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Marketa Zimova
University of Montana

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ADAPTIVE POTENTIAL TO CAMOUFLAGE MISMATCH: PLASTIC AND EVOLUTIONARY RESPONSES TO A CLIMATE CHANGE STRESSOR

By

MARKETA ZIMOVA

B.S., Charles University, Prague, Czech Republic, 2009

M.S., University of Montana, Missoula, MT, 2014

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Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

L. Scott Mills, Ph.D., Chair
Wildlife Biology Program, Office of Research and Creative Scholarship

Paulo Celio Alves, Ph.D.
University of Montana Affiliate, University of Porto, Portugal

Zac Cheviron, Ph.D.
Wildlife Biology Program, Division of Biological Sciences

Solomon Dobrowski, Ph.D.
W.A. Franke College of Forestry & Conservation

J. Joshua Nowak, Ph.D.
Wildlife Biology

Zimova, Marketa, Ph.D., Spring 2019 Fish and Wildlife Biology

Adaptive capacity to camouflage mismatch: Plastic and evolutionary responses to a climate change stressor

Chairperson: L. Scott Mills, Ph.D.

Anthropogenic global environmental change is a significant threat to biodiversity. Whether organisms will go extinct or persist long-term depends, in part, on their capacity to adapt. Quantifying this adaptive capacity is thereby one of the most important challenges ecologists and conservations face today.

Mismatch in seasonal molt for animals that turn white in winter and increasingly confront snowless conditions has emerged as an important example of phenological mismatch directly linked to climate change. Based on my previous work on snowshoe hares (*Lepus americanus*), camouflage mismatch has severe survival costs and can in the absence of adaptive responses result in steep population declines. In my Dissertation, I investigated the capacity of color molting species to adapt to climate change via phenotypic plasticity and evolution.

First, I reviewed published knowledge on the functional, molecular and physiological bases of color molts across bird and mammal species. Next, I tested whether mountain hares (*Lepus timidus*) in Scotland responded adaptively to the long-term declines in snow cover. I repeated historical field studies from the 1950s and found that mountain hares did not shift their phenologies and experienced increased camouflage mismatch. The attenuated selection pressure in Scotland is likely the leading cause for the static phenologies.

Finally, I quantified the intra-specific variation in vulnerability to mismatch across snowshoe hares' range. I integrated remote camera-trap with high-resolution climate data to quantify the environmental drivers of molt phenology, phenotypic plasticity, and the extent of camouflage mismatch in three disjunct populations. I found that only in regions characterized by short snow-season duration, snowshoe hares could not buffer against mismatch via phenotypic plasticity and are most vulnerable under climate change.

Overall, I showed high variation in the adaptive capacity to camouflage mismatch within and among species. Accordingly, adaptive responses are highly site- and population- specific, and findings from one population may not represent adaptive capacity of another or of the species overall. Most importantly, however, climate change will continue to decrease duration of snow cover. I concluded that evolutionary adaptation in molt phenology, phenotypic plasticity and or winter color morph is pivotal for long-term persistence for all color molting species.

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DEDICATION

This dissertation is dedicated to my parents.

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CHAPTER 1

OVERVIEW OF THE DISSERTATION

BACKGROUND

Climate change is creating a myriad of new stressors that threaten the persistence of many wild species (Thomas *et al.*, 2004; Parmesan, 2006; Scheffers *et al.*, 2016). In order to avoid local extinctions, we need to understand how those stressors affect wild populations and their capacity to adapt and persist locally. The adaptive capacity of most species and populations is unknown yet critical for accurate assessments of their vulnerability to climate change (Williams *et al.*, 2008; Dawson *et al.*, 2011; Urban *et al.*, 2016).

Adaptive capacity includes the ability to respond to environmental stressors via movement or dispersal across the landscape, and by local adaptation via phenotypic plasticity and or evolutionary shifts within populations (Hoffmann & Sgrò, 2011; Beever *et al.*, 2016). Both of these in situ mechanisms have been documented in wild populations in response to recent climate change, with rapid and often short-term adaptive responses being more commonly attributed to plasticity (Merilä & Hendry 2014). However, evolutionary adaptation is increasingly anticipated to facilitate long-term persistence under climate change, especially due to accumulating evidence that evolution can occur over much shorter time frames than originally thought (Hendry & Kinnison, 1999; Hendry, 2017).

Among the most common negative ecological consequences of climate change are phenological mismatches- when timing of life-events becomes mistimed with optimal environmental conditions (Parmesan & Yohe, 2003;

Thackeray *et al.*, 2010; Cohen *et al.*, 2018). Phenological mismatches represent one of the most important threats to wild population persistence, and negative consequences of mismatches have been described on individual and population level (Both *et al.*, 2006; Møller *et al.*, 2008; Ozgul *et al.*, 2010; Tafani *et al.*, 2013). It is clear that the ability of populations to adapt will be critical to ameliorate the fitness costs of phenotype – environment mismatches (Charmantier *et al.*, 2008; Charmantier & Gienapp, 2014; Socolar *et al.*, 2017). However, the potential for adaptive responses to phenological mismatches remains rarely understood in the wild.

During my Master's, I began to study a previously not described phenological mismatch; mismatch in seasonal camouflage (Mills *et al.*, 2013). Across temperate and arctic regions, 21 species of birds and mammals molt from summer brown to winter white coat annually to increase crypsis against snow (Mills *et al.*, 2013; Zimova *et al.*, 2018). As with other phenological traits, the molt phenology is dependent on photoperiod (Hofman, 2004; Lincoln *et al.*, 2006; Zimova *et al.*, 2018). Consequently, as snow duration declines in most parts of the northern hemisphere (Vaughan *et al.*, 2013; Kunkel *et al.*, 2016), color molting species become mismatched with their background. The ecological consequences of this temporal loss of camouflage are unknown for many of those species and their communities, but likely severe for most (Zimova *et al.*, 2018).

Previously, I described that snowshoe hares (*Lepus americanus*) in Montana exhibited low phenotypic plasticity in response to camouflage mismatch (Mills *et al.*, 2013; Zimova *et al.*, 2014). That is, hares did not adjust their antipredatory behaviors to reduce mismatch, nor did they shift their molt phenology in response to the shorter snow seasons (Zimova *et al.*, 2014). Subsequently, mismatched snowshoe hares suffered high survival cost (i.e., 4-7% reduction in weekly survival), that would in the absence of future adaptation lead to population declines (Zimova *et al.*, 2016). Subsequent evidence from another, disjunct snowshoe hare population (Wilson *et al.*, 2018) and from least weasels (*Mustela nivalis*; Atmeh *et al.*, 2018) corroborated the high fitness cost of mismatch. Furthermore, multiple studies have shown population contractions or declines linked to camouflage mismatch (mountain hares *Lepus timidus*: Pedersen *et al.*, 2017, snowshoe hares: Diefenbach *et al.*, 2016; Sultaire *et al.*, 2016, rock ptarmigan *Lagopus muta*: Imperio *et al.*, 2013).

The insights on camouflage research from my Master's and from others collectively pointed to the selective costs of camouflage mismatch and to the limited capacity of organisms to buffer against mismatch via phenotypic plasticity. However, it still remained unclear whether snowshoe hares- and the 20 other color molting species- will be able to adapt and persist under climate change. This critical gap in knowledge motivated further research presented in this Dissertation.

DISSERTATION OUTLINE

In my Dissertation, I investigated the capacity of color molting species to adapt to camouflage mismatch via phenotypic plasticity and evolution. To achieve understanding of the adaptive capacity to mismatch beyond single population and species, I extended my research over a temporal (Ch. 3, Ch. 4, App. 2), spatial

(Ch. 4, App. 1, App. 2) and taxonomic scale (Ch. 2, Ch. 3, App. 1). Further, I applied and developed new field and analytical methods to quantify molt phenology and mismatch in the wild (Ch. 3, Ch. 4, App. 3). Overall, my Dissertation manifests the inter-disciplinary, collaborative nature of my research; a prerequisite of effective conservation science in the presence of global environmental change. The results of my Dissertation are disseminated in three primary chapters and three appendices. Below, I summarize the major findings from each chapter:

Chapter 2

My second chapter was an inter-disciplinary literature review on seasonal color molts and adaptive capacity of color molting species to camouflage mismatch. It represented the first comprehensive review of the adaptive value and the molecular and physiological control of the color molts across birds and mammals. Furthermore, this work also outlined the mechanisms of adaptation to mismatch via plastic and evolutionary responses.

Among the most important findings was that across species, the main function of color molts is seasonal camouflage against snow, and photoperiod is the main driver of the molt phenology. Next, it exposed that many underlying mechanisms remain unclear, especially in birds. I detected similarities across color molting mammals in some aspects of hair growth, neuroendocrine control, and the effects of extrinsic factors on molt phenology. The underlying basis between birds and mammal differs profoundly in many aspects, however.

In relation to adaptive responses to mismatch, the review highlighted the limited plasticity in molt phenology, especially in mammal species, and that evolutionary adaptation will be necessary to mediate future camouflage mismatch. I showed that those shifts may be possible due to high variation in the seasonal trait and strong selection for background matching. I concluded that detailed understanding of the color molting will be necessary to manage populations effectively under climate change. Insights gained during this review have complemented other recent major contributions such as the global analysis of adaptive winter color polymorphisms (Appendix 1; Mills et al. 2018).

Chapter 3

Examining whether populations already demonstrate adaptation to recent climate change can help us understand their adaptive capacity and vulnerability in the future (Dawson *et al.*, 2011). My third chapter tested whether a common color molting species in Eurasia, mountain hare, showed adaptive responses to recent climate change.

I repeated historical field studies on molt phenology in Scotland from the 1950s (Watson, 1963; Flux, 1970), and tested for adaptive shifts in molt phenology or phenotypic plasticity in response to declining snow cover. Based on our previous findings from snowshoe hares (Zimova *et al.*, 2016), I expected that mountain hares would have responded to the long-term snow declines across Scotland over the past 60+ years. To the contrary, molt phenology did not shift to align seasonal coat color with reduced snow seasons, nor did phenotypic plasticity in molt phenology buffer against camouflage mismatch. Consequently, the unchanged phenology resulted in 35 additional days of phenotypic mismatch between 1950 and 2016.

The unexpected lack of responses was likely due to an attenuated selection pressure against camouflage mismatch. While hares in Montana confronted a rich suite of predators, mountain hares in north-eastern and central Scotland occupied highly managed heather-moorlands. Therefore, the selective costs of camouflage mismatch were likely much lower than expected, resulting in phenotypic stasis. Importantly, however, the accumulated high degree of camouflage mismatch may have negative fitness consequences if predation pressure increases suddenly and dramatically. The intriguing findings highlighted that predicting responses requires detailed system-specific biological knowledge and caution when inferring adaptive capacity based on seemingly similar natural systems.

Chapter 4

What is the species-wide adaptive capacity to camouflage mismatch and does the vulnerability to climate change vary among populations? To answer this question, in my fourth chapter, I quantified the environmental drivers of color molt phenology, phenotypic plasticity, and the extent of camouflage mismatch in three geographically disjunct snowshoe hare populations.

The three populations occupied areas that ranged over 15 degrees of latitude and collectively spanned nearly full range of environmental conditions experienced by the species. To effectively quantify phenology and mismatch across large spatial and temporal scale, I developed a new method for acquiring phenology data: using by-catch images from remote camera traps. I combined the remotely sensed phenology data with downscaled temperature and snow data to test whether latitude or local climate drove the spatio-temporal variation in molt phenology. To my knowledge, this was the first study to evaluate these processes at such resolution in any seasonally molting species.

Because photoperiod is the principal cue of molt phenology in all color molting species (Zimova *et al.*, 2018), I expected that the variation in molt phenology will be distributed along latitudinal gradients. To the contrary, among the most important findings of this work was that local climate drove spatial and temporal variation in molt phenology across the distributional range of snowshoe hares; populations occupying colder, snowier study areas were associated with earlier fall and later spring molts. Within an area, color molts occurred earlier in the fall and later in the spring during colder and or snowier years (i.e., phenotypic plasticity), but the degree of plasticity varied among areas.

Importantly, the occurrence of camouflage mismatch varied in space and time, but white mismatch (i.e., white hares occurring on snowless background) was more common in areas characterized by shallow, short-lasting snowpack. During very low snow years, adaptive capacity via phenological plasticity could no longer compensate against detrimental camouflage mismatch, suggesting increased vulnerability of those populations to climate change. The main conclusion of this work was that populations vary in their susceptibility to environmental stressors and management efforts should consider this intra-specific variation to prioritize the most threatened populations.

Appendices

Finally, three additional manuscripts fall under the conceptual umbrella of my Dissertation. They are included here as appendices, however, because I am not the (sole) primary author. The first appendix mapped the global distributions of winter color morphs of seasonally color molting species. This effort was based on nearly 2,700 museum specimens and published literature records of eight color molting species. The main drivers of the spatial variation in winter color (i.e., white, brown) were variables characterizing local snow cover duration. Importantly, this manuscript identified areas, where winter brown and white morphs co-occur. Due to their increased genetic variation, these polymorphic zones, indicated areas with elevated evolutionary potential to camouflage mismatch.

The second appendix examined adaptive responses via phenotypic plasticity in response to camouflage mismatch in four snowshoe hare populations in Montana. In agreement with previous studies using smaller samples size, hares did not select microhabitats based on their background color to reduce mismatch nor they preferred denser vegetation when mismatched. Conversely, hares were able to adaptively shift the timing of molt phenology each year in response to snow cover. Despite this adaptive response, hares still became mismatched during low snow years. Hence, the main conclusion was that phenotypic plasticity is unlikely to facilitate adaptive rescue to camouflage mismatch under climate change; a finding that reverberates throughout this Dissertation.

The third appendix evaluated a non-invasive field technique to measure the effect of environmental stressors, e.g., camouflage mismatch, in wild populations. Specifically, it tested the accuracy of fecal glucocorticoid metabolites (FGM) concentrations to index physiological stress responses using captive snowshoe hares. Among the most important findings was that various environmental conditions and the duration of exposure profoundly affected FGM measurements. This appendix provided recommendations on developing protocols for field sampling that yields accurate conclusions regarding populations responses to environmental stressors.

A note on authorship

Throughout the rest of this Dissertation, I use the first-person plural voice, we, to reflect the highly collaborative nature of my Dissertation research. Most of my chapters are in various stages of publication in peer-review journals and I recognize the contributions of my co-authors at the beginning of these chapters. Chapter 2 has been published in *Biological Reviews* and Appendix 1 has been published in *Science*. Chapter 3 is formatted for submission to *Ecography* and Chapter 4 for *Global Ecology and Biogeography.* Appendix 2 is in review in *Ecology* and Appendix 3 is formatted for *Oikos*.

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CHAPTER 2

FUNCTION AND UNDERLYING MECHANISMS OF SEASONAL COLOUR MOULTING IN MAMMALS AND BIRDS: WHAT KEEPS THEM CHANGING IN A WARMING WORLD?

Marketa Zimova[1,2], Klaus Hackländer[2,3], Jeffrey M. Good[4], José Melo-Ferreira[5,6], Paulo Célio Alves[1,5,6], and L. Scott Mills[7]

[1]Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA

[2]Fisheries, Wildlife, and Conservation Biology Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA

[3]Institute of Wildlife Biology and Game Management, BOKU - University of Natural Resources and Life Sciences, Vienna, Austria

[4]Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

[5]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

[6]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 4169-007 Porto, Portugal

[7]Wildlife Biology Program and Office of Research and Creative Scholarship, University of Montana, Missoula, MT 59812, USA

ABSTRACT

Animals that occupy temperate and polar regions have specialized traits that help them survive in harsh, highly seasonal environments. One particularly important adaptation is seasonal coat colour (SCC) moulting. Over 20 species of birds and mammals distributed across the northern hemisphere undergo complete, biannual colour change from brown in the summer to completely white in the winter. But as climate change decreases duration of snow cover, seasonally winter white species (including the snowshoe hare *Lepus americanus*, Arctic fox *Vulpes lagopus* and willow ptarmigan *Lagopus lagopus*) become highly contrasted against dark snowless backgrounds. The negative consequences of camouflage mismatch and adaptive potential is of high interest for conservation. Here we provide the first comprehensive review across birds and mammals of the adaptive value and mechanisms underpinning SCC moulting. We found that across species, the main function of SCC moults is seasonal camouflage against snow, and photoperiod is the main driver of the moult phenology. Next, although many underlying mechanisms remain unclear, mammalian species share similarities in some aspects of hair growth, neuroendocrine control, and the effects of intrinsic and extrinsic factors on moult phenology. The underlying basis of SCC moults in birds is less understood and differs from mammals in several aspects. Lastly, our synthesis suggests that due to limited plasticity in SCC moulting, evolutionary adaptation will be necessary to mediate future camouflage mismatch and a detailed understanding of the SCC moulting in all species will be needed to manage populations effectively under climate change.

INTRODUCTION

Species occupying temperate and polar latitudes have multiple physiological and life-history adaptations that help them cope with harsh winters and high seasonality (Blix, 2016). Those circannual traits include seasonal migration, hibernation and coat colour moults, and their occurrence and optimal phenology is critical for maintaining fitness (Varpe, 2017). However, as global warming alters temperatures and snow cover across much of the northern hemisphere, seasonal adaptations become compromised (Berteaux *et al.*, 2016; Pauli *et al.*, 2013; Williams, Henry & Sinclair, 2015). Understanding whether adaptation in these traits can occur is of intense interest (Helm *et al.*, 2013; Williams *et al.*, 2017) and requires comprehensive knowledge of the traits in question.

A key adaptive trait increasingly compromised by climate change is seasonal coat colour (SCC) moulting from dark pigmented fur or plumage in the summer to white in the winter. Although colour change is found across vertebrates (Duarte, Flores & Stevens, 2017), complete SCC moults to match presence or absence of snow is unique to several bird and mammal species. The biannual phenotypic shifts are controlled with complex physiological mechanisms entrained by photoperiod and optimized to match local conditions. However, as seasonal duration and extent of snow cover declines due to climate change (Kunkel *et al.*, 2016; Vaughan *et al.*, 2013), SCC species become colour mismatched against their surroundings (Mills *et al.*, 2013) (Fig. 2.1B,C).

To understand how seasonally colour changing species may respond to declining snow cover, we must first understand the ecological significance and the underlying mechanisms of this highly specialized trait. Although the ecology and physiology of SCC moults have received substantial attention from naturalists and scientists over the last century, insights from these fields have not been compiled across disciplines and species, or connected to climate change. Herein, we summarize current knowledge on the function and molecular and physiological control of SCC moults in birds and mammals. This cross-disciplinary synthesis and comparison across taxa provides a foundation for study of the effects of climate change on SCC species and their ability to adapt to it in the future.

SPECIES AND FUNCTION OF SCC MOULTS

Most mammal and bird species inhabiting temperate and arctic environments undergo periodical moults one to three times a year. The moults replace worn out and old hairs or feathers and result in new, structurally different coats better suited for the upcoming season (Ling, 1972). In the group of species that we focus on here, brown or greyish brown summer coats are annually shed and replaced with new fully or mostly white fur or plumage. Therefore, in SCC species, individuals are capable of producing multiple phenotypic morphs (polyphenism; Mayr, 1963), i.e. winter white and summer brown colour morphs, and therefore SCC species fundamentally differ from others exhibiting non-seasonal discrete colour polymorphisms (Karell *et al.*, 2011; McLean & Stuart-Fox, 2014).

Seasonal colour moulting has likely independently evolved in 21 species from five families of birds and mammals, although the precise understanding of the evolution of the trait within and across groups of species is limited. SCC species include both prey and predators and collectively occupy much of the northern hemisphere (Mills *et al.*, in press) (Table 2.1). The mammals include six species of leporids (e.g. snowshoe hare *Lepus americanus*), eight muroids (e.g. Siberian hamster *Phodopus sungorus*), three mustelids (e.g. stoat *Mustela erminea*), and one canid (i.e. Arctic fox *Vulpes lagopus*). Birds include three SCC species, all in the genus *Lagopus* (e.g. rock ptarmigan *L. muta*) (Fig. 2.1). Within genera, seasonal moult to white often appears scattered throughout the phylogeny, as can be seen from the phylogenies of hares (Melo-Ferreira *et al.*, 2012) or weasels (Koepfli *et al.*, 2008). Species that seasonally acquire additional to already existing white colouration (e.g. snow bunting *Plectrophenax nivalis*) or undergo winter moults to lighter but not completely white coat [e.g. small vesper mouse *Calomys laucha* (Camargo, Colares & Castrucci, 2006)] are not considered as SCC moulting by our definition.

The function of this phenological trait has long been under debate, with the two primary (not mutually exclusive) adaptive hypotheses centering on camouflage and thermoregulatory properties of the seasonal brown and white coats.

Camouflage

The white coat undoubtedly promotes camouflage against snow, as long proclaimed by many prominent naturalists (e.g. Cott, 1940; Wallace, 1879). Natural selection on cryptic colouration (or camouflage) is prevalent in nature and camouflage is one of the strongest evolutionary forces driving colouration in mammals (Caro, 2005). Strong selection for camouflage and adaptive value of cryptic colouration has been widely demonstrated in birds and mammals; for example, experiments with live predators showed strong selection on protective colouration in mice (Dice, 1947; Kaufman, 1974; Vignieri, Larson & Hoekstra, 2010), and multiple studies have shown local adaptation to reduce predation risk *via* crypsis (e.g. Kiltie, 1989; Slagsvold, Dale & Kruszewicz, 1995; Vignieri *et al.*, 2010). In contrast to the myriad studies demonstrating strong selection on permanent coat colour, the selective advantage of SCC moult has been rarely quantified in the wild (Duarte *et al.*, 2017). However, Zimova, Mills & Nowak (2016) showed a selective advantage of background matching in wild populations of snowshoe hare, as animals mismatched with their background suffer decreased survival. Likewise, rock ptarmigan in the Alps exhibited lower survival during years with delayed autumn snowfall, possibly due to compromised camouflage (Imperio *et al.*, 2013).

A second line of evidence for a camouflage function of SCC moulting is the strong correspondence of moult phenology and local snow cover duration. Inter-population variation in SCC moult has been shown to correlate with local snow duration [e.g. weasels (Hall, 1951; Hewson & Watson, 1979), snowshoe hare (Zimova *et al.*, 2014)] suggesting selection against colour mismatch. Further, different species in the same area appear to have similar moult phenology suggesting convergent evolution; for example, moult timing and duration is similar for sympatric rock ptarmigan and mountain hare (*L. timidus*) in Scotland (Hewson, 1958).

Similarly, SCC species show clinal variation in winter colour in relation to snow cover (Mills *et al.*, in press). Populations found in areas where snow is brief, erratic or absent exhibit patches with pigmented or partially pigmented hairs or feathers [e.g. least weasel *M. nivalis* in the southern portion of their range (Easterla, 1970) or rock ptarmigan in Amchitka Island, Alaska (Jacobsen, White & Emison, 1983)] or winter fur with grey or 'blue' undertones [e.g. mountain hare in Sweden (Thulin, 2003)] (Table 2.1, Fig. 2.2). Further, some individuals acquire a completely brown-pigmented winter coat similar in colour to their summer coat, and we refer to such as colour invariant or non-polyphenic in SCC moult; e.g. snowshoe hare along the Pacific coast of North America (Dalquest, 1942; Nagorsen, 1983), mountain hare in Ireland ['Irish hare' *L. timidus hibernicus* (Flux & Angermann, 1990)], or the subspecies of willow ptarmigan found in Scotland [*L. lagopus scotica*, 'red grouse' (Cramp & Simmons, 1980; Thom, 1986)] (Table 2.1, Fig. 2.2). Populations comprising both winter white and invariant winter brown colour morphs tend to associate with areas of low or unpredictable seasonal snow cover (Mills *et al.*, in press); e.g. Arctic fox (Hersteinsson, 1989), Japanese hare [*Lepus brachyurus* (Nunome *et al.*, 2014)], or stoat (Hewson & Watson, 1979).

Lastly, if seasonal change to white is an adaptation to increase crypsis, animals may be expected to show active maintenance of such crypsis. Many colour-changing invertebrates, fish, and reptiles can perceive their colour and modify their behaviour to increase background matching (Kjernsmo & Merilaita, 2012; Ryer *et al.*, 2008), but the evidence is sparse for SCC species. Snowshoe hares in the USA showed no behavioural plasticity in response to camouflage mismatch, including the degree of hiding behind vegetation, flight-initiation distance and microsite selection (Kumar, 2015; Zimova *et al.*, 2014; but see Litvaitis, 1991). In contrast to mammals, birds exhibit a high degree of self-awareness, potentially due to highly refined sexual selection on plumage colouration (Montgomerie, Lyon & Holder, 2001). Ptarmigan adjust their behaviour to actively maintain camouflage such as resting in areas that match their colouration [rock ptarmigan (Jacobsen *et al.*, 1983; MacDonald, 1970), white-tailed ptarmigan *L. leucurus* (Braun & Rogers, 1971)]. For example, willow ptarmigan in Scandinavia fed in areas that matched their plumage colour even when of inferior nutritious quality (Steen, Erikstad & Hoidal, 1992) and rock ptarmigan in Canada dirtied themselves when mismatched after snowmelt (Montgomerie *et al.*, 2001).

Thermoregulation

Thermoregulatory properties provide a complementary function to crypsis for the white winter pelage or plumage. This is achieved *via* its overall composition and the individual hair/feather microstructure.

