comparability of our method of estimating the body size of species by analysing species body size values obtained from different books and found that body size values of odonates were highly correlated between two important sources ($r^2 = 0.95$, $P < 0.001$; Appendix 4-S2). We used images of female lepidopterans and male odonates because of their availability, and excluded all subspecies and families with only one species, which led to a dataset of 943 species. Body sizes of males and females were highly positively correlated (lepidopterans, $r^2 = 0.86$, $P < 0.001$; odonates, $r^2 = 0.96$, $P < 0.001$; Appendix 4-S3). Therefore, we conclude that our decision to compare female lepidopterans and male odonates does not bias the comparison between the two orders. We averaged body size values if more than one individual of a species was available, with a total of 1867 processed images and an average number of specimens per species of 1.98. We would like to note that our method can also be applied to many other species to obtain metric body size values.

Voltinism data

We assembled data on voltinism of European species of lepidopterans and odonates from existing literature (Appendix 4-S1). In particular, we used the number of generations per year and coded different levels of voltinism into numerical values. We scored uni-, bi- and trivoltinism at values of 1,and 3, respectively. Multi- and polyvoltinism scored 3. Some species of odonates need more than 1 year to complete one generation. Consequently, parti- and semivoltinism were scored at values of 0.33 and 0.5, respectively. If more than one value was available for a single species (228 of 943 species), we averaged the scores. We are aware that this averaging ignores intraspecific variability in the number of generations per year across the range of a species. For example, a species that completes one generation per year in its northern distributional range and two generations per year in its southern distributional range entered our analysis with 1.5 generations per year throughout its distributional range. However, according to the literature, only 18 of 943 species show an intraspecific increase in voltinism with latitude in single parts of their distributional range. We therefore did not run simulation tests for intraspecific variability in voltinism. We conclude that our averaging flattens north–south clines in the number of generations per year and therefore real geographical clines in the number of generations per year might be even steeper than the results of our study suggest, due to intraspecific variability.

Distributional data

Our study covers Europe as defined in Kudrna *et al.* (2011), excluding Belarus, Ukraine, Moldova and Turkey, for which distributional data are not reliable. We divided the study area into 50 km × 50 km grid cells of a polygon mask layer (Common European Chorological Grid Reference System). We excluded all islands except Great Britain and Ireland and grids cells with land coverage < 50% (coastal regions), yielding 1937 grid cells covering 4,833,693 km^2. We then digitized distribution maps of lepidopterans and odonates from existing literature (Appendix 4-S1) with the software WORLDMAP to estimate presence and absence of species within the grid system.

Data on the body size, number of generations per year and distribution were available for 845 species of lepidopterans and 98 species of odonates. We used the ln-transformed body size measures of each species. All assemblage-level analyses of body size were conducted using the arithmetic mean of ln-transformed body size values of all species in each grid cell, unless otherwise stated. All assemblage-level analyses of voltinism were conducted using the arithmetic mean of the number of generations per year of all species in each grid cell.

Environmental data

Besides latitude, three distinct environmental variables were selected to analyse the geographical patterns in the number of generations per year and body size of lepidopteran and odonate assemblages: annual mean temperature (AMT; °C) was chosen because of its direct relationship to the TSR (data from Hijmans *et al.* 2005; available at `http://www.worldclim.org/current`); net primary productivity (NPP; g dry matter m^{-2} $year^{-1}$) was chosen as an indicator of resource availability (data from FAO, available at `http://www.fao.org/geonetwork/srv/en/metadata.show?id536915`, period 1976–2000); growing degree days (GDD; base 5 °C), represents the length of the season and hence the time available for growth in a certain area (data from New *et al.* 1999). We calculated mean values of these variables for each 50 km × 50 km grid cell using GRASS GIS 6.4.3.

Statistical analyses

Regression analyses

In the first step, we used quantile regression to test for a link between the number of generations per year and body size at the species level (R package *quantreg*). Quantile regression is a suitable tool for analysing ecological relationships when the relationships are expected to be influenced by multiple variables (Cade & Noon 2003) and when the variance is not homogeneous across the range of the predictors, as was the case here (Levene's test, $P < 0.05$ for both lepidopterans and odonates). We then used ordinary least-squares regressions to explain the geographical variation in the average number of generations per year and body size of lepidopteran and odonate assemblages with AMT, NPP and GDD as predictors. We built single and multiple regression models and also included species richness, which we calculated from the distribution data for each

grid cell, as a weight in this analysis. We accounted for spatial autocorrelation with autoregressive models (R package *spdep*), in which the error term is predefined from a spatial neighbourhood matrix of a certain distance. This distance was identified using a spatial correlogram (R package *ncf*) and was set to 2000 km for both groups of analysed insects (Appendix 4-S4).

Structural equation modelling

We used structural equation modelling (SEM) to investigate direct and indirect effects of AMT, NPP and GDD on the average number of generations per year and body size within assemblages (R package *lavaan*). SEM allows the calculation of direct and indirect effects of predictor variables in a hierarchical linear regression setting of interconnected predictor and response variables. This setting can be mapped by a path model that represents the assumed causal relationships among the variables (Rosseel 2012). We specified our models based on the hypothesized relationships presented in the Introduction, with the intercorrelated dependent variables generations per year and body size and the intercorrelated explanatory variables AMT, NPP and GDD (Appendix 4-S5).

Randomization tests

We also analysed the possible effect of species richness on the average number of generations per year and the average body size within grid cells with two different randomization tests. First, we created 1000 datasets for lepidopterans and odonates by randomly sampling five species from each grid cell with at least five species (n = 1937 grid cells). We then calculated the average number of generations per year and the average ln-transformed body size within each grid cell and regressed these values against latitude for each dataset. Second, we randomized the assignment of voltinism and ln-transformed body size to lepidopteran and odonate species and calculated the average number of generations per year and the average body size within grid cells for the randomized data sets. We then calculated the standardized effect size as the observed average number of generations per year and body size within grid cells minus the expected values from the randomizations, divided by the standard deviation of the expectation. We repeated this procedure 1000 times and calculated the average effect size for each grid cell. The average effect size within grid cells was then regressed against AMT, NPP and GDD for lepidopterans and odonates (Appendix 4-S6).

Simulation of intraspecific variability of body size

To test for the possible influence of intraspecific variability on the general results of our study we set up two scenarios of body size variations within species across their distributional range. In each scenario, we first assumed that all species show intraspecific clines in body size and iteratively increased the relative level of intraspecific variability (in %). We then determined the percentage of intraspecific variability up to which our main results are robust and compared these obtained thresholds with known levels of intraspecific variability given in the original data sources. In both scenarios, we simulated intraspecific variability by calculating the intraspecific body size range for each species as

ln (body size ± (body size × variability × 0.5)). Subsequently, we divided the intraspecific body size range of each species into quantiles. These quantiles were calculated for each species by the number of grid cells in the north–south direction of the distributional range. Each grid cell in the distributional range was then assigned the corresponding value of the body size quantiles according to latitude – as either increasing or decreasing with latitude. In the first scenario (*random clines*), we randomly chose the geographical direction of body size clines within each species (increasing or decreasing with latitude). This random selection is reasonable because intraspecific clines in body size are not consistent in direction for either lepidopterans or odonates (see the Discussion). We then calculated simulated datasets for intraspecific variability between 1% and 100% in steps of 1%. Each step was repeated 100 times and the results were averaged per grid cell for each step. In the second scenario (*fixed clines*), we set the geographical direction of the body size clines within each species as increasing with latitude for lepidopterans and decreasing with latitude for odonates. These fixed intraspecific clines are contrary to the observed macroecological trends and the full range of intraspecific variability runs exactly counter to the observed macroecological trends; hence, this scenario is very conservative. We then calculated simulated datasets for intraspecific variability between 1% and 50% in steps of 1%. For both scenarios, we analysed the slopes of the regressions between ln body size and latitude of each level of intraspecific variability.

All calculations were performed in R version 3.2.3.

RESULTS

At the species level, lepidopterans had an average of 1.38 ± 0.59 (SD, n = 845) generations per year and an average body size of 43 ± 47 mm^3, ranging between 1.4 mm^3 (*Cleta perpusillaria*) and 447 mm^3 (*Papilio alexanor*). Odonates had an average of 0.98 ± 0.63 (SD, n = 98) generations per year and an average body size of 604 ± 571 mm^3 (SD, n = 98), ranging between 27 mm^3 (*Nehalennia speciosa*) and 2507 mm^3 (*Anax imperator*; Appendix 4-S7). Lepidopterans had on average more generations per year and a smaller body size than odonates (t-test, $P < 0.001$). Body size decreased with increasing number of generations per year for both groups of insect species (all slopes were negative for quantiles between 0.02 and 0.98 in steps of 0.01; Fig. 6.2.1.1). However, for a given number of generations within one year, odonates are able to develop to a body volume about ten times larger than lepidopterans.

The average number of generations per year within assemblages decreased clearly with latitude from southern to northern Europe, for both lepidopterans ($r^2 = 0.76$, $P < 0.001$) and odonates ($r^2 = 0.86$, $P < 0.001$; Fig. 6.2.1.2). In contrast, body size trends differed between lepidopterans and odonates: the average body size in assemblages of lepidopterans decreased with latitude ($r^2 = 0.30$, $P < 0.001$), whereas the average body size in assemblages of odonates increased with latitude ($r^2 = 0.35$ $P < 0.001$; Fig. 6.2.1.2).

Consistently throughout all models (linear, linear weighted, spatial), AMT, NPP and GDD were positively correlated with the average number of generations per year in lepidopteran and odonate assemblages (Table 6.2.1.1). Body size was positively correlated with AMT, NPP and GDD in all models of lepidopteran assemblages, and negatively correlated with AMT, NPP and GDD in all models of odonate assemblages. AMT explained the largest amount of variance in most of the models. However, GDD explained a slightly higher amount of variance than AMT in the linear and spatial models for the average number of generations per year in lepidopteran assemblages, and NPP explained a slightly higher amount of variance than AMT in the spatial model for the average body size in lepidopteran assemblages (Table 6.2.1.1). Note that for the average body size within assemblages, the amount of variance explained by all variables together was lower than for the number of generations per year (multiple linear models; Lepidoptera, $0.15 \leq R2 \leq 0.22$; Odonata, $0.29 \leq R2 \leq 0.41$; Table 6.2.1.1) but was still higher than what would be expected in interspecific studies of body size variation of insects along environmental gradients (mean = 0.17, median = 0.06, n = 50 studies; calculated with data from Shelomi 2012).

Our structural equation models also showed a strong positive influence of AMT on the average number of generations per year within lepidopteran and odonate assemblages (Fig. 6.2.1.3). The average number of generations per year and body size co-varied positively in lepidopteran assemblages and negatively in odonate assemblages. Hence, AMT had an indirect positive effect on the body size of lepidopterans and an indirect negative effect on the body size of odonates via the average number of generations per year. In addition to this indirect effect of AMT on body size, AMT also had a direct positive effect on body size in lepidopteran assemblages and a direct negative effect on body size in odonate assemblages. NPP had a direct positive effect on body size in lepidopteran assemblages, and GDD had a direct positive effect on the number of generations per year in lepidopteran assemblages (Fig. 6.2.1.3).

An increase in body size with latitude was also found in assemblages of odonates when only species with voltinism < 1 (more than one year per generation) were considered ($r^2 = 0.24$, $P < 0.001$; Fig. 6.2.1.4). However, the average body size in assemblages decreased clearly with latitude for species with voltinism > 1 (more than one generation per year) for both lepidopterans ($r^2 = 0.42$, $P < 0.001$) and odonates ($r^2 = 0.70$, $P < 0.001$; Fig. 6.2.1.4) and AMT explained the largest amount of variance in almost all models (Appendix 4-S8).

Randomization tests showed that the observed latitudinal trends in voltinism and body size are also consistent for a random subset of species from each grid cell. Additionally, the effect sizes calculated with randomized data revealed trends highly similar to those obtained with the original data (Appendix 4-S6).

Simulation of intraspecific variability showed that the latitudinal trends in body size were robust ($P < 0.001$; no change in the direction of the relationship) up to 100 % intraspe-

cific variability according to the random clines scenario and up to 21 % for lepidopterans and 30 % for odonates according to the fixed clines scenario (Fig. 6.2.1.5). The average intraspecific variability in body size obtained from the literature was 26 % for lepidopterans (variability in wingspan, n = 516 species) and 14 % for odonates (variability in body length, n = 98 species).

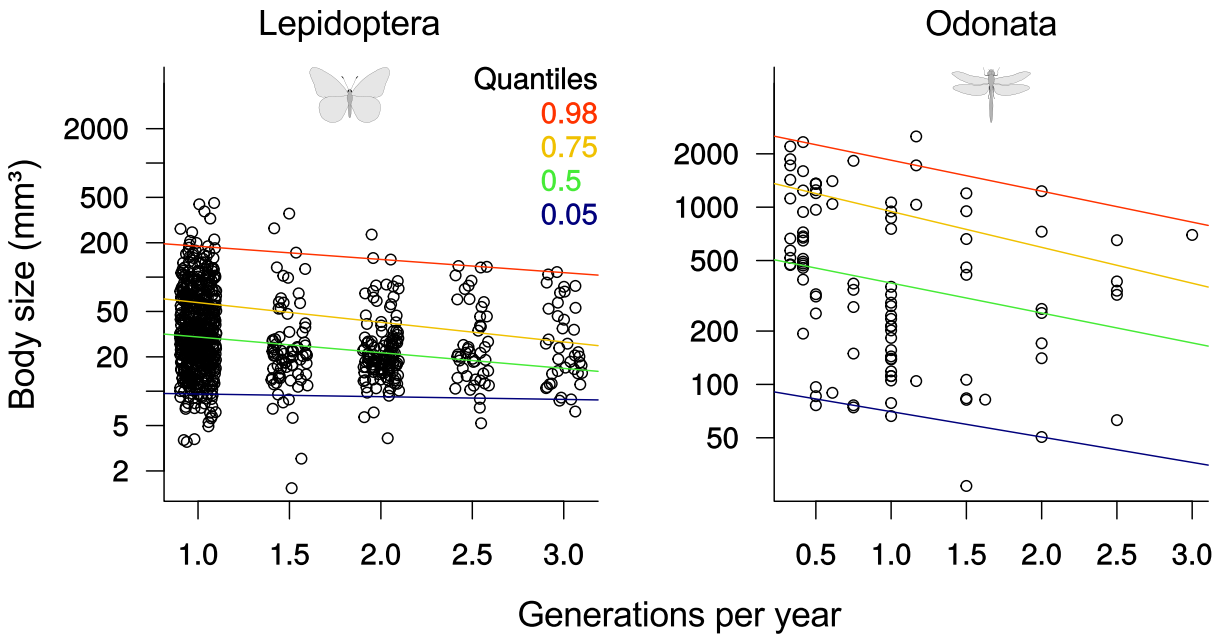

Figure 6.2.1.1: Relationship between body size and number of generations per year (voltinism) for lepidopteran (n = 845) and odonate species (n = 98) in Europe. Each dot represents one species. Jitter was added to the generations per year for lepidopterans to better distinguish single species. Note that body size decreases with increasing number of generations in both groups (quantile regressions, all slopes negative for quantiles between 0.02 and 0.98 in steps of 0.01). Note also that species with few generations per year can be both small and large, whereas species with more generations per year must be small.

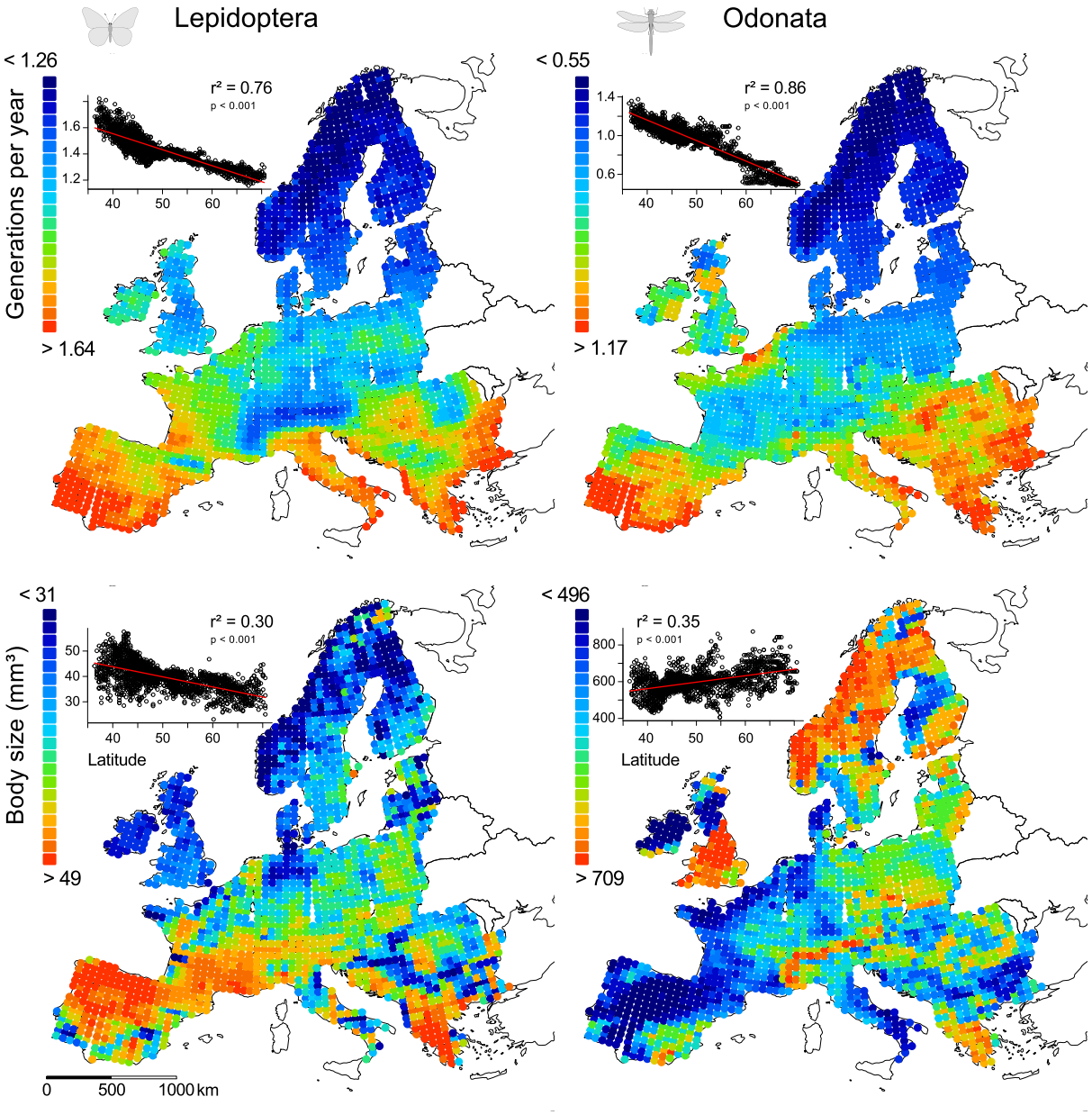

Figure 6.2.1.2: Average number of generations per year (voltinism) and body size within lepidopteran and odonate assemblages across Europe. The colour code was set according to an equal-frequency classification; red indicates assemblages with more species having many generations per year or large body size and blue indicates assemblages with more species having few generations per year or small body size (n = 1937 grid cells; EPSG 3575; Lambert azimuthal equal area projection). Note that the average number of generations per year decreases clearly from south to north in both groups. Note also that the average body size within lepidopteran assemblages decreases with increasing latitude, whereas the average body size in odonate assemblages increases with increasing latitude. Values of r^2 and p are from ordinary least-squares regressions with ln-transformed body size data.

Table 6.2.1.1: Models explaining geographical variation in the average number of generations per year (voltinism) and body size of lepidopteran and odonate assemblages in Europe. AMT, annual mean temperature; NPP, net primary productivity; GDD, growing degree days. Statistics were calculated with linear regressions (linear), regressions weighted with the number of species in each assemblage (linear weighted) and models to account for spatial autocorrelation (spatial). The explained variance is given for single variables (r^2, highest value of each model in bold, calculated with ln-transformed body size data) and for the full model (R^2). +/−, positive/negative relationship. Lepidoptera: n = 845 species across 1937 assemblages. Odonata: n = 98 species across 1937 assemblages. Note that directions of the relationships are consistent for voltinism but contrast for body size between lepidopterans and odonates. *p/P < 0.001; †p/P < 0.01; ‡p/P < 0.05. [1]Nagelkerke R^2.

			r^2			
Assemblage	Variable	Model	AMT	NPP	GDD	R^2
Lepidoptera	Voltinism	Linear	(+) 0.82*	(+) 0.15*	(+) 0.84*	0.87*
		Linear weighted	(+) 0.78*	(+) 0.06*	(+) 0.78*	0.83*
		Spatial[1]	(+) 0.82*	(+) 0.46‡	(+) 0.85*	0.88*
	Body size	Linear	(+) 0.17*	(+) 0.16*	(+) 0.12*	0.21*
		Linear weighted	(+) 0.11*	(+) 0.12*	(+) 0.06*	0.15*
		Spatial[1]	(+) 0.18*	(+) 0.23*	(+) 0.20†	0.22*
Odonata	Voltinism	Linear	(+) 0.78*	(+) 0.29*	(+) 0.62*	0.78*
		Linear weighted	(+) 0.67*	(+) 0.11*	(+) 0.54*	0.67*
		Spatial[1]	(+) 0.80*	(+) 0.58*	(+) 0.72*	0.80*
	Body size	Linear	(−) 0.37*	(−) 0.21*	(−) 0.28*	0.38*
		Linear weighted	(−) 0.28*	(−) 0.10*	(−) 0.21*	0.29*
		Spatial[1]	(−) 0.40*	(−) 0.34*	(−) 0.35*	0.41*

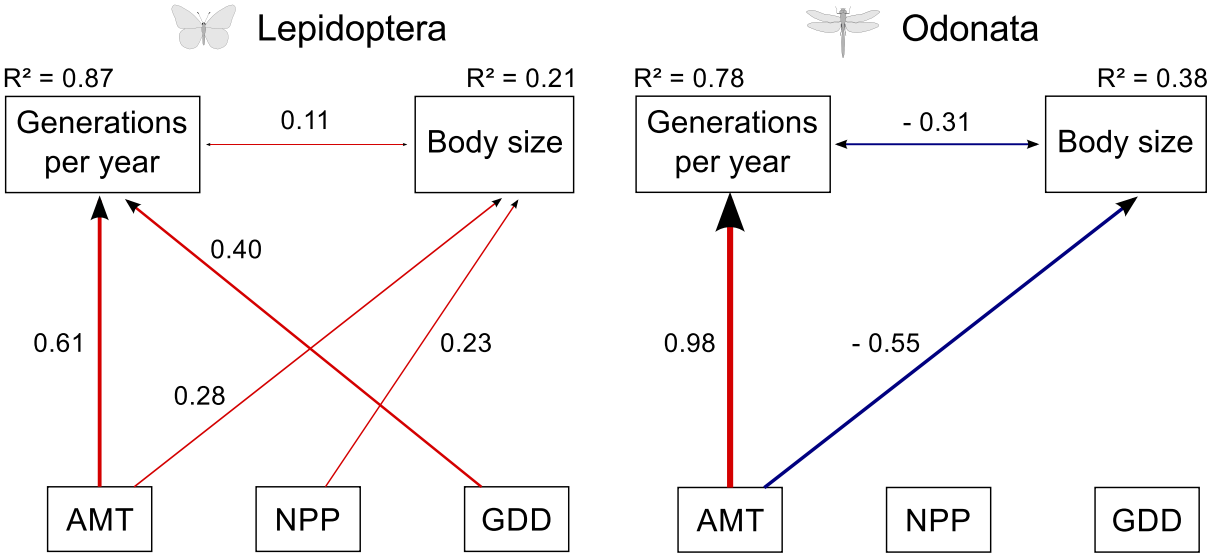

Figure 6.2.1.3: Structural equation models of the relationships between average generations per year (voltinism) and body size of European lepidopteran and odonate assemblages and three environmental variables. AMT, annual mean temperature; NPP, net primary productivity; GDD, growing degree days. Arrows indicate the direction and strength of the effects, and standardized coefficient estimates are given for each path. For convenience, only paths with $p < 0.001$ and predictor estimates > 0.15 are shown. Full model R^2 values for generations per year and body size are given for each group. Note that AMT strongly influences voltinism, which in turn positively covaries with lepidopteran body size and negatively covaries with odonate body size. Note also that the direct effects of AMT on body size of lepidopterans and odonates contrast in sign.