Microstructure

The major structural difference between the winter and summer coat is that the winter white guard hairs contain more air (as opposed to pigment), which increases their insulative ability. Russell & Tumlison (1996) compared the microstructure between winter and summer guard hairs (i.e. the long coarse hairs forming a layer over the short wooly underfur) of multiple SCC species (stoat, least weasel, Arctic hare *L. arcticus*, snowshoe hare, and Arctic fox) and found considerable interspecific variation in how increased insulation might be achieved. The most common differences between the two different coloured hairs were that the hair shaft of the white guard was wider or consisted of more air-filled cells, both of which allowed for increased air trapped within the shaft. Additionally, all species (except least weasel) exhibit a thinner lower part of the guard hair, which could increase coat density irrespective of their hair colour (i.e. by providing room for more down hairs or by increasing the volume of air trapped in the bottom layer of the fur).

Composition

In most animals occupying temperate and arctic regions, fur/feather composition changes seasonally and provides greater insulation, regardless of the winter colour (Hart, 1956; Walsberg, 1991). Mammalian winter fur is denser and or longer in most species in those regions (e.g. Al-Khateeb & Johnson, 1971; Nixon *et al.*, 1995), which may overwhelm the thermal effects of concurrent changes in hair microstructure and pigmentation (Dawson, Webster & Maloney, 2014). For SCC species, white winter fur is also denser and longer [snowshoe hare (Grange, 1932; Hewson, 1958; Lyman, 1943), Arctic fox (Underwood & Reynolds, 1980), collared lemming *Dicrostonyx groenlandicus* (Maier & Feist, 1991; Reynolds, 1993), Siberian hamster (Kuhlmann, Clemen & Schlatt, 2003)]. Furthermore, winter fur changes with latitude and temperature; e.g. snowshoe hares have longer, denser, and warmer coats in northern as opposed to southern parts of their range (Gigliotti, Diefenbach & Sheriff, 2017).

The thermoregulatory function of SCC moults is less important in weasels and ptarmigans. Weasels have short fur year-round, perhaps facilitating locomotion, and the length of the dorsal fur (including guard hairs) does not increase in winter in least weasels and stoats (Casey & Casey, 1979). Although structural differences exist between white and brown hairs in these species (Russell & Tumilson, 1996), the two seasonal coats have the same thermal conductance (Casey & Casey, 1979). It is possible that weasels thermoregulate in winter by increasing their metabolism (Szafranska, Zub & Konarzewski, 2007), and by resting in subnivean nests of lemmings lined with rodent fur [least weasel and stoat (MacLean, Fitzgerald & Pitelka, 1974)]. In rock ptarmigan, winter white plumage may be longer and denser than summer plumage (Salomonsen, 1939), but no difference in mass was found in rock ptarmigan in Norway (Mortensen, Nordøy & Blix, 1985). Furthermore, density or plumage depth were similar between white willow ptarmigan and the year-round brown subspecies red grouse, implying no differences in thermal resistance and minimal differences in heat gain (Ward *et al.*, 2007).

Optical properties

Although some authors have argued that white hairs offer a thermal advantage *via* their spectral properties, these hypotheses have been rejected. One idea suggests that white coats are warmer because they lose less heat *via* radiation than dark pigmented coats (Stullken & Hiestand, 1953). Importantly, arctic and winter white animals appear white only in the visible spectrum; however, most of the radiative exchange occurs in the far infra-red (IR) and therefore white and darkly coloured animals experience equally high emission of the absorbed heat (Hammel, 1956; Johnsen, 2012). Additionally, for SCC species occupying open polar regions (e.g. Arctic hare, Arctic fox), radiative loss to the environment is further augmented by the increased exposure to clear sky and extensive snow and ice.

The observation that white arctic and SCC animals appear black under ultra-violet (UV) light (Leblanc *et al.*, 2016; Reynolds & Lavigne, 1981) led to a premise that they would be warmer because the white hairs act as optical fibres that transmit UV heat to the skin below (Grojean, Sousa & Henry, 1980). This idea has been rejected based on the evidence that low UV reflection of the fur is instead due to high absorption by the hair protein keratin (Bohren & Sardie, 1981; Koon, 1998), and because UV represents only about 1% of the solar spectrum. Therefore, no strong evidence supports thermal benefits of winter white coats due to their spectral properties.

HAIR AND FEATHER GROWTH

Regenerative cycling and moulting in mammals

SCC moults arise from complete shedding and regrowth of brown or white hairs as opposed to colour bleaching. The complete moults are accomplished by the regenerative ability of hair follicles, facilitating life-long growth of new hairs (Schneider, Schmidt-Ullrich & Paus, 2009). The hair growth cycle in all mammals can be described by four main stages: active hair proliferation (anagen), regression (catagen), rest (telogen), and shedding (exogen) (Stenn & Paus, 2001). The exogen and anagen stages often overlap, and the old hair shaft may not shed until the new hair is fully grown, as occurs during some SCC moults.

The moulting process and the level of synchrony between individual hair follicles varies across mammalian taxa, and the controlling mechanisms are still not fully understood. Some species [e.g. guinea pig *Cavia porcellus* (Chase, 1954)] show minimal synchrony in anagen entry between neighbouring hair follicles and steady asynchronous moulting. In mice and most domesticated mammals, however, hair cycling is a highly synchronized, endogenously controlled process. Hair regeneration starts in a certain region where all hair follicles enter anagen synchronously and from there moves posteriorly and dorsally as moulting waves (Chase, 1954; Plikus & Chuong, 2008; Whiteley & Ghadially, 1954).

The physiology and phenology of hair follicle cycling has been rarely described in seasonally moulting species. It has been suggested that seasonal moults also proceed in growth waves, but unlike in mice, waves are restricted to short moulting periods initiated by changing day length; during winter and summer, hair follicles rest in the telogen stage (Geyfman *et al.*, 2015; Nixon *et al.*, 1995). A gene expression study on skin tissues of the snowshoe hare is compatible with this suggestion, as expression waves in the spring moult mostly represent the mechanism of hair shedding and regrowth, although there is a lag between gene expression and visible coat colour (Ferreira *et al.*, 2017). Although no histological studies have described hair regeneration throughout the year in any SCC species, a wave-like regeneration process controlled by externally stimulated hormonal levels is supported by multiple studies of captive stoats (e.g. Rust, 1962). Further, the winter white fur period is long and fur most likely rests in telogen in most SCC species; for example, in snowshoe hares (Severaid, 1945; D. J. R. Lafferty & L. S. Mills, unpublished data) and stoats (Feder, 1990) hair growth only begins after the removal of hair follicles (as opposed to mere shaving or cutting).

A different model of hair regeneration was described in hamsters, for whom recurrent moulting waves seem to replace lost and damaged hairs as a continuous and endogenous process independent of photoperiodic changes. Siberian hamsters exhibit waves independent of photoperiod and the skin of the winter and summer coats contains moulting patches and follicles at different stages including anagen (Kuhlmann *et al.*, 2003; Paul *et al.*, 2007). Therefore, it has been argued that hamsters do not undergo seasonal moults, but rather a continuous, endogenously controlled moulting combined with seasonal, photoperiodically controlled changes in hair composition and colour. Thus, moulting and colour change may be independently controlled processes, with only colour change being controlled by changing prolactin levels (Badura & Goldman, 1992; Kuhlmann *et al.*, 2003).

Moulting patterns in SCC mammals

Differences in the moult pattern exist among taxa, but closely related species show similar patterns across the body, proceeding in the reverse order in the spring compared with the autumn. For example, the autumn moult in SCC leporids progresses in a distinct pattern, with brown hairs not shed until the white hairs are nearly or fully grown [mountain hare (Flux, 1970; Hewson, 1958; Slotta-Bachmayr, 1998), snowshoe hare (Grange, 1932; Lyman, 1943; Severaid, 1945), Arctic hare (Best & Henry, 1994), white-tailed jackrabbit *Lepus townsendii* (Lim, 1987)] (Fig. 2.3A). The moult starts on some parts of the head and feet, continues onto the shoulders and thighs, and subsequently spreads dorsally and along the sides. The area along the posterior dorsal midline and some portions of the face turn white last. In the spring, old hairs are shed as the new brown hairs begin their growth [snowshoe hare (Lyman, 1943; Severaid, 1945), mountain hare (Hewson, 1958), Arctic hare (Nelson, 1909)], causing a reverse and less defined pattern. Shedding may potentially be accelerated by grooming [mountain hare (Flux, 1970), Arctic hare (Best & Henry, 1994)]. In addition, a third seasonal moult (sometimes referred to as ‘summer’ or ‘preliminary fall moult’) has been described only in leporids (Hewson, 1958; Lyman, 1943); it occurs prior to the white moult and results in another brown coat.

In mustelids, the autumn moult is faster and the pattern more diffused than in the spring moult (Rothschild, 1942). It starts on the belly, chest and throat and progresses upward and toward the back [stoat (Bissonnette & Bailey, 1944; Feder, 1990; Hamilton, 1933; Rust, 1962; Van Soest & Van Bree, 1969), least weasel (King, 1979; Smith, 1980), long-tailed weasel *Mustela frenata* (Glover, 1942; Sheffield & Thomas, 1997)] (Fig. 2.3C). The area between the ears and shoulders becomes white last, or sometimes stays brown throughout the winter (Fig. 2.2A). In the spring, brown hairs first appear along the posterior dorsal midline and continue ventrally to the belly, breast and throat, in a definite moult line. The last to become brown are some parts of the head and the belly. As in hares, shedding in the spring may potentially be accelerated by grooming [long-tailed weasel (Glover, 1942)].

In Arctic foxes, the autumn moult has a diffused and non-sequential pattern and the exact progress of this moult is unclear (Chesemore, 1970; Moberg, 2017). Studies provide conflicting evidence on whether the white hairs appear and spread uniformly across the whole body (Pocock, 1912), or instead grow first on the belly or the head (Chesemore, 1970; Lavrov, 1932; Underwood & Reynolds, 1980). The last regions to turn fully white are the centre of the back and tail. Spring moult usually starts on the legs and some parts of the face (Pocock, 1912; Underwood & Reynolds, 1980). Then shedding usually occurs rapidly along the mid-dorsal line on the back, and in large patches on the flanks and legs, with the tail being the last to change. The moult in the winter blue colour morph in the spring and autumn occurs in the same sequence and manner as in the white morph (Moberg, 2017; Pocock, 1912).

In murids, moult patterns appear different between lemmings and hamsters. In collared lemmings, autumn moult begins ventrally and spreads from the flanks dorso-anteriorly (Degerbøl & Møhl-Hansen, 1943; Hansen, 1959). The hair growth progresses with the gradual appearance of white patches within the dark summer fur, or the fur along the mid-dorsal line gradually becomes lighter until it reaches full white. The last area to gain winter pelage is usually the head. The head and shoulders are the first to start moulting in the spring and the new growth progresses posteriorly and ventrally. In Siberian hamsters, the autumn moult starts on the face and on two spots on the posterior part of the back (Figala, Hoffmann & Goldau, 1973; Kuhlmann *et al.*, 2003). These spots then spread over the dorsal part of the body towards the neck and then in a posterior direction towards the tail, while the belly and flanks become increasingly whiter. A remnant of the dorsal line usually remains dark throughout the winter. In the spring, animals become increasingly more greyish-brown all over the body.

Regenerative cycling and moulting in birds

Despite the significance of moults for birds, avian moulting remains relatively poorly studied (Payne, Farner & King, 1972; Rohwer, Thompson & Young, 1992). In general, however, feather regeneration is analogous to hair regeneration in mammals, and feather follicles facilitate lifelong feather growth. The feather growth cycle has the same stages of growth (anagen, catagen, telogen and exogen) and equally resumes each time it is shed or plucked (Chen *et al.*, 2015; S.G. Lin *et al.*, 2013). Moulting itself occurs in a regular and highly synchronized pattern; it always begins at certain points on the body and progresses from these in a wavelike pattern (Payne *et al.*, 1972).

Most adult bird species undergo one (i.e. pre-basic moult, also called post-nuptial) or two moults (i.e. pre-basic and pre-alternate moult, also called pre-nuptial) (Humphrey & Parkes, 1959; Pittaway, 2000). The pre-basic moult is the more complete moult that occurs following the breeding season or laying cycle and results in ‘basic’ pelage. The pre-alternate moult results in ‘alternate’ or ‘breeding’ plumage, which is brighter and more colourful in many species, while almost identical in others.

Moulting patterns in SCC birds

Ptarmigans are unusual among birds in that they have three moults: pre-basic, pre-alternate and pre-supplemental. The terminology, however, differs among authors; first, studies disagree on identifying moult homologies between ptarmigans and other grouse species (Cramp & Simmons, 1980; Watson & Moss, 2008). Second, white feathers are occasionally retained in spring and summer at high latitudes (Watson & Moss, 2008). Lastly, inter-sexual differences in feather growth and moult phenology suggest that moults may have evolved separately in each sex (Pyle, 2007). Despite these nuances, we refer to the moults as 'spring', 'summer' and 'autumn' as all species clearly undergo three major annual moults. The only exception is the willow ptarmigan subspecies, red grouse, that omits the autumn brown to white moult (Cramp & Simmons, 1980).

In rock ptarmigan, the autumn moult starts on the underparts and spreads upwards to finish on the back and head, with white feathers appearing among old pigmented ones (Salomonsen, 1939; Watson, 1973). Some populations (e.g. Scotland, Amchitka Island) show dark-pigmented or partially coloured feathers (Cramp & Simmons, 1980; Jacobsen *et al.*, 1983; Watson, 1973) during winter. In the spring, new feather growth does not proceed in a sharp distinguishable line, but generally starts on the crown and moves down to the breast and back, finishing on the underparts (Watson, 1973). In arctic populations, the spring moult occurs much later in males than in females; male breeding plumage is white which is likely a consequence of sexual selection for bright and conspicuous plumage during breeding season (Montgomerie *et al.*, 2001). Interestingly, during the summer brown to brown moult, some white feathers are already acquired in certain areas (e.g. wings, legs, belly) (Hewson, 1973; Salomonsen, 1939; Watson, 1973).

In willow ptarmigan, autumn moults are similar to rock ptarmigan with mosaic-like growth of white feathers among pigmented feathers (Cramp & Simmons, 1980) (Fig. 2.3B). In the spring, however, willow ptarmigan males develop conspicuous, partially pigmented breeding plumage. In this partial moult [not recognized by some as a pre-alternate moult (Höhn & Braun, 1980)], pigmented feathers develop in males on the head, upper neck, upper anterior breast and lastly on the back, while the ventral and wing feathers remain white (Cramp & Simmons, 1980). At the end of the breeding period, males begin to moult into a pigmented summer plumage. Females start to grow pigmented feathers later in the spring, and feather replacement is not as sequential, although it still starts on the head and upper breast, and finishes with the back and tail. After brooding, a summer moult occurs when brown summer pelage is grown in both sexes, although great variation exists among individuals.

White-tailed ptarmigan moults are similar to those of willow ptarmigan, with males having partially pigmented plumage during the breeding season (Braun, Martin & Robb, 1993). But unlike other ptarmigan species, males retain a white lower breast and abdomen throughout the year and both sexes have white primaries and white tail feathers year-round.

MOLECULAR CONTROL OF SCC MOULTS

Pigmentation

Although the genetic and physiological bases of colour pigment synthesis and deposition have been widely studied in vertebrates (Dupin & Le Douarin, 2002; Slominski *et al.*, 2004), the development and regulation of seasonal colour moults is poorly understood. Colouration in mammals and birds is mainly caused by endogenously produced melanin pigments, the products of complex biochemical events starting from the amino acid tyrosine and its metabolite dopa (Prota, 1995; McGraw, 2006; Slominski *et al.*, 2004). Tyrosinase, the main enzyme regulating melanin synthesis (i.e. melanogenesis) is encoded by the TYR locus and is highly conserved among species (Del Marmol & Beermann, 1996).

Melanogenesis occurs exclusively during the anagen stage of hair/feather growth and is restricted to pigment-producing cells (i.e. melanocytes) in hair/feather follicles (Stenn & Paus, 2001). Melanins are transferred *via* melanosomes into keratinized cells (i.e. keratinocytes) of the developing hair or feathers (Chen *et al.*, 2015; Slominski *et al.*, 2004).

The two main types of melanins are eumelanin (black or brown pigments) and phaeomelanin (yellow to red pigments) and their ratio along individual hairs and feathers and spatial distribution across the body determines an animal's overall coat colour (S. Lin *et al.*, 2013; Slominski *et al.*, 2004). The production of a specific melanin type in each cell is regulated primarily by melanocortin-1 receptor (MC1R) in melanocytes and its two ligands: α-melanocyte stimulating hormone (α-MSH), secreted from the pituitary, and the agouti signalling protein

(ASIP) (Chen *et al.*, 2015; Slominski *et al.*, 2004). When activated by α-MSH, melanocytes produce dark eumelanin. Binding of the other ligand ASIP, inhibits MC1R activity and causes a switch to light phaeomelanin production (Barsh, 1996; Vrieling *et al.*, 1994). The relative contributions of ASIP and other endocrine signals (see Sections V.2 and V.3) on the production of white colouration in SCC species is not yet fully understood. However, in many species the winter white appearance is achieved by the absence of pigment in the guard hairs or feathers (Dyck, 1979; Hadwen, 1929; Paul *et al.*, 2007; Russell & Tumlison, 1996).

Genetics of coat colour

The genetic underpinnings of non-seasonal variation in melanin-based colouration have been extensively described in a number of well-studied model systems. Over 150 genes that affect animal colour and patterning have been identified but only a few appear to serve as major contributors to colour variation across a wide array of animal taxa (Protas & Patel, 2008; San-Jose & Roulin, 2017). *Mc1r* and its antagonist *Agouti* are the most widely studied pigmentation genes in vertebrates (Hubbard *et al.*, 2010; Manceau *et al.*, 2010). *Mc1r* is highly conserved and many studies have shown a link between variation in *Mc1r* and pigmentation across vertebrates (Hoekstra, 2006; Roulin, 2004). The majority of these studies have identified one or more amino acid changes associated with a discrete colour polymorphism (e.g. Hoekstra *et al.*, 2006; McRobie *et al.*, 2014; Uy *et al.*, 2009). The large number of instances in which colouration differences are explained by protein-coding mutations in *Mc1r* is likely due to its minimal pleiotropic effects, large mutational target size, and high mutation rate (Hubbard *et al.*, 2010).

Several *Agouti* mutations have also been associated with colour variation (e.g. Carneiro *et al.*, 2014; Hiragaki *et al.*, 2008). Unlike the *Mc1r* mutations, which usually involve protein-coding changes that are inferred to be directly causative, coat colour variation linked to *Agouti* is usually associated with genetic variants that impact gene expression; for example, increased expression of *Agouti* results in light-coloured deer mice *Peromyscus maniculatus* (Linnen *et al.*, 2009; Manceau *et al.*, 2011). Fewer causative mutations have been identified at *Agouti*, likely due to a more complex exon and regulatory structure relative to *Mc1r*, and the general inherent difficulties of identifying causative regulatory variants. Additionally, several other pigmentation genes have been shown to play important roles in determining colour variation in animals, including genes encoding tyrosinase and tyrosinase-related proteins [e.g. *Tyrp1* (Delmore *et al.*, 2016), *Tyr* (Ekblom *et al.*, 2012)] and other components of the melanocortin pathway (Anderson *et al.*, 2009). Collectively, intra-specific colour variation may have different genetic bases (i.e. different genes or mutations), yet remarkable genetic convergence with *Mc1r* and *Agouti* underlies adaptive coat colour evolution across a broad range of species. Recent high-throughput sequencing technologies will allow extending such studies to a broader number of species and colouration traits (San-Jose & Roulin, 2017).

Genetics of SCC moults

The genetic basis of seasonal colouration is poorly known. Yet it is expected that genes and pathways are similar to non-seasonal colour variation due the highly conserved nature of pigmentation in vertebrates. The strong genetic control of SCC moult has long been evident from translocation experiments and common garden studies. For example, winter white and winter brown snowshoe hares from a polymorphic population in Washington, USA continued changing to their winter colours when exposed to identical temperature and light condition in a 'common garden' environment (L. S. Mills & D. J. R. Lafferty, unpublished data), and mountain hares from Austria maintain their regular seasonal changing cycle over several years when translocated to Portugal (P.C. Alves, unpublished data). Similar observations were described in other mammal transplants [long-tailed weasel (Hall, 1951), stoat (Feder, 1990; but see: King & Powell, 2007; Rothschild & Lane, 1957), white-tailed jackrabbit (Hansen & Bear, 1963)].

The mechanisms underlying the evolution of SCC remain unknown, but some studies have focused on the genetics of colour variation in SCC species. Notably, extensive research into the basis of colour polymorphism in wild white and blue Arctic foxes has been driven by their economic importance. The white morph is completely white in winter, but in summer it is brownish grey dorsally and light grey ventrally (Fig. 2.1D). By contrast, the blue morph lacks the light brown during winter and is uniformly chocolate brown during the summer (Fig. 2.2C) (Chesemore, 1970). The blue colour morph is inherited as a simple Mendelian trait that is dominant to white and caused by two cysteine substitutions in the *Mc1r* gene (Våge *et al.*, 2005). Importantly, however, the mutations resulting in colour polymorphism affect both summer and winter pelage and therefore are not seasonally expressed *per se*. Also, major dominant genes may determine the winter grey/blue morph in Swedish mountain hares [called 'heath-hares' (Fig. 2.2D) (Bergengren, 1969)] and the winter brown *M. nivalis vulgaris* morph in least weasels (Stolt, 1979), but particular gene mutations were not investigated. Nunome *et al*. (2014) found no differentiation between Japanese hare populations of winter white and winter brown morphs in three candidate genes (*Agouti*, *TYR*, *Mc1r*). Similarly, no association has been found between colour polymorphism and *Mc1r* and three other candidate genes (*Tyr*, *Tyrp1*, and *Dct*) in willow ptarmigan (Skoglund & Hoglund, 2010).

Very few studies have investigated the regulatory underpinnings of SCC moults (as opposed to colour polymorphism) and of their phenology. In snowshoe hares, Ferreira *et al*. (2017) pinpointed three known pigmentation genes, *Agouti*, *Myo7a* and *Spns2*, that were differentially expressed during spring moult and thus may play important functional roles in regulating the seasonal production of brown pelage. This suggests that known pigmentation pathways with transient regulation may underlie SCC. Another study using hybrids of Siberian and Campbell's dwarf hamsters (*P. campbelli*, a sister species that is brown year-round) suggested recessive heritability of SCC moulting and a potentially simple genetic basis (Clare-Salzler, 2017).

PHYSIOLOGICAL REGULATION OF SCC MOULTS

Photoperiodism

The phenology of SCC moults is coordinated by the same mechanisms that controls the annual timing of other life events such as reproduction, hibernation, and migration. In vertebrates, these mechanisms are based on an internal circannual rhythm synchronized with external stimuli (*Zeitgeber*), most commonly day length (photoperiod) (Gwinner, 2003; Hofman, 2004). Organisms in polar and temperate regions depend on their ability to assess and use day length (photoperiodism) to time seasonal events optimally (Bradshaw & Holzapfel, 2007).

Mammals and birds have central clocks or pacemakers that are entrained to the 24 h light cycle and provide a 'reference clock' for the reading of calendric information. Melatonin is the chief humoral signal that translates photoperiodic information and induces a wide variety of effects on the animals' physiology. Mammals have one central circadian clock located in the brain in the suprachiasmatic nucleus (SCN) that synchronizes clock genes expression in other tissues (Balsalobre, 2002). By contrast, birds have a system of at least three independent yet interacting circadian clocks in the SCN, the retina of the eyes and the pineal gland (Kumar *et al.*, 2010).

Despite advances in understanding the circadian clock in mammals and birds, the molecular basis of circadian time measurement and how it is used to measure photoperiod and regulate circannual timing is still not fully understood (Schwartz & Andrews, 2013). The circadian timing is generated endogenously by a cell autonomous mechanism involving a small number of core clock genes, with transcription factor genes *Clock* and *Bmal1*, and *Period* and *Cryptochrome* genes playing crucial roles (Ono, Honma & Honma, 2015; Reppert & Weaver, 2002). Their expression in the master pacemakers as well as in most cells throughout the body (Balsalobre, 2002; Reppert & Weaver, 2002) including the skin, plays a role in the regulation of hair cycling (Geyfman *et al.*, 2015; Lin *et al.*, 2009). Recently, Ferreira *et al*. (2017) detected expression waves of circadian rhythm genes in snowshoe hare skin during the moulting process.

Circannual rhythms in mammals

The degree to which animals rely on internal clock *versus* photoperiodic stimuli varies among species and two primary long-term timekeeping mechanisms or circannual rhythms are recognized (Paul, Zucker & Schwartz, 2008). Type I rhythm is common among many short-lived temperate and boreal mammals and works like an hourglass timer measuring the lapse of time since a photoperiodic cue (Goldman, 2001). In many long-lived species, the internal circannual cycle predominates (Type II rhythm), with continued expression of circannual cyclicity in the absence of annual change in day length or other external stimuli over mutliple years.