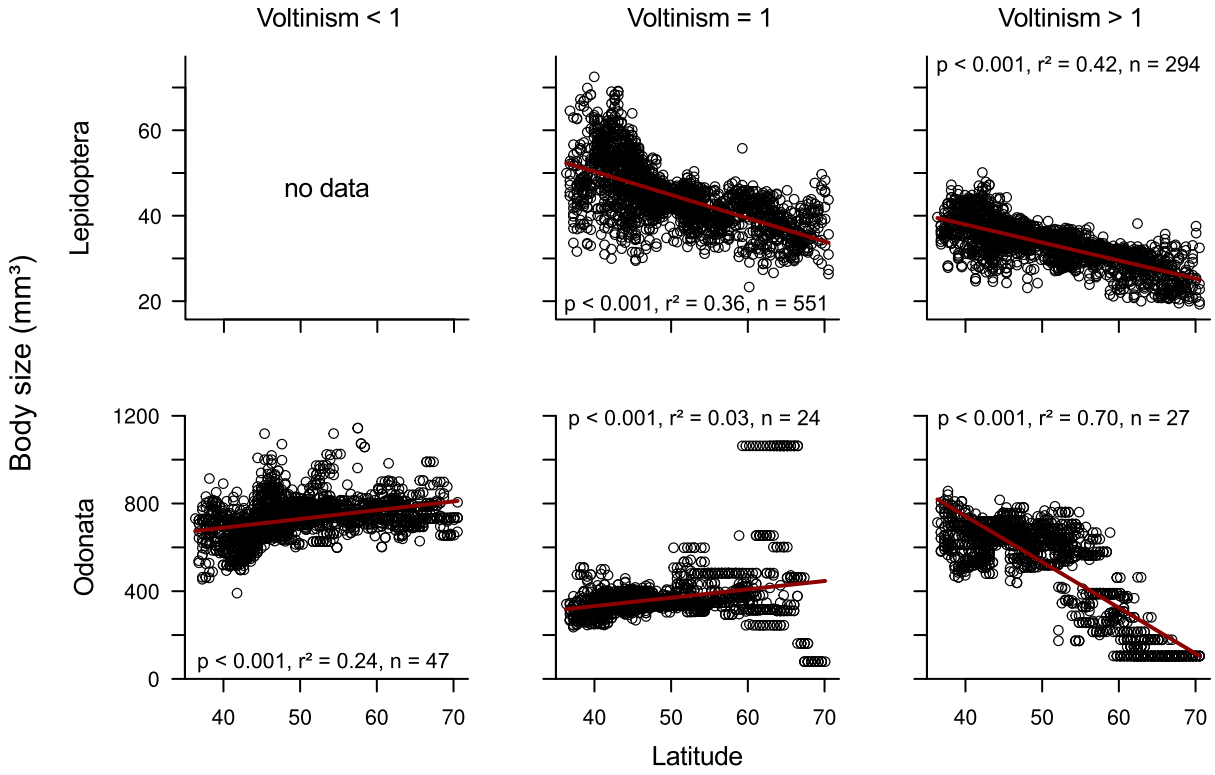

Figure 6.2.1.4: Scatterplots between latitude and average body size in assemblages of lepidopterans and odonates categorized by different levels of voltinism. Voltinism < 1, only assemblages of species which need more than one year per generation; voltinism $= 1$, only assemblages of species with one generation per year; voltinism > 1, only assemblages of species with more than one generation per year. Note that the dataset of this study does not contain lepidopteran species with voltinism < 1. Values of r^2 and p are from ordinary least- squares regressions with ln-transformed body size data; n refers to the number of species in the analyses. Note also that the average body size in assemblages of both lepidopteran and odonate species with voltinism > 1 decreases with latitude.

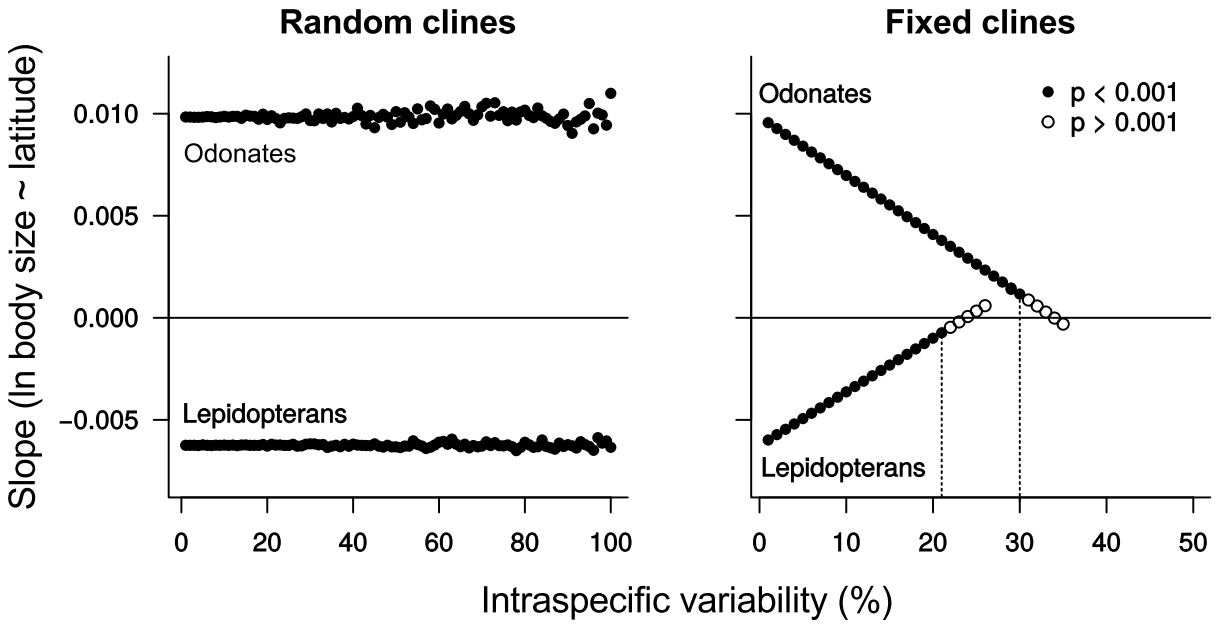

Figure 6.2.1.5: Slopes of regressions between body size and latitude for different levels of simulated intraspecific variability. Left panel: the north-south direction of intraspecific clines was selected randomly for each species as either increasing or decreasing with latitude. Right panel: intraspecific clines were set to be fixed and opposite to the observed macroecological trends for lepidopterans and odonates, i.e. increasing body size with latitude for lepidopterans and decreasing body size with latitude for odonates. Note that our observed geographical trends in body size are robust ($p < 0.001$, no change in the direction of the relationship) to intraspecific variability when the direction of intraspecific clines is randomly selected. Note also that our observed geographical trends in body size are robust to intraspecific variability up to 21 % for lepidopterans and 30 % for odonates (vertical lines) when all intraspecific variability of each species is simulated to be directly opposite to the observed macroecological patterns.

DISCUSSION

First, we found that voltinism constrains the body size of lepidopteran and odonate species (Fig. 6.2.1.1). This suggests that there is an upper limit for body size given a particular number of generations per year for species of both lepidopterans and odonates, even though odonates are much larger than lepidopterans. The most important aspect of this finding, however, is that not all species use the limits of the available time: many species produce one generation within a year but nevertheless grow only to a small body size.

We furthermore showed that the average number of generations per year in insect assemblages increases strongly with increasing ambient temperature: species living at high latitudes or in cool climates have a lower number of generations per year than species found at low latitudes or in warm climates. This result was consistent for both insect orders studied. However, we found contrasting geographical patterns of body size for lepidopteran and odonate assemblages across Europe: assemblages in northern Europe are dominated by relatively small lepidopteran species and relatively large odonate species. In addition, direct effects of temperature, productivity and season length on body size contrasted in sign between lepidopterans (positive) and odonates (negative), with temperature having the strongest effect in both groups. Hence, our results support the idea that body sizes of terrestrial and aquatic insects show contrasting geographical patterns because they are differently affected by temperature, resources and time constraints.

Our simulations of intraspecific variability showed that our main findings for body size are robust to within-species variability up to at least 100 % in the more likely random clines scenario (Fig. 6.2.1.5). Even in the most unlikely scenario (fixed clines), i.e. when all intraspecific variability of all species was modelled to be exactly opposite to the observed macroecological trends, our main results for body size were robust up to 21 % intraspecific variability for lepidopterans and up to 30 % intraspecific variability for odonates. Known levels of intraspecific variability taken from the literature are 26 % for lepidopterans (wingspan) and 14 % for odonates (body length); this variability also includes sexual dimorphism for both groups and variation in wing morphology for lepidopterans (see Van Dyck & Matthysen 1999). The results of our simulations showed that our main findings for body size are to some extent sensitive to intraspecific variability, but we conclude that this extent is too low to seriously bias our results and conclusions.

A possible explanation for the contrasting geographical patterns of body size of lepidopterans and odonates may lie in the different characteristics of their habitats, especially of their larvae, and associated life histories. Larvae of butterflies and moths are terrestrial herbivores that rely on plant tissue for growth, whereas larvae of dragonflies and damselflies are carnivores that feed on a comparatively nitrogen-rich diet. In addition, odonates often need several years to reach the adult stage, whereas lepidopterans often complete several genera- tions within a single year. Hence, odonates on average dedicate a larger proportion of their life cycle to growth. Following this line of argument,

the body size of odonates is less affected by resource and time constraints than the body size of lepidopterans.

We conclude that the average body size of co-occurring species of lepidopterans as well as odonates is influenced by temperature, productivity and season length, but the effects differ in strength and direction. For lepidopterans, we suggest that the temperature effect (TSR) is overwritten by resource and time limitations, especially in northern areas. Furthermore, the life history of lepidopterans is dependent on the phenology of their host plants and plant defence mechanisms (Cizek *et al.* 2006, Altermatt 2010), which might in part explain the stronger relationship between season length and the number of generations per year for lepidopterans compared with odonates and the high number of small univoltine species of lepidopterans. For odonates, we suppose that the temperature effect is strong because their larvae are more dependent on ambient temperature in the aquatic part of their life history due to the physical properties of water, where heat conduction is high and oxygen consumption is strongly determined by temperature (Verberk *et al.* 2011). However, a lot of unexplained variance in body size remained in this correlative study. As body size is involved in or constrained by many ecological processes, the low explanatory power of environmental variables for predicting the average body size of assemblages is not surprising. We also did not consider physiological traits relevant for the energetic budget of insects, for example their coloration (e.g. Zeuss *et al.* 2014). Given our finding that temperature is both a direct and indirect driver of body size, more detailed energetic considerations might be a promising future research direction for a more holistic understanding of variation in insect body size.

Our results of the average body size in lepidopteran assemblages support the findings of García-Barros (2000), who found a positive correlation between body size and temperature in a global interspecific analysis of 496 species of the superfamilies Papilionoidea and Hesperioidea. In addition, Barlow (1994), who analysed 1058 Australian and 2605 African species of the superfamily Papilionoidea, found a decrease in wingspan with increasing latitude, similar to the findings of Hawkins & Lawton (1995). These latter authors, however, found no consistent relationships between latitude and body size for European species, which might be a consequence of the authors' mid-point approach and the division of Europe into coarse latitudinal bands. A decrease in lepidopteran wingspan with increasing latitude in Sweden was reported by Nylin & Svärd (1991) in an intraspecific analysis of nine species. By contrast, Karl & Fischer (2008) showed that the body size of *Lycaena tityrus* increases with decreasing developmental temperatures when reared in the laboratory without food and time limitations. The authors' explanation for this result is increased food intake and higher efficiency in converting food into body matter at low temperatures. This intraspecific trend contrasts with the decrease in lepidopteran body size with increasing latitude found in our interspecific study of species assemblages. However, the absence of food and time limitations may be the reason for their results. Our finding that the geographical clines in voltinism and body size match in sign for lepidopterans shows that butterflies and moths cannot always compensate for food and

time limitations in northern areas because their life history does not allow them to grow for more than one season. We suggest that this is the major reason for the small average body size in northern assemblages of lepidopterans compared with southern areas, where lepidopterans tend to have both more generations per year and larger body sizes.

To the best of our knowledge, no macro-scale analysis of the body size of odonates is currently available. There are, however, several intraspecific studies, for example the study of Hassall *et al.* (2008), who found that the body mass of *Coenagrion puella* increases with latitude. Similarly, Hassall *et al.* (2014) found a positive correlation between latitude and body size of *Erythromma viridulum*, and Bried (2009) concluded from an intraspecific analysis of four species in Mississippi, USA, that on average larger and heavier species of odonates are found in the northern parts of the state. By contrast, Śniegula *et al.* (2012) found that individuals of *Coenagrion puella* and *Coenagrion pulchellum* collected in Poland are larger than those from Sweden, which indicates a negative intraspecific relationship between body size and latitude. The differences in the findings for *C. puella* between Hassall *et al.* (2008) and Śniegula *et al.* (2012) may lie in different collection times during the year, as the body size of odonates declines with the date of emergence (summarized in Corbet 2004). However, the above-mentioned studies on odonates differ from our study as they exclusively deal with intraspecific variability, whereas our analyses were at the assemblage level.

Finally, although the average number of generations per year within lepidopteran and odonate assemblages consistently decreased with decreasing temperature, the average body size showed contrasting geographical patterns for both groups (Fig. 6.2.1.2). We suppose that species with the ability to extend their generation time over multiple years can overcome this constraint, which allows a relatively large body size in cold areas. In addition, our finding that the average body size of species with more than one generation per year strongly decreased with latitude in assemblages of both lepidopterans and odonates underlines the importance of voltinism-body size trade-offs for the large-scale geographical variation in insect body size.

Our study also has implications for the consequences of global warming – with increasing temperature, large aquatic insect species should retreat to colder areas and species with the ability to increase their annual number of generations should benefit and extend their distribution to higher latitudes. These shifts in the distribution and probably also abundance of species will lead to a change in the phenology and overall body size structure of terrestrial and aquatic ecosystems. Attempts to forecast effects of climate warming must hence consider the strong temperature dependence of insect voltinism and its relationship to body size of terrestrial and aquatic species.

Bergmann's and Allen's rules in native European and Mediterranean Phasmatodea

with
Matan Shelomi
in revision in Frontiers in Ecology and Evolution

Bergmann's and Allen's Rules in Native European and Mediterranean Phasmatodea

ABSTRACT

Bergmann's rule states that organisms at higher latitudes should be larger and thicker than those closer to the equator to better conserve heat, and Allen's rule states that they will have shorter and thicker limbs at higher latitudes. Alternative explanations for latitudinal size clines include plant productivity and seasonality. The rules generally hold in endotherms, but in insects different species within the same genus can respond to latitude in unpredictable ways. We present the first biogeographical analysis of these rules in stick insects (Phasmatodea), using four European species. Their long and thin bauplan makes the Phasmatodea ideal for ecomorphological studies of body length, which could identify the evolutionary drivers of their remarkable size range (including the world's longest insects). Using preserved specimens from collections across Europe; body segment and limb measurements were taken for both genders of the species *Bacillus rossius*, *Clonopsis gallica*, *Leptynia attenuata*, and *Pijnackeria hispanica*. Lengths and volumetric features were compared to latitude as well as annual mean temperature, net primary productivity, and season length (growing degree days), using weighted linear regressions and ANOVA analyses. Most variation in size could be attributed to annual mean temperature. *C. gallica* showed a weak Bergmann cline. Both genders of *B. rossius* had strong converse-Bergmann clines, and *B. rossisus* females and both genders of *L. attenuata* had longer limbs in warmer regions. *P. hispanica* showed converse-Allen clines only. Our results highlight the complexity of body size evolution in Phasmatodea, and identifying which rules hold true in tropical Phasmatodea or in multi-species analyses of the order is a promising topic for future studies.

Body size of species rich moths increases along a large tropical elevational gradient

with
Gunnar Brehm
in preparation for Ecography

Body size of species rich moths increases along a large tropical elevational gradient

ABSTRACT

The body size of an animal is probably its most important functional trait. For arthropods, however, environmental drivers of body size variation are still poorly documented and understood, especially in tropical regions. Here, we use a large and unique dataset of two species rich phylogenetically independent moth taxa (Lepidoptera: Geometridae, Arctiinae) to investigate body size variation along a large tropical elevational gradient in Costa Rica. Forewing lengths (FWLs) as a proxy for body size were measured and analysed using two approaches: mean FWLs of each species at each site and mean FWLs of complete local assemblages. Analyses were carried out at the level of higher taxa (family, subfamilies) and at the species level. We analysed potential differences in FWLs between males and females and incorporated wing loadings in our analyses. Linear and multiple regressions were calculated with ambient temperature, rainfall and enhanced vegetation index as predictors for body size variation in addition to elevation. In total, 15,047 specimens (794 species) of Geometridae and 4,167 specimens (308 species) of Arctiinae were analysed. Between species, FWLs increased significantly with elevation in both sexes, both taxa and both approaches, either linearly or asymptotically. Within species, the FWLs of between 60 and 75% of the species with at least 10 individuals in the dataset showed a significant increase in FWL with elevation. Wing loading increased with FWL, and thus with elevation, in both taxa. FWLs were significantly negatively correlated with temperature ($-0.98 < r < -0.74$), whereas the contribution of the other environmental variables was inconsistent. We conclude that the body size of lepidopterans is strongly influenced by environmental temperature, which is in accordance with predictions from the temperature-size rule. Our results indicate that the temperature-size rule might be an important mechanism underlying body size variation in arthropods also in tropical regions.

Part III

Synthesis and outlook

Although I identified important environmental drivers of colour and size in insects, several knowledge gaps remain in the current literature – especially for the drivers of colouration in tropical regions and through time, and for the interdependencies between colour and size.

It is still unknown if the temperature-dependency of colour lightness is also valid in tropical environments at large spatial scales. This is particularly important because factors influencing the colouration of insects may differ between tropical and adjacent regions. Furthermore, it is unknown how the colour lightness of insects changes with elevation in tropical regions. This is particularly important because different processes may lead to the evolution of colour lightness along elevational gradients in the tropics. Finally, it is unknown how the colour lightness of insects has changed over longer time spans at local and regional scales. This is particularly important because knowledge of past changes in colour lightness might help to predict consequences of climate warming on future insect distributions.

Considering that geographical gradients in the colour lightness of insects are shaped by several processes simultaneously, and that different environmental factors may be involved in tropical and temperate regions, along elevational gradients, and through time (Gaston *et al.* 2009, Bastide *et al.* 2014), it will be necessary to extent the previous work to the above mentioned scales and to non-heliothermic taxa to gain a deeper understanding of the mechanistic drivers of colour lightness in insects.

If the response of colour lightness to temperature is consistent in both non-tropical and tropical regions and beside other functions of insect colouration than thermal melanism, then I expect **a**) a double hump-shaped relationship between colour lightness and latitude with darker coloured insects found towards the poles and with a peak of colour lightness in hot deserts (Fig. 6.2.3.1a), **b**) a decrease in colour lightness with increasing elevation (Fig. 6.2.3.1b), and **c**) an increase in colour lightness through time during the last century due to climate warming (Fig. 6.2.3.1c).

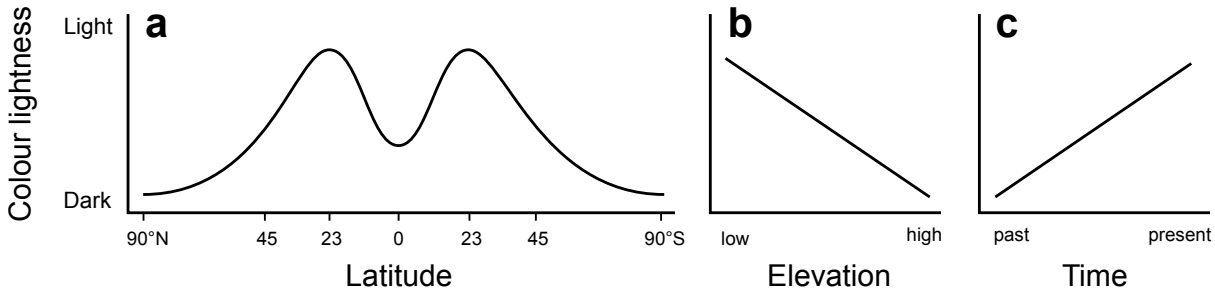

Figure 6.2.3.1: Expected simplified relationships between the colour lightness of insects and latitude, elevation, and time.

For insect body size, it has been shown that the effects of temperature, productivity and season length differ in strength and direction between aquatic and terrestrial species

(Horne *et al.* 2015, Zeuss *et al.* 2017), with warming-induced reductions in body size being larger in aquatic than terrestrial species (Forster *et al.* 2012). A reduction in body size has even been termed to be a universal response to climate warming (Gardner *et al.* 2011, Sheridan & Bickford 2011), as predicted by the temperature-size rule. While the ultimate causes underlying the temperature-size rule are still debated, it seems to be clear that metabolic rate and its effect on life history play a central role in determining adult body size in insects (Gillooly *et al.* 2001, Kozlowski *et al.* 2004). A life-history perspective might hence be suitable to integrate the drivers of colour and size into one framework

Melanin pigments and body size are related to the fitness of insects – especially survival and growth – but are costly to produce. Investments in melanin and body size are hence likely to be traded-off against investments in reproduction. At cold temperatures, the lifetime investment in survival and growth is large (long lifetime with a large proportion dedicated to growth and high investment in survival) leading to dark colours and large body sizes – dark colours because of their benefits for thermoregulation and survival (e.g. pathogen resistance), and large body sizes according to the temperature-size rule. At warm temperatures, the lifetime dedicated to growth is relatively short. Resources might therefore be primarily invested in reproduction, leading to light colours and small sizes (Fig. 6.2.3.2). This framework is of course very simplified but might be a starting point to be extended in the future.

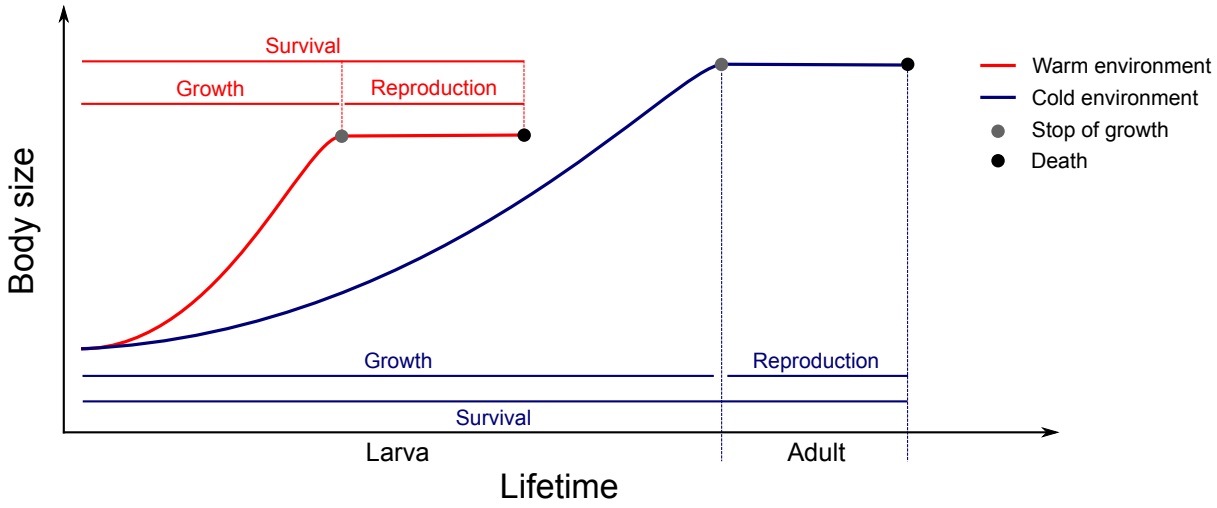

Figure 6.2.3.2: Schematic change of insect body size with lifetime. Curves are depicted for warm (red) and cold (blue) environments, divided into phases of survival, growth and reproduction. Each curve represents the life of an individual of an univoltine holo- or hemimetabolic species without food and time (season length) limitations. Note that environmental temperature accelerates the development rate more than the growth rate, leading to the adult stage being reached earlier and at a smaller body size in warm environments. Note also that the lifetime in a cold environment is relatively long with a large proportion dedicated to growth and high lifetime investment in survival.

I would like to conclude this thesis with two predictions that arise from its results and

considerations: Firstly, it seems that thermal melanism and other fitness benefits imposed by melanin pigments generally constrain the colouration of insects in cold climates. Consequently, the colourfulness of insects could increase from the poles towards the tropics. Secondly, if the temperature-dependency of insect metabolic rates lead to relatively short lifetimes in warm environments, then life-history trade-offs might result in long-lived insects being more dark coloured than short-lived insects, including non-basking insect species.

Finally, it is important to keep in mind that the geographical distribution of species, which is the focal unit in this macroecological thesis, is the manifestation of complex relationships between various traits of individuals and climatic conditions and also involves biotic interactions (Liebig 1840, Hutchinson 1957, Brown *et al.* 1996, Lomolino *et al.* 2010). Despite this complex interplay between multiple abiotic and biotic factors, basic relationships and general patterns as identified in this thesis, may help to understand the present distribution of species and to even predict the broad impact of climate warming on ecosystems in future studies.

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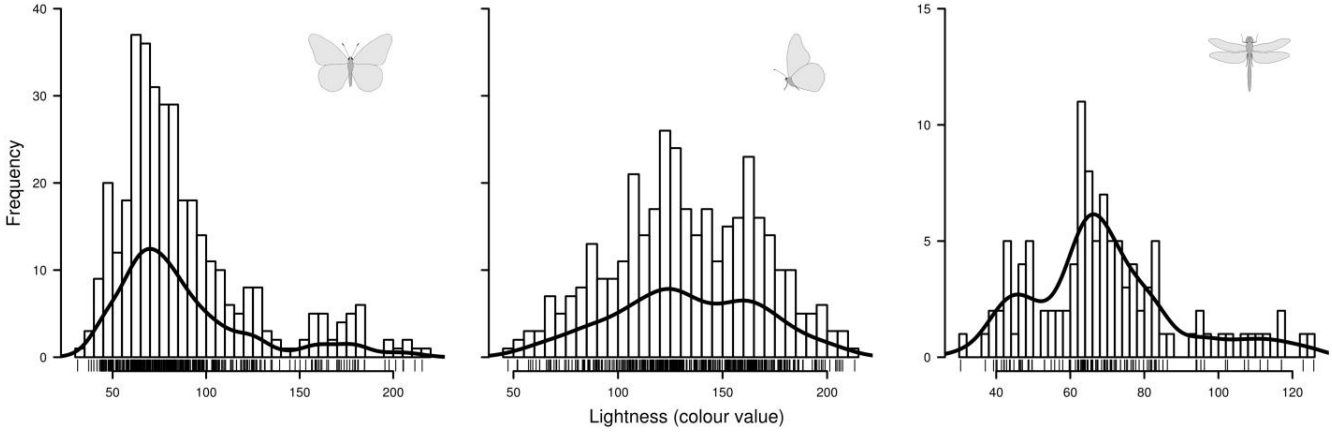
Part IV

Appendix

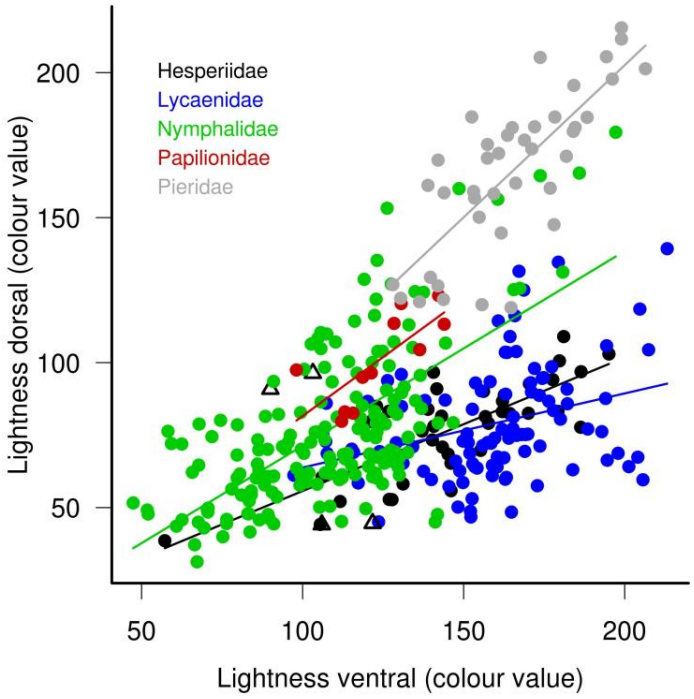
Supporting information

Global warming favours light-coloured insects in Europe

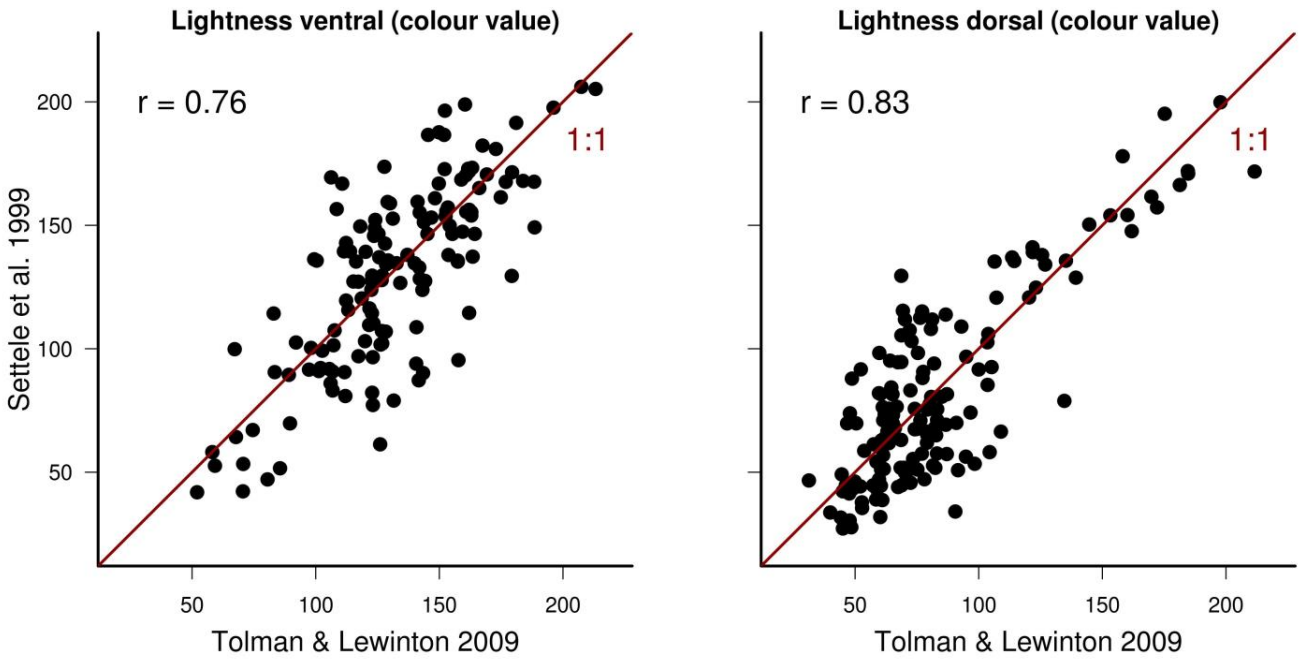
Supporting Information



Supplementary Figure 1 | Distribution of colour values for dorsal and ventral butterfly wings and for dragonfly bodies. We measured the colour value of the basal wing area of 366 species of butterflies and the thorax and abdomen of 107 species of dragonflies and converted the values to a grey scale (see Methods). Rugs at the abscissa indicate observed values.

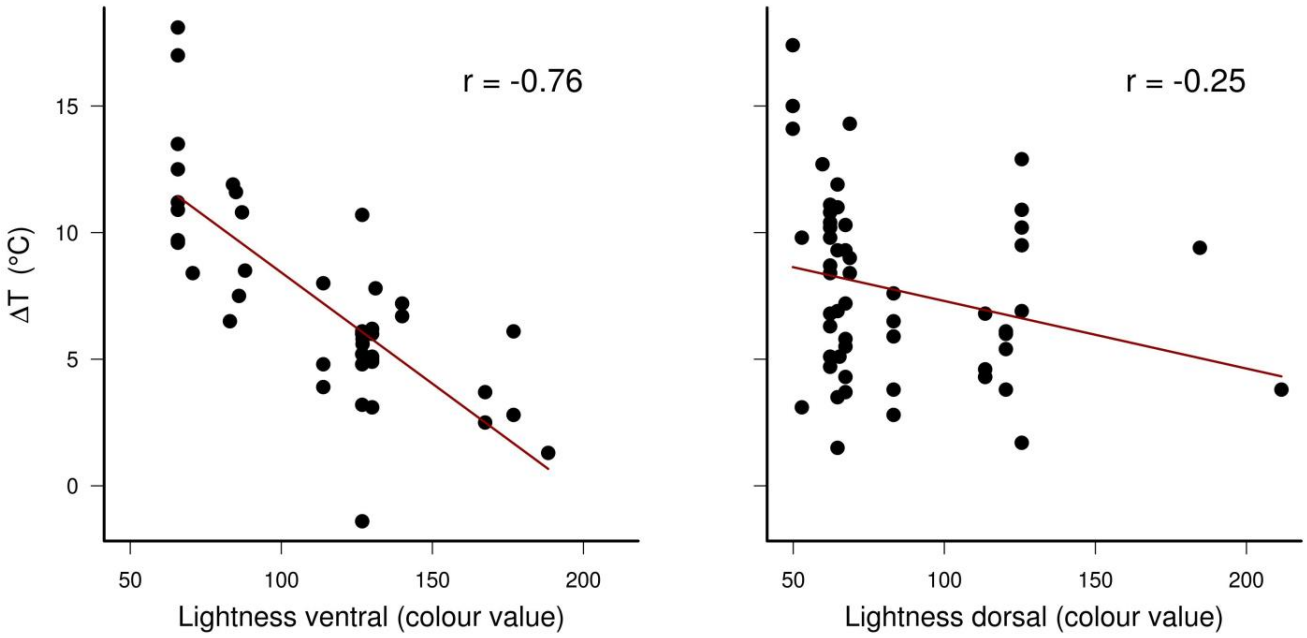


Supplementary Figure 2 | Correlation of ventral versus dorsal wing colour lightness of butterflies. For families with more than 10 considered species (see Supplementary Table 1), we also plotted a regression line. Black triangles indicate species in genera with less than 10 species. Note the clear difference in the colour value as well as the variation of the slope across families of butterflies.

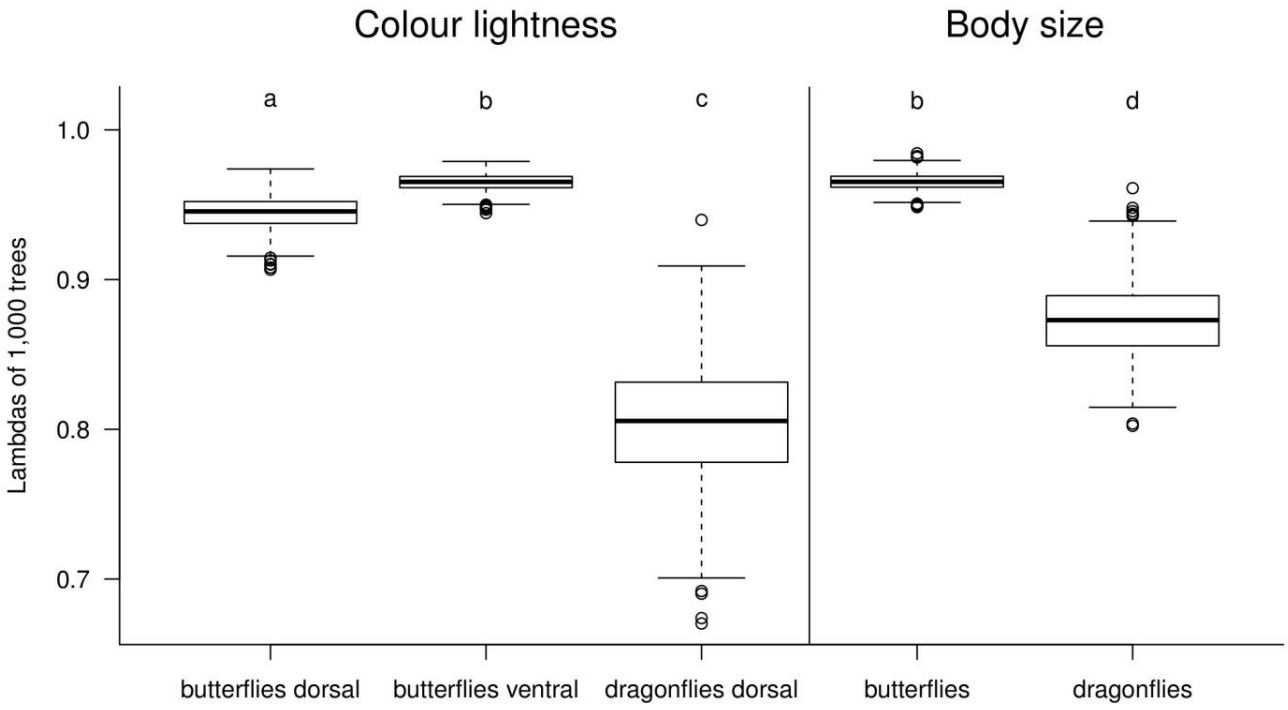


Supplementary Figure 3 | Plot of mean colour values of butterfly species illustrated in refs. 1 and 2. These plots show that the determined colour values remained similar when different sources of illustrated species were used (see Supplementary Methods).

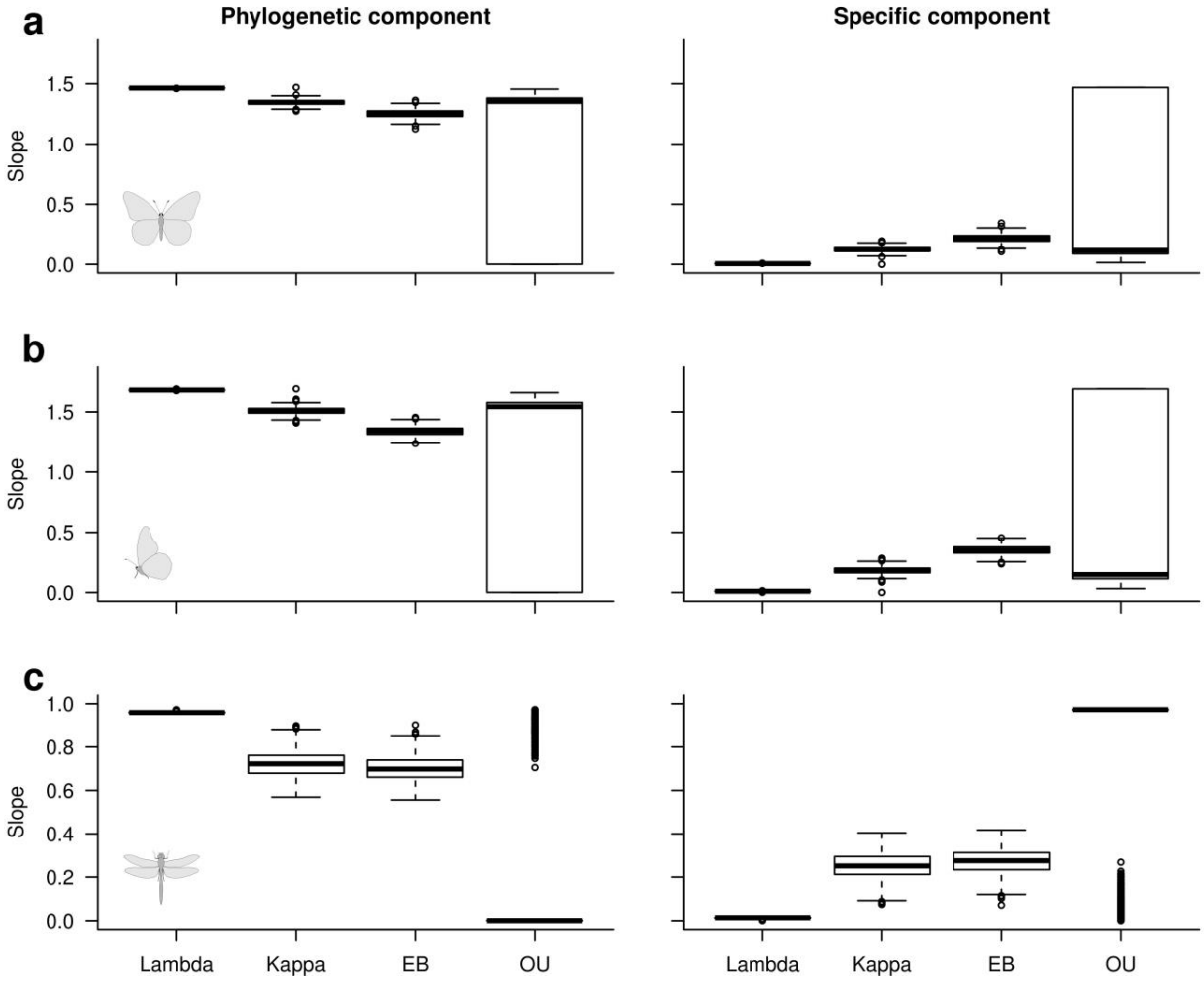
Supplementary Figure 4 | Measurement of the surface temperature of basal wing parts with an infrared thermometer "testo 845". Note that exact surface temperature is measured when and where the two red dots on the wings of the Apollo butterfly (*Parnassius apollo*) overlap while simultaneously ambient temperature of the instrument is recorded (Supplementary Methods).



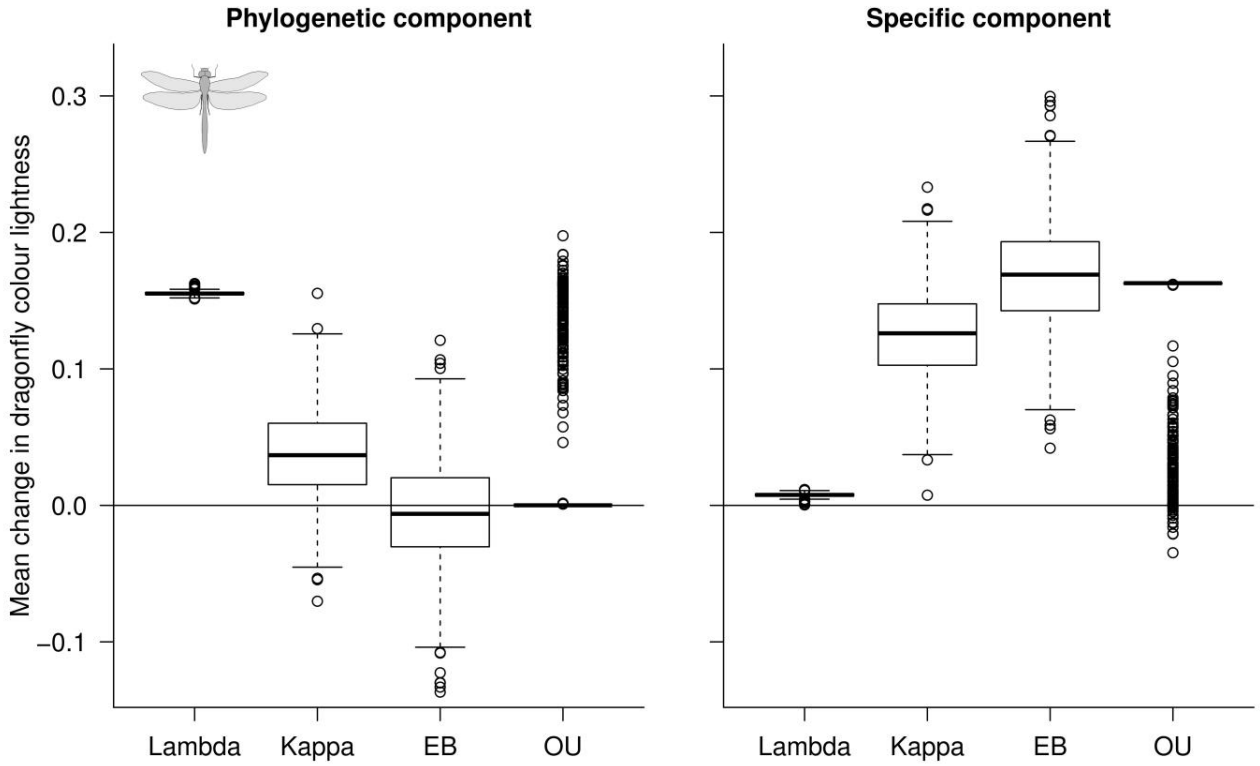
Supplementary Figure 5 | Correlations between the difference of butterfly surface temperature and ambient temperature (ΔT) and the corresponding colour values of the species. We measured the wing surface temperature of 166 individuals out of 18 species with a ultraredanalyzor ”testo 845” (Supplementary Methods). Note that dark-coloured species (low colour value) reached higher surface temperatures in relation to ambient temperature than light-coloured species.