Siberian hamsters are one of the best-studied animal models for seasonality. Type I rhythm clearly governs their seasonal changes including moults and reproduction (Wade, Bartness & Alexander, 1986). A short photoperiod during the autumn increases melatonin concentration and subsequent prolactin suppression, triggering an endogenous interval timer. The same pathway also drives the loss of a response to day length (i.e. photorefractoriness) in the spring and reversal of the initial physiological response without additional external stimuli (Lincoln, Andersson & Loudon, 2003). Multiple studies with captive hamsters demonstrated that the brown to white autumn moults are triggered by decreasing day lengths (Kuhlmann *et al.*, 2003; Paul *et al.*, 2007). Subsequently, white to brown spring moult occurs independently of external stimuli; when exposed to a prolonged period of short days, hamsters show a characteristic refractory response as they revert to the summer phenotype (i.e. brown pelage, reproductive status, decreased body mass) within 38 weeks (Duncan & Goldman, 1984; Paul *et al.*, 2007). On the contrary, they remain indefinitely in the summer pelage until exposure to short day lengths, resetting their circannual clock.

Although the role of endogenous mechanisms on any seasonal moults has not been intensively studied in other species, the relative importance of external stimuli seems to be higher than in Siberian hamsters. For example, in snowshoe hares, autumn moults can be arrested by an artificially long photoperiod, and the refractory response occuring in the spring is not as striking as in hamsters; after prolonged exposure to short days, hares still underwent spring moult, but it was delayed or incomplete (Lyman, 1943). However, both autumn and spring moults can be initiated by changes in photoperiod at any time of the year in hares (Lyman, 1943) and collared lemmings (Gower, Nagy & Stetson, 1993; Maier & Feist, 1991; Nagy, Gower & Stetson, 1993). In long-tailed weasels and stoats both spring and autumn moults were induced and reversed using photoperiod manipulation (Bissonnette & Bailey, 1944; Rust, 1962, 1965). Importantly, however, endogenous control is still evident, because reversions can be made only after a latent period of about three months after the change in photoperiod (Bissonnette & Bailey, 1944).

Neuroendocrine and endocrine regulation in mammals

The photoperiod cue controlling the major humoral signal (melatonin) responsible for the phenology of many annual events, including moults, is received solely by the eyes in mammals [snowshoe hare (Lyman, 1943), mountain hare (Novikov & Blagodatskia, 1948)]. Specialized photosensitive ganglion cells in the eye retina forward the signal *via* a neural pathway to the SCN located directly above the optic chiasm in the hypothalamus. From the SCN the signal is conveyed to the pineal gland, which produces the hormone melatonin (Moore, 1995) (Fig. 2.4). Melatonin is produced exclusively at night at a rate inversely proportional to day length. The circadian and circannual variation in melatonin signal duration is then used as a critical input signal for the pituitary gland and its pars tuberalis (PT) that regulate seasonal prolactin secretion (Lincoln *et al.*, 2006). Prolactin is mainly associated with reproduction and lactation, but also influences hair growth (Foitzik, Langan & Paus, 2009; Paus *et al.*, 2014).

Melatonin and prolactin

Melatonin acts *via* inhibition of prolactin production and this hormonal interaction serves as the main humoral signal controlling the phenology of many seasonal events including reproduction, migration and moult (Lincoln *et al.*, 2006; Paul *et al.*, 2008) (Table 2.2, Fig. 2.4). In SCC species, prolactin plays an important role in regulating moults, but the exact mechanisms remain unclear. Increased prolactin concentrations are associated with initiation of reproduction and spring moult [collared lemming (Gower *et al.*, 1993), stoat (Rust, 1965), snowshoe hare (Lyman, 1943)]. Administration of prolactin can induce or artificially maintain pigmentation of the fur in Siberian hamsters despite changing day lengths (Duncan & Goldman, 1984, 1985). On the contrary, artificially suppressed prolactin secretion induces moult to the white winter pelage [Siberian hamster (Badura & Goldman, 1992; Niklowitz, Hoffmann & Klaus, 1988), collared lemming (Gower *et al.*, 1993), stoat (Feder, 1990; Rust & Meyer, 1969), Arctic fox (Nieminen *et al.*, 2004)], delays spring moult [Arctic fox (Smith *et al.*, 1987)] or results in the growth and maintenance of white coat regardless of photoperiod [stoat (Rust, 1965)].

Despite the overwhelming evidence that melatonin affects moult phenology indirectly *via* changing prolactin levels, the hormone may also directly affect hair pigmentation. Melatonin inhibits enzymes in the tyrosine to melanin production pathway or directly affects melanin by local production in the skin, leading to white colouration (Logan & Weatherhead, 1978; Slominski *et al.*, 2002). However, the direct effects of melatonin on hair melanization are not understood.

Other hormones

SCC moults are chiefly regulated by the interactions between melatonin and prolactin, but other circulating hormones play a role (Table 2.2). α-MSH, released by the pituitary, signals MC1R leading to the production of dark eumelanin, while

ASIP is an antagonist that leads to the production of lighter phaeomelanin (see Section IV.1). Whether and how the production of ASIP is regulated throughout the year in SCC species remains unknown. Additional endocrines involved with melanization and hair regeneration include other hormones of the hypothalamic–pituitary–gonadal (HPG) axis (most notably gonadal steroids), thyroid hormones, and glucocorticoids as described below.

Gonadal steroids

Gonadal steroids, chiefly testosterone and oestradiol have long been known to affect reproduction as well as hair growth (Johnson, 1958*a*; Wright, 1942), but are only secondary drivers of SCC moults. They affect hair growth cycle and pigmentation largely *via* stimulation of circulating prolactin levels. In experiments comparing groups with different levels of gonadal steroids, animals with lower levels moulted more rapidly and more completely in the autumn [collared lemming (Gower *et al.*, 1993; Gower, Nagy & Stetson, 1994), Siberian hamster (Duncan & Goldman, 1984)], and exhibited heavier or earlier spring moults [snowshoe hare (Lyman, 1943), least weasel (King, 1979)].

Thyroid hormones and glucocorticoids

Hormones of the thyroid gland or adrenal cortex are known to be involved in hair regeneration (Maurel, Coutant & Boissin, 1987; Paul *et al.*, 2007), but controlled experiments failed to find effects on SCC moults [snowshoe hare (Lyman, 1943), stoat (Rust, 1965), Siberian hamster (Barrett *et al.*, 2007; Scherbarth & Steinlechner, 2010)]. Hormones of the adrenal cortex, mainly glucocorticoids, inhibit moulting by suppressing the anagen hair growth stage, whereas adrenalectomy accelerates the growing process (Johnson, 1958*b*; Rose & Sterner, 1992). However, no effect of cortisol on the induction of moult or pigmentation was found in hypophysectomized weasels (Rust, 1965).

Neuroendocrine and endocrine regulation in birds

In birds as in mammals, seasonal moults are stimulated by photoperiod (Dawson *et al.*, 2001; Höst, 1942), but the photoperiodic control in birds is less clear, partially because they have at least three independent pacemakers (Gwinner, 2003; Kumar, Singh & Rani, 2004). In fact, light cues are not only sent and perceived by the melatonin-producing pineal gland (Cassone *et al.*, 2009), but also through photoreceptors located in the mediobasal hypothalamus and in the eyes (Ikegami & Yoshimura, 2012). Because of these two extra pacemakers/receptors, birds do not rely as critically on the pineal gland (and melatonin) for the photoperiodic response. Moult and reproduction are timed such that moult starts immediately after breeding; if breeding activity continues beyond the photoperiodic timing of moult, the start of moult is delayed, but then occurs at a faster rate (Dawson, 1998).

Although avian mechanisms of seasonal regulation are not fully understood, it is clear that moults are largely controlled by hormones released from the pituitary and thyroid glands (Chen *et al.*, 2015; Payne *et al.*, 1972) (Table 2.2). Administration of pituitary hormones, including α-MSH, follicle stimulating hormone (FSH), and luteinizing hormone (LH), can induce unseasonal feather pigmentation in winter in willow and white-tailed ptarmigan (Höhn & Braun, 1980). Additionally, different studies showed that captive willow ptarmigan, kept at low winter temperatures, can be induced to moult prematurely into pigmented breeding or summer plumage (and gonadal activity) by being artificially exposed to a long-day photoperiod (Höst, 1942; Novikov & Blagodatskia, 1948; Stokkan, 1979*b*).

Prolactin

Hormones of the HPG axis are strongly involved with moult regulation. In general, moult initiation is associated with high prolactin levels and moults can be experimentally induced by prolactin administration (Dawson, 2006; Kuenzel, 2003). Further, prolactin is likely involved in pigmentation as well; Höhn & Braun (1980) demonstrated that several hormones of the pituitary, mainly prolactin and LH, were involved in feather pigmentation in white-tailed ptarmigan.

Gonadal steroids

In most birds, moulting starts after the end of reproduction and the inhibitory effects of gonadal steroids on moult contribute to this separation in time (Payne *et al.*, 1972). Experimental studies have shown that implants of testosterone can delay, prevent or interrupt moults (Dawson, 1994; Nolan *et al.*, 1992). Such a mechanism would delay the start of moult until the time of gonadal regression; for example, in male rock ptarmigan which moults to brown summer plumage after breeding, testicular hormones likely suppress initiation of the moult (MacDonald, 1970; Salomonsen, 1950). The influence of gonadal steroids on moult and pigmentation is unclear in willow ptarmigan, but likely differs due to the overlap of moulting and breeding activity (Höst, 1942); some studies reported spring moult advancement in response to increased testosterone (Hannon & Wingfield, 1990; Stokkan, 1979*a*,*b*), but others found no effect on moulting between castrated and intact birds (Braun & Höhn, 1977; Höhn & Braun, 1980).

Thyroid hormones and glucocorticoids

Thyroid hormones and hormones of the adrenal cortex (i.e. glucocorticoids) appear to be important regulators of avian moult phenology, more so than in mammals. Similarly to gonadal steroids, glucocorticoids are elevated during the breeding season in birds and decrease during moulting (Romero, 2002). By contrast, thyroid hormones can stimulate moults (Kuenzel, 2003) and removal of the thyroid gland prevents them (Yoshimura, 2013); however, this may not reflect a direct effect of thyroid hormones since they influence prolactin and corticosterone secretion (Goldsmith & Nicholls, 1984). For SCC birds, similar effects of thyroid hormones have been described; increased thyroid concentration was associated with all three moults in white-tailed ptarmigan (Höhn & Braun, 1977). However, whether thyroid affects feather regeneration alone or also pigmentation is unclear; while Höhn & Braun (1980) found no effect of thyroid hormones on pigmentation in willow and white-tailed ptarmigan, Novikov & Blagodatskia (1948) reported that excessive administration of thyroid powder induced feather pigmentation in willow ptarmigan.

OTHER FACTORS INFLUENCING SCC MOULTS

Temperature and snow cover

Exogenous cues, particularly temperature and snow cover, have modifying effects on the rate and sometimes completeness of SCC moults (Table 2.3). As previously described, photoperiod regulates the onset of moults; without shifts in photoperiod, temperature alone cannot induce moulting in captive animals [snowshoe hare (Lyman, 1943), weasels (Bissonnette & Bailey, 1944; Feder, 1990; Rust, 1962)]. Similarly, wild populations show nearly identical date of moult onset each year [mountain hare (Jackes & Watson, 1975; Watson, 1963), snowshoe hare (Zimova *et al.*, 2014; but see Kumar, 2015)], confirming the overwhelming effect of photoperiod. While temperature and probably snow appear to modify the rate of moults, the physiological connection between those exogenous cues and most seasonal traits including SCC moults has not been established (Caro *et al.*, 2013).

Once the white moult has been initiated in the autumn, cold temperatures can accelerate growth of the white coat in both mammals [mountain hare (Jackes & Watson, 1975; Watson, 1963), stoat (Rothschild, 1942), Siberian hamster (Larkin, Freeman & Zucker, 2001), collared lemming (Degerbøl & Møhl-Hansen, *et al.*, 1943)], and birds [rock ptarmigan (Salomonsen, 1939; Watson, 1973)]. The modifying effect of temperature is strong in weasels; captive stoats kept under cold temperatures completed the brown to white moult in three days while animals exposed to warm temperatures took 10–20 days (Rothschild, 1942). Furthermore, in ptarmigan, temperature can also affect the completeness of the autumn moult; individual captive rock ptarmigan grew more dark-pigmented winter feathers when exposed to warm winter temperature (Hewson, 1973) or when translocated to a warmer locality (Salomonsen, 1939).

In the spring, warm temperatures can accelerate the white to brown moults [snowshoe hare (Kumar, 2015; Zimova *et al.*, 2014), stoat (Rothschild, 1942)]. Rust (1962) found that stoats exposed to warm temperatures in the spring changed their entire dorsum in less than two days and moulting was more diffuse, with an undefined line of new brown fur, than in animals exposed to cold temperatures. The effects of snow are uncertain due to challenges in separating its effects from temperature. However, multiple field studies indicate some influence of snow cover on moult rate. Based on both population means and individuals observed over multiple years, animals exhibited a slower rate of moult during colder and snowier springs [mountain hare (Clinging, 1982; Flux, 1970; Watson, 1963), snowshoe hare (Kumar, 2015; Zimova *et al.*, 2014), rock ptarmigan (Watson, 1973)]. In another study, Jackes & Watson (1975) found that the brown to white moult was related initially to air temperature, particularly days with a minimum of 0°C or less, and later in the season to snow cover. However, no phenotypic plasticity in response to cooler temperature or more snow cover was observed in the autumn moult in mountain (Flux, 1970) and snowshoe hares (Zimova *et al.*, 2014). In Arctic foxes, the evidence for plasticity is mixed; minimal plasticity in response to temperature or snow has been described in both moults (Hersteinsson, 1989) (but see Moberg, 2017).

Intrinsic factors

All SCC species exhibit large intra-population variation in moult phenology. Some of this variation can be explained by gender, age and to a lesser degree condition and social rank (Table 2.3), all of which are correlated with underlying hormonal levels.

Sex

Gender-specific differences in phenology have been described for multiple species in both moults. Inter-sexual differences in moult phenology in the spring are likely related to varying levels of gonadal hormones, which spike at different times in each sex during reproduction. In most mammal species, females initiated the spring moults earlier than males [mountain hare (Bergengren, 1969; Flux, 1970; Hewson, 1973), snowshoe hare (Aldous, 1937; Severaid, 1945; Zimova *et al.*, 2014), collared lemming (Degerbøl & Møhl-Hansen, 1943), Arctic hare (Banfield, 1974), stoat (Van Soest & Van Bree, 1969), Arctic fox (Moberg, 2017)], but no sex differences were observed in least weasels (King, 1979) and Siberian hamsters (Butler & Zucker, 2009). In ptarmigan, the breeding plumage and consequently effect of sex on phenology differs among species. Male willow and white-tailed ptarmigan initiate the pigmented moult earlier than females (Braun *et al.*, 1993; Stokkan, 1979*b*; Watson & Moss, 2008). Female rock ptarmigan, however, exhibited more complete summer plumage with less winter white retained on the underparts and no white feathers on the back (Watson & Moss, 2008). Male rock ptarmigan in Scotland begin the moult first but finish at about the same time as females (Hewson, 1973; Watson, 1973), but in subarctic and arctic regions remain white much longer than females (Jacobsen *et al.*, 1983; Montgomerie *et al.*, 2001; Salomonsen, 1939).

Differential moult phenology between the sexes has been described in the autumn as well, although physiological mechanisms including links to changes in gonadal steroid levels are less clear. Hewson & Watson (1979) suggested that the smaller sex, presumably more susceptible to cold (e.g. females in stoat and ptarmigan; males in hares) initiates the autumn moult earlier. Accordingly, male mountain hares (Bergengren, 1969; Flux, 1970), Arctic hares (Manning, 1943) and Siberian hamsters (Butler & Zucker, 2009) initiated the moult earlier than females. By contrast, female snowshoe hares (Aldous, 1937; Severaid, 1945) and stoats (Hewson & Watson, 1979) initiated the autumn moult before males. Furthermore, in stoats, a higher proportion of the brown or piebald winter phenotype are females (Flintoff, 1935; Hall, 1951; Hamilton, 1933; Hewson & Watson, 1979; King & Moody, 1982). Also, female mountain hares appeared more patchy than males during spring and autumn moults (Flux, 1970). In rock ptarmigan, Salomonsen (1939) showed that both sexes completed the moult at the same time across their range, but some exceptions were recorded in milder climates; e.g. in Scotland and Amchitka Island, females completed moulting first but males achieved whiter plumage (Hewson, 1973; Jacobsen *et al.*, 1983; Watson, 1973). White-tailed ptarmigan males initiated moult before females (Braun *et al.*, 1993).

Age

Age may also affect moult phenology and the completeness of the moult, especially in the autumn. In some species younger animals tend to exhibit delayed phenology [mountain hare (Watson, 1963), snowshoe hare (Grange, 1932;

Severaid, 1945), Arctic fox (Chesemore, 1970), ptarmigan spp. (Hewson, 1973), willow ptarmigan (Stokkan & Steen, 1980), white-tailed ptarmigan (Braun *et al.*, 1993)] or acquire a greyish hue in winter [mountain hare (Flux, 1970; Watson, 1963)]. Further, later birth date correlates with later moult in the following autumn; juvenile mountain hares born in early summer moulted to white earlier than juveniles born later (Bergengren, 1969).

By contrast, juvenile collared lemmings initiated autumn moults before adults (Degerbøl & Møhl-Hansen, 1943) and in captive-bred blue foxes older cubs initiated the autumn moult earlier than younger cubs, but finished at the same time (Blomstedt, 1998). The effect of age is unclear in Siberian hamsters, with some evidence for juveniles skipping autumn moults and some for no effect of age (Butler & Zucker, 2009). In mustelids, juveniles vary much more widely in moult pattern than older individuals but phenology is not considerably different (Hamilton, 1933; King, 1979).

Body mass and condition

Condition is a function of factors such as nutrition, disease, and stress, and so might logically belong in the exogenous factors section above. However, the effects of condition are similar to the effects of age and may explain some of its effects (Flux, 1970). In general, lighter, injured or sick animals as well as females that reproduced late show delayed autumn moults compared to healthier individuals in mountain hares (Bergengren, 1969; Hewson, 1958). In the spring, no effect of body condition (e.g. body mass, fat ratio) on moult phenology was found in mountain hares except for very sick individuals that show delayed moult

(Flux, 1970). Notably, more-aggressive mountain hare males moulted earlier than less-aggressive, lighter males (Verkaik & Hewson, 1985), perhaps due to differences in testosterone level. Lastly, evidence from non-SCC species connects diet to melanin production (McGraw, 2003), but more research is required.

ADAPTIVE POTENTIAL TO CAMOUFLAGE MISMATCH

Research investigating the ecology and underlying basis of SCC moults has declined since the 1980s and many aspects of SCC moulting remain unresolved. Recently, the direct connection between winter white coats and anthropogenic climate change has led to renewed interest in SCC species (Imperio *et al.*, 2013; Mills *et al.*, 2013; Pedersen, Odden & Pedersen, 2017; Sultaire *et al.*, 2016; Zimova *et al.*, 2016). Both snow cover duration and extent are projected to decrease dramatically across most of the northern hemisphere, especially during spring and autumn (Danco *et al.*, 2016; Musselman *et al.*, 2017; Ning & Bradley, 2015), driving increased camouflage mismatch and population declines in the absence of an adaptive response (Zimova *et al.*, 2016). Below, we synthesize the current understanding of adaptive potential to camouflage mismatch *via* phenotypic plasticity and evolution.

Phenotypic plasticity

Despite a strong influence of photoperiod on moult phenology, SCC species show some ability to adjust moult phenology in response to temperature and snow conditions and reduce camouflage mismatch [e.g. snowshoe hare (Zimova *et al.*, 2014)]. Furthermore, ptarmigan may also adjust the presence and amount of dark pigmented feathers in their winter plumage [e.g. rock ptarmigan (Hewson, 1973)].

Overall, however, little is known about the extent or mechanisms through which abiotic factors or behaviours may mediate moult phenology or winter coat colour. Further, we do not understand to what degree plasticity is limited by correlations with other life-history traits that share the same basic physiological pathway [e.g. reproduction (Paul *et al.*, 2008)], and how those correlations affect potential adaptation *via* plasticity. Carefully designed physiological studies in the future may resolve the neuroendocrine and endocrine mechanisms that regulate SCC moults and are critical for predicting future adaptive responses.

Evolutionary shifts

Strong selection connected with camouflage mismatch [recently quantified in snowshoe hares (Zimova *et al.*, 2016)] combined with variation in SCC moulting suggests adaptive capacity *via* evolutionary shifts in SCC moult phenology or in the resulting winter colour. First, past adaptive evolution *via* moult phenology is evident across SCC species; for example, snowshoe hare (Grange, 1932; Zimova *et al.*, 2014), mountain hare (Couturier, 1955; Watson, 1963), Arctic fox (Lavrov, 1932), weasels (Feder, 1990; Hamilton, 1933; King & Powell, 2007), and ptarmigan (Cramp & Simmons, 1980; Salomonsen, 1939) exhibit range-wide inter-population variation in moult phenology correlated with altitude, winter onset and winter duration as opposed to latitude. Further, intra-population variation is equally common; during spring or autumn moults a full range of moult stages (ranging from brown to white) can be observed on the same day within a population [e.g. mountain hare (Bergengren, 1969; Clinging, 1982), Arctic fox (Underwood & Reynolds, 1980), rock ptarmigan (Salomonsen, 1939;

Watson, 1973)]. The molecular basis of variation in moult phenology remains unknown, but the extensive genetic machinery is amenable to genomic and transcriptomic approaches across taxa.

Microevolution in winter colour phenotype (instead of or in combination with microevolution in moult phenology) is also possible in response to climate change. Local variation in winter coat colour is tightly linked with local climate and especially snow duration across all SCC species (Mills *et al.*, in press). Furthermore, some populations are polymorphic in their winter colour phenotype (Mills *et al.*, in press) (Table 2.1) and rapid evolutionary shifts may have potentially occurred, for example in mountain hares in the Faroe Islands (Degerbol, 1940). The strong underlying molecular basis of winter colour phenotype is evident from latitudinal translocations, common garden experiments [e.g. stoat (Feder, 1990)] and a several genetic studies [e.g. Arctic fox (Våge *et al.*, 2005)]. Because adaptation will be critical to allow persistence under climate change due to limited plasticity, we need to understand the genetic basis in all SCC species, including correlations with phenology of other circannual traits.

CONCLUSIONS

(1) Although SCC moults have a dual function of increased crypsis and thermoregulation, camouflage against snow in the winter appears to be more significant. First, strong natural selection for cryptic colouration has been demonstrated across taxa, including some SCC species. Second, all SCC species show clinal distributions of winter coat colour phenotypes across snow gradients, including the loss of winter white in moderate climates. Third, some species (i.e.

ptarmigan) exhibit behavioural plasticity to maintain camouflage when mismatched against their background. Lastly, white winter coats of some SCC species are not warmer (i.e. weasels, ptarmigan), confirming the primary benefits of increased camouflage.

(2) SCC hair/feather regeneration is similar across species, with some differences in the details of moulting and moulting patterns. The underlying hair growth and moult processes are complex and relatively unknown. Some SCC mammals (e.g. weasels, hares) conform to the standard view of seasonal moulting: short periods of photoperiod-stimulated hair proliferation and shedding, alternating with longer periods of hair follicle inactivity. Others (i.e. Siberian hamsters) undergo endogenously regulated continuous moulting with periodical changes in hair structure and pigmentation. In ptarmigan, SCC moulting varies among species and the moult patterns and processes are less understood than in mammals.

(3) The molecular basis of SCC moults remains mostly unknown. To date, research has focused on the genetic basis of invariant winter brown morphs, and simple genetic differences have been described for the different winter colour morphs in SCC mammals (e.g. Arctic fox, mountain hare). Major candidate colouration genes (i.e. *Mc1r* and *Agouti*) are likely involved in SCC moults in both mammals and birds, but other genes such as those regulating hair regeneration and melanization, and seasonal rhythms (e.g. ‘clock’ genes) likely play important roles too.

(4) The endocrine basis of SCC moults is not fully understood in any taxa. Generally, moults are stimulated by photoperiod, triggering a network of neural

and hormonal pathways that initiate moulting. The hormonal signals remain important throughout the moult duration; for example, moulting can be arrested or reversed by photoperiod manipulation alone. Mammals have one central clock entrained to photoperiod and the physiological basis of moults is largely driven by the interaction of melatonin and prolactin. In birds, multiple pacemakers direct prolactin, thyroid and gonadal hormones. In both groups, the same hormonal pathways regulating moults and pigmentation also drive other seasonal events such as reproduction. Therefore, any shifts in moult phenology may have strong epistatic effects on the other circannual traits and impact fitness.

(5) Several external and internal factors can modify moult phenology in all SCC species. Temperature and potentially snow cover can accelerate or decelerate moult phenology, although the evidence for snow effects is less clear. The physiological mechanisms by which those two environmental factors modify moults are unknown, considerably obstructing our understanding of their role on moult phenology. Similarly, several intrinsic factors contribute to inter-individual variation in phenology, probably interacting with gonadal steroids. These intrinsic factors include sex, age and to lesser degree mass and condition.

(6) Current climate change leads to increased colour mismatch between SCC and background colour. Adaptation could occur either in the polyphenic SCC trait (*via* shifts in moult phenology) or *via* complete loss of winter whitening. Adaptation *via* phenotypic plasticity could occur, but to date its capacity appears limited, especially in mammals. Adaptive shifts via microevolution in moult phenology or winter colour morph are possible due to high variation in the SCC trait and strong

selection for background matching. A detailed understanding of the ecological, physiological, and molecular bases of SCC moult will be needed to manage populations effectively under climate change.

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Table 2.1. Species exhibiting seasonal coat colour (SCC) moult, their trophic level, references describing intra-specific winter colour polymorphisms, and their geographical distribution. Taxonomy follows the IUCN Red List.

Common name	Latin name	Trophic level	Winter colour polymorphism	Distribution
Leporidae				
Alaskan hare	*Lepus othus*	Prey		W and SW Alaska
Arctic hare	*Lepus arcticus*	Prey		N Canada, Canadian Arctic islands, Greenland
Japanese hare	*Lepus brachyurus*	Prey	Nunome *et al.* (2014)	Japan (except Hokkaido)
Mountain hare	*Lepus timidus*	Prey	Bergengren (1969); Jackes & Watson (1975); Flux & Angermann 1990); Thulin (2003); Watson (1963)	Palearctic; N Eurasia, isolated mountain ranges and some islands
Snowshoe hare	*Lepus americanus*	Prey	Dalquest (1942); Nagorsen (1983)	Canada, N and mountainous USA
White-tailed jackrabbit	*Lepus townsendii*	Prey	Hansen & Bear (1963)	W and central North America
Muridae				
Collared lemming	*Dicrostonyx groenlandicus*	Prey		N Canada, N Alaska, N and NE Greenland, some Arctic islands
Nelson's collared lemming	*Dicrostonyx nelsoni*	Prey		W and SW Alaska
Ogilvie mountains collared lemming	*Dicrostonyx nunatakensis*	Prey		NC Yukon Territory, Canada
Palearctic collared lemming	*Dicrostonyx torquatus*	Prey		N Russia and some Arctic islands
Richardson's collared lemming	*Dicrostonyx richardsoni*	Prey		NC Canada

Siberian hamster	*Phodopus sungorus*	Prey		SW Siberia, E Kazakhstan
Ungava collared lemming	*Dicrostonyx hudsonius*	Prey		N Quebec and Labrador, Canada
Wrangel Island collared lemming	*Dicrostonyx vinogradovi*	Prey		Wrangel Island, Russia
Mustelidae				
Least weasel	*Mustela nivalis*	Prey, predator	Easterla (1970); King (1979); Stolt (1979); Abramov & Baryshnikov (2000); Zima & Cenevova (2002)	Europe, N Africa, N Asia, N North America, introduced to New Zealand and other islands
Long-tailed weasel	*Mustela frenata*	Prey, predator	Hamilton (1933); Hall (1951)	North America from S Canada to N South America
Stoat	*Mustela erminea*	Prey, predator	Hamilton (1933); Rothschild (1942); Hall (1951); Gaiduk (1977); Hewson & Watson (1979); Van Soest & Van Bree (1969); King & Moody (1982); Feder (1990)	Europe, Asia, North America, Greenland and S Arctic islands, introduced to New Zealand and other islands
Canidae				
Arctic fox	*Vulpes lagopus*	Prey, predator	Braestrup (1941); Hersteinsson (1989)	Arctic North America and Asia, Arctic Islands, Greenland
Tetraonidae				
Rock ptarmigan	*Lagopus muta*	Prey	Cramp & Simmons (1980); Hewson (1973); Jacobsen, White & Emison (1983); Salomonsen (1939)	Arctic and mountainous Eurasia, Canada, Alaska, Greenland, Arctic Islands
White-tailed ptarmigan	*Lagopus leucurus*	Prey		Mountains in S Alaska, W Canada, and W USA
Willow ptarmigan	*Lagopus lagopus*	Prey	Cramp & Simmons (1980); Thom (1986)	Eurasia, North America, some S Arctic islands

Table 2.2. Effect of hormones on spring and autumn moult phenology in seasonal coat colour (SCC) species.

Hormone	Species	Effect on spring moult	Effect on autumn moult	References
Cortisol	Stoat	No effect	–	Rust (1965)
Prolactin	Arctic fox	Strong association	Suppression	Nieminen *et al.* (2004); Smith *et al.* (1987)
	Collared lemming	Strong association	Suppression	Gower, Nagy & Stetson (1993)
	Stoat	Strong association	Suppression	Rust (1965); Rust & Meyer (1969); Feder (1990)
	Siberian hamster	Pigmentation stimulation, maintenance	Suppression	Badura & Goldman (1992); Duncan & Goldman (1984); Duncan & Goldman (1985); Niklowitz, Hoffmann & Klaus (1988)
	Snowshoe hare	Strong association	–	Lyman (1943)
	White-tailed ptarmigan	Strong association	–	Höhn & Braun (1980)
	Willow ptarmigan	Strong association	–	Höhn & Braun (1980)
Sex steroids	Collared lemming	–	Suppression (testosterone)	Gower, Nagy & Stetson (1993); 1994)
	Least weasel	Suppression (oestrogen)	–	King (1979)
	Rock ptarmigan	Suppression (testosterone)	–	Salomonsen (1950); MacDonald (1970)
	Siberian hamster		Suppression (testosterone)	Duncan & Goldman (1984)
	Snowshoe hare	Lighter moult (testosterone)	No effect	Lyman (1943)

	Willow ptarmigan	Stimulation (testosterone)	No effect	Stokkan (1979*a*); Braun & Höhn (1977)
Thyroid	Stoat	No effect	No effect	Rust (1962)
	Snowshoe hare	No effect	No effect	Lyman (1943)
	White-tailed ptarmigan	No effect or stimulation	Stimulation	Höhn & Braun (1980); Braun & Höhn (1977)
	Willow ptarmigan	No effect or stimulation, pigmentation	–	Novikov & Blagodatskia (1948); Höhn & Braun (1980)

Table 2.3. Effect of extrinsic and intrinsic factors on spring and fall moult phenology in seasonal coat colour (SCC) species.

Factor	Species	Effect on spring moult	Effect on fall moult	References
Temperature	Arctic fox	No effect	No effect	Hersteinsson (1989)
	Collared lemming	–	Cold accelerated	Degerbøl & Møhl-Hansen (1943)
	Mountain hare	Cold slowed down	Cold and snow accelerated, or no effect	Flux (1970); Jackes & Watson (1975); Watson (1963); Clinging (1982)
	Rock ptarmigan	Cold and snow slowed down	Cold accelerated, and whiter winter plumage	Hewson (1973); Watson (1973); Salomonsen (1939)
	Stoat	Cold slowed down	–	Rust (1962)
	Siberian hamster	–	Cold accelerated	Larkin, Freeman & Zucker (2001)
	Snowshoe hare	Cold and snow slowed down	No effect	Zimova *et al.* (2014); Kumar (2015)
Sex	Arctic hare	Females earlier	Males earlier	Manning (1943); Banfield (1974)
	Collared lemming	Females earlier	–	Degerbøl & Møhl-Hansen (1943)
	Least weasel	No effect	–	King (1979)
	Mountain hare	Females earlier	Males earlier	Flux (1970); Bergengren (1969); Hewson & Watson (1979)
	Rock ptarmigan	Males earlier, females earlier, or no effect	Females earlier, males whiter, or no effect	Hewson (1973); Watson (1973); Jacobsen, White & Emison (1983); Montgomerie, Lyon & Holden (2001); Salomonsen (1939)
	Stoat	Females earlier	Females earlier	Hewson & Watson (1979); Van Soest & Van Bree (1969)

	Siberian hamster	No effect	Males earlier	Butler & Zucker (2009)
	Snowshoe hare	Females earlier	Females earlier or no effect	Zimova *et al.* (2014); Mills *et al.* (2013); Aldous (1937); Severaid (1945)
	White-tailed ptarmigan	Males earlier	Males earlier	Braun *et al.* (1993)
	Willow ptarmigan	–	Males earlier	Stokkan (1979*b*)
Age	Arctic fox	–	Juveniles later	Chesemore (1970); Blomstedt (1998)
	Collared lemming	–	Juveniles earlier	Degerbøl & Møhl-Hansen (1943)
	Least weasel	–	No effect, or late-born juveniles later	King (1979)
	Mountain hare	–	Juveniles later and darker winter fur	Flux (1970); Watson (1963) Bergengren (1969); Hewson & Watson (1979)
	Rock ptarmigan	–	Juveniles later	Hewson (1973)
	Siberian hamster	No effect	No effect	Butler & Zucker (2009)
	Snowshoe hare	–	Juveniles later	Severaid (1945); Grange (1932)
	White-tailed ptarmigan	–	Juveniles later	Braun *et al.* (1993)
Condition	Mountain hare	Less aggressive and sick later, or no effect	Sick or injured later	Flux (1970); Hewson (1958)

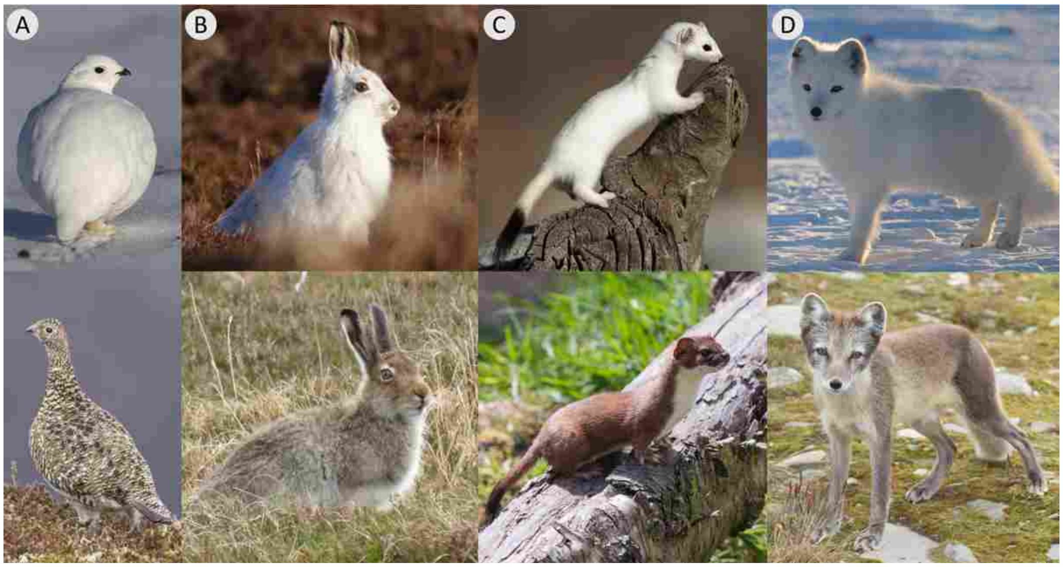

Figure 2.1. Seasonal coat colour (SCC) species in their winter (top row) and summer (bottom row) coats. (A) Rock ptarmigan; (B) mountain hare; (C) stoat; (D) Arctic fox. Photos by stock.adobe.com: Pilipenko D, Paul Carpenter, Stephan Morris, Diego Cottino; Mills lab research photo, and Seoyun Choi.

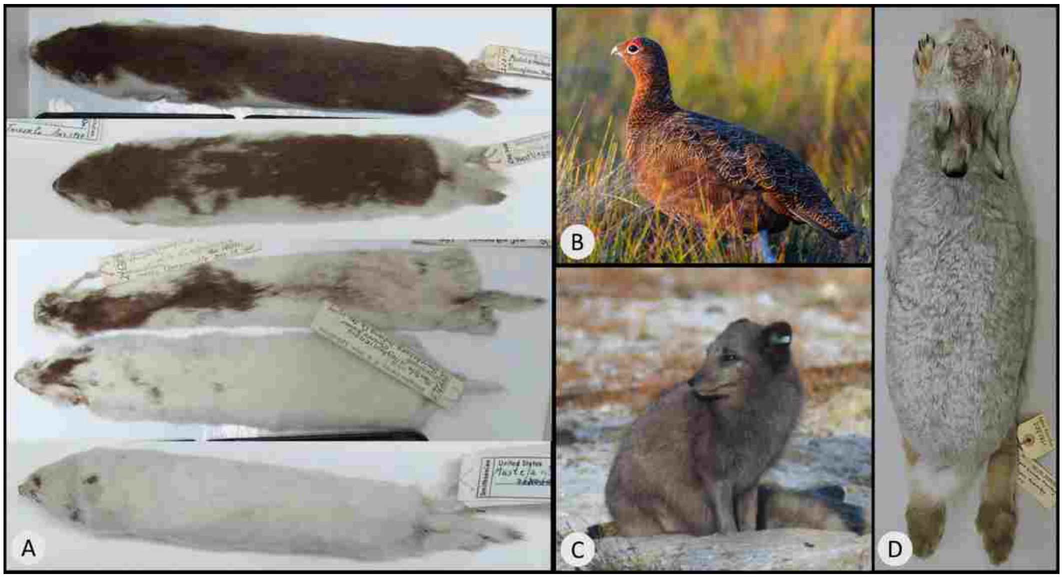

Figure 2.2. Different winter colour morphs. (A) Different winter colour morphs of least weasels, eastern United States, (B) red grouse, UK, (C) blue morph of Arctic fox, Norway, (D) blue morph of mountain hare, Sweden. Access to specimens provided by National Museum of Natural History, Washington DC, and Swedish Museum of Natural History, Stockholm. Photos by Mark Medcalf/stock.adobe.com, Seoyun Choi, and Mills lab research photo.

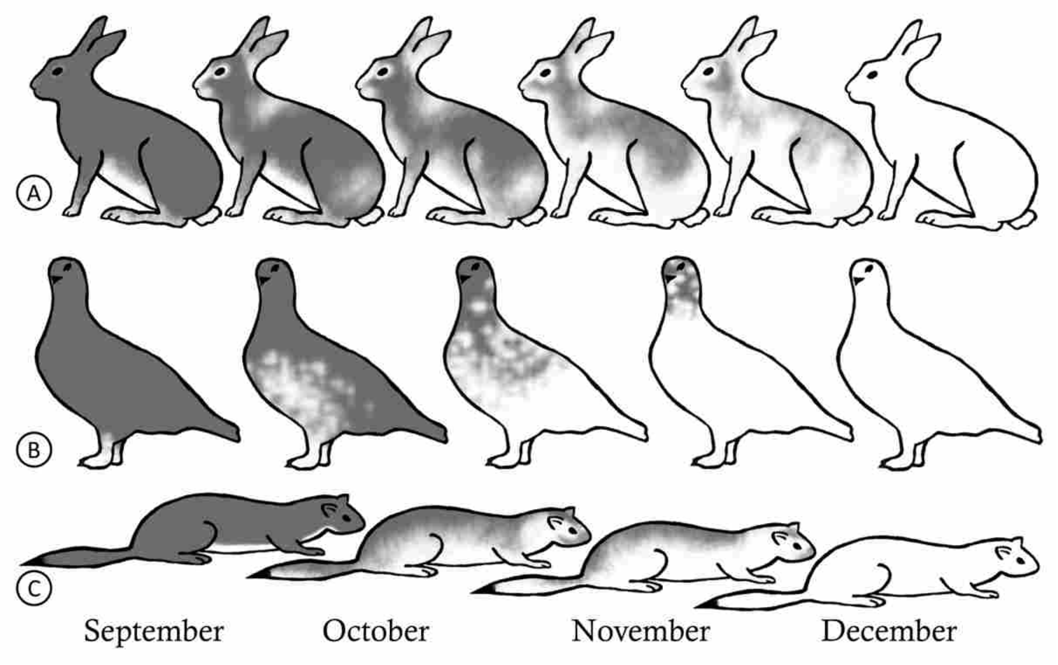

Figure 2.3. Typical autumn moult progression in (A) mountain hare, (B) willow ptarmigan, and (C) stoat.

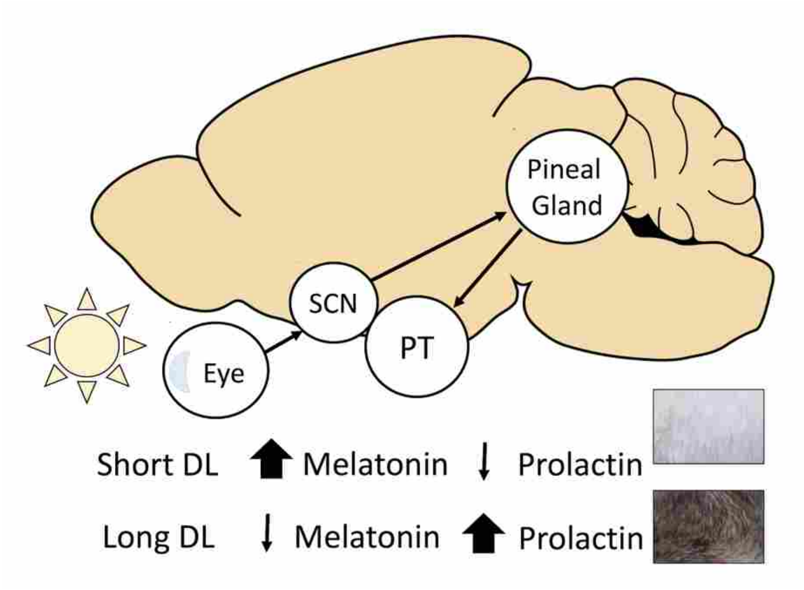

Figure 2.4. Main hormonal pathway regulating seasonal coat colour (SCC) moults in mammals. The photoperiodic signal enters the brain *via* the eye and is forwarded to the suprachiasmatic nucleus (SCN) and endocrine glands. Short day length (DL) yields high production of pineal melatonin leading to low secretion of prolactin by the pars tuberalis (PT) of the pituitary gland, resulting in white winter fur. The opposite is true for the production of brown summer fur.

CHAPTER 3

STATIC MOLT PHENOLOGY AND INCREASED CAMOUFLAGE MISMATCH IN MOUNTAIN HARES

Marketa Zimova[a], Sean T. Giery[b], Scott Newey[c], J. Joshua Nowak[a], Michael Spencer[d], L. Scott Mills[e]

[a] Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA.

[b] Department of Biology, The Pennsylvania State University, University Park, PA, 16801, USA.

[c] The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK.

[d] Scotland's Rural College, King's Buildings, Edinburgh, EH9 3JG, Scotland.

[e] Wildlife Biology Program and Office of Research and Creative Scholarship, University of Montana, Missoula, MT 59812, USA

ABSTRACT

Understanding whether organisms will be able to adapt to the myriad of human-induced stressors currently endangering their existence is an urgent priority. Multiple species distributed across the northern hemisphere seasonally moult from brown summer coat to white winter coat to maintain camouflage against snowy landscapes. But snow cover duration is decreasing due to climate change resulting in camouflage mismatch. White animals mismatched against dark snowless background suffer higher predation rates and adaptive responses will be critical for species persistence in the altered environments. We took advantage of a historical dataset to test for adaptive responses to climate change in a widespread, colour moulting mammal, the mountain hare (*Lepus timidus*). We repeated historical (1950s) field studies of moult phenology in Scotland and tested whether population moult phenology shifted to align seasonal coat colour with shorter snow seasons. We found that population moult phenology did not change despite the snow season being shortened by 37 days, nor did phenotypic plasticity buffer against increased camouflage mismatch. The lack of responses resulted in 35 additional days of mismatch between 1950 and 2016, whereby white hares confronted snowless backgrounds. Multiple factors might have contributed to the static moult phenology, but the unexpectedly low local selection pressure against camouflage mismatch is likely the leading cause. We suggest that future declines in snow cover, combined with potential changes in biotic interactions, make mountain hares in Scotland highly vulnerable to future alterations in selection pressures. More generally, our findings highlight the important role of both biotic and abiotic conditions in shaping scope for adaptive responses to anthropogenic stressors.

INTRODUCTION

Recent climate change has already subjected wild populations to large changes in environmental conditions (IPCC 2013). Failure of populations to sufficiently track these changes will result in local declines and extinctions (Thomas et al. 2004, Bellard et al. 2012, Scheffers et al. 2016). Some populations have responded adaptively via phenotypic plasticity and or evolution (Buckley et al. 2012, Kovach et al. 2012, van Asch et al. 2013, Phillips et al. 2016). However, others have failed to track climate change or seem to have responded in non-adaptive ways (Both et al. 2010, Ozgul et al. 2010, Todd et al. 2011, Lane et al. 2012, Plard et al. 2014, Mayor et al. 2017, Evans et al. 2018). Predicting population responses to climate change remains challenging, in part because many interacting factors determine future trajectories of inherently complicated natural systems

(Chevin et al. 2010, Giery et al. 2015, Senner et al. 2017a, Nosil et al. 2018). Yet understanding whether and how populations will respond to climate change is one of the most urgent challenges facing biologists (Dawson et al. 2011, Moritz and Agudo 2013, Smith et al. 2014, Urban et al. 2016).

In response to climate change snow cover duration is decreasing in many parts of the northern hemisphere (Vaughan et al. 2013, Kunkel et al. 2016), and consequently imposing changing and potentially novel selection pressures on organisms adapted to seasonally changing environments (Pauli et al. 2013, Williams et al. 2015, Varpe 2017, Berger et al. 2018). A diverse group of birds and mammals moult from summer brown to winter white coat annually to increase crypsis against snow (Beltran et al. 2018, Zimova et al. 2018). As snow duration declines due to climate change, colour moulting species become increasingly mismatched with their background (Mills et al. 2013). Research on snowshoe hares (*Lepus americanus*) and least weasels (*Mustela nivalis*) shows that mismatched individuals suffer higher predation rates (Zimova et al. 2016, Atmeh et al. 2018); for example, snowshoe hares experience 7-14% decreased weekly survival when mismatched against their background (e.g., white hare against a snowless ground, (Zimova et al. 2016, Wilson et al. 2018). Given this strong selection against mismatch, persistence of colour moulting species will require adapting to future changes in global snow cover (Krasting et al. 2013, Danco et al. 2016, Mills et al. 2018)

Climate-mediated phenotypic plasticity, described in several colour moulting species can, theoretically, buffer against camouflage mismatch (Zimova et al. 2018). However, previous studies that investigated plasticity in response to climate change showed that current levels of plasticity are insufficient to prevent mismatch; snowshoe hares, least weasels, and Arctic foxes (*Alopex lagopus*) became mismatched during years with fewer days of snow cover (Mills et al. 2013, Zimova et al. 2014, Moberg 2017, Atmeh et al. 2018). This suggests that adaptive evolution of moult phenology and or moult plasticity – evolutionary rescue – may be crucial for the persistence of colour moulting species. Whether evolutionary shifts in moult phenology or plasticity can occur is unknown. However, existing intrapopulation variation in moult phenology and strong selection favouring cryptic colouration suggest evolutionary rescue is possible (Caro 2005, Merilaita and Stevens 2011, Zimova et al. 2016, Mills et al. 2018).

Historical phenological studies provide some of the only opportunities to test whether organisms have already responded to climate change. Unfortunately, such datasets are rare for moult phenology. Fortunately, Watson (1963) and Flux (1970a) described seasonal moult of wild mountain hares (*Lepus timidus scoticus*) in the north-east and central highlands of Scotland over spring and autumn seasons during the 1950s and 1960s. To our knowledge, this effort represents the longest-running systematic historical survey of moult phenology in any species. The studies documented intra-population variation in hares' moult phenology each year and population-level phenotypic plasticity in response to ambient temperature, especially in the spring (Watson 1963, Flux 1970a). The adaptive capacity of mountain hares to buffer against camouflage mismatch via phenotypic plasticity is unknown, however. Similarly, the selective costs of camouflage mismatch in the highly managed Scottish Highlands, have not been investigated. But based on the insights from other colour moulting species (e.g., Zimova *et al.*, 2016; Atmeh *et al.*, 2018), mountain hare populations might have already responded and avoided increases in camouflage mismatch arising from widespread reductions in snow cover (Barnett et al. 2006, Prior and Perry 2014).

In this study, we assessed the potential of a wild population of a common, seasonally colour moulting species to adaptively track climate change. We took advantage of the detailed historical surveys of mountain hare moult phenology in the Scottish Highlands to examine population responses to decreasing snow cover over the past 65 years. First, we quantified population mean moult phenologies and tested whether they have shifted since the 1950s. We hypothesised that mountain hares should have aligned moult phenologies in ways that reduce camouflage mismatch by moulting to white winter fur later in the autumn and to brown summer fur earlier in the spring. Second, we quantified population-level phenotypic plasticity to examine whether it contributes to any potential shifts in mean phenology. Third, we quantified historical and present-day frequency of mismatch and evaluated species vulnerability to future environmental change. We end with general conclusions on some key considerations when predicting adaptive responses of wild populations to climate change.

METHODS

Study areas

Historical surveys were carried out at six sites in the north-east and central highlands of Scotland, UK from 1951 to the end of 1961 (Watson 1963, Flux 1970a). We were unable to resurvey the same sites due to changes in land management, access restrictions to private land, and loss of mountain hares from some historic sites (Watson and Wilson 2018). We therefore surveyed different, yet very similar sites in terms of topography, land management practices and vegetation type. The current sites were located in the upland areas of the north-east and central highlands of Scotland and spanned a similar elevational range as the historical sites (Table 3.1, Appendix 3 Table 3.S1). All historic and current sites were dominated by dwarf heath and subalpine plant communities that dominate the Scottish uplands and represent the habitat type preferred by mountain hares in the geographic area (Raymond Hewson, 1984; Patton et al., 2010).