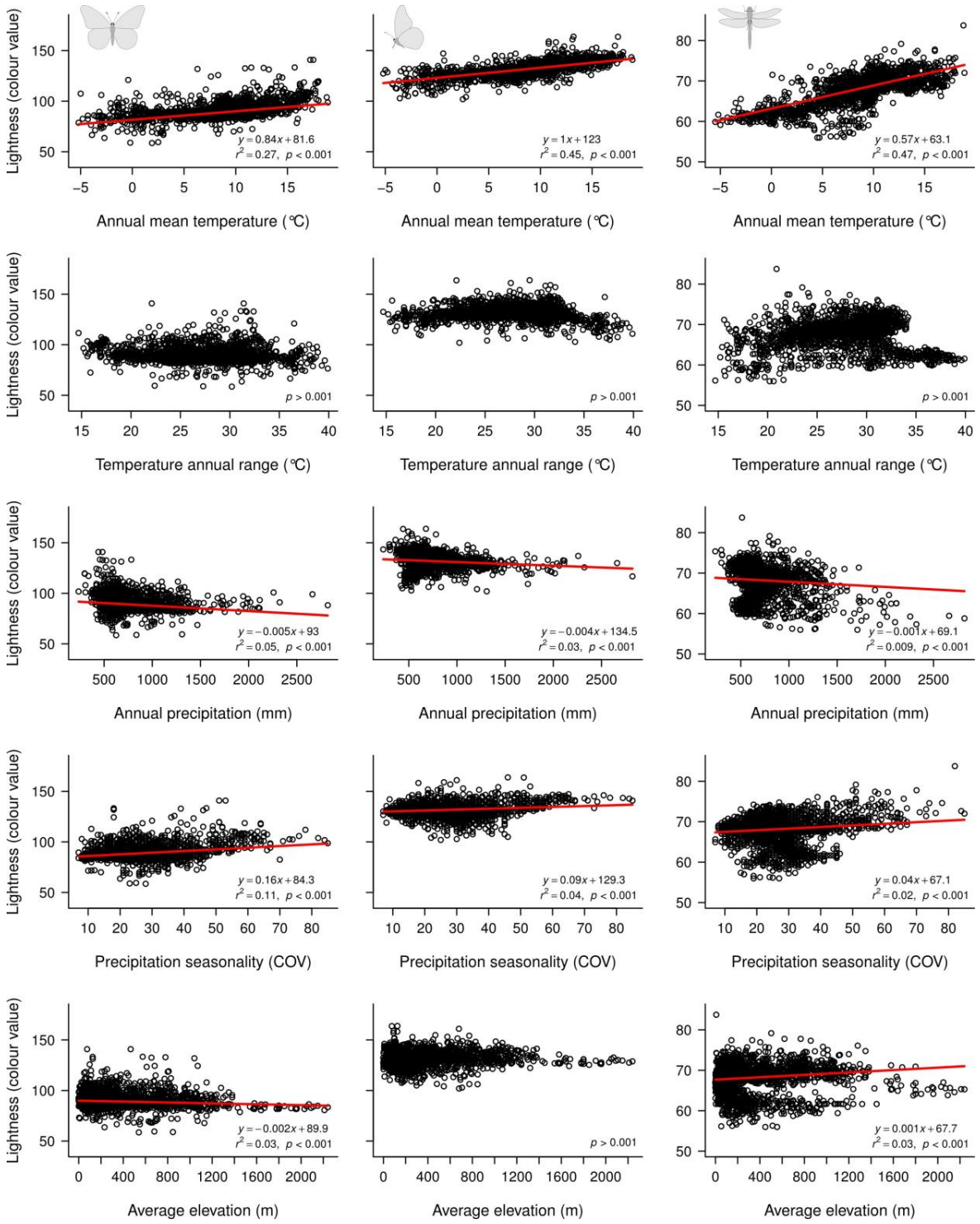
Supplementary Figure 6 | Phylogenetic signal (lambda) calculated for colour lightness and body size of European butterflies and dragonflies. Each boxplot represents lambdas of 1,000 phylogenetic trees in which polytomies were resolved (see Methods). We found high values of lambda (median and mean > 0.80 for all traits), indicating that colour lightness is highly phylogenetically conserved, with significantly higher values for butterflies than for dragonflies and with the ventral colour lightness of butterflies being phylogenetically as conserved as their body size (Tukey multiple comparisons of means with 95% family-wise confidence level). Boxes indicate first and third quartiles, whiskers indicate lowest and highest values within 1.5 times the interquartile range.



Supplementary Figure 7 | Slopes of regressions between phylogenetic and specific parts of colour lightness and thermal component 1 calculated with different evolutionary model transformations and alternative phylogenetic trees. Transformations for (**a**) dorsal and (**b**) ventral colour lightness of butterflies and (**c**) dorsal colour lightness of dragonflies were performed for 1,000 phylogenetic trees with the models Lambda, Kappa, Early-burst (EB) and Ornstein-Uhlenbeck (OU) (see Methods). Every single regression was highly significant (t-test, $p < 0.001$) with positive slopes throughout the models, indicating that different evolutionary models do not alter the positive relationship between colour lightness and the thermal environment. Boxes indicate first and third quartiles, whiskers indicate lowest and highest values within 1.5 times the interquartile range.

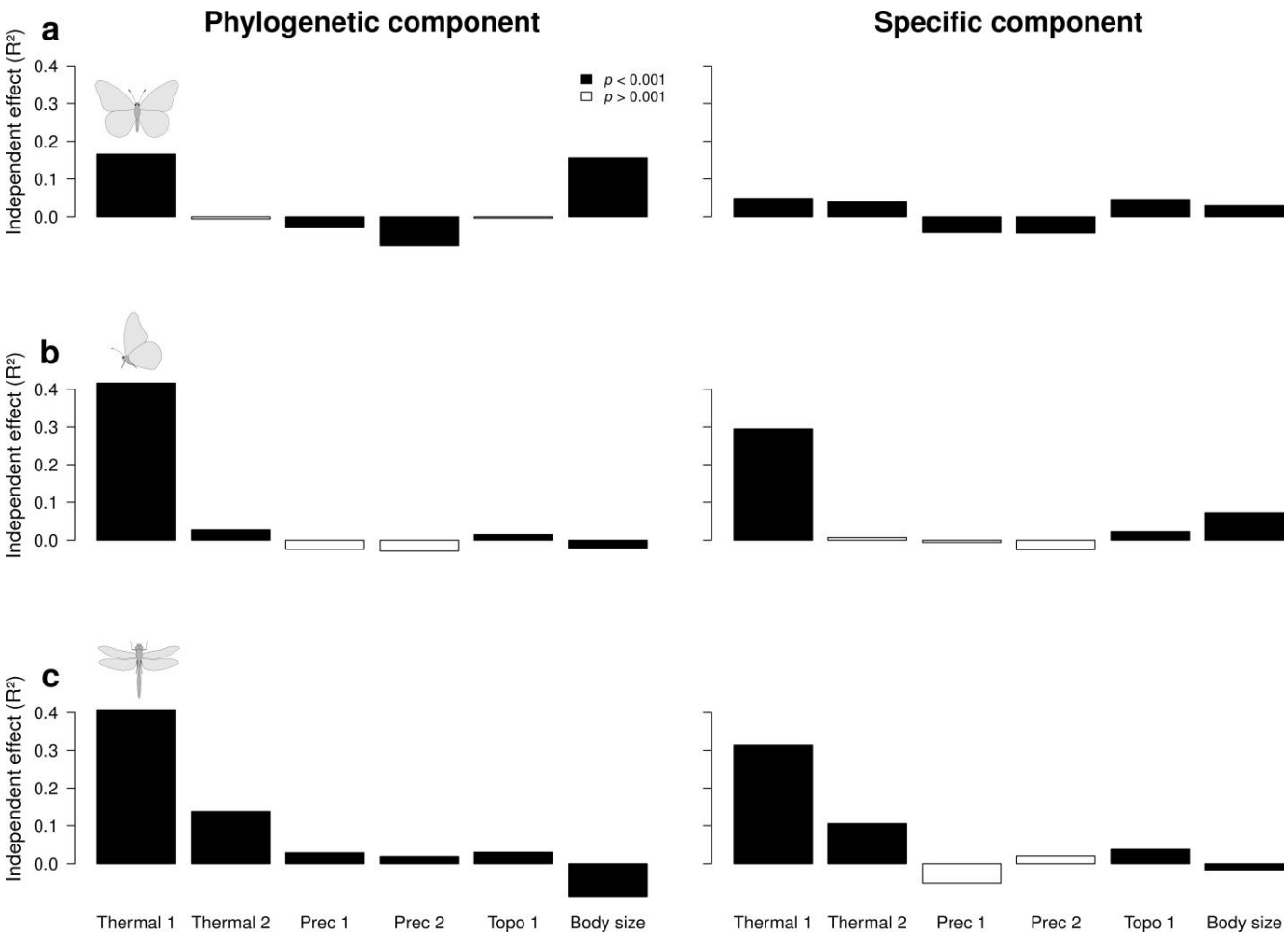


Supplementary Figure 8 | Mean change in the colour lightness of dragonfly assemblages across Europe between 1988 and 2006 under different evolutionary models. Phylogenetic and specific parts of colour lightness were calculated for 1,000 phylogenetic trees that were transformed according to the models Lambda, Kappa, Early-burst (EB) and Ornstein-Uhlenbeck (OU) (see Methods). Note that most values are positive — especially for the specific component, indicating that different evolutionary models do not alter the overall shift towards lighter coloured dragonfly assemblages. Boxes indicate first and third quartiles, whiskers indicate lowest and highest values within 1.5 times the interquartile range.



Supplementary Figure 9 | Illustration of the directions of ecological associations.

Simple weighted regressions between selected variables of each principal component and the dorsal and ventral colour lightness of butterflies and the dorsal colour lightness of dragonflies to illustrate the directions of ecological associations (butterflies: n = 1,825; dragonflies: n = 1,845).



Supplementary Figure 10 | Independent contribution of different predictors in explaining the variance of colour lightness of butterfly and dragonfly assemblages across Europe. Statistics were obtained from hierarchical partitioning for a multivariate regression setting of 6 predictors against the assemblage-based phylogenetic and specific components of mean (**a**) dorsal and (**b**) ventral colour lightness of butterflies and (**c**) dorsal colour lightness of dragonflies. The bars show the independent contribution of each predictor to the explanation (R-squared) of the variance in the spatial patterns of colour lightness. For details see Methods. Negative relationships are indicated with negative R-squared values, non-significant (t-test, $p > 0.001$) predictors are indicated with white bars (butterflies: n = 1,825; dragonflies: n = 1,845).

Supplementary Table 1 | Species richness of families of butterflies occurring in Europe and the mean colour value across species within families.

Family	Genera	Species	Species analysed	Colour value	
				dorsal	ventral
Hesperiidae	12	42	39	77.8	147.8
Papilionidae	5	11	11	100.8	123.7
Pieridae	12	47	40	166.0	165.2
Lycaenidae	38	107	98	78.9	159.1
Riodindae	1	1	1	44.6	121.7
Libytheidae	1	1	1	44.2	106.0
Nymphalidae	43	195	174	75.4	109.1
Danaidae	1	2	2	93.6	96.6

In ref. 1, 406 species and 113 genera are listed; 366 species entered our analysis. The mean colour value of dorsal and ventral wings across species within families (mean colour value of dorsal wing area 89 ± 36; mean colour value of ventral wing area 133 ± 36; see Supplementary Figs. 1 and 2) ranges from 0 (totally black) to 255 (totally white).

Supplementary Table 2 | Species richness of families of dragonflies occurring in Europe and the mean colour value of the dragonfly body across species within families.

Family	Genera	Species	Colour value
Aeshnidae	6	17	65.9
Calopterygidae	1	3	58.1
Coenagrionidae	7	20	56.6
Cordulegastridae	1	3	56.7
Corduliidae	5	9	71.4
Euphaeidae	1	1	101.9
Gomphidae	5	12	80.2
Lestidae	2	8	72.1
Libellulidae	11	31	73.1
Platycnemididae	1	3	85.2

In ref. 3, 107 species and 40 genera are listed. The mean colour value of dragonflies on a grey scale was 69 ± 20. These values range from 0 (totally black) to 255 (totally white).

Supplementary Table 3 | Butterfly species in ref. 1 not considered in the analysis.

Species	Reason for exclusion from the analysis
Artogeia balcana	Not considered in ref. 4
Pontia edusa	Taxa not separated in ref. 4
	P. daplidice complex
Euchloe crameri, E. simplonia	Taxa not separated in ref. 4
	E. ausonia complex
Elphinstonia charlonia	Not considered in ref. 4
Maculinea rebeli	Taxa not separated in ref. 4
	M. alcon complex
Agrodieateus agenjoi	Not considered in ref. 4
Plebejus loewii	No insolation data available for occupied grids
Lysandra philippi	Not considered in ref. 4
Polyommatus andronicus	Not considered in ref. 4
Polyommatus menelaos	Not considered in ref. 4
Pseudochazara hippolyte hippolyte	Subspecies not considered in ref. 4
Pseudochazara mamurra	Not considered in ref. 4
Hipparchia pellucida	No insolation data available for occupied grids
Maniola chia	Not considered in ref. 4
Maniola telmessia	No insolation data available for occupied grids
Coenonympha elbana	Not considered in ref. 4
Coenonympha darwiniana	Taxa not separated in ref. 4
	C. gardetta complex
Ypthima asterope	No insolation data available for occupied grids
Carcharodus stauderi	No insolation data available for occupied grids
Pelopidas thrax	Not considered in ref. 4

Supplementary Table 4 | Butterfly species and number of individuals with measurements of wing surface temperatures.

Family	Species	Individuals analysed	
		dorsal	ventral
Hesperiidae	*C. palaemon*	3	5
	O. venatus	5	7
Papilionidae	*I. podalirius*	4	0
	P. apollo	10	1
Pieridae	*G. rhamni*	0	1
	P. brassicae	2	4
	P. rapae	1	7
Lycaenidae	*P. icarus*	10	2
	L. phlaeas	3	1
Nymphalidae	*A. urticae*	11	0
	I. io	12	8
	N. c-album	2	0
	A. levana	2	0
	A. paphia	10	9
	A. hyperanthus	3	9
	M. jurtina	3	12
	M. galathea	9	2
	E. ligea	6	4

Supplementary Table 5 | Results from principal component analyses of variables assigned to the categories thermal environment, precipitation, and topography.

Thermal environment	PC1	PC2	PC3
INS	0.83	0.27	0.22
AMT	0.96	0.14	−0.20
MDTR	0.44	0.70	0.44
IT	0.84	−0.21	0.23
TS	−0.66	0.73	0.04
MTWM	0.79	0.59	−0.03
MTCM	0.95	−0.23	−0.20
TAR	−0.45	0.86	0.22
MTWeQ	−0.05	0.66	−0.67
MTDQ	0.89	−0.12	0.24
MTWaQ	0.82	0.50	−0.18
MTCQ	0.98	−0.15	−0.15
Eigenvalue	7.12	3.03	0.99
Cumulative proportion	0.59	0.85	0.93
Precipitation	**PC1**	**PC2**	**PC3**
AP	0.98	−0.15	0.06
PWM	0.88	−0.44	−0.10
PDM	0.88	0.44	0.03
PS	−0.31	−0.88	−0.32
PWeQ	0.89	−0.43	−0.10
PDQ	0.91	0.38	0.10
PWaQ	0.69	0.37	−0.62
PCQ	0.79	−0.43	0.41
Eigenvalue	5.38	1.85	0.69
Cumulative proportion	0.67	0.90	0.99
Topography	**PC1**	**PC2**	
AE	0.95	−0.23	

LE	0.60	−0.78
HE	0.98	0.09
ER	0.94	0.29
SDE	0.84	0.40
Eigenvalue	3.80	0.92
Cumulative proportion	0.76	0.95

Loadings, eigenvalues and cumulative proportion of variance are shown for the first three eigenvectors (PC) in each category. For definition of the abbreviations see Methods.

Supplementary Methods

Selection of butterfly species

We used the figures in ref. 1 to estimate the colour value (see Methods), and the distribution maps of European butterflies[4] to estimate the range in Europe. We restricted our analyses to European butterfly species because only for this fauna data on distribution, colour lightness and phylogeny at the species-level are hitherto available. Ref. 1 presents 440 species. We excluded all species occurring only in North Africa because these species are not considered in ref. 4. Species occurring exclusively on the Canaries and Azores (e.g. *Azanus ubaldus, Catopsilia florella, Cyclyrius webbianus, Euchloe eversi, Euchloe grancanariensis, Hipparcia gomera, Thymelicus christi*) are not illustrated in ref. 1. Furthermore, the distributional data for Belarus, Ukraine, Moldova and Russia are still poor (ref. 4, see also ref. 2), and therefore we also excluded species occurring only in these areas. We then checked the remaining list of European species considered in ref. 1 for the availability of distributional data in ref. 4; 16 species illustrated in ref. 1 were not considered in ref. 4. Five species occurring on the islands along the coast of Turkey were also excluded because no insolation data[5] were available for the grid cells occupied by these species (Supplementary Table 3). Some species listed in ref. 4 are not illustrated in ref. 1: *Hipparchia christenseni, Pseudochazara hippolyte williamsi, Maniola megala*, and *Maniola halicarnassus*. Three of these four species occur along the Turkish coast. Another group of species listed in ref. 4 but not illustrated in ref. 1 consists of subspecies with uncertain taxonomic status (e.g. *Euphydryas glaciegenita*). Finally, we transferred all distribution data to Worldmap IV Europe, which uses a Lambert azimuthal equal area projection.

Phylogeny of butterfly and dragonfly species

We constructed the phylogenetic tree of butterflies using information given in the Tree of Life (see web page http://tolweb.org/Ditrysia/11868, accessed December 2010; refs. 6–12). Since the illustrations in ref. 1 are the basis of our study, the nomenclature in the tree follows that of ref. 1. We were forced to introduce a number of multifurcations, particularly for species within species-rich genera. Therefore, we are aware that the tree we constructed is due to the limited data availability only a first approximation. Furthermore, within the Tree of Life, several genera with their corresponding species were grouped together, e.g. the genera *Polyommatus*, *Agriades*, *Lysandra*, *Neolysandra*, *Agrodiaetus*, *Cyaniris*, *Meleageria*, *Plebicula* in ref. 1 with the genus *Polyommatus*. We retained the taxonomy of ref. 1 and introduced these genera as a group of related genera for the respective genus in the Tree of Life. We used two approaches to introduce branch length. First, we set all branch lengths to 1, and second, we estimated branch length according to the procedure of Grafen[13] using the function compute.brlen in *ape*[14] (version 2.6.). For the present analyses, we concentrated on the second approach.