Field surveys

We followed the original historical field survey methods (Watson 1963, Flux 1970a); one surveyor walked along a predetermined route (~3-6 km long). Hares were detected as they were either flushed (moved) in response to the surveyor or in reaction to other hares, or less frequently, as the surveyor thoroughly scanned the surroundings with a telescope/binoculars. Hares are largely inactive during the day and the majority of hares were detected as they flushed. Our experience and that reported in the literature is that hares tend not to flush until an observer is very close to them, or unless disturbed by other fleeing hares; therefore the large majority of detections were within <50 m of the observer (Shewry et al. 2002, Newey and al. 2003, Newey et al. 2018). For all hares detected within 200 m of the observer, we recorded coat colour (described below). Surveys were repeated twice a month during the main periods of the historical surveys (October-January and March-June) for a total of 5-11 surveys in the autumns and springs of 2016 at the Findhorn sites and 2015 and 2016 at the Lecht site. Surveys were always undertaken in clear and dry conditions so as to reduce the possible effects of weather on detecting hares. The risk of repeat observations of the same individuals within a survey was minimized by monitoring flushed individuals as far as possible. Each survey was completed within a single 4-5 hour session.

Moult phenology

We recorded, and where possible photographed, the coat colour for each observed hare using the moult score protocol developed by Watson (1963). Each hare was ranked in one of five colour categories; DD (completely dark), D (mostly dark), LD (half-dark and half-white), L (mostly white), or LL (completely white) by the surveyor. Observations accompanied by photographs (>80%) were later verified by a single observer (MZ) (Appendix 3 Fig. 3.S1). Historical surveys from the Roar Hill site used a slightly different method (Flux 1970a) to determine five colour categories (=colour was scored independently for seven body parts and averaged), but interchangeability of the two scoring methods was confirmed by the site's observer (J. Flux, Pers. Comm) and by agreement with records from similar dates and sites (Watson 1963). Finally, to reduce potential bias between observers (three field observers conducted recent surveys) and to simplify parameter estimation, we reduced the initial five colour categories into three: white (=LL, L), moulting (=LD) and brown (=D, DD) for all analyses (Appendix 3 Fig. 3.S1).

Statistical analyses

We used R (version 3.5.2; R Core Team 2016) for all statistical analyses.

Climate variables and analysis

To characterize climate in the study region we calculated temperature and snow cover variables over the past 65 years. Mean seasonal temperature was calculated for each year from 1950–2016 using gridded 5 km x 5 km resolution monthly average temperature data (Met Office UKCP09; Perry and Hollis 2005) for each study site. Days with snow cover (snow days) were summed for each season of each year; for 1960-2011 snow days were those days when snow water equivalent (SWE) >0 mm based on daily gridded 5 km x 5 km resolution data (Spencer 2016a). Because this dataset became unavailable after 2011, for 2012-2016 snow days were days when grid cells were >50% snow covered based on daily 1.5 km x 1.5 km resolution data (Dietz et al. 2015). We combined the two different snow datasets to span the entire period of interest and visually verified the compatibility of the two datasets by comparing the period of overlap (2000-2011; Appendix 3 Fig. 3.S2). Additionally, we calculated the number of transitions as the number of occurrences snow fell and melted completely at each site by summing the number of times snow days were followed by days without snow and vice versa each year and season. The resulting 'number of transitions' is a measure of snow cover transiency with snow cover repeatedly falling and melting multiple times during each snow season.

Although snow data do not exist for the study sites prior to 1960, we assumed that the 1950s data were comparable to the 1960s and used the 1960s data as a proxy to calculate historical mismatch (see below). We validated this assumption by comparing number of snow days during 1951-1960 and 1961-1970 which were collected during the Snow Survey of Great Britain (SSGB) (Spencer et al. 2014, Spencer 2016b). Only records from stations lying within 40 km of any of our study sites and that recorded daily snow cover for at least 6 years during both decades (n=7) were included in the comparison. We found no difference between number of snow days during water years (October 1-May 31) between the two decades using a Wilcoxon rank sum test with continuity correction (*P*=0.45, W=1684).

Changes in mean temperature, number of snow days and number of transitions between snow presence and absence were quantified using mixed effects models. Mean seasonal temperature, number of snow days, and number of transitions were used as response variables, year as a fixed effect and site as a random effect using the *lmer* function from the *lme4* package in R. The seasons for temperature variables were defined as spring (March 1-May 31) and autumn (September 1-November 30), autumn snow-fall period (October 1-December 31) and the autumn-to-spring main snow fall period (October 1-May 31; hereafter referred to as 'water year').

Moult phenology

We developed a hierarchical multinomial logistic regression analysis within a Bayesian framework to describe moult phenology and its phenotypic plasticity. For all models, we estimated the probability of a hare being in colour category *i* at site *j* on a Julian day *d* as:

$$\Pr(y = i) = \frac{e^{\alpha_i + \beta 1_i * \mathrm{d} + s_{i,j}}}{1+\sum_{k=1}^{i-1} e^{\alpha_i + \beta 1_i * \mathrm{d} + s_{i,j}}}$$

Coat colour was treated as a categorical variable, such that a hare on day *d* was either brown (p_{brown}), white (p_{white}), or moulting (p_{moult}) and $\Sigma(p_{1:3,j,d}) = 1$. Site was coded as a random covariate $s_{i,j}$ to reflect the hierarchical structure of the dataset and admit repeat measures. α_i was the intercept and $\beta 1_i$ was the effect of Julian day on the probability of being either brown, white or moulting. Autumn and spring moults were modelled separately. Hereafter, we refer to this model without additional covariates as the basic model.

To compare moult phenology between the time periods we combined colour observations from all years and sites in one dataset and added a fixed effect of time period $\beta 2_i$ (1950s or 2010s) on the probability of being in a certain colour category to the basic model. We used the estimated probabilities to derive approximate dates when hares initiated and completed the moults as 'initiation and 'completion dates during each time period. Autumn initiation was specified as the first Julian day when mean $p_{brown}<0.9$ and completion date when mean $p_{white}>0.9$; the opposite condition was used to estimate the spring dates (i.e., initiation$_d$ $p_{white}<0.9$ and completion$_d$ $p_{brown}>0.9$).

Next, we investigated the role of phenotypic plasticity in moult phenology in response to ambient temperature. Because ambient temperature is thought to moderate mountain hare phenology (Watson 1963, Flux 1970a), and thereby improve a model's ability to detect differences between time periods, we constructed an additional set of models with temperature as an additional covariate ($tavg_{j,e}$). $tavg_{j,e}$ was the average seasonal temperature at site j during year e and was added as a fixed effect $\beta3_i$ to the basic model containing time period $\beta2_i$ described above. We standardized tavg to have a mean of 0 and SD of 1. Additionally, to explicitly test the effect of tavg, we constructed a univariate model with a single fixed effect $\beta3_i$ ($tavg_{j,e}$). The resulting $\beta3_i$ coefficients were the slopes of reaction norms of the probabilities of being brown ($\beta3_{brown}$) or white ($\beta3_{white}$) on tavg.

For all models, we obtained posterior distributions of all parameters along with their 95% credible intervals (CRI) using Markov Chain Monte Carlo (MCMC) implemented in JAGS (version 4.0.1), which we called using the R2jags package (Su and Yajima 2012). Model convergence was assessed using the Gelman–Rubin statistic, where values <1.1 indicated convergence (Gelman and Rubin 1992). We generated three chains of 300,000 iterations after a burn-in of 150,000 iterations and thinned by three. Parameters α_i, $\beta1_i$, and $\beta3_i$ received a vague prior of N(0, 0.001), while $\beta2_i$ and the standard deviation of random effect $s_{i,j}$ received uniform priors of U(-10, 10) and U(0, 100), respectively.

Phenotypic mismatch

To examine the occurrence of mismatch between hare winter coat colour and an increasingly snow free ground between 1951 and 2016, we calculated the number of days of mismatch at each site each year and season. Mismatch occurred on days when hares were white and snow was absent at each site. We defined white hares when mean p_{white}>60% based on the basic model, as this threshold would include completely (LL) and mostly white (L) hares (Appendix 3 Fig. 3.S1) and the resulting mismatch definition conforms with previously established methods (Mills et al. 2013). To test for increase in the mismatch over the 60+ years of climate change, we ran a univariate linear mixed model (*lme4* package, function *lmer*) with mismatch days as the response variable, year as a fixed effect, and site as a random effect. Finally, to explore the sensitivity of the definition of white threshold, we repeated the analysis with an alternative threshold at mean p_{white}>90%.

RESULTS

Climate change

Over the 1950-2016 period, temperature increased and snow cover duration decreased for all sites and seasons. Seasonal average temperature (tavg) increased by a mean (±SD) of 0.17 (±0.02)°C/decade during spring and 0.1 (±0.02)°C/decade during autumn (Appendix 3 Fig. 3.S3). This led to increases in average seasonal temperature of 1.15°C in the spring and 0.84°C in the autumn between 1950 and 2016.

The number of snow days decreased during both seasons by a mean of -2.8 (±0.3) days/decade in spring, and -1.7 (±0.3) days/decade in autumn (Appendix 3 Fig. 3.S4) and by a mean of -6.5 (±0.7) snow days/decade for the entire water year (October 1-May 31; Fig. 3.1). This led to an average of 37 fewer days of annual snow cover at our sites between 1960 and 2016.

Lastly, we found no change in the number of transitions between bare ground and snow cover during the water year (β=-0.02, SE=0.01, P=0.17) or autumn seasons (β=-0.01, SE=0.01, P=0.33). In the spring there was a significant decrease in the number of transitions (β=-0.03, SE=0.01, P<<0.001), although this effect size is small (1.63 fewer transitions between 1960 and 2016), likely due to the confounding effect of the decreasing number of springtime snow days (Fig. 3.2, Appendix 3 Fig. 3.S5).

Moult phenology

We did not detect any significant shifts in spring or autumn moult phenology between 1951 and 2016 (Table 3.2a,b). The effect of the time period covariate on the probabilities of being brown ($\beta2_{\text{brown}}$) or white ($\beta2_{\text{white}}$) overlapped zero for both seasons in models with (Table 3.2b), or without seasonal temperature (tavg; Table 3.2a, Fig. 3.2). Next, mean population moult initiation dates did not differ significantly between moult phenology of the 1950s and 2010s in spring or autumn as indicated by the overlapping 95% credible intervals (Fig. 3.2); hares initiate autumn moults in late October (mean Julian date=296) and spring moults around mid-March (mean Julian day=78). Similarly, the estimated completion dates have not changed between the two time periods for either season (Fig. 3.2).

We found some evidence for the presence of phenotypic plasticity in response to annual variation in temperature tavg (Table 3.2b,c). In the spring, the effect of tavg ($\beta 3_i$) was significant and stronger than in the autumn (Table 3.2c), indicating that moults occurred later during colder springs. In the autumn, tavg had significant effect only on the probability of being white ($\beta 3_{white}$, Table 3.2c), suggesting molts occurred earlier during colder autumns.

Phenotypic mismatch

Estimated mismatch in coat colour increased between 1950-2016 at all sites and seasons. The increases were steepest over the entire snow season (Oct 1-May 31) (β_{Year}=0.52, P<<0.001) and evident when moulting seasons were considered separately (autumn β_{Year}=0.14, P<<0.001; spring β_{Year}=0.18, P<<0.001; Fig. 3.3). Since the 1960s, from when gridded snow data are available, the regression slopes translate to 29.7 more days with white hares (mean p_{white}>60%) against snowless background than in the 2010s, with an additional 5.2 days when data are extrapolated to 1950s (Fig. 3.3b). Across all the sites, average number of days of mismatch increased from 44.3 (SD=24.8) days during 1950s and 1960s to 69.9 (SD=30.1) during 2010s (Fig. 3.3a). The results were similar when an alternative threshold of mismatch (=mean p_{white}>90%) was used (Appendix 3 Table 3.S2).

DISCUSSION

Across the north-east and central highlands of Scotland seasonal temperatures have increased and the number of snow days has declined since the 1950s, a trend seen across the northern hemisphere (Kunkel et al. 2016, Easterling et al. 2017). Despite the directionality and magnitude of the observed climate shift documented here, our results show that hare moult phenology did not appear to track the shortening snow seasons to prevent camouflage mismatch. Further, temperature-mediated phenotypic plasticity in moult phenology was not sufficient to buffer against camouflage mismatch. Altogether, this resulted in 35 additional days of phenotypic mismatch whereby mostly white hares confronted snowless backgrounds. As snow cover is expected to decline by up to 50% by 2100 across Scotland (Barnett et al. 2006, Trivedi et al. 2007, Bell et al. 2016), mountain hares in the Scottish uplands are likely to experience further phenotypic mismatch in the future.

The lack of adaptive phenological responses in mountain hares was unexpected for two main reasons. First, phenotypic plasticity has been commonly documented to buffer against phenological mismatches across taxa (Merilä and Hendry 2014, Fox et al. 2019) and plasticity in moult phenology has been observed in several seasonally colour-moulting species (Zimova et al. 2018). Yet, the current levels of plasticity were apparently not sufficient to have prevented increases in camouflage mismatch. Second, strong natural selection against camouflage mismatch has been documented in other colour moulting species (Zimova et al. 2016, Atmeh et al. 2018, Wilson et al. 2018) and is common in other similar wild systems (e.g. lizards and mice inhabiting light colored substrates: Rosenblum, 2006; Barrett *et al.*, 2019). Therefore, evolutionary shifts in moult phenologies is a plausible, if not expected, response to reduced snow cover. Given that these two components of adaptive capacity are so widely observed, our results provide a striking contrast to evidence for possible adaptive shifts observed in similar systems (Zimova et al. 2016, Atmeh et al. 2018, Mills et al. 2018).

Multiple factors may have contributed to the lack of climate-mediated alteration of moult phenology in mountain hares. In the next paragraphs we discuss the potential contributions of environmental stochasticity, potentially low genetic variance, and attenuation of selection pressure against camouflage mismatch in Scotland. We also discuss how the increasing duration of camouflage mismatch in mountain hares might influence these populations in the future.

Adaptive tracking of decreasing snow cover could be slowed or stalled if temporally-varying selection pressures prevent the generation of stable optimal phenotypes via phenotypic plasticity or evolutionary adaptation (Gienapp et al. 2014, Nadeau et al. 2017, Senner et al. 2017b). The climate of Scotland's highlands is extremely variable and unpredictable in time and space, subjecting mountain hares to high environmental stochasticity. Although temperature exerts major control over snow cover and depth in Scotland (Harrison et al. 2001, Kay 2016), snowfall is often associated with frontal systems and a cold winter does not necessarily mean a snowy one (Spencer and Essery 2016). Indeed, hares experience high variability in snow cover during each winter, with an average of 14.2 snow cover transitions per winter during our study period. However, the high stochasticity in climate has not increased over the past 60 or more years (Appendix 3 Fig. 3.S5), so environmental stochasticity seems unlikely to be a primary inhibitor of recent adaptive responses.

For moult phenology or its plasticity to evolve by natural selection, sufficient heritable genetic variation must exist in the trait and population must be large enough that selection is not overwhelmed by genetic drift (Wright 1969, Barrett and Schluter 2008, Hoffmann et al. 2017). The genetic basis of moult phenology and its plasticity is unknown, but phenological traits are often heritable (e.g., reproduction, migration, hibernation: van Asch *et al.*, 2007; Lane *et al.*, 2011; Tarka *et al.*, 2015; Dobson *et al.*, 2017). Indeed, studies of trait genetic architecture, and common garden and translocation experiments, show a genetic basis for winter colour morph and potentially for moult phenology in *Lepus* spp. (Bergengren 1969, Ferreira et al. 2017, Jones et al. 2018, Zimova et al. 2018). However, even if heritability in phenology or its plasticity is high, genetic shifts may be compromised by the stochastic processes of genetic drift in small populations. Recent (i.e., since 1990s) population reductions have been reported for some areas in the north-east and central highlands of Scotland (Massimino et al. 2018, Watson and Wilson 2018). Furthermore, the extent of overall genetic variation in Scotland populations is unknown, but some evidence suggests it is lower than in other mountain hare populations in Europe (Melo-Ferreira et al. 2007, Zachos et al. 2010). However, without better information on effective population size and genetic variance in Scottish hares we cannot infer whether low genetic variation and subsequent genetic drift might have contributed to the lack of response in moult phenology.

We believe that the main contributing factor for the unchanged moult phenology in mountain hares in Scotland is attenuation of selection pressure. Natural selection for cryptic colouration is one of the strongest drivers of adaptive evolution (Wallace 1879, Caro 2005, Duarte et al. 2017), with examples including peppered moths (*Biston betularia*) in Great Britain (Saccheri et al. 2008, Walton and Stevens 2018), fire melanism in Swedish grasshoppers (Forsman et al. 2011), and seasonal colour moults in birds and mammals (Beltran et al. 2018, Mills et al. 2018, Zimova et al. 2018). However, relaxed selection can lead to a loss of functional traits or diminished phenotypic plasticity

(Darwin 1859, Lahti et al. 2009, Oostra et al. 2014, Tseng and O'Connor 2015). Because the main adaptive advantage of the winter-white moult is predator avoidance (thermoregulatory properties are overwhelmingly controlled by changes in hair length and density, not hair colour; Zimova *et al.*, 2018), high predation risk for poor camouflage would be required to facilitate evolutionary shifts in colour moult phenology.

In Scotland, mountain hares are prey for a range of species including red fox (*Vulpes vulpes*), wild cat (*Felis silvestris*), stoat (*Mustela erminea*), weasel, otter (*Lutra lutra*), common buzzard (*Buteo buteo*), hen harrier (*Circus cyaneus*) and golden eagle (*Aquila chrysaetos*) (Flux 1970b, Hewson 1976, Watson et al. 1993). However, in the north-east and central highlands of Scotland, mountain hares are associated with heather-dominated moorlands managed for commercial shooting of red grouse (*Lagopus lagopus scoticus*) (Raymond Hewson, 1984; Patton et al., 2010). Predator numbers and diversity are severely depressed across these lands due to legal and illegal predator control over the last century (Langley and Yalden 1977, Thirgood et al. 2000, Whitfield et al. 2006, Thompson et al. 2016, Sainsbury et al. 2019). Thus, while decreased snow cover should lead to increased mismatch costs, natural selection against mismatch in these areas is likely very weak compared to regions with intact predator communities such as in the Rocky Mountains in Montana, USA (Zimova et al. 2016). Given the highly altered selection regime on intensively managed moorlands, camouflage mismatch might have little-to-no fitness costs for mountain hares in our study system, now, and in the recent past.

Interestingly, the increase in days of coat colour mismatch that we observed for mountain hares in Scotland (37 days) is virtually identical to the increased mismatch that we projected for snowshoe hares in Montana over the next half century in the absence of adaptation (Mills et al. 2013). However, the ecological differences between the systems mean that the lack of response in Scotland is not a harbinger of lack of future adaptive responses in Montana (or elsewhere). Specifically, the climate change in Scotland coincides with local attenuation of predation risk that ameliorates the costs of mismatch, undercutting adaptation and resulting in increasingly mismatched hares. However, if ecological conditions change such that fitness consequences of camouflage mismatch return, hares may face negative fitness consequences via increased predation mortality (Zimova et al. 2016). For example, based on our results we predict that the accumulating degree of camouflage mismatch in these hares would threaten population persistence if generalist predators were to increase in response to land use change (Brooker et al. 2018). This ‘latent maladaptation’ is therefore worth considering when assessing the species vulnerability to climate and land use change (Rehnus et al. 2018, Watson and Wilson 2018). We recommend management efforts that favour evolutionary rescue (i.e., large connected populations that harbour high genetic diversity; Carlson et al. 2014) to achieve evolutionary resilience and long-term persistence in the face of future biotic and abiotic changes (Sgrò et al. 2011, Carroll et al. 2014, Mills et al. 2018). This recommendation is especially relevant for the mountain hare populations in the north-east and central highlands of Scotland, where there is evidence of recent population declines (Massimino et al. 2018, Watson and Wilson 2018) and that face additional stressors related to game bird management and woodland/forestry expansion (Harrison et al. 2010, Gilbert 2016, Mustin et al. 2018, Watson and Wilson 2018).

For at least 21 species across the northern hemisphere, seasonal coat colour has been shaped directly by climate (Mills et al. 2018). The general consensus is that as decreasing snow duration continues to cause winter white animals to be found against dark snowless backgrounds, adaptation will be necessary to mitigate the negative effects of increasing camouflage mismatch (Mills et al. 2013, Zimova et al. 2016). However, here we documented unchanged moult phenology in mountain hares in Scotland despite directional climate change over the past 60 or more years. While more study is necessary to understand why moult phenology has been static over this time period, we suggest that relaxed selection for camouflage, potentially coupled with low genetic variance, is a reasonable explanation. Duration of snow cover in Scotland is expected to continue to decrease (Brown et al. 2010, Bell et al. 2016), and eventually become absent from altitudes below 900 m by the end of the century (Trivedi et al. 2007). Our findings suggest that these Scottish mountain hares will experience increasing mismatch. We conclude that the fitness consequences of these static phenotypes will depend not only on future climate change, but also on future community assembly and the consequent predation pressure. Altogether, our findings underline that wildlife adaptive responses to anthropogenic stressors will ultimately depend on both abiotic and biotic conditions.

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TABLES

Table 3.1. Historical and present study sites in north-east and central Scotland, average elevation at the sites, years when surveys were carried out, latitude and longitude.

Region	Site	Elevation (m)	Survey years	Latitude	Longitude
Historic Surveys					
Angus Glens	Glen Esk high	610	1957-1961	56.957	-2.839
Angus Glens	Glen Esk low	270	1957-1961	56.943	-2.835
Deeside/Strathdon	Corndavon	450	1951, 1955, 1957-1959	57.068	-3.234
Deeside/Strathdon	Glen Muick	380	1958-1959	57.022	-3.046
Deeside/Strathdon	Punchbowl	310	1957-1959	56.860	-2.730
Deeside/Strathdon	Roar Hill	450	1958-1959	57.129	-2.999
Current Surveys					
Highland	Findhorn high	640	2016 (spring & autumn)	57.235	-4.136
Highland	Findhorn low	430	2016 (spring & autumn)	57.206	-4.102
Deeside/Strathdon	Lecht	730	2015 (autumn)-2016 (spring)	57.193	-3.240

Table 3.2. Absence of shifts in moult phenology from 1951 to 2016 and some phenotypic plasticity in mountain hares in the highlands of Scotland in autumn and spring. Mean effect sizes and 95% credible intervals (CRI) estimates for slopes for models including a) time period only, b) time period and seasonal average temperature (tavg), c) tavg only covariate(s). β2i indicates the effect of time period and β3i indicates the effect of seasonal tavg on the probability of brown (β3brown) and white (β3white). Asterisks indicate CRIs not overlapping zero.

a)	**Moult season**	**$\beta 2_{brown}$ (CRI)**	**$\beta 2_{white}$ (CRI)**
	Autumn	-0.62 (-2.12, 0.89)	0.62 (-0.26, 1.56)
	Spring	-0.23 (-0.83, 0.35)	0.01 (-1.00, 1.00)

b)	**Moult season**	**$\beta 2_{brown}$ (CRI)**	**$\beta 2_{white}$ (CRI)**	**$\beta 3_{brown}$ (CRI)**	**$\beta 3_{white}$ (CRI)**
	Autumn	-0.34 (-1.68, 1.00)	0.06 (-0.68, 0.92)	0.25 (-0.05, 0.54)	-0.46* (-0.71, -0.20)
	Spring	-0.37 (-1.56, 0.60)	-0.05 (-1.30, 0.93)	1.00* (0.87, 1.14)	-0.78* (-0.92, -0.64)

c)	**Moult season**	**$\beta 3_{brown}$ (CRI)**	**$\beta 3_{white}$ (CRI)**
	Autumn	0.270 (-0.018, 0.554)	-0.456* (-0.676, -0.288)
	Spring	1.005* (0.870, 1.144)	-0.766* (-0.906, -0.627)

FIGURES

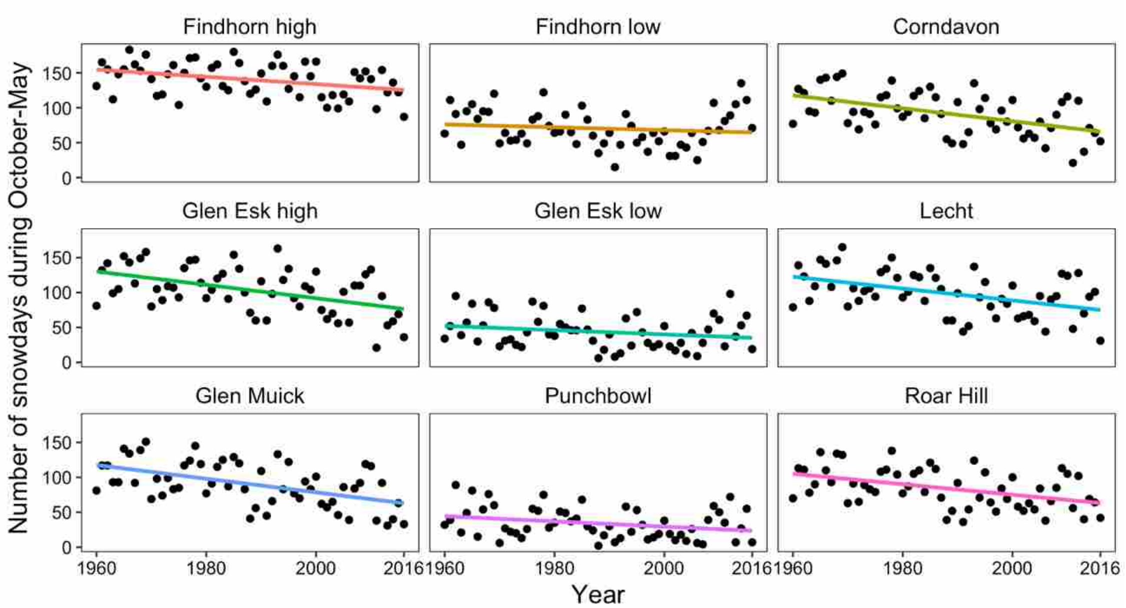

Figure 3.1. Number of snow days during the snow season (October-May) at the study site between 1960-2016. Coloured lines are based on linear regression at each site

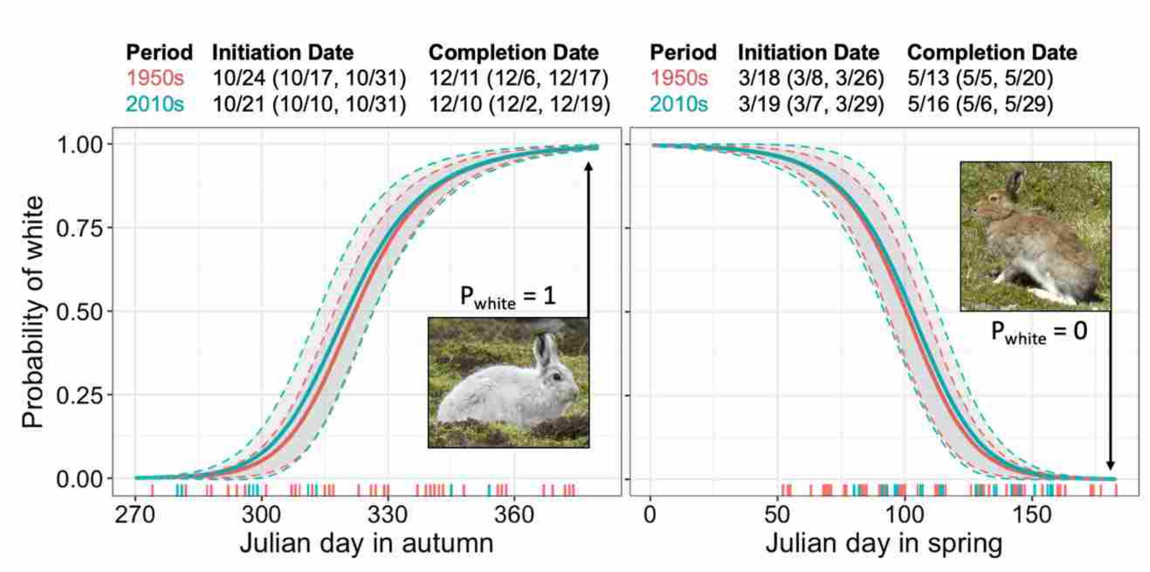

Figure 3.2. Similar mean mountain hare moult phenologies during 1950s (red) and 2010s (blue) in the highlands of Scotland. Solid lines depict predicted probabilities of being white over time based on the basic model including seasonal average temperature. The shaded areas and dashed lines show 95% credible intervals (CRI) and the perpendicular hash marks along the x-axis depict survey dates, colour coded for each time period. Photographs show mountain hares when probability of being in white pelage is 100% (left) and 0% (right). Dates above plots indicate mean initiation and completion dates and CRIs.