The tree is given in newick format, which can be imported into various programs for plotting phylogenetic trees. To shorten the tree, we abbreviated the genus names with initials according to ref. 1:

((((P.alveus alveus,P.andromedae,P.armoricanus,P.cacaliae,P.carlinae carlinae, P.cirsii,P.carthami carthami,P.centaureae,P.cinarae,P.malvae,P.onopordi, P.serratulae,P.sidae sidae,P.warrenensis),((E.tages,E.marloyi),(((C.alceae, C.boeticus,C.flocciferus,C.lavatherae lavatherae,C.orientalis),(S.orbifer, S.phlomidis,S.sertorius sertorius),(M.cribrellum,M.proto,M.tessellum)))),(((C.palaemon,C.silvicolus),H.morpheus),((B.borbonica,(G.nostrodamus,G.pumilio)),((T.acteon,T.hyrax,T.lineola,T.sylvestris),(H.comma,O.venatus))))),(((P.alexanor, P.hospiton,P.machaon gorganus),I.podalirius,((P.mnemosyne,(P.apollo,P.phoebus)),(A.apollinus,((Z.rumina,Z.polyxena),A.cerisy)))),(((L.sinapis,L.morsei,L.duponcheli),(((G.cleopatra,G.rhamni,G.farinosa),(C.alfacariensis,C.aurorina,C.caucasia, C.chrysotheme,C.crocea,C.erate,C.hecla,C.hyale,C.myrmidone, C.nastes,C.palaeno, C.phicomone)),(C.evagore,((E.penia,((A.belia euphenoides,A.cardamines,A.damone, A.gruneri),(Z.eupheme,((E.insularis,E.tagis tagis),(E.ausonia,E.belemia))))),(A.crataegi,((P.callidice,P.chloridice,P.daplidice),(P.brassicae,A.bryoniae,A.ergane, A.krueperi,A.mannii,A.napi napi,A.rapae)))))),((((L.celtis,((D.plexippus,D.chrysippus), (((((((C.pamphilus,C.thyrsis),(C.tullia tullia,C.rhodopensis),C.dorus dorus)),((C.arcania,C.gardetta),(C.hero,C.leander leander)),C.corinna)),C.glycerion

glycerion), C.oedippus),((L.megera,L.maera maera,L.petropolitana),P.aegeria aegeria,
L.achine,(K.climene,K.roxelana)),(((M.jurtina jurtina,M.nurag),(P.tithonus,P.cecilia,
P.bathseba),A.hyperantus),(M.arge,M.galathea,M.ines,M.lachesis,M.larissa, M.occitanica occitanica,M.russiae
cleanthe),((H.alcyone,H.aristaeus aristaeus, H.cretica,H.fagi,H.neomiris,H.semele,H.syriaca,H.volgensis),(N.fatua,
N.statilinus statilinus,P.fidia),((M.dryas,(S.actaea,S.ferula),((C.briseis,C.prieuri),(P.anthelea
anthelea,P.cingovskii,P.geyeri,P.graeca graeca,P.orestes))),((O.bore, O.glacialis,O.jutta,O.norna),(K.circe,A.arethusa
arethusa)))),(((((E.calcaria, E.cassioides,E.nivalis,E.tyndarus),E.hispania),E.ottomana), E.aethiopella,E.aethiops,
E.alberganus,E.christi,E.claudina,E.disa,E.embla,E.epiphron silesiana,E.epistygne, E.eriphyle,E.euryale
euryale,E.flavofasciata,E.gorge,E.gorgone,E.lefebvrei lefebvrei,E.ligea,E.manto manto,E.medusa
medusa,E.melampus,E.melas schanerdae, E.meolans meolans,E.mnestra,E.montana montana,E.neoridas,E.oeme
oeme, E.orientalis,E.palarica,E.pandrose,E.pharte pharte,E.pluto pluto,E.polaris, E.pronoe
pronoe,E.rhodopensis,E.scipio,E.sthennyo,E.stirius,E.styx styx, E.sudetica sudetica,E.triaria
triaria,E.zapateri),P.afra),(H.lupina,H.lycaon))),C.jasius),
((((N.sappho,N.rivularis),(L.camilla,L.populi,L.reducta)),((B.aquilonaris,B.graeca, B.napaea,B.pales
pales),P.eunomia eunomia,(C.dia,C.freija,C.frigga,C.selene, C.thore
thore,(C.polaris,C.euphrosyne,(C.chariclea,C.titania titania))),(I.lathonia,((A.aglaja
aglaja,A.laodice,(A.paphia,A.pandora),(A.adippe adippe,A.niobe,A.elisa)),(
B.hecate,(B.daphne,B.ino))))))),(((((E.aurinia,E.desfontainii),(H.cynthia,H.iduna,
H.intermedia,H.maturna)),((M.cinxia,(M.diamina diamina,((M.varia,(M.parthenoides,(
M.asteria,M.aurelia))),(M.athalia athalia,(M.britomartis,M.deione deione))))),(M.trivia trivia,M.didyma
didyma,(M.arduinna,(M.aetheriae,M.phoebe phoebe))))),(((V.cardui,V.virginiensis),V.atalanta),
A.levana,((A.urticae,I.io),((N.antiopa, N.polychlorus,N.vaualbum,N.xanthomelas),(P.c-album,P.egea))))), (A.ilia
ilia,A.iris,A.metis))))))),((H.lucina,((L.boeticus,C.marshalli,L.pirithous,(T.balcanicus, T.theophrastus),
Z.knysna,C.argiolus,((C.carswelli,C.lorquinii,C.minimus,
C.osiris),(E.alcetas,E.argiades,E.decoloratus)),((G.melanops,G.alexis),T.endymion,(M.alcon,M.arion
arion,M.nausithous,M.teleius),S.orion lariana,(P.abencerragus, P.barbagiae,P.baton,P.bavius
macedonicus,P.panoptes,P.vicrama),I.iolas),((((P.argyrognomon,P.idas idas),P.argus argus,P.pylaon
sephirus),C.trochylus, A.orbitulus,(K.eurypilus,K.psylorita),E.eumedon,P.nicias,(A.agestis,A.artaxerxes,
A.morronensis),A.glandon glandon,U.anteros,V.optilete),((P.icarus icarus,P.eros,
P.eroides),(L.bellargus,L.albicans,L.coridon,L.hispana), N.coelestina, (A.amanda, A.aroaniensis,A.galloi,
A.humedasae,A.nephohiptamenos, A.pyrenaicus pyrenaicus, A.thersites,A.violetae,(A.damon,((A.iphigenia,(A.dolus
dolus,(A.ainsae, A.fabressei)),(A.admetus,A.ripartii ripartii))))),C.semiargus semiargus,M.daphnis,(
A.escheri,P.dorylas,P.golgus golgus,P.nivescens)))),(L.phlaeas,L.dispar,L.helle,(L.alciphron
alciphron,L.ottomana,L.tityrus tityrus,L.virgaureae),(L.candens, L.hippothoe
hippothoe),(L.thetis,L.thersamon)),(((T.ballus,T.nogelii),(C.avis,C.rubi),
(S.acaciae,S.esculi,S.ilicis,S.pruni,S.spini,S.w-album)),(T.betulae,L.roboris),Q.quercus)))))))));

We compiled phylogenetic species-level data to construct the tree for dragonflies using information from refs. 15–19. The tree is given in newick format, which can be imported into various programs for plotting phylogenetic trees:

(((((((Erythromma.najas,Erythromma.viridulum,Cercion.lindeni,
Pyrrhosoma.nymphula),(Enallagma.cyathigerum,((Ischnura.elegans,
Ischnura.graellsi),Ischnura.pumilio))),((Coenagrion.caerulescens,
Coenagrion.scitulum),(Coenagrion.mercuriale,((Coenagrion.hastulatum,
Coenagrion.lunulatum),(Coenagrion.ornatum,(Coenagrion.puella,
Coenagrion.pulchellum))),(Coenagrion.armatum,Coenagrion.johanssoni))))),(
Calopteryx.splendens,(Calopteryx.haemorrhoidalis,Calopteryx.virgo)),(
Epallage.fatime,((Platycnemis.acutipennis,(Platycnemis.latipes,
Platycnemis.pennipes)),(Ceriagrion.tenellum,Nehalennia.speciosa)))),((
Sympecma.annulata,Sympecma.fusca),(Lestes.barbarus,Lestes.dryas,

Lestes.macrostigma,Lestes.sponsa,Lestes.virens,Lestes.viridis))),((
Epitheca.bimaculata,(Cordulia.aenea,(Somatochlora.alpestris, Somatochlora.arctica,Somatochlora.flavomaculata,
Somatochlora.metallica, Somatochlora.sahlbergi))),(Oxygastra.curtisi,Macromia.splendens,(((
Leucorrhinia.albifrons,Leucorrhinia.caudalis,Leucorrhinia.dubia, Leucorrhinia.pectoralis, Leucorrhinia.rubicunda),
(Sympetrum.sanguineum, Sympetrum.striolatum,Sympetrum.vulgatum,Sympetrum.danae,
Sympetrum.depressiusculum,Sympetrum.flaveolum,Sympetrum.fonscolombei,
Sympetrum.pedemontanum)),(Crocothemis.erythraea,Diplacodes.lefebvrei),
Brachythemis.leucosticta,(((Pantala.flavescens,Trithemis.annulata),(
Zygonyx.torridus,Selysiothemis.nigra)),((Libellula.depressa,Libellula.fulva,
Libellula.quadrimaculata),(Orthetrum.albistylum,Orthetrum.brunneum,
Orthetrum.cancellatum,Orthetrum.chrysostigma, Orthetrum.coerulescens,
Orthetrum.nitidinerve,Orthetrum.ramburi,Orthetrum.trinacria))))))),(
Lindenia.tetraphylla,((Ophigomphus.cecilia,(Onychogomphus.costae,
Onychogomphus.forcipatus,Onychogomphus.uncatus)),(Gomphus.flavipes,
Gomphus.graslini,Gomphus.pulchellus,Gomphus.schneideri,Gomphus.simillimus,
Gomphus.vulgatissimus),Paragomphus.genei))),(((Boyeria.irene,
Caliaeschna.microstigma),(Brachytron.pratense,((Aeshna.affinis,Aeshna.caerulea,Aeshna.crenata,Aeshna.cyanea,Ae
shna.grandis,Aeshna.isoceles,Aeshna.juncea,
Aeshna.mixta,Aeshna.serrata,Aeshna.subarctica,Aeshna.viridis),((Anax.imperator, Anax.parthenope),
Hemianax.ephippiger)))),(Cordulegaster.boltoni, Cordulegaster.heros,Cordulegaster.picta)));

Test of reliability of colour values

To test the robustness of our procedure to estimate the colour value as well as our decision to use the illustrations in ref. 1, we present a scatterplot (Supplementary Fig. 3) between estimated colour values using illustrations in ref. 1 as well as photographs of specimens from scientific collections published in ref. 2. Ref. 2 presents only species from Germany, and therefore the number of species in the two graphs is lower that the number of species used in our final analysis of colour value across Europe (n = 366). Furthermore, ref. 2 does not present the ventral part of the wings of all species. Therefore, the plot of the ventral colour value consists of 138 species, and the plot of the dorsal colour value consists of 148 species. Note that for the two measures of wing colour value, the data scatter around the bisecting line (red line: intercept = 0 and slope = 1, Supplementary Fig. 3).

To confirm that the extracted colour values represent the physical ability of the species to absorb and reflect radiation energy, we measured surface temperatures of butterfly wings of 167

individuals out of 18 species (see Supplementary Table 4) and ambient temperature with an infrared thermometer ”testo 845” (see Supplementary Fig. 4), calculated the difference (ΔT), and related them to the corresponding colour values (Supplementary Fig. 5). We found the expected negative correlation between ΔT and the colour values, that is dark-coloured species reached higher surface temperatures in relation to ambient temperature than light-coloured species.

Supplementary References

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Colour lightness of dragonfly assemblages across North America and Europe

Supporting Information

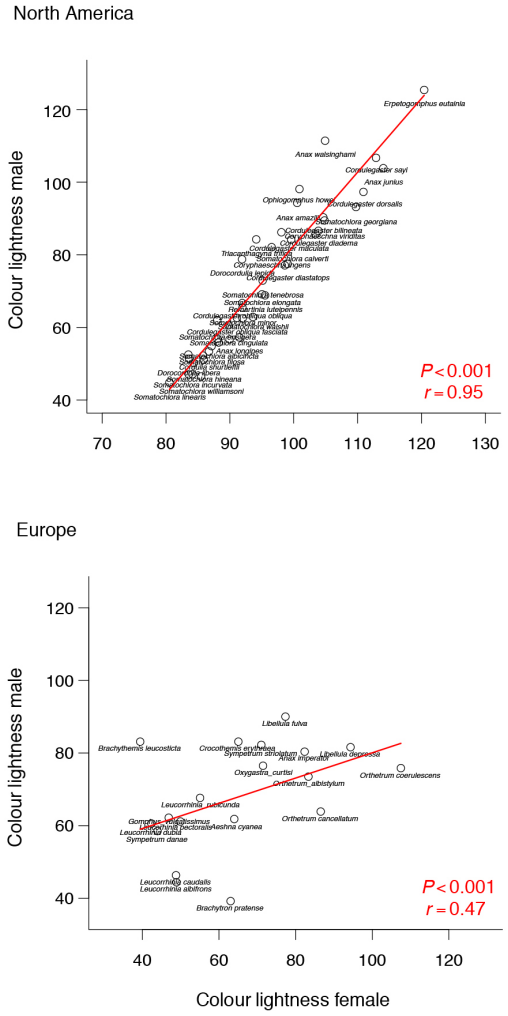


Figure A1. Scatterplots between female and male colour lightness of 44 North American (Needham et al. 2000) and 19 European (Askew 1988) dragonfly species. Note that colour lightness of females and males is highly correlated.

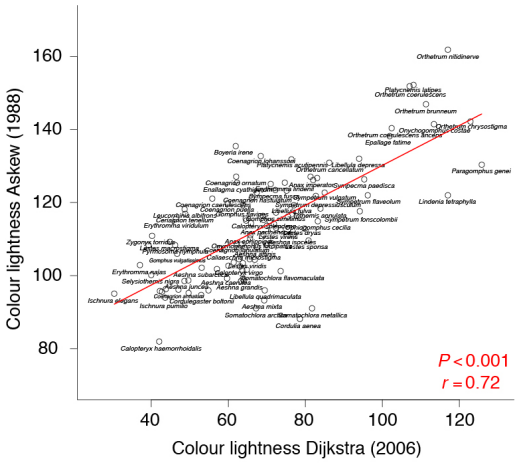


Figure A2. Correlation of the average colour lightness of European dragonfly species illustrated in both Askew (1988) and Dijkstra and Lewington (2006). Average colour lightness ranges from 0 (absolute black) to 255 (pure white). Note that the extracted colour values of dorsal dragonfly drawings from both sources are highly correlated.

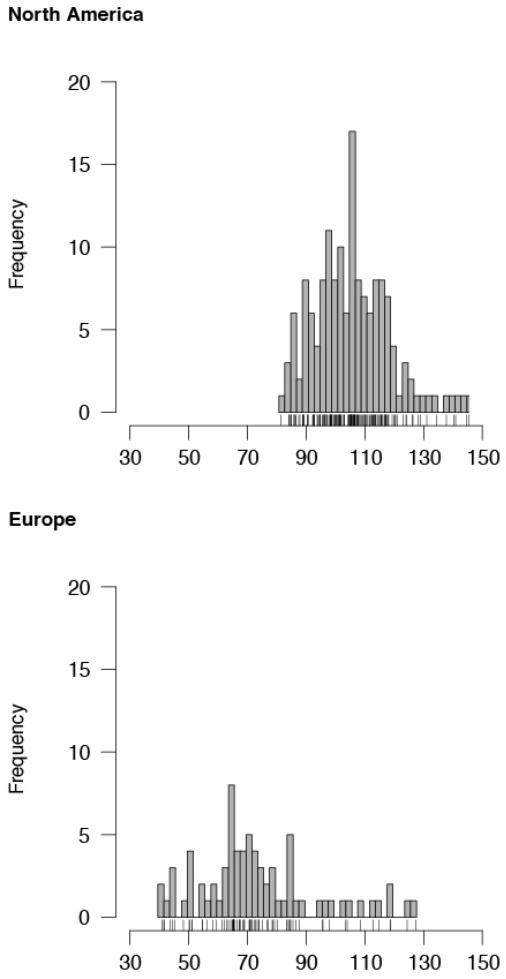


Figure A3. Frequency distribution of the average colour lightness of 152 North American and 74 European dragonfly species. Average colour lightness ranges from 0 (absolute black) to 255 (pure white). Rugs at the abscissa indicate the value of each species. Note that colour values are from different sources (North America: Needham et al. 2000, Europe: Askew 1988), and hence absolute values are not directly comparable.

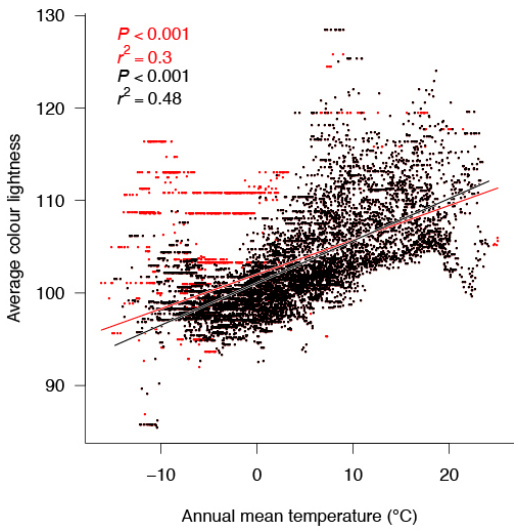


Figure A4. Scatterplots of single ordinary least-squares regressions between average colour lightness of 8,127 North American dragonfly assemblages and mean temperature of the warmest quarter. Red dots represent assemblages that were excluded from the analysis because they contained less than five species. Note that those assemblages that were excluded scatter more than those with more than five species (c.f. the coefficients of determination) due to the inherent effect of very low sampling sizes.

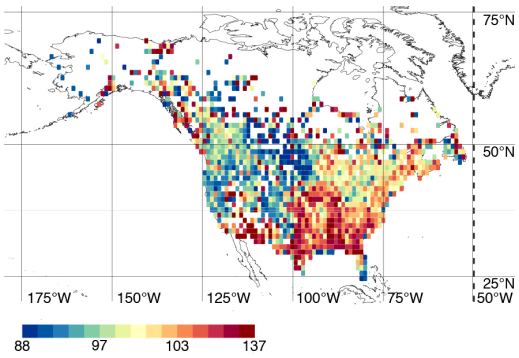


Figure A5. Map of the spatial variation in average colour lightness of North American dragonfly assemblages. Colour lightness ranges from 0 (absolute black) to 255 (pure white). Colour scale intervals follow an equal-frequency classification, ranging from blue (darkest) to red (lightest). The dataset represents occurrence records provided by OdonataCentral.org (Abbott 2006), which were resampled to 1,373 one-degree grid cells (EPSG: 4326).

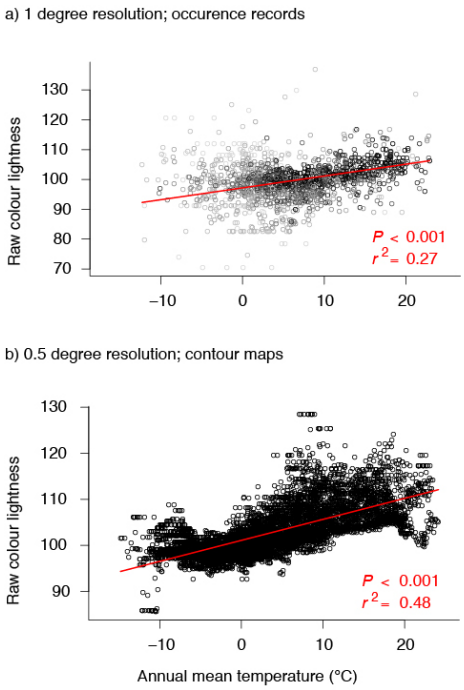
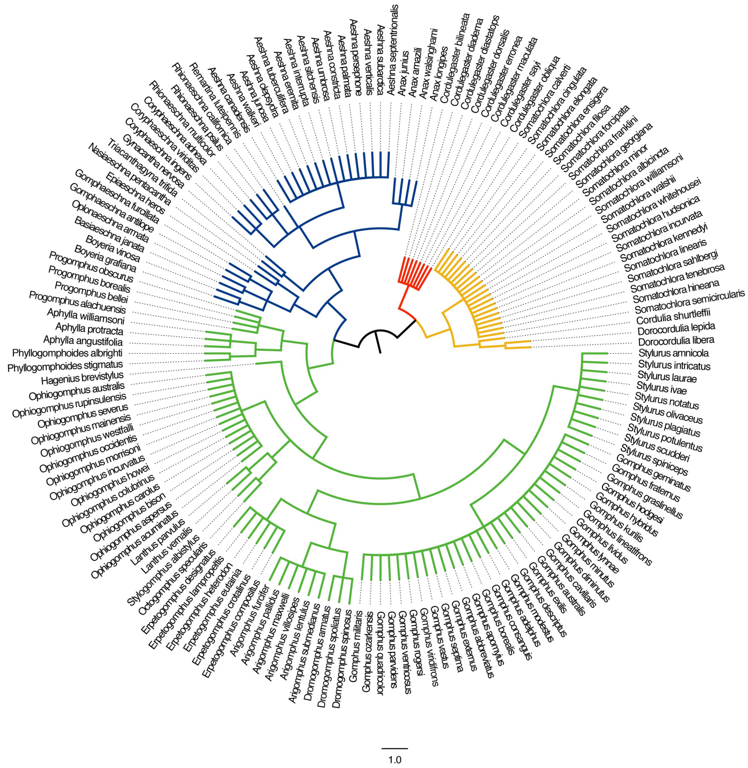
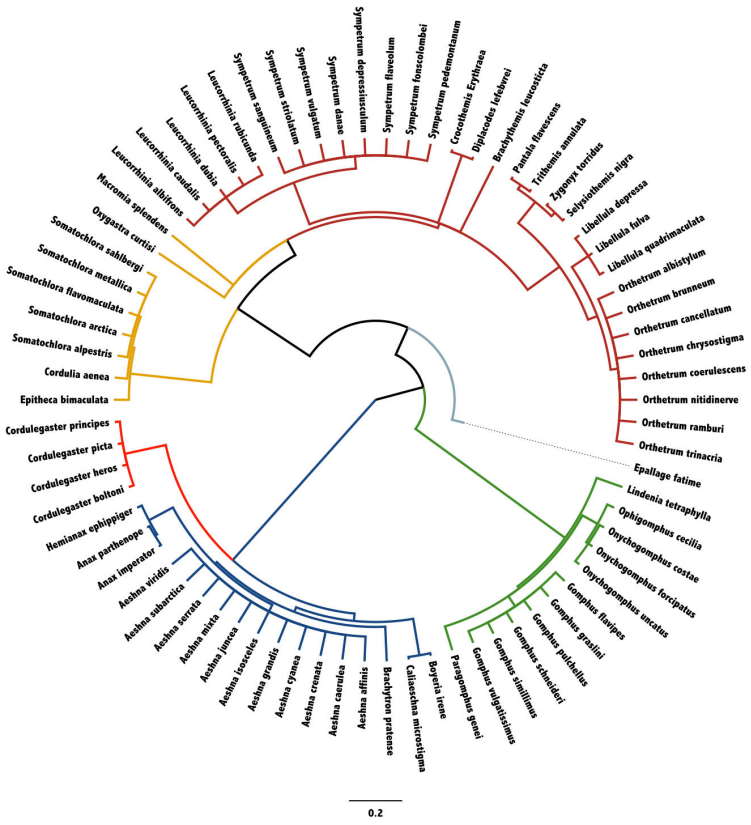


Figure A6. Scatterplots showing the correlation of the spatial variation in average colour lightness. **a)** Average colour lightness was based on occurrence records that were resampled to one-degree resolution (weighted by sampling density, i.e. the number of records). **b)** Average colour lightness was based on contour maps of distribution ranges that were resampled to half-degree grid cells against the main predictor annual mean temperature (see main text). Colour lightness ranges from 0 (absolute black) to 255 (pure white). Dot colours follow an equal-frequency classification, ranging from light grey (low sampling density) to black (high sampling density). Note that data from both occurrence records and contour maps basically support the thermal melanism hypothesis, but they also indicate that the correlation between average colour lightness and temperature is less strong and the slope is less steep for dragonflies in North American than for dragonflies in Europe.