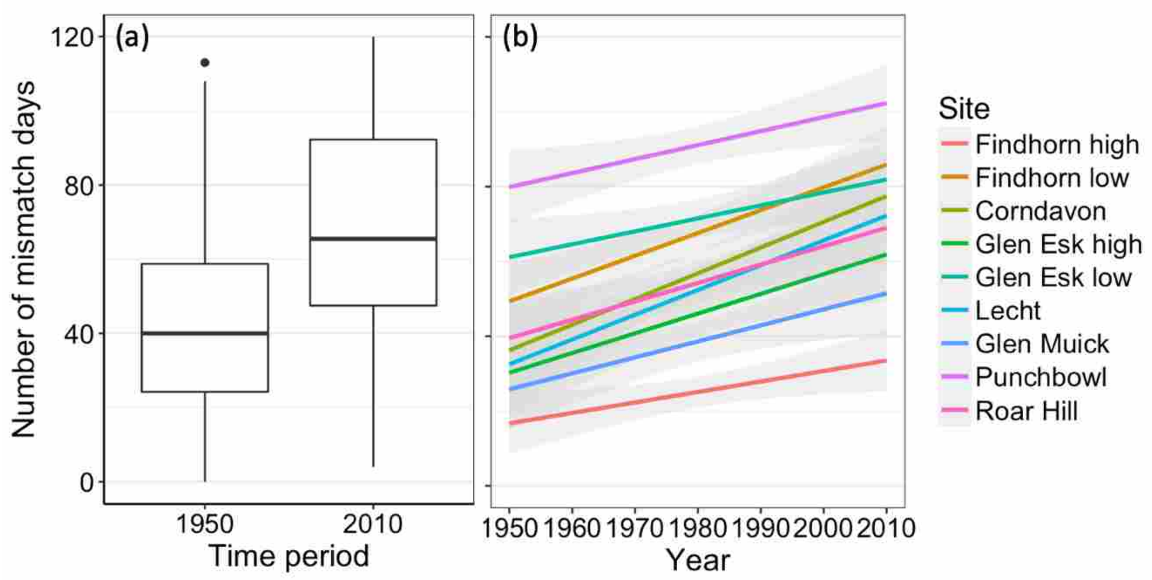

Figure 3.3. Estimated number of days when white mountain hares would be found mismatched against snowless background from 1950 to 2016 in the highlands of Scotland. Number of mismatch days are calculated over the entire snow season (October 1-May 31) for each year. (a) boxplots show number of mismatch days in 1950s and 2010s. Horizontal lines within the boxes denote the medians, boxes the first and third quartiles, whiskers extend to the largest and smallest value within 1.5×the interquartile range and the point represents an outlier. (b) coloured lines show linear regression slopes for each site with 95% confidence intervals depicted in grey.

APPENDIX 3. SUPPLEMENTAL MATERIAL

Figure 3.S1. Coat colour categories used to score mountain hares in the field and in the analysis (based on Watson 1973). Each category is given two example photos to illustrate range of variation.

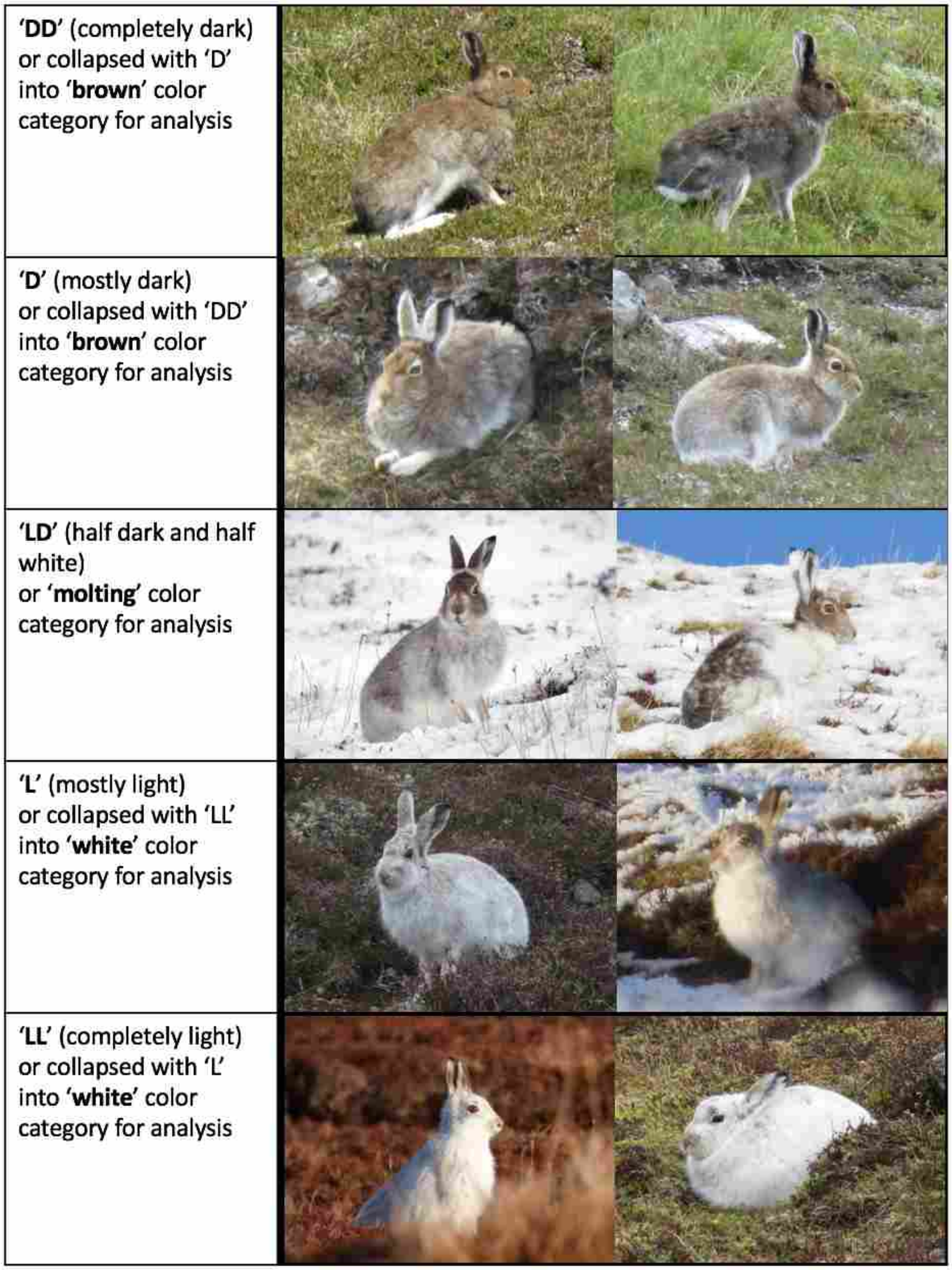

Figure 3.S2. Comparison between two different snow cover datasets for the period 2000-2011. The points show proportion of snow days each month (October-May) at each study site.

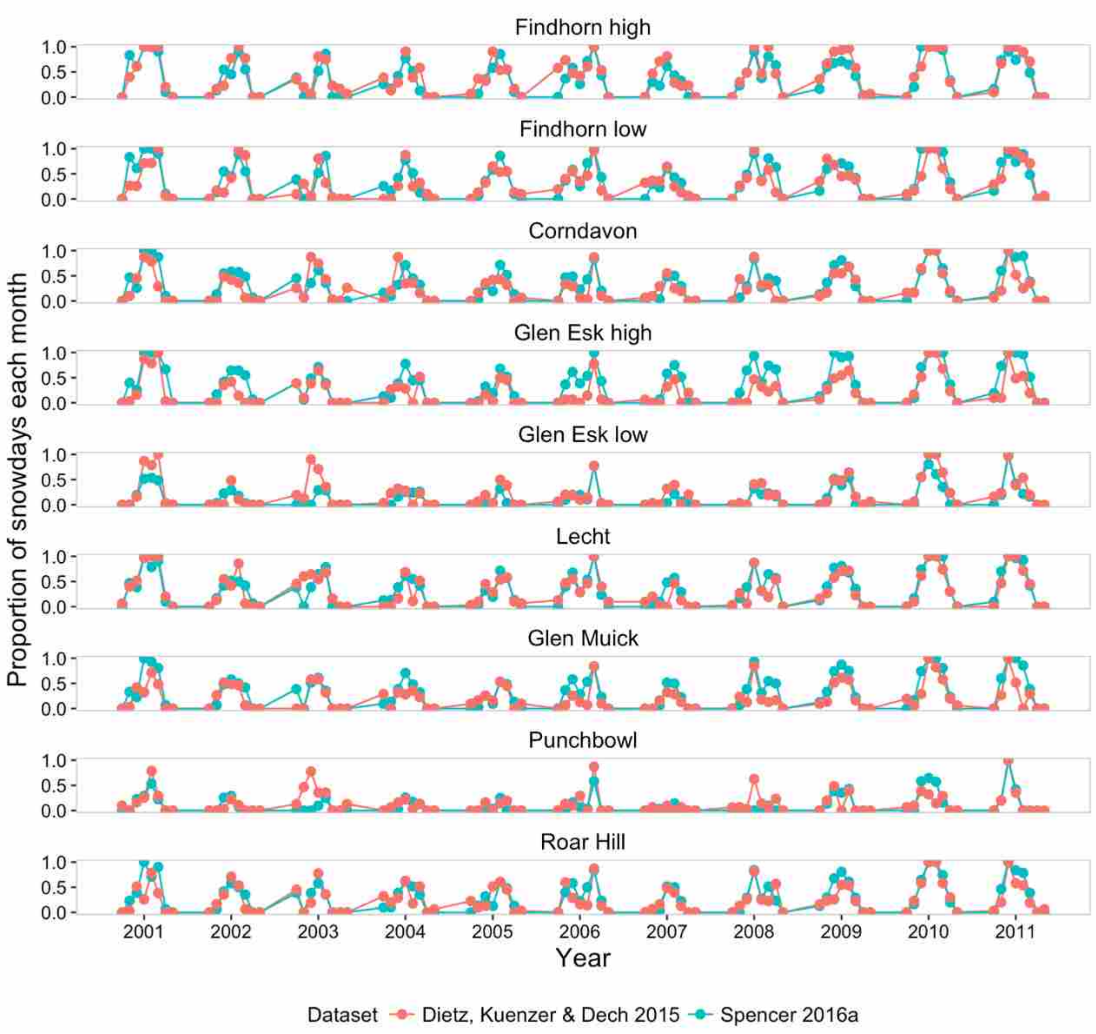

Figure 3.S3. Average spring (top) and autumn (bottom) seasonal temperature (tavg) at the study site between 1950-2016. Dashed line is based on linear regression at each site.

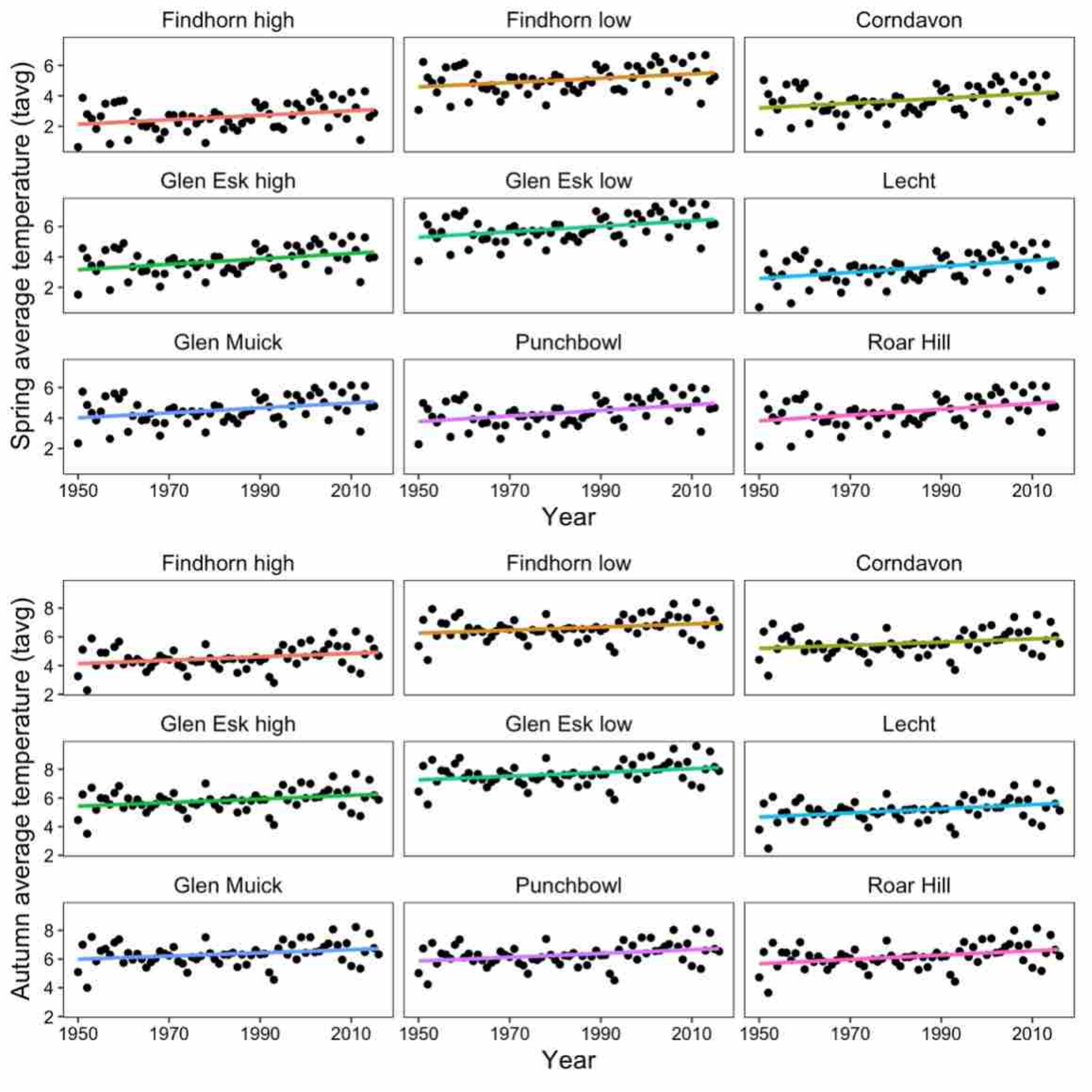

Figure 3.S4. Number of snow days during spring (top) and autumn (bottom) seasons at study sites in the highlands of Scotland between 1960-2016. Coloured lines are based on linear regressions at each site.

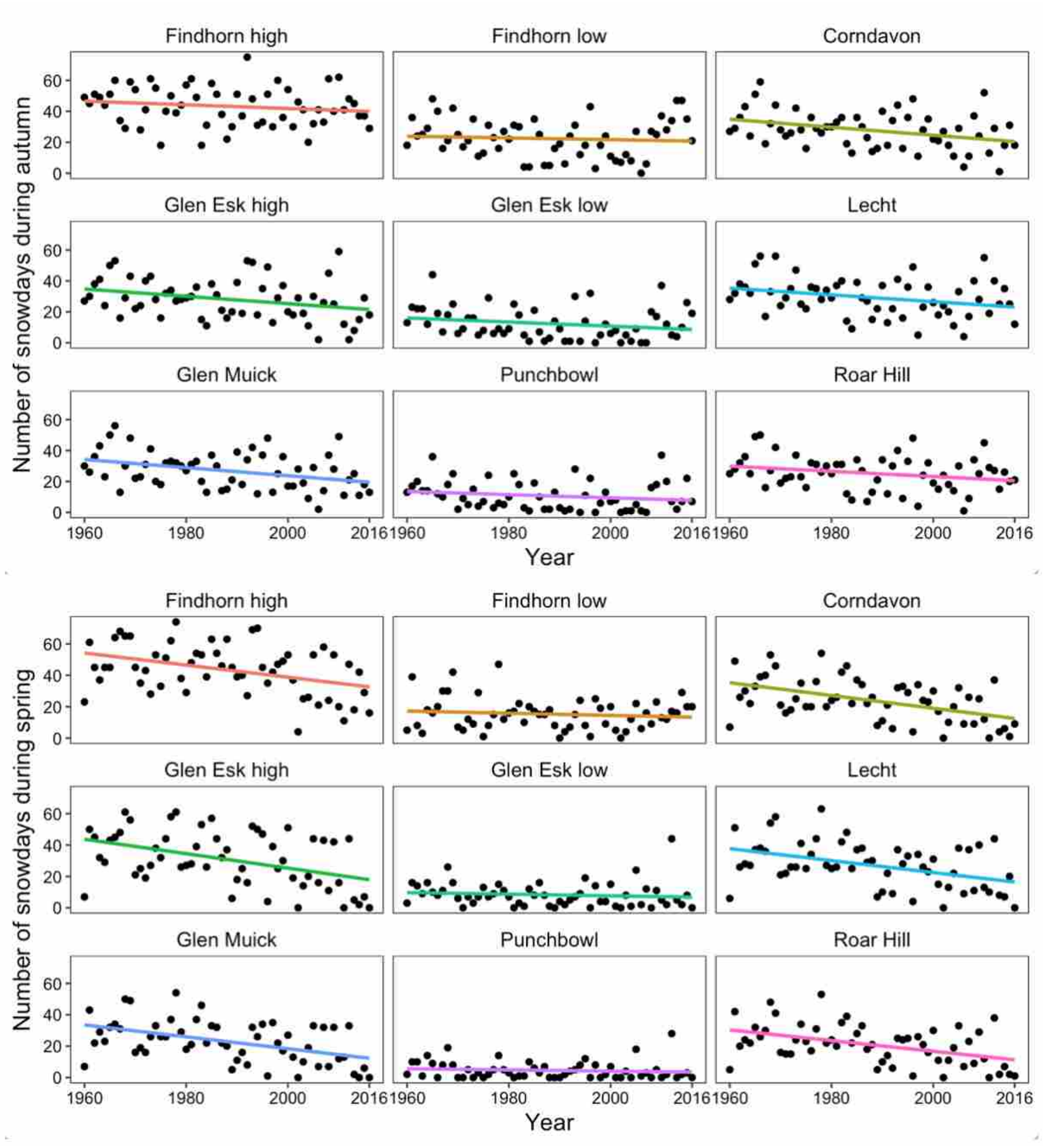

Figure 3.S5. Number of transitions between bare and snow-covered ground during spring (a), autumn (b) and snow season (c) at the study sites between 1960-2016. Coloured lines are based on linear regressions at each site.

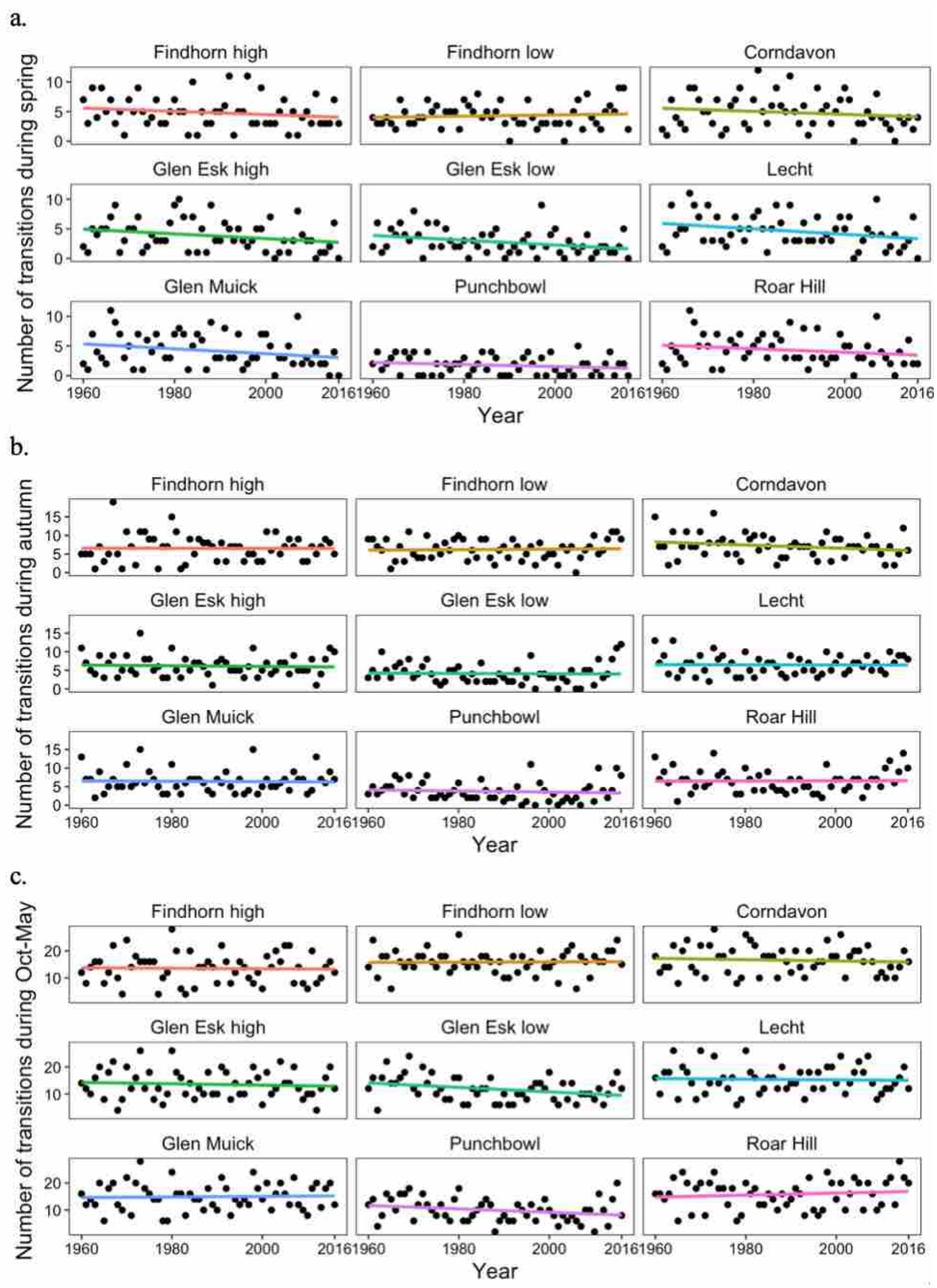

Table 3.S1. Present and historical survey sample sizes. Number of surveys carried out at each site during particular year and season. Last three columns give mean number of hares, its standard deviation and minimum number of hares observed during a survey that season.