((((((((Stylurus amnicola,Stylurus intricatus,Stylurus laurae,Stylurus ivae,Stylurus notatus,Stylurus olivaceus,Stylurus plagiatus,Stylurus potulentus,Stylurus scudderi,Stylurus spiniceps),(Gomphus geminatus,Gomphus fraternus,Gomphus graslinellus,Gomphus hodgesi,Gomphus hybridus,Gomphus kurilis,Gomphus lineatifrons,Gomphus lividus,Gomphus lynnae,Gomphus minutus,Gomphus diminutus, Gomphus cavillaris,Gomphus australis,Gomphus exilis,Gomphus descriptus,Gomphus modestus,Gomphus adelphus,Gomphus consanguis,Gomphus borealis,Gomphus apomyius,Gomphus abbreviatus,Gomphus externus,Gomphus septima,Gomphus vastus,Gomphus viridifrons,Gomphus rogersi,Gomphus ventricosus,Gomphus parvidens,Gomphus quadricolor,Gomphus ozarkensis,Gomphus militaris)),(((Dromogomphus spinosus,Dromogomphus spoliatus,Dromogomphus armatus),(Arigomphus submedianus,Arigomphus lentulus,Arigomphus villosipes,Arigomphus maxwelli,Arigomphus pallidus,Arigomphus furcifer)),(Erpetogomphus compositus,Erpetogomphus crotalinus,Erpetogomphus eutainia,Erpetogomphus heterodon,Erpetogomphus lampropeltis,Erpetogomphus designatus))),((Octogomphus specularis,Stylogomphus albistylus),(Lanthus vernalis,Lanthus parvulus)),(Ophiogomphus acuminatus,Ophiogomphus aspersus,Ophiogomphus bison,Ophiogomphus carolus,Ophiogomphus colubrinus,Ophiogomphus howei,Ophiogomphus incurvatus,Ophiogomphus morrisoni,Ophiogomphus occidentis,Ophiogomphus westfalli,Ophiogomphus mainensis,Ophiogomphus severus,Ophiogomphus rupinsulensis,Ophiogomphus australis)),Hagenius brevistylus),(((Phyllogomphoides

stigmatus,Phyllogomphoides albrighti),(Aphylla angustifolia,Aphylla protracta,Aphylla williamsoni)),(Progomphus alachuensis,Progomphus bellei,Progomphus borealis,Progomphus obscurus)))),((((Boyeria grafiana,Boyeria vinosa),(Basiaeschna janata,Oplonaeschna armata),(Gomphaeschna antilope,Gomphaeschna furcillata)),(Epiaeschna heros,Nasiaeschna pentacantha)),((Triacanthagyna trifida,Gynacantha nervosa),(((Coryphaeschna ingens,Coryphaeschna viriditas,Coryphaeschna adnexa),(Rhionaeschna multicolor,Rhionaeschna psilus,Rhionaeschna californica),Remartinia luteipennis,(Aeshna canadensis,Aeshna walkeri,Aeshna juncea,Aeshna clepsydra,Aeshna tuberculifera,Aeshna eremita,Aeshna interrupta,Aeshna sitchensis,Aeshna umbrosa,Aeshna constricta,Aeshna palmata,Aeshna persephone,Aeshna verticalis,Aeshna subarctica,Aeshna septentrionalis)),(Anax junius,Anax amazili,Anax walsinghami,Anax longipes))))),((Cordulegaster bilineata,Cordulegaster diadema,Cordulegaster diastatops,Cordulegaster dorsalis,Cordulegaster erronea,Cordulegaster maculata,Cordulegaster sayi,Cordulegaster obliqua),((Somatochlora calverti,Somatochlora cingulata,Somatochlora elongata,Somatochlora ensigera,Somatochlora filosa,Somatochlora forcipata,Somatochlora franklini,Somatochlora georgiana,Somatochlora minor,Somatochlora albicincta,Somatochlora williamsoni,Somatochlora walshii,Somatochlora whitehousei,Somatochlora hudsonica,Somatochlora incurvata,Somatochlora kennedyi,Somatochlora linearis,Somatochlora sahlbergi,Somatochlora tenebrosa,Somatochlora hineana,Somatochlora semicircularis),(Cordulia shurtleffii,(Dorocordulia lepida,Dorocordulia libera)))));



(((((Epitheca bimaculata, (Cordulia aenea, (Somatochlora alpestris, Somatochlora arctica, Somatochlora flavomaculata, Somatochlora metallica, Somatochlora sahlbergi))), (Oxygastra curtisi, Macromia splendens, (((Leucorrhinia albifrons, Leucorrhinia caudalis, Leucorrhinia dubia, Leucorrhinia pectoralis, Leucorrhinia rubicunda), (Sympetrum sanguineum, Sympetrum striolatum, Sympetrum vulgatum, Sympetrum danae, Sympetrum depressiusculum, Sympetrum flaveolum, Sympetrum fonscolombei, Sympetrum pedemontanum)), (Crocothemis erythraea, Diplacodes lefebvrei), Brachythemis leucosticta, (((Pantala flavescens, Trithemis annulata), (Zygonyx torridus, Selysiothemis nigra)), ((Libellula depressa, Libellula fulva, Libellula quadrimaculata), (Orthetrum albistylum, Orthetrum brunneum, Orthetrum cancellatum, Orthetrum chrysostigma, Orthetrum coerulescens, Orthetrum nitidinerve, Orthetrum ramburi, Orthetrum trinacria)))))), Epallage fatime), (Lindenia tetraphylla, ((Ophigomphus cecilia, (Onychogomphus costae, Onychogomphus forcipatus, Onychogomphus uncatus)), (Gomphus flavipes, Gomphus graslini, Gomphus pulchellus, Gomphus schneideri, Gomphus simillimus, Gomphus vulgatissimus), Paragomphus genei))), (((Boyeria irene, Caliaeschna microstigma), (Brachytron pratense, ((Aeshna affinis, Aeshna caerulea, Aeshna crenata, Aeshna cyanea, Aeshna grandis, Aeshna isosceles, Aeshna juncea, Aeshna mixta, Aeshna serrata, Aeshna subarctica, Aeshna viridis), ((Anax imperator, Anax parthenope), Hemianax ephippiger)))), (Cordulegaster boltoni, Cordulegaster heros, Cordulegaster picta, Cordulegaster principes)));

Figure A7. Phylogenetic hypotheses for the 152 North American and 74 European dragonfly species, compiled from different sources (see Methods). Branch colours on the tree represent the families: Aeshnidae (dark blue), Cordulegastridae (bright red), Corduliidae (ochre), Gomphidae (green), Libellulidae (dark red) and Euphaeidae (bright blue). Note that both continents share six families, but colour data for North American dragonflies was only available for four of them (Aeshnidae, Cordulegastridae, Corduliidae, Gomphidae).

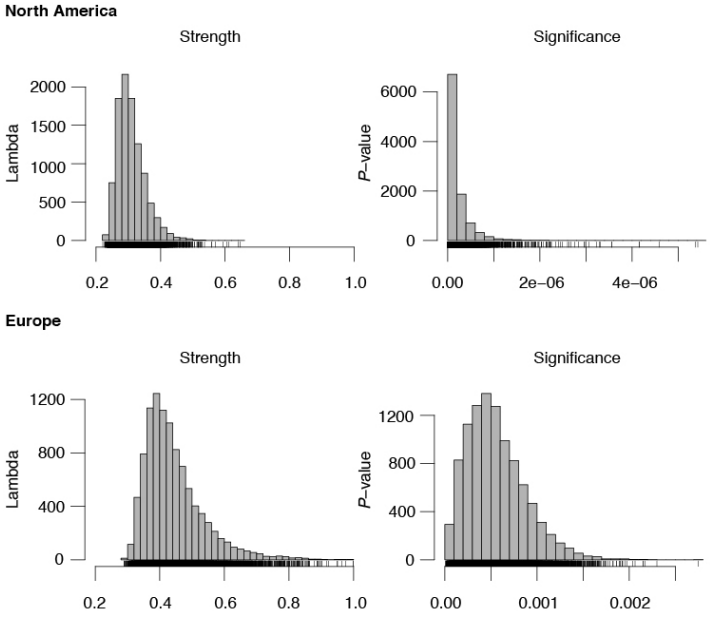


Figure A8. Frequency plots of strength and significance of the phylogenetic signal in colour lightness of 152 North American and 74 European dragonfly species. Pagel's lambda was repeatedly calculated for 10,000 randomly resolved phylogenetic trees (see Methods). Note that all of the alternative trees had a significant phylogenetic signal ($P < 0.05$).

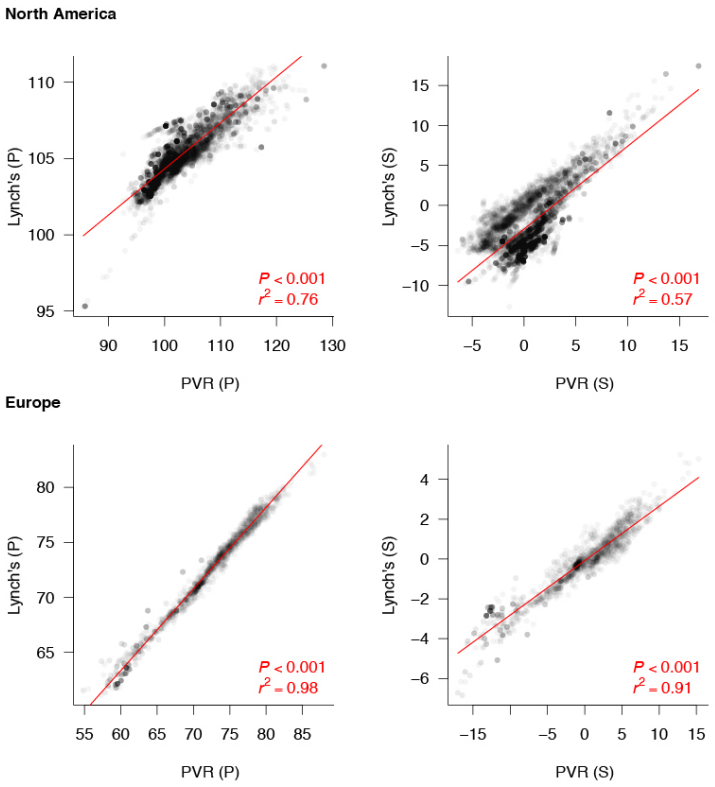


Figure A9. Correlations between phylogenetic components (P) and between specific components (S) of the colour lightness of 8,127 North American and 1,839 European dragonfly assemblages obtained from Lynch's comparative method (Lynch's) and phylogenetic eigenvector regression (PVR). Partly transparent dots indicate data density. Both methods were used to partition the phylogenetically predicted part of colour lightness (P) of dragonfly species based on 10,000 randomly resolved phylogenetic trees (see Methods) and the species-specific deviation from this prediction (S). However, because the results of the two approaches were highly similar only the spatial variation of the P and S components obtained from Lynch's comparative method was discussed in the main text.

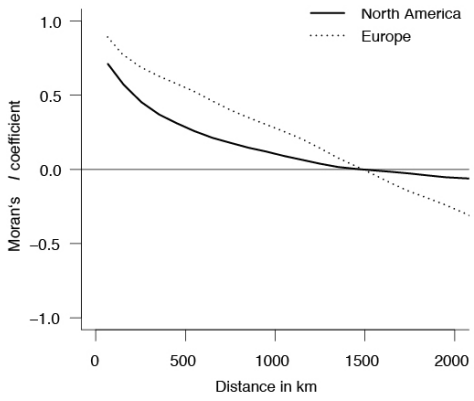


Figure A10. Spatial autocorrelation (Moran's *I* correlation coefficient) of the residuals from the model of average colour lightness of 8,127 North American and 1,839 European dragonfly assemblages and six environmental variables (mean annual temperature, temperature seasonality, mean temperature of the warmest quarter, mean altitude, mean annual precipitation and mean precipitation of the warmest quarter). Note the spatial autocorrelation up to about a distance of 1,400 km in North America and 1,500 km in Europe.

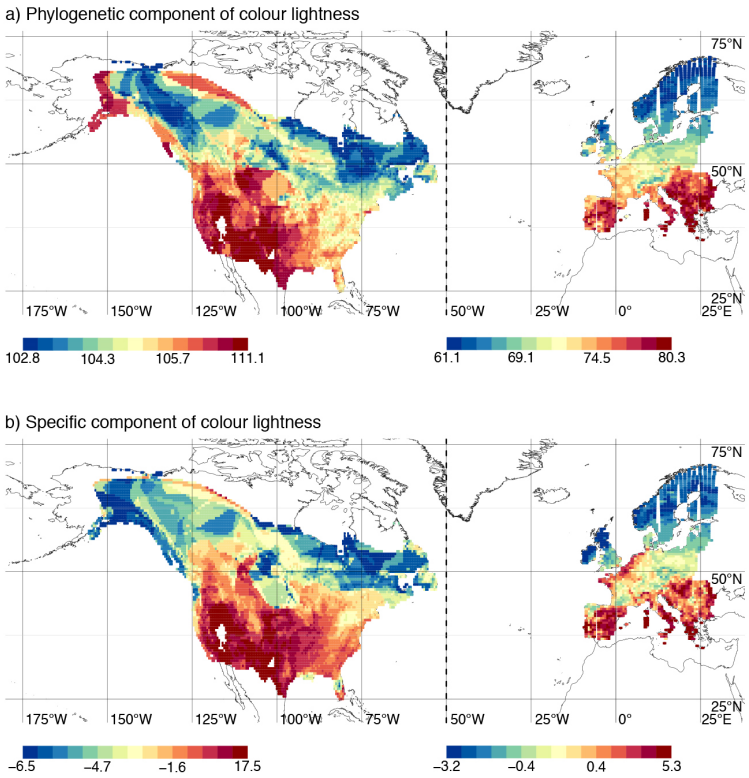


Figure A11. Maps of the spatial variation of the average phylogenetic (P) and specific (S) components of the colour lightness of North American and European dragonfly assemblages. Raw colour lightness, ranges from 0 (absolute black) to 255 (pure white), and was divided into two components with Lynch's comparative method to differentiate the influence of phylogenetic autocorrelation in the data (see Methods). We randomly resolved multifurcations of the original tree 10,000 times, calculated P and S components for each alternative phylogenetic tree and averaged these values for each species. **a)** P component, i.e. the proportion of colour lightness explained by the species' ancestral relations. **b)** S component, i.e. the deviation from the ancestral predicted colour lightness. Colour scale intervals follow an equal-frequency classification, ranging from blue (darkest) to red (lightest). Because of different data sources for North America and Europe, only the classes but not the values can be directly compared. Note that the colour lightness decreases in both continents towards northern and increases towards warmer regions. The datasets comprise 8,127 half-degree grid cells in North America and 1,839 approximately half-degree grid cells in Europe (EPSG: 4326 and EPSG: 3537; rectangular latitude and longitude grid).

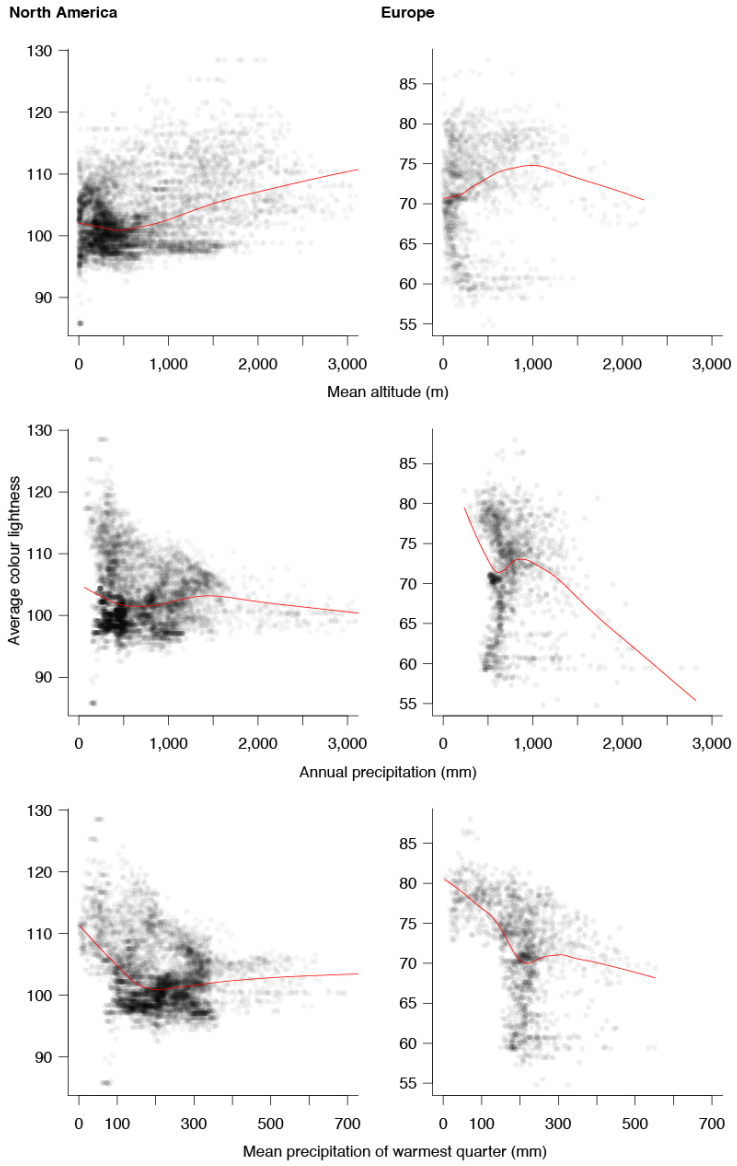


Figure A12. Scatterplots of single ordinary least-squares regressions between average colour lightness of 8,127 North American and 1,839 European dragonfly assemblages and three environmental variables (mean altitude, annual precipitation, mean precipitation of warmest quarter). Partly transparent dots indicate data density. Red lines were fitted with spline-based smoothed regressions. Note that correlation trends between average colour lightness and the environmental variables in the two continents are similar.

Table A1. Total sums of squares loadings, proportion of explained variance and cumulative explained variance of three principal components that characterise major trends of a set of six biologically relevant environmental variables for each continent based on a correlation matrix (highest contributions are in bold).

	Continent					
	Europe			North America		
Variable	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Annual mean temperature	0.90	0.36	–0.10	0.96	0.24	–0.09
Temperature seasonality	–0.31	–0.82	–0.04	–0.62	–0.51	0.53
Mean temperature of warmest quarter	**0.92**	0.01	–0.13	**0.97**	0.00	0.19
Mean altitude	–0.14	0.12	**0.98**	–0.08	–0.30	**–0.89**
Annual mean precipitation	–0.42	**0.85**	0.19	0.17	**0.95**	0.04
Precipitation of warmest quarter	–0.83	0.29	0.09	0.08	0.83	0.35
SS loadings	2.65	1.63	1.04	2.30	2.01	1.26
Proportion of variance	0.44	0.27	0.17	0.38	0.33	0.21
Cumulative variance	0.44	0.71	0.89	0.38	0.72	0.93

Table A2. Individual slopes from a multiple regression model between colour lightness of 8,127 North American and 1,839 European dragonfly assemblages and three environmental variables. Models were controlled for spatial autocorrelation using trend surface generalized additive models, i.e. a smoothing term for longitude and latitudes (effective degrees of freedom = 29). Differences in slopes between the two continents that were significant at $P < 0.001$ are shaded grey. Variables: TWaQ, mean temperature of the warmest quarter; A, mean altitude; and AP, mean annual precipitation. Note that the individual slopes of the correlations between colour lightness and environmental variables are significantly steeper in Europe than in North America, with mean temperature of the warmest quarter having the steepest slopes in each continent. All results, except for the relationship of colour lightness of North American dragonflies and AP that was corrected for spatial autocorrelation, were significant at $P < 0.001$.

Model	Variable	Individual slopes ± SE		Intercept ± SE
		North America	Europe	
None	TWaQ	7.3×10^{-1} $\pm 6.0 \times 10^{-3}$	1.5×10^{0} $\pm 1.9 \times 10^{-2}$	
	A	4.4×10^{-3} $\pm 6.1 \times 10^{-5}$	5.2×10^{-3} $\pm 2.0 \times 10^{-4}$	8.8×10^{1} $\pm 1.3 \times 10^{-1}$
	AP	-6.1×10^{-4} $\pm 7.8 \times 10^{-5}$	-1.9×10^{-3} $\pm 2.8 \times 10^{-4}$	
Corrected	TWaQ	5.6×10^{-1} $\pm 2.3 \times 10^{-2}$	-1.7×10^{-1} $\pm 4.2 \times 10^{-2}$	
	A	2.1×10^{-3} $\pm 1.4 \times 10^{-4}$	-2.7×10^{-3} $\pm 2.8 \times 10^{-4}$	8.9×10^{1} $\pm 4.3 \times 10^{-1}$
	AP	-3.4×10^{-5} $\pm 1.1 \times 10^{-4}$	-2.0×10^{-3} $\pm 2.5 \times 10^{-4}$	

Supplementary references

Abbott, J. C. 2006. OdonataCentral: An online resource for the distribution and identification of Odonata. - Digital resource at <http://www.odonatacentral.org>, accessed 28 Nov 2012.

Askew, R. R. 1988. The Dragonflies of Europe. - 2nd ed., Harley Books, Colchester, UK.

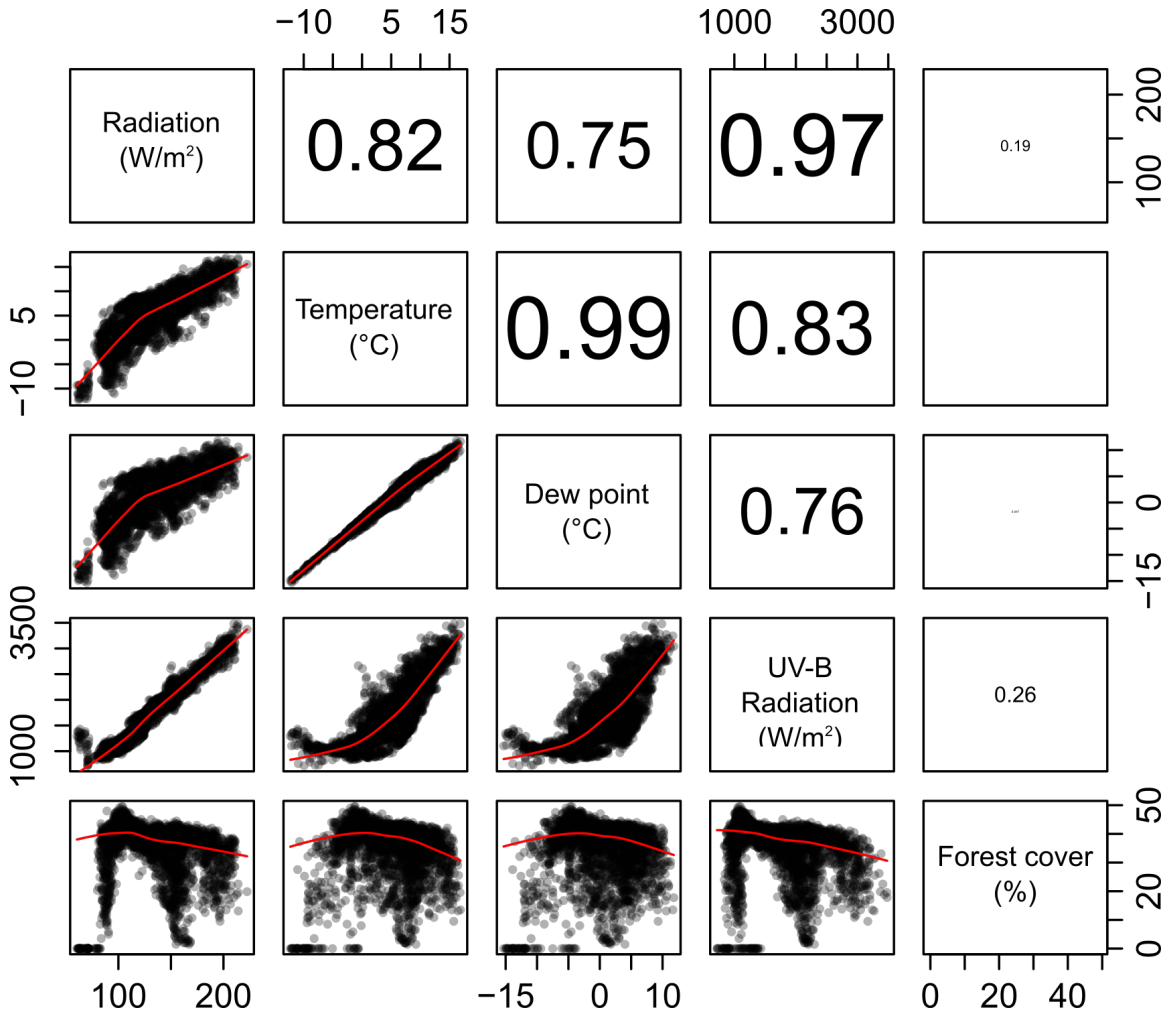
Dijkstra, K.-D. B. and Lewington, R. 2006. Field Guide to the Dragonflies of Britain and Europe. British Wildlife Publishing, Gillingham, UK.