Site	**Season**	**Year**	**n surveys**	**mean n hares/ survey**	**sd n hares/ survey**	**min n hares/ survey**
Findhorn high	spring	2016	10	37.1	11.5	27
Findhorn high	autumn	2016	6	39.7	9.4	30
Findhorn low	spring	2016	11	26.7	4.9	19
Findhorn low	autumn	2016	6	14.0	10.3	6
Lecht	spring	2015	6	46.3	7.5	36
Lecht	autumn	2015	5	37.4	6.9	30
Lecht	spring	2016	6	38.5	5.5	31
Lecht	autumn	2016	6	36.5	10.4	23
Corndavon	spring	1951	4	15.3	5.7	8
Corndavon	spring	1955	4	30.3	41.3	6
Corndavon	spring	1957	4	410.3	413.3	36
Corndavon	autumn	1957	3	49.7	42.5	7
Corndavon	spring	1958	4	114.8	43.5	75
Corndavon	autumn	1958	3	78.7	28.7	48
Corndavon	spring	1959	2	38.5	17.7	26
Glen Esk high	autumn	1957	8	60.8	55.1	17
Glen Esk high	spring	1958	9	116.9	100.2	10
Glen Esk high	autumn	1958	7	44.0	24.9	18
Glen Esk high	spring	1959	8	73.5	32.2	26
Glen Esk high	autumn	1959	9	66.7	49.0	15
Glen Esk high	spring	1960	4	61.0	33.0	18
Glen Esk high	autumn	1960	6	56.5	57.2	9
Glen Esk high	spring	1961	5	70.0	42.5	19
Glen Esk low	autumn	1957	7	26.3	18.9	8
Glen Esk low	spring	1958	9	47.2	63.4	11
Glen Esk low	autumn	1958	7	24.9	12.6	15
Glen Esk low	spring	1959	8	37.3	13.0	22
Glen Esk low	autumn	1959	7	17.9	7.5	6
Glen Esk low	spring	1960	2	13.0	11.3	5
Glen Esk low	autumn	1960	6	12.5	8.0	2
Glen Muick	spring	1958	2	22.0	5.7	18
Glen Muick	autumn	1958	2	21.5	0.7	21
Glen Muick	spring	1959	2	36.0	15.6	25
Glen Muick	autumn	1959	1	56.0	NA	56
Punchbowl	autumn	1957	2	26.5	6.4	22
Punchbowl	spring	1958	3	98.7	29.4	67
Punchbowl	autumn	1958	2	18.5	7.8	13
Punchbowl	spring	1959	1	35.0	NA	35
Roar Hill	spring	1958	9	27.8	8.3	25
Roar Hill	autumn	1958	9	25.0	0.0	25
Roar Hill	spring	1959	12	25.0	0.0	25
Roar Hill	autumn	1959	9	25.0	0.0	25

Table 3.S2. Observed and predicted occurrence of mismatch (in days) with white hares defined either as mean $p_{white}> 60\%$ or mean $p_{white}> 90\%$. The first five columns show mean number of mismatch days during 1950s and 2010s, their standard deviations, and the observed difference in mismatch days between the two time periods means. The last three columns show slopes, their standard errors (SE) and the predicted increase in mismatch days based on the mixed linear regression.

mean $p_{white} > 60\%$

Season	**mean 1950s**	**SD**	**mean 2010s**	**SD**	**Observed increase since 1950s**	**β_{Year}**	**SE**	**Predicted increase since 1950s**
Snow season	44.3	24.8	69.9	30.1	25.6	0.521	0.004	34.9
Autumn	13.7	9.7	19.5	11.3		0.138	0.003	
Spring	14.6	10.8	25.7	22.6		0.184	0.003	

mean $p_{white} > 90\%$

Season	**mean 1950s**	**SD**	**mean 2010s**	**SD**	**Observed increase since 1950s**	**β_{Year}**	**SE**	**Predicted increase since 1950s**
Snow season	23.7	17.4	38.2	22.3	14.5	0.289	0.003	19.4
Autumn	4.9	4.6	8.2	7.0		0.059	0.002	
Spring	3.1	5.1	5.8	7.1		0.034	0.001	

CHAPTER 4

LOCAL CLIMATE DETERMINES CAMOUFLAGE MISMATCH IN SNOWSHOE HARES

Marketa Zimova[a], Alexej P. K. Sirén[b,c], Joshua Nowak[a], Alexander M. Bryan[c,d], Jacob Ivan[e], Toni Lyn Morelli[b,c], J. Skyler Suhrer[a], Jesse Whittington[f], L. Scott Mills[a,g]

[a] Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA.

[b] Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, USA.

[c] U.S. Geological Survey, Northeast Climate Adaptation Science Center, Amherst, MA 01003, USA.

[d] Integrated Environmental Data, LLC, Berne, NY 12023, USA.

[e] Colorado Parks and Wildlife, Fort Collins, CO 80526, USA.

[f] Parks Canada, Banff National Park Resource Conservation, Banff, Alberta, Canada T1L 1K2.

[g] Wildlife Biology Program and Office of Research and Creative Scholarship, University of Montana, Missoula, MT 59812, USA.

ABSTRACT

Aim: Phenological mismatches, when timing of life-events becomes mistimed with optimal environmental conditions, have become increasingly common under climate change. Organisms' susceptibility to mismatches depends on how phenology and phenotypic plasticity vary across a species distributional range. Here we quantify the environmental drivers of color molt phenology, phenotypic plasticity, and the extent of phenological mismatch in seasonal camouflage to assess susceptibility of a common North American mammal.

Location: North America.

Time period: 2010-2017.

Major taxa studied: Snowshoe hares (*Lepus americanus*).

Methods: We used >5,500 by-catch photographs of snowshoe hares from 448 remote camera trap sites at three independent study areas. To quantify molt phenology and phenotypic plasticity, we used multinomial logistic regression models that incorporated geospatial and high-resolution climate data. We estimated occurrence of camouflage mismatch between hares coat color and the presence and absence of snow over seven years of monitoring.

Results: Spatial and temporal variation in molt phenology depended on local climate conditions rather than latitude. First, hares in colder, snowier areas molt earlier in the fall and later in the spring. Next, hares exhibited phenotypic plasticity in molt phenology in response to annual variation in temperature and snow duration, especially in the spring. Finally, the occurrence of camouflage mismatch varied in space and time; white hares on dark, snowless background occurred primarily during low-snow years in regions characterized by shallow, short-lasting snowpack.

Main Conclusions: Long-term climate and annual variation in snow and temperature determine coat color molt phenology in snowshoe hares. In most areas, climate change leads to shorter snow seasons, but the occurrence of camouflage mismatch varies across the species range. Our results underscore the population-specific susceptibility to climate-

induced stressors and the necessity to understand this variation to prioritize the populations most vulnerable under global environmental change.

INTRODUCTION

As a result of anthropogenic climate change, plant and animal populations are increasingly confronting environmental conditions different from the ones to which they are adapted. Organisms occupying seasonal environments have evolved mechanisms to time their life cycles (i.e., phenology) to match with optimal environmental conditions and resources at their location (Bradshaw & Holzapfel, 2007; Williams *et al.*, 2017). When phenology and favorable environmental conditions become asynchronized, organisms can suffer negative fitness costs (Both *et al.*, 2006; Post & Forchhammer, 2008; Lane *et al.*, 2012; Zimova *et al.*, 2016; Senner *et al.*, 2017). Such phenological mismatches are becoming increasingly common under climate change (Parmesan & Yohe, 2003; Thackeray *et al.*, 2010; Williams *et al.*, 2015), which in the absence of adaptive responses could lead to population declines and local extinctions (Visser & Gienapp, 2019). Thus, there is a pressing need to understand the degree to which climate change leads to phenological mismatches and the capacity of wild populations to withstand attendant fitness costs.

Understanding the variation in phenology and its environmental drivers is fundamental for assessing current and future species' susceptibility to phenological mismatches. For the majority of traits in plants and animals in temperate regions, photoperiod serves as the principal cue for phenology, with temperature and other environmental factors exerting lesser influence (Hofman, 2004; Bradshaw & Holzapfel, 2007). Because photoperiod, latitude, and climate covary across most species’ ranges, variation in phenology is often distributed along latitudinal gradients. Northern populations typically experience harsher and colder climates that correspond with later initiation of spring and earlier initiation of fall events compared to southern populations (Bradshaw & Holzapfel, 2007; Hut *et al.*, 2013). However, latitude may not be a reliable predictor of phenology for two reasons. First, the covariance between latitude and climate is imperfect when other geographical factors such as elevation also affects climatic conditions (Chuine, 2010; Visser *et al.*, 2010). Secondly, species show year-to-year *in situ* variation in phenological traits in response to annual variation in climate. This temporal phenological variation is referred to as "population-level" phenotypic plasticity, and is different from between-individual level phenotypic plasticity (Phillimore *et al.*, 2010; Gienapp & Brommer, 2014). Overall, both spatial and temporal variation in phenology may have consequences for susceptibility to phenological mismatch on both the local population and broader species levels.

Seasonal coat color molt, a key phenological trait, has received increased attention as a trait directed shaped by climate. Across the Northern Hemisphere, 21 species of birds and mammals change coat or plumage color from brown in the summer to white in the winter to match snow-covered landscapes (Mills *et al.*, 2018; Zimova *et al.*, 2018). As with other phenological traits, photoperiod serves as the principal cue for molt phenology, with some evidence that year-to-year variation in winter climate modulates the progression of molt (Hofman, 2004; Zimova *et al.*, 2018). However, decreasing duration of snow cover due to climate change may result in phenological mismatch, whereby winter white animals become color mismatched against dark, snowless backgrounds (Mills *et al.*, 2013). Field studies indicate that mismatch in seasonal coat color and snow presence or absence leads to high fitness costs due to increased predator-induced mortality (Zimova *et al.*, 2016; Atmeh *et al.*, 2018) and may have already contributed to range contractions for several species including *Lagopus* and *Lepus* spp. (Imperio *et al.*, 2013; Diefenbach *et al.*, 2016; Sultaire *et al.*, 2016; Pedersen *et al.*, 2017).

The snowshoe hare (*Lepus americanus*), a key prey species of the boreal forest of North America (Krebs *et al.*, 2001), exhibits seasonal color molts in the majority of its range (Nagorsen, 1983; Gigliotti *et al.*, 2017; Mills *et al.*, 2018). Because of their broad distribution (Fig. 4.1a), hares inhabit a large range of environmental conditions, making them an ideal species for investigating variation in molt phenology and camouflage mismatch. In the only two areas where molts have been recently investigated in relation to climate change (i.e., Montana and Wisconsin, USA), phenotypic plasticity is not sufficient to prevent camouflage mismatch (e.g., Mills *et al.*, 2013; Wilson *et al.*, 2018). For example, hares in Montana experience about a week of mismatch whereby hares are in the wrong coat color in relation to their background (i.e., white hares on snowless background or brown hares on snow; Mills *et al.*, 2013; Zimova *et al.*, 2014, 2016). Because hares rely heavily on their camouflage for survival, mismatch has strong negative fitness costs (i.e., 7-12% reduced weekly survival) and may, in the absence of evolutionary shifts, lead to steep population declines and local extinction (Zimova *et al.* 2016, Wilson *et al.* 2018). To date, no study has evaluated the phenological drivers and the susceptibility to camouflage mismatch across the heterogeneous snowshoe hare range in a unified analytical framework.

To understand snowshoe hares susceptibility to camouflage mismatch, we quantified the spatial and temporal variation in color molt phenology, phenotypic plasticity, and the occurrence of camouflage mismatch across three disjunct, climatically and geographically distinct populations. First, we hypothesized that the spatial variation in molt phenology would be determined by latitude and local climate. We tested two predictions: i) populations in more northern sites molt earlier in the fall and later in the spring, ii) populations in colder and snowier sites molt earlier in the fall and later in the spring. Second, we hypothesized that hares exhibit temperature- and snow-mediated phenotypic plasticity in molt phenology and we predicted that molts occur earlier in fall and later in spring during colder and or snowier years. Third, we quantified the occurrence of camouflage mismatch at each population in spring and fall and assessed which snowshoe hare populations may be the most susceptible to camouflage mismatch.

MATERIALS AND METHODS

Study Areas

Our analysis integrated three regional monitoring studies in North America: the Canadian Rockies, the San Juan Mountains in Colorado, and northern New England (Fig. 4.1a). Together, these areas encompass wide latitudinal, altitudinal and habitat variation across the distributional range of snowshoe hares (Table 4.1). Our northernmost study area in the Canadian Rockies included three national parks (Banff, Yoho, and Kootenay National Parks) and was characterized by rugged, forested mountainous regions with a long snow season. This area is located within a homogeneous boreal habitat at the core of the snowshoe hare distribution range (Cheng *et al.*, 2014). The southernmost study area was located in the San Juan Mountains of southwest Colorado; an isolated patch of high-elevation southern boreal forest near the southern range boundary. The northern New England study area, also near the southern edge of hare distribution, encompassed portions of the Green Mountains in Vermont and the White Mountains in New Hampshire. This area stretched across a transition zone between the northern hardwood and boreal forests and had, on average, the mildest climate of the three areas. Major hare predators at all areas included coyotes (*C. latrans*), red fox (*Vulpes vulpes*), Canada lynx (*Lynx canadensis*), bobcats (*L. rufus*), American martens (*Martes americana*), weasels (*Mustela spp*.), northern goshawks (*Accipter gentilis*), and great-horned owls (*Bubo virginanus*). The study area in Canada also had wolves (*Canis lupus*) and grizzly bears (*Ursus arctos*).

Camera trapping design

We obtained our snowshoe hare phenology data as by-catch photos from three long-term independent studies of forest carnivores. A combination of motion-triggered camera models was used across the sites, but all produced comparable high-quality images during the day and night. The camera spacing differed between regions, but at any given time the minimum distance between cameras was >1 km; spacing was achieved using grids that varied by study area (10 × 10 km in Canada, 4 × 4 km in Colorado, and 2 × 2 km in New England). Each camera site was stationary and remained within a grid cell for a minimum duration of one year. We combined all observations for the cameras that were moved between years within <1 km distance and assigned them an average latitude, longitude and elevation. The 1-km threshold was chosen because it far exceeds average hare seasonal movement and home range size at all three areas (Hodges, 1999; Mills *et* *al.*, 2005; Ivan, 2011), and matches with the spatial resolution of the climate datasets used in this study (i.e., Daymet, SNODAS).

Coat color monitoring

Coat color was visually estimated from the by-catch hare photographs by one observer, following a standardized protocol for color classification and image quality filtering. Briefly, hares were classified as 1) white when >90% of the body (excluding belly and feet) was white, 2) brown when <10% of the body was white, and 3) molting for all other instances. As per previously developed classification methods (Zimova *et al.*, 2014), we excluded the feet and belly color as these stay white all year in all three areas (Grange, 1932). Because hares cannot be individually identified from photos, we considered photos spaced by >24 hours as independent events, unless different individuals were distinguishable (e.g., two hares in one photo, a hare in a different molt stage recorded within 24 hrs).

In Canada, 121 cameras operated continuously from September 2011 to November 2016, yielding 1,888 independent coat color observations. In Colorado, 206 cameras operated from September 2010 to June 2011 and continuously from January 2014 to August 2017, yielding 2,027 independent observations. In New England, 121 cameras operated continuously from January 2014 to January 2018, yielding 1,608 independent observations.

Statistical analysis

We used R (version 3.4.3) (R Core Team, 2016) for all statistical analyses.

Climate variables

To characterize annual and long-term climate conditions at each camera site, we prepared a set of temperature and snow cover variables relevant to molt phenology (Mills *et al.*, 2018; Zimova *et al.*, 2018). Annual minimum (tmin), and maximum (tmax) temperature during spring and fall was calculated for each year of monitoring (2010-2017) at each camera site using daily gridded 1 x 1 km resolution data (Daymet; Thornton *et al.*, 2018). We used the same dataset to calculate mean seasonal tmin and tmax during the 30-year period 1980-2009 to describe long-term climate at each camera site. The seasons were defined as spring (March 1- May 31) and fall (September 1- November 30), because at all areas the majority of molting occurs during those months.

We used modeled snow water equivalent (SWE) to quantify the duration of the continuous snow season and the total number of snowdays for the spring and fall. For the years of monitoring (2010-2017), we used daily gridded 1 x 1 km resolution data by Snow Data Assimilation (hereafter SNODAS: Barrett, 2003). Because SNODAS is unavailable prior to 2000, we used daily gridded 6 x 6 km resolution data (Livneh *et al.*, 2015) to describe the mean long-term snow conditions during the 30-year period (1980-2009). The duration of spring snow season each year was calculated as the number of days between January 1st and snowmelt date, i.e. the first day when snow is absent (SWE= 0) at a camera site for a minimum of 7 days. Fall snow season was estimated as the number of days following the summer (i.e., the longest snowless period at each camera site each year) between snow onset date (i.e., first day when SWE >0 for a minimum of 7 days) and December 31st. We used the 7-day buffers to discard spurious early spring snowmelts followed by further extended snow season and to account for spurious snow flurries in the fall. The total number of snowdays was calculated as the sum of days with SWE >0 from January 1st to August 31st for the spring and from September 1st to December 31st for the fall.

Molt phenology

We used a hierarchical multinomial logistic regression analysis within a Bayesian framework to describe molt phenology and its phenotypic plasticity. For all models, we estimated the probability of a hare being in color category *i* at a camera site *j* on a Julian day *d* as:

$$\Pr(y = i) = \frac{e^{\alpha_i + \beta 1_i * \mathrm{d} + s_{i,j}}}{1+\sum_{k=1}^{i-1} e^{\alpha_i + \beta 1_i * \mathrm{d} + s_{i,j}}}$$

Coat color was treated as a categorical variable, such that a hare on day *d* was either brown (p_{brown}), white (p_{white}), or molting (p_{molt}) and $\Sigma(p_{1:3,j,d}) = 1$. Camera site was coded as a random covariate $s_{i,j}$ to reflect the hierarchical structure of the dataset and allow for repeated measurements. α_i was the intercept and $\beta 1_i$ was the effect of Julian day on the probability of being either brown, white, or molting. Fall and spring molts were modeled separately.

Spatial variation in molt phenology

First, to quantify average molt phenology of each population (Canada, Colorado, New England), we combined color observations from all years at that area and ran the model separately for each. We used the estimated probabilities to derive approximate dates when hares initiated and completed their molts as ‘initiation’ and ‘completion’ dates at each area. Fall start was specified as the first Julian day when mean $p_{\text{brown}}<0.9$ and end date when mean $p_{\text{white}}>0.9$; the opposite condition was used to estimate the spring dates (i.e., start when $p_{\text{white}}<0.9$ and end when $p_{\text{brown}}>0.9$).

Next, to test the effect of local environmental covariates on phenology, we combined color observations from all years and populations in one dataset and constructed a set of univariate models. Each model included a single fixed effect of an environmental covariate $\beta2_i$ on the probability of being brown, white, or molting. The environmental covariates were latitude, elevation, and the 30-year long-term temperature and snow conditions (i.e., tmin and tmax, duration of snow season) in spring and fall during each season at each camera site. We used univariate models to avoid problems associated with multicollinearity as most environmental covariates were highly correlated (Pearson correlation coefficients > |0.60|; Appendix 4 Table 4.S1). To facilitate comparisons between models, all covariates were standardized to a mean of 0 and SD of 1.

The resulting β coefficients represented the increase in the probability of being in a certain color category on the multinomial-logit scale for every one-unit (SD) change in the covariate. Because we were not interested in quantification of the effect size *per se*, but rather on the direction and the relative sizes of the different covariates, we did not convert the coefficients to normal scale. We primarily focused on the covariate effects on probability of the season's final color (i.e., $\beta2_{\text{white}}$ in the fall, $\beta2_{\text{brown}}$ in the spring) not the initial color or molting color category, in part to simplify the reporting of results. Furthermore, since photoperiod is known to strongly control the hormonal cascade that triggers the molt, we expected the effects of climate to be more apparent in the final rather than the initial stage of the molt (i.e., follicle stimulation and hair growth initiation versus the appearance of the newly grown hairs and shedding of the old hairs; Zimova *et al.*, 2018). Finally, in most cases, the significance or absolute effect size of initial and final color probabilities were similar (all β coefficients shown in Appendix 4 Table 4.S2 and S3).

Temporal variation in molt phenology

Next, to test the effect of annual variation in temperature and snow season duration, we constructed an alternative set of univariate models. Each model included a single fixed effect of climate covariate $\beta2_i$ on the probability of being in a certain color category to avoid multicollinearity issues (Appendix 4 Table 4.S1). The covariates included mean annual tmin, tmax, and duration of snow season in spring and fall at each camera site. The resulting $\beta2_i$ coefficients were the slopes of reaction norms of the climate covariates on probabilities of being brown ($\beta2_{\text{brown}}$) or white ($\beta2_{\text{white}}$).

For all models, we obtained posterior distributions of all parameters along with their 95% credible intervals (CRI) using Markov chain Monte Carlo (MCMC) implemented in JAGS (version 4.0.1), which we called using the R2jags package (Su & Yajima, 2012). Model convergence was assessed using the Gelman–Rubin statistic, where values <1.1 indicated convergence (Gelman & Rubin, 1992). We generated three chains of 300,000 iterations after a burn-in of 150,000 iterations and thinned by three. Parameters α_i, $\beta1_i$, and $\beta2_i$ received a vague prior of N(0, 0.001), and the standard deviation of random effect $s_{i,j}$ received uniform prior of U(0, 100).

Camouflage mismatch

Camouflage mismatch was calculated based on the daily presence or absence of snow and the modeled coat color at each camera site. Snow was present at a camera site when SWE>0 mm, and absent when SWE=0 mm, based on daily gridded 1 x 1 km resolution data (SNODAS, for validation of dataset see Sirén *et al.*, 2018). Next, we defined white hares as when mean $p_{white} \geq 60\%$ and brown hares as $p_{brown} > 60\%$ as these thresholds included mostly white or brown hares, respectively, when compared to observations. The camera days with brown and white hares were calculated using color probabilities from the models that included the best annual climate predictor (effect sizes in Appendix 4 Table 4.S3) in order to account for inter-annual variation in phenology.

To quantify the frequency of camouflage mismatch within each population, we calculated the number of days at all camera sites (camera days) when the color of hares would either match or mismatch against the background color. 'White mismatch' occurred when hares were white and snow was absent at the site. 'Brown mismatch' occurred when hares were brown and snow was present (Mills *et al.*, 2013). 'Match' occurred on days when hares were white (brown) and snow was present (absent). The proportion of white mismatch occurrence, for example, was calculated as the sum of all camera days when hares would experience white mismatch, divided by the total number of camera days (i.e., number of camera sites at each area multiplied by the total number of days in a season). We calculated the mismatch occurrence for the main spring (February 1- May 31) and fall snow seasons (September 1- December 31), that covered the main time periods when mismatch might occur at all three study areas. All proportions were multiplied by 100 for interpretation in %.

RESULTS

Spatial variation in molt phenology

Snowshoe hare molt phenology varied across the three study areas (Fig. 4.1). In the fall, populations in Colorado and Canada initiated fall molts in early October, with some evidence that hares in Canada molted faster (32 days total) than hares in Colorado (42 days;

Fig. 4.1b). The hare population in New England initiated fall molts three weeks later and took the longest to complete (46 days). In the spring, hares in New England initiated the white-to-brown molt first, in late March, followed two weeks later by populations in Canada and four weeks later by the southernmost population in Colorado. The Colorado population took the shortest to complete the transition (44 days) and became brown only 2 and 3 weeks later than the populations in Canada and New England, respectively (Fig. 4.1b).

Variation in molt phenology between populations did not follow the north-south latitudinal gradient as we predicted. Among populations, latitude had a significant effect on the spring molt phenology, but the effect was negative; hares at higher (i.e., northern) latitudes became brown earlier than hares in lower latitudes (Table 4.2). In the fall, latitude had no effect on molt phenology (Table 4.2).

Local climate and elevation were strong predictors of molt phenology and always in the predicted direction in both seasons; earlier fall and later spring molts were associated with areas that are generally colder, snowier and located in higher elevation. Elevation and long-term minimum temperature had the strongest effect on molt phenology in both seasons (Table 4.2).

Temporal variation in molt phenology

We found evidence of temperature- and snow-mediated plasticity in molt phenology. All annual temperature and snow covariates affected molt phenology in the predicted direction; molts occurred later in the spring and potentially earlier in the fall during colder and or snowier years (Table 4.3). In the spring, this annual variation in temperature and snow duration resulted in two- to three-week differences in mean population initiation and completion dates between some years in Canada and New England (Fig. 4.2). In contrast, we found no significant differences between spring initiation and completion dates in the Colorado population. Furthermore, we did not detect any differences in the fall molt phenology start or end dates in any population (Appendix 4 Fig. 4.S3).

Camouflage mismatch

The occurrence of camouflage mismatch varied between study areas and years (Fig. 4.3, Table 4.4, Appendix 4 Fig. 4.S1). White mismatch (white hare against snowless background) was relatively infrequent at all sites and occurred only during low snow years in some areas. Hares in New England experienced the highest frequency of white mismatch during both seasons (Table 4.4), with the highest proportions of camera days with white mismatch in fall 2015 (15%) and in spring 2016 (9%); seasons with 37-40 fewer snow days than observed during 1980-2009 (Fig. 4.3). Brown mismatch was rare in the New England population, with the exception of fall 2016, with 6% brown mismatch (Appendix 4 Fig. 4.S1). Snowshoe hares in Colorado had lower proportions of white mismatch days than in New England but reached 7% in the fall of 2016 with very short snow season (Fig. 4.3). In the springs brown mismatch was more common than white mismatch in Colorado (Table 4.4). In Canada, white mismatch was very rare, exceeding 1% in only 3 out of the 9 seasons of observations (Fig. 4.3), but brown mismatch was frequent (Table 4.4, Appendix 4 Fig. 4.S1).