Needham, J. G. et al. 2000. Dragonflies of North America. - 2nd ed., Scientific Publishers, Gainesville, Florida, USA.

The dark side of Lepidoptera: A continental gradient in the colour lightness of assemblages of geometrid moths

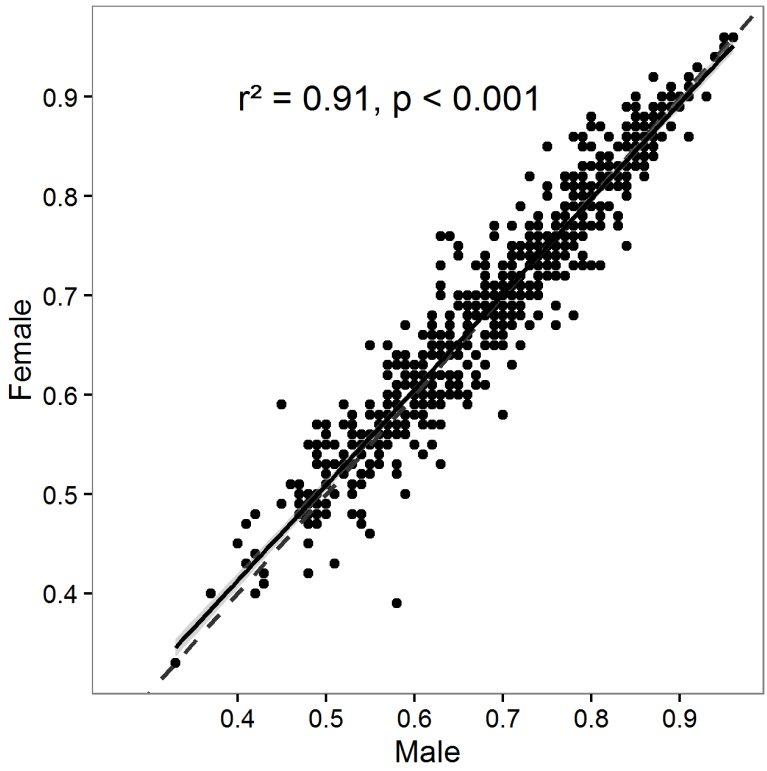
Supporting Information

APPENDIX S1 Environmental variables used in the study



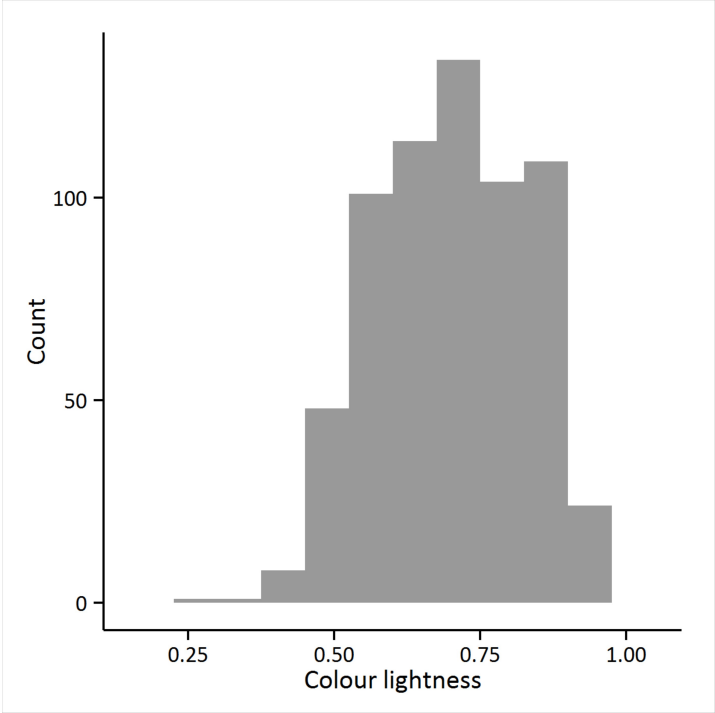
Scatterplot matrix of the used environmental variables within the generalized least square models and within the structure equation model, with Pearson R^2 indices in the right half. To avoid multicollinearity within the climatic variables, we chose solar radiation and the dew point temperature because i) they have the lowest correlation between each other and ii) radiation is a good proxy for both temperature and UV-B radiation.

APPENDIX S2 Male and female colour lightness

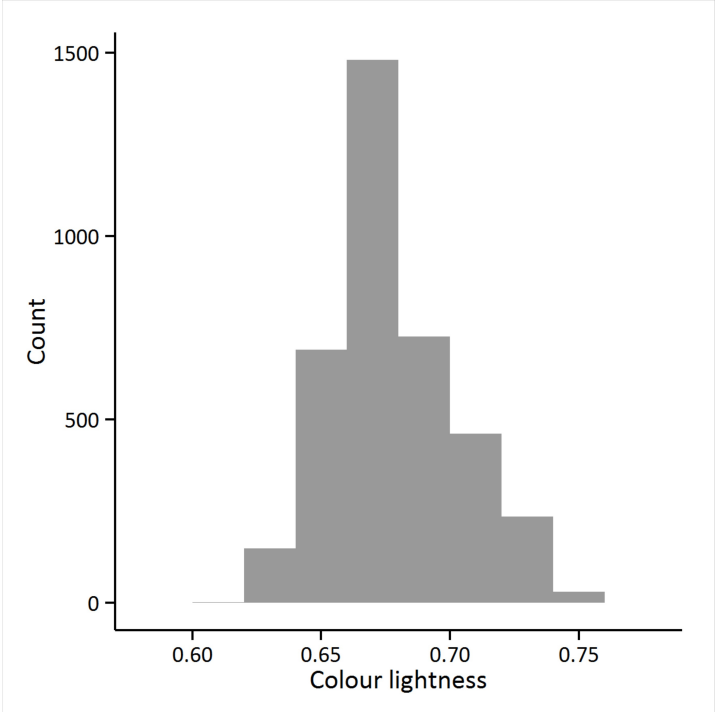


We compared colour lightness of female and male geometrid moths. High values indicate light-coloured species, and low values indicate dark-coloured species. The colour lightness of males and females did not significant differ (t-test, $p = 0.66$). r^2 and p-value are from an ordinary linear regression. The colour lightness values of the two sexes were highly correlated ($p < 0.001$, $r^2 = 0.91$). The slope of the linear regression differed slightly from a slope of 1 (estimate = −0.04, $p = 0.003$), and the intercept differed slightly from 0 (estimate = 0.03, $p = 0.002$). Note, however, that within the data range, the bisectrix (dashed, grey line) is fully covered by the regression. Therefore, we assume that there are no biological meaningful differences between the colour lightness of males and the colour lightness of females.

APPENDIX S3 Colour lightness histograms

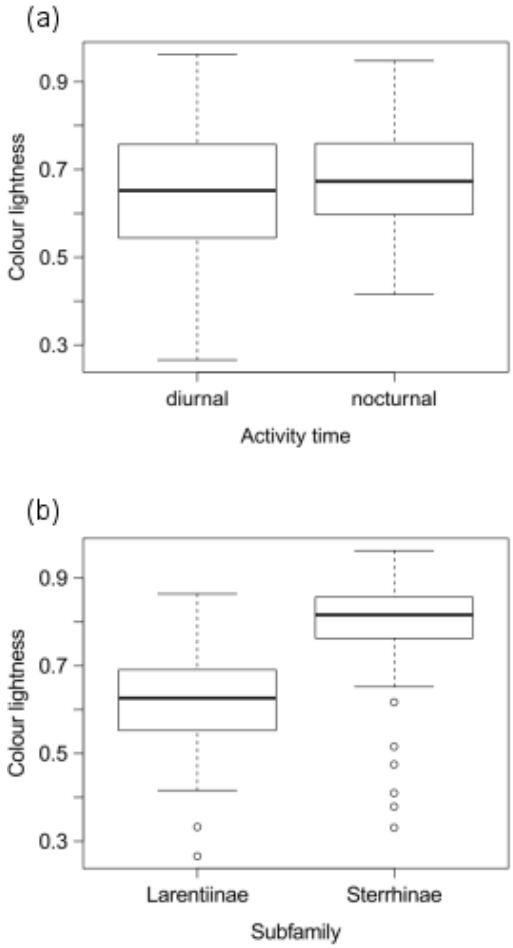


Histogram of the colour lightness of geometrid moth species



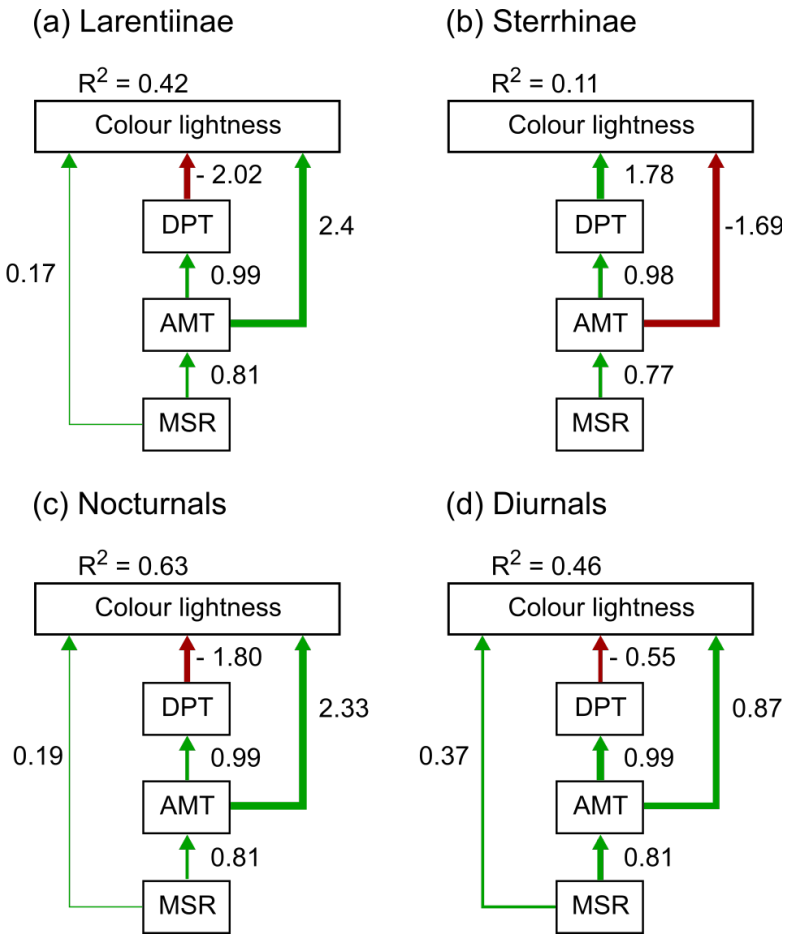
Histogram of the colour lightness of geometrid moth assemblages

APPENDIX S4 Colour lightness of species per subset



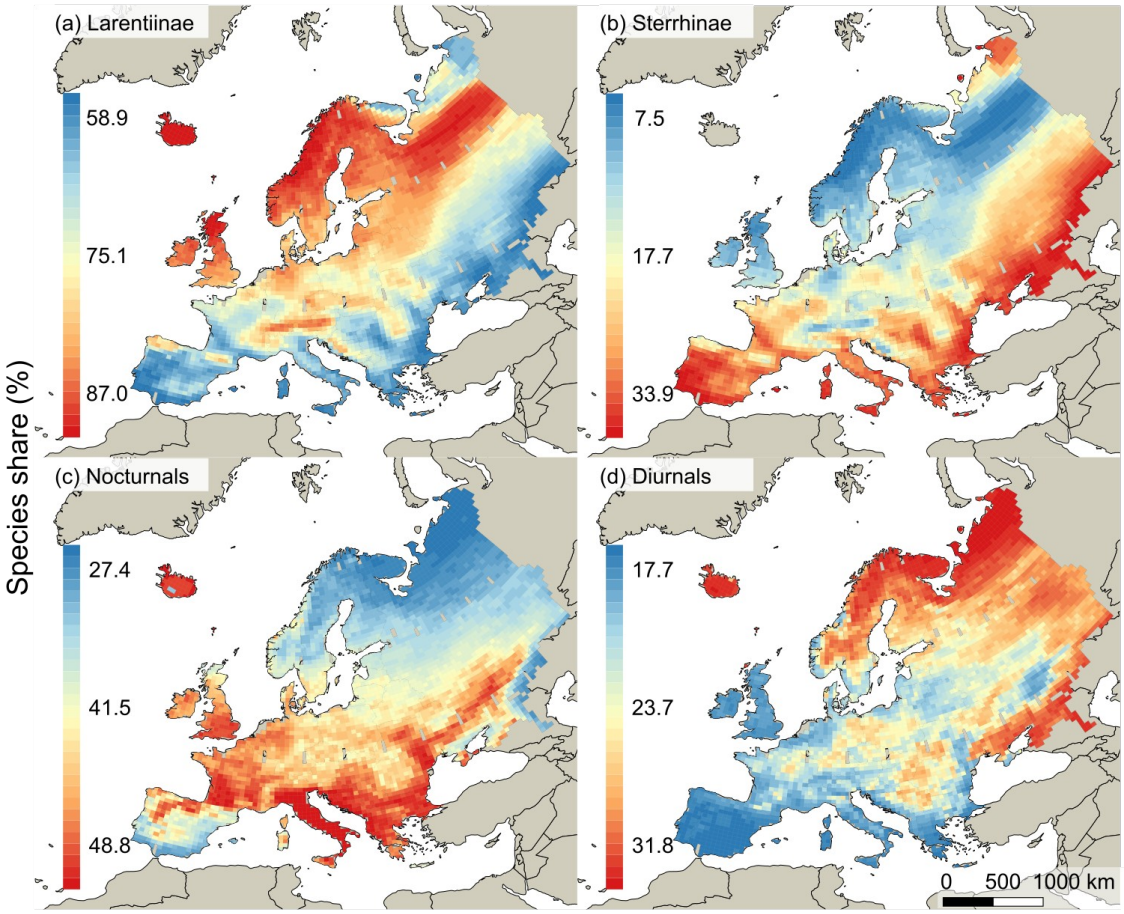
Mean colour lightness of **(a)** species of Larentiinae (n = 386) and Sterrhinae (n = 196), and **(b)** of nocturnal (n = 163) and diurnal (n = 102) species. Colour lightness ranging from 0 (black) to 1 (white). Larentiinae were more darkly coloured than Sterrhinae (t-test, $p < 0.01$), whereas diurnal and nocturnal species did not differ in their colour lightness (t-test, $p = 0.17$).

APPENDIX S5 Structural equation models of the subsets



Results of the differentiation of the thermal aspect of solar radiation and dew point temperature via structure equation modelling for subsets of geometrid moths; **a)** Larentiinae (CFI fit 0.85), **b)** Sterrhinae , **c)** strictly nocturnal moths and **d)** (partially) diurnal moths. The amount of explained variance in colour lightness is shown within the 'colour lightness' box. Arrows represent causal paths, the thickness is proportional to path coefficients, and dashed lines represent non-significant pathways ($p > 0.001$). All standardized coefficients shown were significant.

APPENDIX S6 Share of the species subsets within the assemblages per grid



Share of **(a)** Larentiinae, **(b)** Sterrhinae, **(c)** strictly nocturnal moths and **(d)** (partially) diurnal moths within the assemblages. The colouration represents equal-frequency classes (quantiles), with blue representing low and red representing high. The share of Larentiinae and diurnal species increases with latitude, whereas the share of Sterrhinae and nocturnal species decreases with latitude.

Environmental drivers of voltinism and body size in insect assemblages across Europe

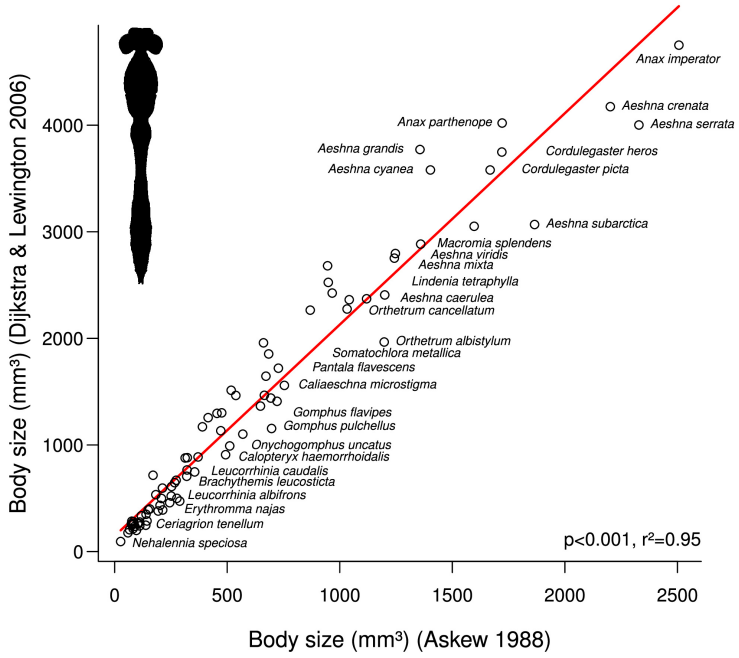
Supporting Information

Appendix S1: Data sources.

Data sources used for body size, number of generations per year (voltinism) and distribution of lepidopterans and odonates (see also Supplementary References).

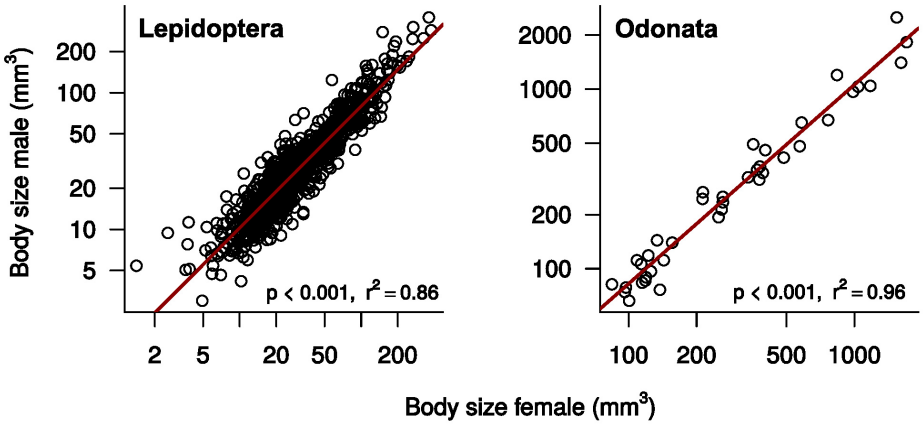
	Data source		
Order	Body size	Voltinism	Distribution
Lepidoptera	Hausmann (2001) Hausmann (2004) Hausmann & Viidalepp (2012) Mironov (2003) Tolman & Lewington (2009)	Hausmann (2001) Hausmann (2004) Hausmann & Viidalepp (2012) Mironov (2003) Tolman & Lewington (2009)	Hausmann (2001) Hausmann (2004) Hausmann & Viidalepp (2012) Kudrna *et al.* (2011) Mironov (2003)
Odonata	Askew (1988)	Corbet *et al.* (2006) Wildermuth & Martens (2014)	Dijkstra & Lewington (2006)

Appendix S2: Comparability of body size values.



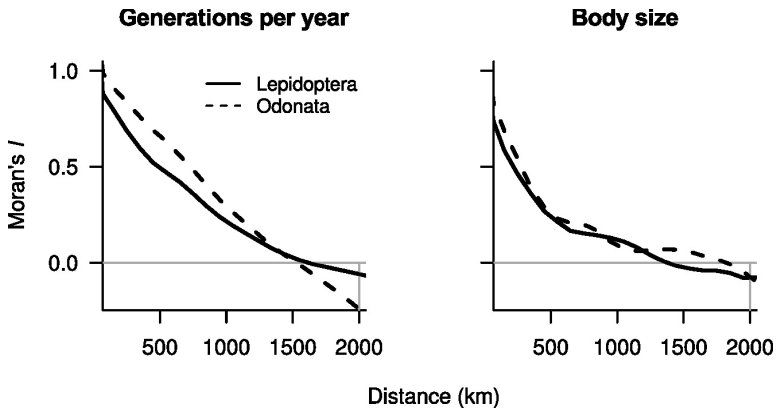
We compared body size values obtained from digital-image analysis of 86 male dragonfly species depicted in both Askew (1988) and Dijkstra & Lewington (2006). Body size values between the two sources were highly correlated (OLS regression: $p < 0.001$, $r^2 = 0.95$, red line), which indicated that our method of calculating body size values is a suitable measure for interspecific body size variation. For details, see Material and Methods.

Appendix S3: Male vs. female body size.



We compared body size values of male and female lepidopterans and odonates. For direct comparability, we processed an additional 1798 specimen from Askew (1988), Hausmann (2001, 2004), Mironov (2003), Tolman & Lewington (2009) and Hausmann & Viidalepp (2012) according to the method described in the main text. We found that for a subset of species, body sizes were highly correlated between the sexes (lepidopterans: $r^2 = 0.86$, $p < 0.001$, $n = 801$; odonates: $r^2 = 0.96$, $p < 0.001$, $n = 40$; OLS regressions, red lines). We hence conclude that the comparison of female lepidopterans and male odonates in the main text is not strongly biased by sexual dimorphism.

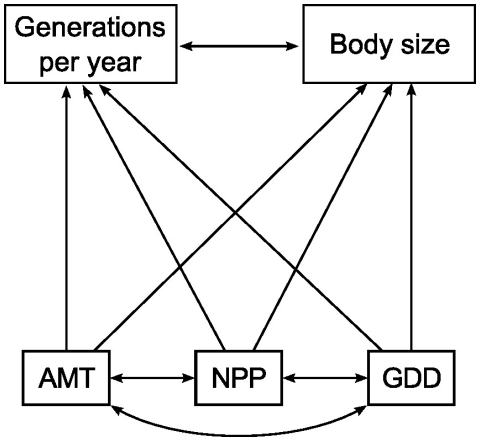
Appendix S4: Spatial autocorrelation profiles.



Spatial autocorrelation. Spatial autocorrelation profiles of average generations per year and body size within lepidopteran and odonate assemblages across Europe. Spatial similarity (Moran's *I*) was calculated using 50 km × 50 km grid cells (n = 1937 grid cells) and the ln-transformed body size measures of each species. Note that spatial independence (Moran's $I = 0$) was reached at about 2000 km distance for both variables.

Appendix S5: Structural equation modelling.

We used structural equation modelling to investigate direct and indirect effects of annual mean temperature (AMT), net primary productivity (NPP) and growing degree days (GDD) on the average number of generations per year and body size of European lepidopteran and odonate assemblages. Models were run with maximum-likelihood estimation and ln-transformed body size data (R package *lavaan*; Rosseel, 2012).



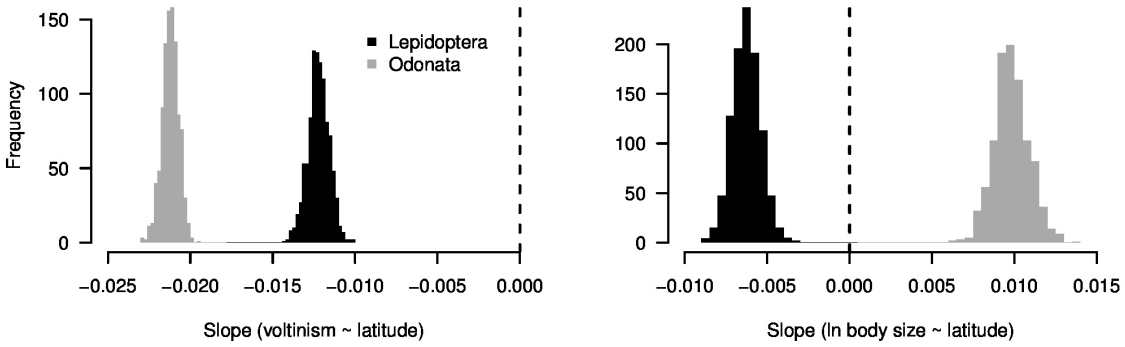
Model specification. Path diagram of the model that was fit to both lepidopterans and odonates based on the hypothesized relationships presented in the main text. Arrows indicate the assumed direction of the effects.

Model results. Parameter estimates and standard errors for the specified model. *, significant at p < 0.001; n.s., p > 0.001; ~~, correlated with; ~, regressed on.

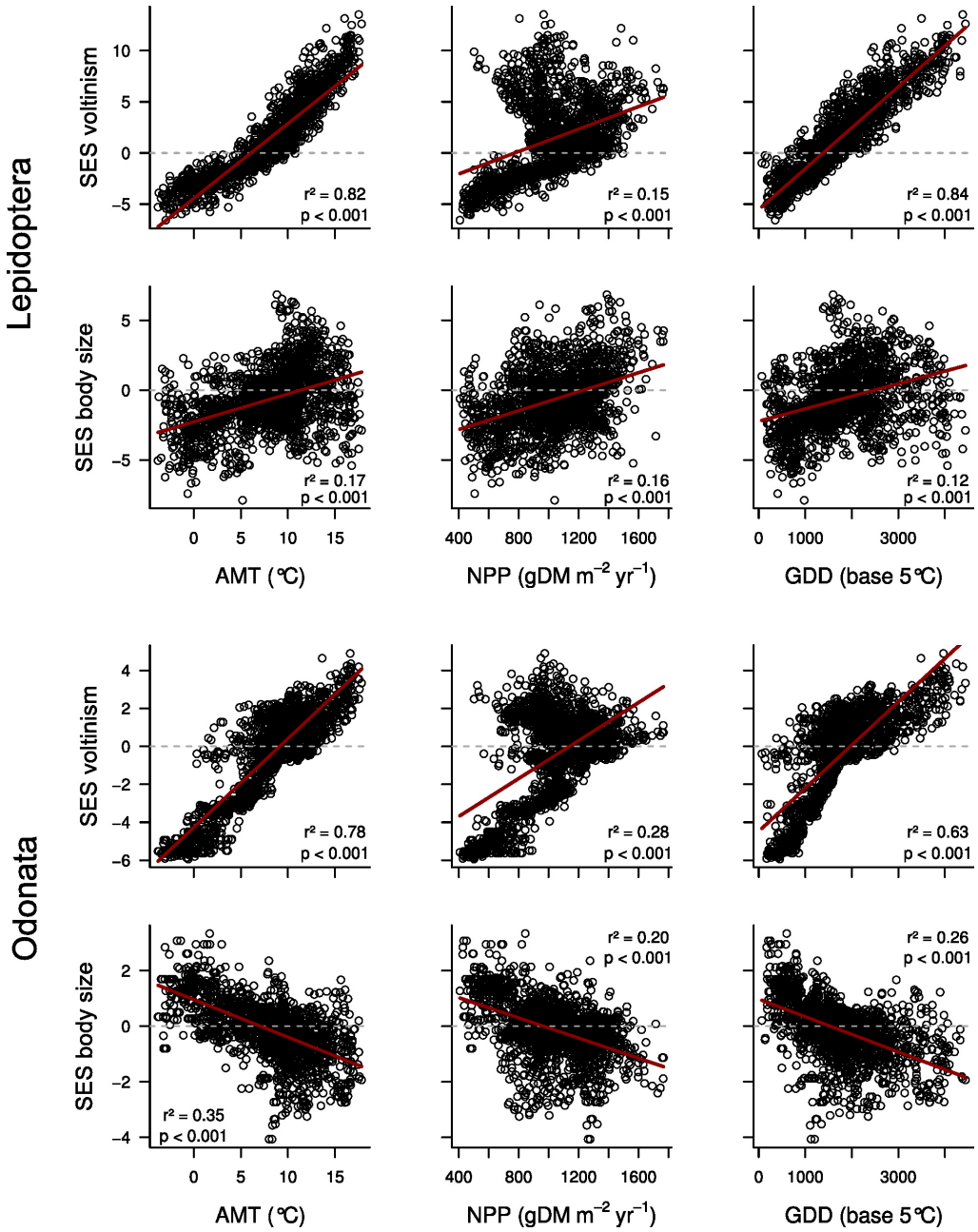
			Lepidoptera		Odonata	
Response	Operator	Predictor	Estimate	± SE	Estimate	± SE
Generations per year	~~	Body size	0.11*	0.01	–0.31*	0.01
Generations per year	~	AMT	0.61*	0.03	0.98*	0.03
	~	NPP	–0.13*	0.01	n.s.	
	~	GDD	0.40*	0.02	–0.11*	0.03
Body size	~	AMT	0.28*	0.07	–0.55*	0.06
	~	NPP	0.23*	0.03	–0.15*	0.02
	~	GDD	n.s.		n.s.	

Appendix S6: Randomization tests.

We ran two different randomization tests to analyse whether the species richness distributions of lepidopteran and odonate species across Europe could generate through random processes alone the observed geographic trends in voltinism and body size.



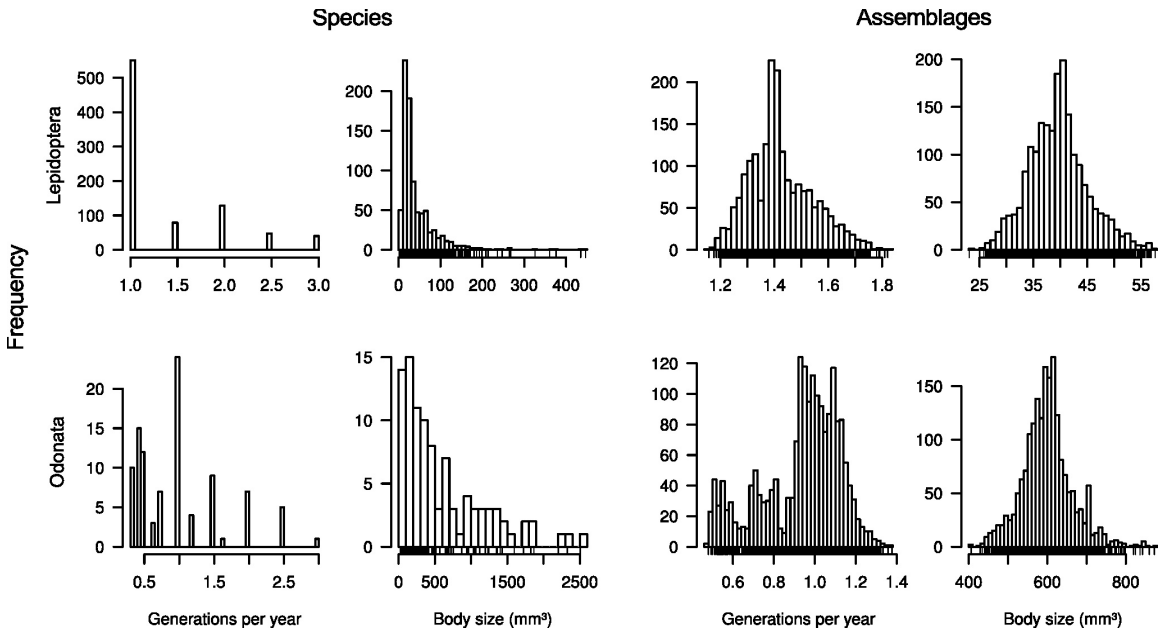
Sub-sampling. First, we created 1000 datasets for lepidopterans and odonates by randomly sampling five species from each grid cell with at least five species (n = 1937 grid cells). We then calculated the average number of generations per year and the average ln-transformed body size within each grid cell and regressed these values against latitude. For the average generations per year, all slopes were negative for both lepidopterans and odonates (0 indicated by dashed line). For the average body size, all slopes were negative for lepidopterans and positive for odonates. The results indicate that the number of generations per year in both lepidopteran and odonate assemblages increased from north to south, whereas lepidopteran assemblages consisted of larger species in southern Europe and odonate assemblages consisted of larger species in northern Europe. These results of our sub-sampling analysis are basically the same as the results presented in the main text. This indicates that unequal species numbers among grid cells do not account for the observed geographic trends in voltinism and body size.



Effect size. Second, we randomized the assignment of voltinism and ln-transformed body size to lepidopteran and odonate species and calculated the average number of generations per year and the average body size within grid cells for the randomized data sets. The spatial structure of the original data was retained in this analysis, i.e. species distributions and species numbers in each grid cell. We then calculated the standardized effect size (SES) as the observed average number of generations per year and body size within grid cells minus the expected values from the randomizations, divided by the standard deviation of the expectation. We repeated this procedure 1000 times and calculated the average effect size for each grid cell (positive effect size: more

generations per year or larger body size than expected by chance; negative effect size: fewer generations per year or smaller body size than expected by chance; dashed line: observed values = expected values). The average effect size within grid cells was then regressed against annual mean temperature (AMT), net primary productivity (NPP), and growing degree days (GDD) for lepidopterans and odonates. R^2, p values, and red lines are from ordinary least-squares regressions. The results of this analysis are highly similar to the results obtained with the original data (see Table 1 in the main text). We conclude that random processes alone are very unlikely to generate the observed geographic trends in voltinism and body size.

Appendix S7: Generations per year and body size distributions.



Histograms of generations per year and body size of lepidopterans and odonates at species and assemblage levels. Histograms are given for 845 lepidopteran species (top row) and 98 odonate species (bottom row) and for 1937 assemblages across Europe. Rugs at the abscissa indicate observed values. At the assemblage level, lepidopterans had an average of 1.42 ± 0.12 (SD, n = 1937) generations per year and an average body size of 39.6 mm^3 ± 5.4 mm^3, and odonates had an average of 0.93 ± 0.19 (SD, n = 1937) generations per year and an average body size of 600 mm^3 ± 63 mm^3.

Appendix S8: Environmental drivers of body size by voltinism levels.

Models explaining geographic variation in the average body size of lepidopteran and odonate assemblages in Europe categorized by different levels of voltinism. Voltinism < 1, only assemblages of species which need more than one year per generation; voltinism = 1, only assemblages of species with one generation per year; voltinism > 1, only assemblages of species with more than one generation per year. AMT, annual mean temperature; NPP, net primary productivity; GDD, growing degree days. Statistics were calculated with linear regressions (linear), regressions weighted with the number of species in each assemblage (linear weighted) and models to account for spatial autocorrelation (spatial). The explained variance is given for single variables (r^2, highest values of each model in bold, calculated with ln-transformed body size data) and for the full model (R^2). +/–, positive/negative relationship. *, $p < 0.001$; ‡, $p < 0.05$; n.s., $p > 0.05$; §, Nagelkerke-R^2.

					r^2			
Assemblage	Voltinism	$n_{species}$	$n_{assemblages}$	Model	AMT	NPP	GDD	R^2
Lepidoptera	= 1	551	1937	linear	(+) **0.23***	(+) 0.20*	(+) 0.17*	0.27*
				linear weighted	**(+) 0.20***	(+) 0.16*	(+) 0.14*	0.24*
				spatial§	(+) 0.22*	**(+) 0.29***	(+) 0.26*	0.30*
	> 1	294	1937	linear	**(+) 0.31***	(+) 0.18*	(+) 0.23*	0.32*
				linear weighted	**(+) 0.15***	(+) 0.08*	(+) 0.10*	0.17*
				spatial§	**(+) 0.32***	(+) 0.29*	(+) 0.30*	0.34*
Odonata	< 1	47	1937	linear	**(–) 0.24***	(–) 0.10*	(–) 0.21*	0.24*
				linear weighted	**(–) 0.22***	(–) 0.10*	(–) 0.20*	0.23*
				spatial§	**(–) 0.25***	(–) 0.17*	(–) 0.22*	0.25*
	= 1	24	1833	linear	(–) 0.03*	n.s.	**(–) 0.07***	0.10*
				linear weighted	(–) 0.05*	(+) 0.00‡	**(–) 0.09***	0.10*
				spatial§	(–) 0.04*	(+) 0.03‡	**(–) 0.08***	n.s.
	> 1	27	1937	linear	**(+) 0.62***	(+) 0.31*	(+) 0.45*	0.64*
				linear weighted	**(+) 0.24***	(+) 0.02*	(+) 0.17*	0.25*
				spatial§	**(+) 0.68***	(+) 0.54*	(+) 0.58*	0.70*

SUPPLEMENTARY REFERENCES

Askew, R.R. (1988) *The Dragonflies of Europe*. Harley Books, Colchester.

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Other manuscripts

Chromosome numbers in three species groups of freshwater flatworms increase with increasing latitude

with
Sven Lorch, Roland Brandl & Martin Brändle
published in Ecology and Evolution

Chromosome numbers in three species groups of freshwater flatworms increase with increasing latitude

ABSTRACT

Polyploidy in combination with parthenogenesis offers advantages for plasticity and the evolution of a broad ecological tolerance of species. Therefore, a positive correlation between the level of ploidy and increasing latitude as a surrogate for environmental harshness has been suggested. Such a positive correlation is well documented for plants, but examples for animals are still rare. Species of flatworms (Platyhelminthes) are widely distributed, show a remarkably wide range of chromosome numbers, and offer therefore good model systems to study the geographical distribution of chromosome numbers. We analyzed published data on counts of chromosome numbers and geographical information of three flatworm "species" (*Phagocata vitta*, *Polycelis felina* and *Crenobia alpina*) sampled across Europe (220 populations). We used the mean chromosome number across individuals of a population as a proxy for the level of ploidy within populations, and we tested for relationships of this variable with latitude, mode of reproduction (sexual, asexual or both) and environmental variables (annual mean temperature, mean diurnal temperature range, mean precipitation and net primary production). The mean chromosome numbers of all three species increased with latitude and decreased with mean annual temperature. For two species, chromosome number also decreased with mean precipitation and net primary production. Furthermore, high chromosome numbers within species were accompanied with a loss of sexual reproduction. The variation of chromosome numbers within individuals of two of the three species increased with latitude. Our results support the hypothesis that polyploid lineages are able to cope with harsh climatic conditions at high latitudes. Furthermore, we propose that asexual reproduction in populations with high levels of polyploidization stabilizes hybridization events. Chromosomal irregularities within individuals tend to become more frequent at the extreme environments of high latitudes, presumably because of mitotic errors and downsizing of the genome.

Evolutionary processes, dispersal limitations and climatic history shape current diversity patterns of European dragonflies

with
Stefan Pinkert, Klaas-Douwe Dijkstra, Christoph Reudenbach,
Roland Brandl & Christan Hof
in review in Ecography

Evolutionary processes, dispersal limitations and climatic history shape current diversity patterns of European dragonflies

ABSTRACT

We investigated the effects of contemporary and historical factors on the spatial variation of European dragonfly diversity. Specifically, we tested whether patterns of endemism and phylogenetic diversity of European dragonfly assemblages are structured by (i) phylogenetic conservatism of thermal adaptations and (ii) differences in the ability of post-glacial recolonization by species adapted to running waters (lotic) and still waters (lentic). We investigated patterns of dragonfly diversity using digital distribution maps and a phylogeny of 122 European dragonfly species, which we constructed by combining taxonomic and molecular data. We calculated total taxonomic e distinctiveness and mean pairwise distances across 4,192 50 km × 50 km equal-area grid cells as measures of phylogenetic diversity. We compared species richness with corrected weighted endemism and standardized effect sizes of mean pairwise distances or residuals of total taxonomic distinctiveness to identify areas with higher or lower phylogenetic diversity than expected by chance. Broken-line regression was used to detect breakpoints in diversity-latitude relationships. Dragonfly species richness peaked in central Europe, whereas endemism and phylogenetic diversity decreased from warm areas in the south-west to cold areas in the north-east and with an increasing proportion of lentic species. Except for species richness, all measures of diversity were consistently higher in formerly unglaciated areas south of the 0 °C isotherm during the last glacial maximum than in formerly glaciated areas. Phylogenetic conservatism of thermal adaptations and differences in recolonization ability after the last glacial maximum between lentic and lotic species in concert with the climatic history of the European continent shaped current diversity patterns of dragonflies in Europe. These findings highlight the importance of integrating climatic and evolutionary history with contemporary ecological data to understand the processes driving the geographical variation of biological diversity.

Understanding the drivers of cross-taxon congruence

with
Stefan Pinkert, Klaas-Douwe Dijkstra, Jens Kipping,
Viola Clausnitzer, Katherine Bannar-Martin & Roland Brandl
in preparation for Global Ecology and Biogeography

Understanding the drivers of cross-taxon congruence

ABSTRACT

Analyses of intra-taxon and cross-taxon congruence of diversity patterns are a key element of strategic conservation planning. Current conservation networks are, however, only based on our knowledge of a few well-known taxa that serve also as surrogates for other taxa. Here we investigate the degree of cross-taxon congruence in species richness and endemism in six major taxa across Africa. We used high-resolution distribution data of 8,138 species of mammals, birds, amphibians, freshwater fishes and dragonflies – the first complete set of distribution data for a group of insects of a tropical continent. We assessed the importance of biogeographic history and environmental factors for observed patterns of cross-taxon congruence in diversity by controlling for 20 variables describing temperature, precipitation and spatially autocorrelated latent factors. We found that overall diversity patterns were highly congruent across all taxa, except fishes. Mammals and birds also had substantial overlap of species richness and endemism hotspots, whereas hotspots of fishes, odonates and amphibians were not congruent. Overall diversity patterns of all taxa were highly correlated with environmental factors ($0.41 < R^2 < 0.86$). After controlling for environmental factors, cross-taxon congruence significantly decreased, especially for odonates and fishes. Our results suggest that the diversity patterns of the African fauna are similar across taxa, but the determinants of cross-taxon congruence differ considerably between groups. Mammals, birds and amphibians have largely similar biogeographic patterns, whereas the high cross-taxon congruence of odonates is entirely determined by their strong response to environmental factors. This mismatch between hotspots of terrestrial vertebrate diversity – those areas that currently have the highest relevance for conservation planning – and freshwater diversity is even higher, which stresses the need to adopt taxon-specific conservation priorities.

Inferring biotic interactions from macro-ecological data: a review

with
Carsten Dormann, Maria Bobrowski, Matthias Dehling, Florian Hartig, Heike Lischke, Marco Moretti, Jörn Pagel, Stefan Pinkert, Matthias Schleuning, Susanne Schmidt, Christine Sheppard, Manuel Steinbauer & Casper Kraan

in preparation for Global Ecology and Biogeography

Inferring biotic interactions from macro-ecological data: a review

ABSTRACT

Recent studies increasingly incorporate biotic interactions in macro-scale species distribution models (SDMs). This is clearly an important link between community ecology and macro-ecology, but not a simple step to make, because many aspects need to be taken into account when considering biotic interactions in SDMs. We present a set of questions that analysts and reviewers should ask in order to avoid too rash attribution of species association patterns to biotic interactions. We include studies from plants, birds, reptiles, fish, insects, and macrobenthic fauna to draw relevant conclusions. We review studies of biotic interactions to evaluate if conclusions on the presence of biotic interactions are supported by implemented modelling approaches. Irrespective of the method used, studies that test for biotic interactions also tend to find them. Yet, when compared to our list of questions, few hold up to scrutiny. This does not dismiss the presence of biotic interactions, but merely highlights that more work is needed to detect and interpret them correctly. Including biotic interactions in macro-ecological models is a highly topical and promising field of research. Such approaches allow moving from species to communities, as well as integrating species traits into studies of biodiversity-ecosystem functioning. We call upon future studies on biotic interactions to adhere to our list of questions to define if they actually consider biotic interactions, or co-occurrences, or shared habitat preferences, or rather something that prevents unravelling of biotic interactions due to confounding factors.

Erklärung

Ich versichere, dass ich meine Dissertation

Environmental drivers of colour and size in insects – A macroecological perspective

selbständig und ohne unerlaubte Hilfe angefertigt habe und mich keiner als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe. Diese Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, den 23.2.2017

Dirk Zeuss