DISCUSSION

Using by-catch photographs from remote camera traps, we quantified the spatial and temporal variation in snowshoe hare molt phenology and camouflage mismatch across nearly the full range of environmental conditions experienced by the species. To our knowledge, this was the first study to evaluate these processes at such resolution in any seasonally molting species. First, spatial and temporal variation in molt phenology was determined by local climate conditions, not latitude; hares in colder and snowier sites and in higher elevations molt later in the spring and earlier in the fall. Second, hares exhibited temperature- and snow-mediated plasticity in molt phenology; we found strong evidence of later spring- and suggestive evidence of earlier fall-molts during colder and or snowier years. Finally, the occurrence of camouflage mismatch varied in space and time, but white mismatch was more common in areas characterized by shallow, short-lasting snowpack. Our findings collectively suggest that, in those areas, phenotypic plasticity in molt phenology does not compensate against camouflage mismatch during very low snow years, resulting in increased vulnerability under climate change for these populations.

Local climate drives variation in molt phenology

Our analysis of three widely spaced populations showed local climate drives the spatial variation in molt phenology, as opposed to the variation distributed along a latitudinal (i.e., north-south) gradient (Fig. 4.1). We were able to show this because the southernmost study area, Colorado, was as cold as the northernmost area, removing the pervasive correlation between climate and latitude. As a consequence, the effects of climate were strong and in the predicted direction in both seasons, while the effects of latitude were nonexistent or, in the case of spring phenology, in the opposite direction (i.e., during the spring molt, the probability of being brown increased with latitude, Table 4.2). These results show that latitude, although correlated with the proximate phenological cue, photoperiod, cannot be universally used to directly predict molt phenology across a species range. Thus, it appears that at each location, hares have evolved to respond differently to the same photoperiod cue, likely to optimize molt phenology with local climate conditions.

In contrast to latitude, mean phenology in both seasons structured strongly along elevational, temperature, and snow cover gradients. Similar findings were described for other phenological traits across taxa (e.g., migration, reproduction, and hibernation) where phenology correlates with local variation in climate (e.g., Fielding *et al.*, 1999; Sheriff *et al.*, 2011; While & Uller, 2014; Duursma *et al.*, 2018). As for seasonal color molt phenology, local climatic factors have been previously described to determine phenology in color molting species including snowshoe hares (Grange, 1932; Zimova *et al.*, 2014), mountain hare (Watson, 1963), stoats (Feder, 1990), and rock ptarmigan (Salomonsen, 1939), although all were examined over relatively small spatial scales or conclusions were based on opportunistic observations and low sample sizes. Likewise, for multiple color molting species, the global distribution of genetically determined winter brown and winter white coat color morphs is driven by variation in snow cover duration (Mills *et al.*, 2018).

Phenotypic plasticity in response to temperature and snow

We found that annual temperature and snow affects the molt phenology. However, this variation resulted in differences in initiation and completion dates between years only in the spring (Fig. 4.2). For example, hares in Canada became brown 26 days earlier in spring 2015, which was on average 3.6°C warmer (tmax) with a snow season 25 days shorter than spring 2016 (Fig.4. 2, Appendix 4 Fig. 4.S2). Similar differences were observed between the same two years in New England, where the 1°C increase in spring tmax and 23-day decrease in snow season corresponded with a 17- and 13-day advance in molt initiation and completion dates, respectively (Fig. 4.2, Appendix 4 Fig. 4.S2). In contrast, we did not detect any differences between the spring molt phenologies in the Colorado population (Fig. 4.2). However, annual temperature and snow season length were less variable in Colorado than the other two study areas (Appendix 4 Fig. 4.S2). For example, the 11-day difference in snow duration between the two most extreme springs might not have been sufficient to observe significant differences between phenology dates (Fig. 4.2). To determine whether hares in Colorado have lower phenotypic plasticity or whether this finding was caused by lower inter-annual variation will require additional years across a wider range of climatic conditions.

We found some evidence for phenotypic plasticity in the fall molt phenology (Table 4.3), but we did not detect significant differences between initiation or completion dates in any study area (Appendix 4 Fig. 4.S3). We do not think that this is a result of lower annual variation in climate, however, as may be the case above. Indeed, the inter-annual variation in temperature and snow season duration during the course of this study was comparable between spring and fall seasons (Appendix 4 Fig. 4.S2). For example, snow season duration differed by up to 44 days and tmax by 2.3°C between the most extreme falls in New England (Appendix 4 Fig. 4.S2 and 4.S3), yet we observed no differences between molt phenology dates. Furthermore, minimal plasticity in the fall and similar level of phenotypic plasticity in the spring as observed here were previously described for snowshoe hares in the Rocky Mountains in Montana (Mills *et al.*, 2013; Zimova *et al.*, 2014). However, in this study, the number of independent snowshoe hare color observations were lower during the falls than in the spring (Table 4.1), which may have resulted in reduced statistical power to detect differences during this season.

Spatial variation in camouflage mismatch

We observed high variation in the number of snow days each year but, overall, snow cover duration has decreased since the 1980s in all three areas (Fig. 4.4, Appendix 4 Fig. 4.S4). The declines in snow cover manifested differently in each population, however. In the Canadian population, white mismatch was very low each year, while the number of snow days ranged from 1 to 28 fewer days than observed on average during 1980-2009 (Fig. 4.3). This is likely due to the very deep, long-lasting snowpack in the study area (Table 4.1, Fig. 4.4). For example, during the springs of monitoring, the snow season ended on average on June 6 (Fig. 4.4), about two weeks after hares finished their spring molts (i.e., molts initiated on April 5 and completed on May 23; Fig. 4.1b). Therefore, in this study area, even strong declines in snow season duration that may exceed the limits of phenotypic plasticity will not substantially increase the frequency of white hares on snowless background.

In contrast, in Colorado and New England, the proportion of white mismatch increased with fewer snow days, especially once the snow days anomaly exceeded 21 days (Fig. 4.3). Beyond this three-week threshold, hares began to experience elevated white mismatch (i.e., 15% and 9% in New England; 7% in Colorado). This suggests that phenotypic plasticity in molt phenology is insufficient to buffer against the snow declines in those marginal areas where snow season is already short (Table 4.1, Fig. 4.4). To contrast the New England site with the previous example from Canada, the snow season ended on average on April 15 (Fig. 4.4) but hares underwent spring molts from March 23 to May 13 (Fig. 4.1b). Therefore, with mean snowmelt occurring before hares are barely halfway through the molt, early snow melt years (e.g., 2016 seasonal snow melted on April 1; Appendix 4 Fig. 4.S2) result in steep increases in white mismatch.

The pattern observed with white mismatch was somewhat mirrored by that of brown mismatch. In both seasons in New England and during the falls in Colorado, white mismatch was more frequent than brown mismatch as snow cover was more likely to be absent when hares were white than vice versa (Fig. 4.4). In contrast, during springs in Canada and Colorado, hares experienced long periods when they had already molted to summer brown pelage, but snow was still present on the ground (Fig. 4.4). Furthermore, the onset of snow during fall often occurred prior to hares completing their brown to white molts in Canada. Importantly, climate change will lead to shorter snow duration across most of the snowshoe hare range (Easterling *et al.*, 2017; Fyfe *et al.*, 2017) and therefore increasingly favor white over brown mismatch.

The relative fitness costs of white versus brown mismatch are unknown, but we suspect that white mismatch has a higher survival cost than brown mismatch based on our experience in the field while locating radio-collared hares. First, brown animals and objects (e.g., branches, tree trunks, brown animals) are relatively common year-round, but white animals and objects are rare outside of winter. Perhaps due in part to this frequency difference in the two mismatch types, a white hare against a snowless background appears far more conspicuous than a brown hare resting on snow. Previous quantifications of survival costs were carried out for ‘absolute mismatch’, that is both white and brown mismatch combined (Zimova *et al.*, 2016; Wilson *et al.*, 2018). Nonetheless, as documented here and elsewhere (Zimova *et al.*, 2016; Wilson *et al.*, 2018), white mismatch is already high in some populations and will increase under climate change (Mills *et al.*, 2013). Therefore, fitness costs of white and brown mismatch must be quantified to inform conservation efforts, notably *in situ* management actions that foster evolutionary rescue, or genetic rescue by assisted gene flow of individuals with preadapted molt phenologies or winter coat color (Mills *et al.* 2018).

Understanding the spatial and temporal variation in phenological traits is critical for understanding the impact of climate change and species' adaptive potential to environmental stressors. Here we showed that snowshoe hare molt phenology is determined by local climate, but populations vary in their susceptibility to camouflage mismatch. Snowshoe hares responded to annual variation in temperature and snow via some adjustments in molt phenology, but the potential buffering effects of plasticity were diminished in populations distributed along the southern edge of their range. In those areas, characterized by mild climate and shallow, short-lived snowpack, climate change mediated snow declines led to higher phenological mismatch. More generally, our results underscore that populations vary in their susceptibility to environmental stressors and management efforts must consider this intra-specific variation to identify populations most vulnerable under global environmental change.

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TABLES

Table 4.1. Geospatial and long-term climate details regarding the camera trap networks in the study areas. Number of camera sites and of independent coat color observations are given for each season and area. Other values give mean values across all camera sites within a study area with standard deviation in parentheses. Long-term mean minimum (tmin) and maximum (tmax) temperature and snow season duration are based on 1980-2009 period.

			Fall					**Spring**				
Study Area	**Latitude** (degrees)	**Elevation** (m)	**Camera sites** (n)	**Obs.** (n)	**tmin** (°C)	**tmax** (°C)	**Snow season** (days)	**Camera sites** (n)	**Obs.** (n)	**tmin** (°C)	**tmax** (°C)	**Snow season** (days)
Canada	51.38 (0.33)	1850.98 (266.20)	98	963	-5.41 (1.33)	5.22 (1.86)	93.08 (10.93)	91	921	-6.62 (1.68)	5.17 (2.33)	168.69 (18.18)
Colorado	37.63 (0.30)	3200.79 (215.46)	110	308	-4.68 (1.09)	9.70 (1.78)	72.83 (6.91)	183	1,705	-7.24 (1.28)	8.03 (1.71)	155.70 (11.07)
New England	44.54 (0.51)	627.18 (284.59)	65	476	0.25 (0.89)	10.61 (1.63)	52.07 (7.30)	105	1,122	-3.44 (0.93)	8.35 (1.84)	125.01 (12.49)

Table 4.2. Effect of latitude, elevation and long-term climate covariates on snowshoe hare molt phenology. Mean effect sizes and 95% credible interval (CRI) estimates for slopes for univariate models including data from all years and populations combined. Betas indicate effects of covariates on the probability of the molt's final color category ($\beta 2_{brown}$ in the spring, $\beta 2_{white}$ in the fall). Snow is the duration of continuous snow season (days), tmax and tmin are the mean minimum and maximum temperature (°C) in springs and falls during 1980-2009. Asterisks indicate CRIs not overlapping 0. Values reflect standardized data.

Covariate	**Fall $\beta 2_{white}$**	**Spring $\beta 2_{brown}$**
Latitude	0.566 (-0.136, 1.293)	0.689* (0.376, 1.012)
Elevation	2.165* (1.450, 3.033)	-1.325* (-1.631, -1.039)
Snow	0.446 * (0.214, 1.123)	-0.809* (-1.143, -0.492)
tmax	-1.855 (-2.479, -1.288)	0.776* (0.440, 1.123)
tmin	-2.370* (-2.894, -1.909)	1.280* (0.998, 1.579)

Table 4.3. Effect of annual temperature and snow season duration on molt phenology in snowshoe hares. Betas are the slopes of reaction norms β2 (=mean effect size of annual climate covariate) and their 95% credible intervals (CRI) on the probability of the molt's final color category. Asterisks indicate CRIs not overlapping zero. Values reflect standardized data.

Covariate	**Fall $\beta 2_{white}$**	**Spring $\beta 2_{brown}$**
Snow annual	1.466* (1.009, 1.929)	-1.627* (-1.1969, -1.303)
tmax annual	-2.070* (-2.850, -1.432)	1.587* (1.208, 2.003)
tmin annual	-2.344* (-2.943 -1.845)	1.273* (0.921, 1.655)

Table 4.4. Modeled mean percent of camera days with white and brown mismatch at each study area. Mean percent were calculated based on annual estimates (Canada: 6 falls and 5 springs, Colorado and New England: 4 springs and 4 falls). Standard deviations are given in parentheses.

	Canada		**Colorado**		**New England**	
	white	brown	white	brown	white	brown
spring	0.56 (0.55)	11.26 (4.31)	1.74 (1.34)	6.39 (0.68)	2.41 (4.36)	0.98 (0.73)
fall	0.55 (0.47)	9.54 (3.60)	2.69 (2.80)	1.11 (0.66)	4.72 (7.00)	2.42 (2.68)

FIGURES

Figure 4.1. Camera site locations and snowshoe hare molt phenologies and molt dates in the Canada, Colorado and New England study areas. (a) snowshoe hare range is colored and shaded by the mean annual number of snowdays. Colored points represent camera trap sites. (b) bold lines depict predicted probabilities of being in the final color category (brown in the spring and white in the fall) over time based on models without environmental covariates. The dashed lines show 95% credible intervals. The horizontal dashed lines at 0.90 intersect with population means to identify molt completion dates. Population mean molt initiation and completion dates are depicted as a date range in the bottom right corners, with the completion dates in bold. The dates were estimated for each season and population based on the model without covariates.

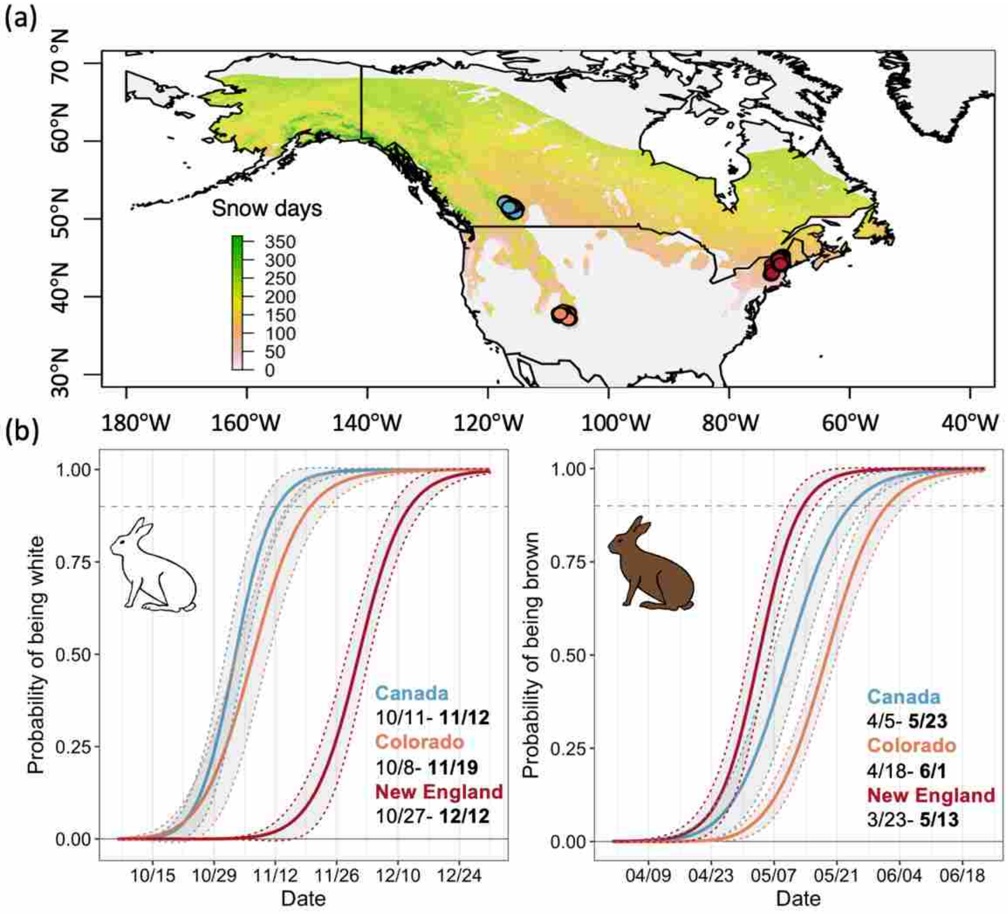


Figure 4.2. Estimated annual spring molt initiation (left) and completion (right) dates in the studied hare populations in Canada, Colorado and New England. Points show mean date estimates and are colored by the annual duration of spring snow season (in days). Horizontal lines show 95% credible intervals (overlapping CRIs identify same dates

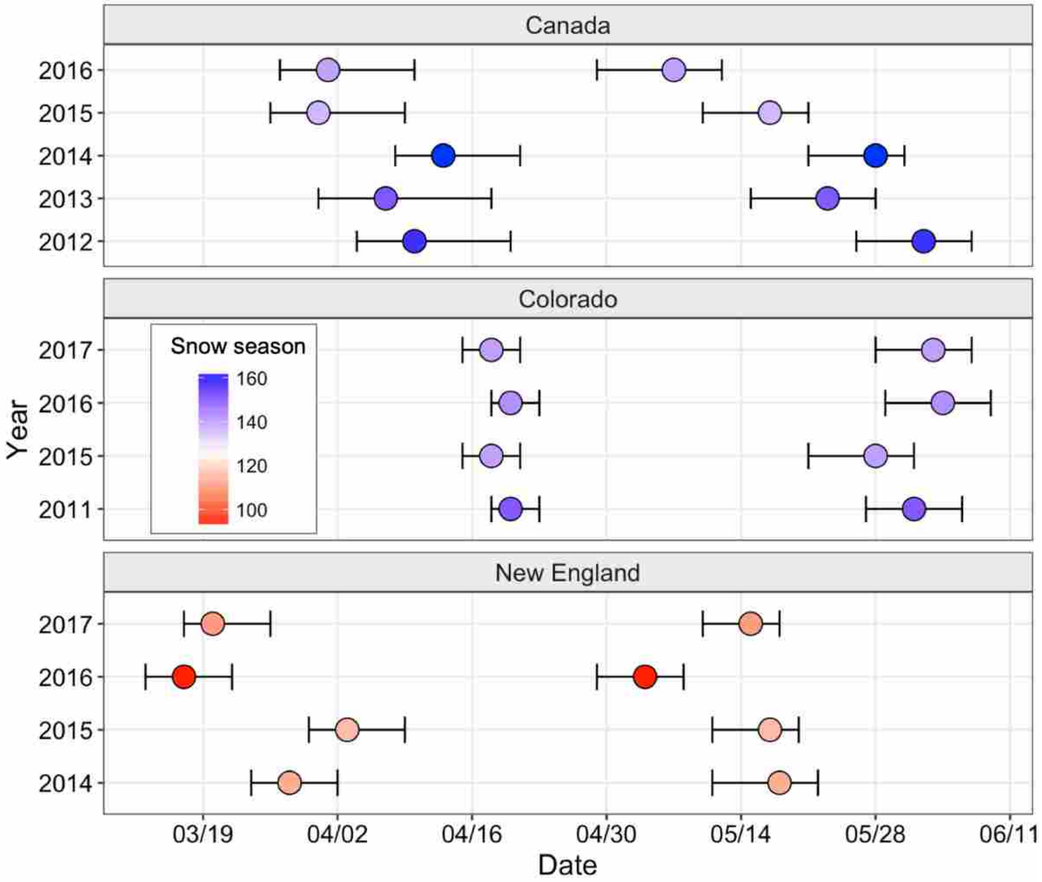


Figure 4.3. Annual proportions of camera days with white mismatch occurrences (in %) plotted against anomalies in the number of snow days each season in Canada, Colorado and New England. Study area-specific anomaly in the number of snow days was calculated for each year as the difference between the mean number of snow days during each season and the mean number of snow days during 1980-2009 at all camera sites. Photo in the corner depicts white mismatched hare.

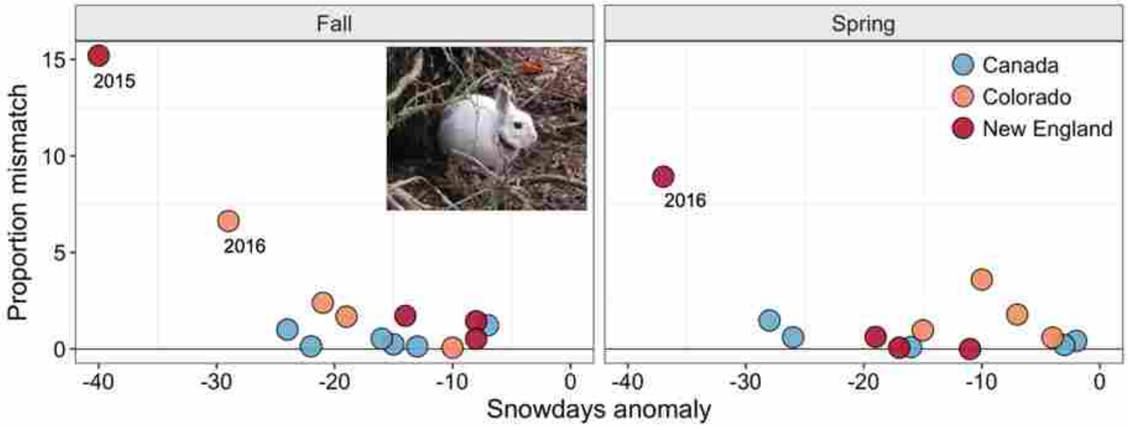
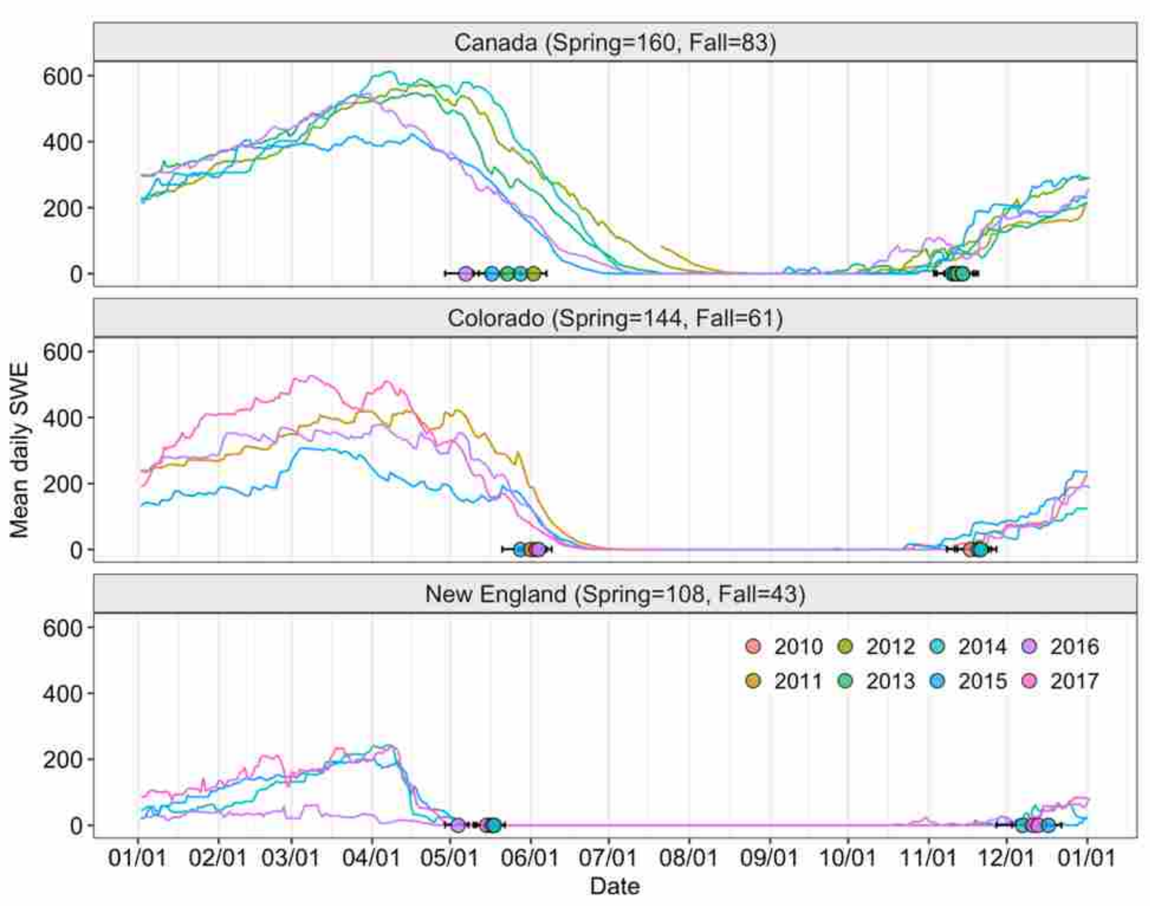


Figure 4.4. Mean daily snow water equivalent (SWE; m) at the remote camera sites for the years of molt phenology monitoring. Colored circles along the x axes indicate the population' mean molt completion dates for each year, with spring molts on the left and fall molts on the right. Mean number of snow days for years of monitoring is given in facet titles for spring and fall.



APPENDIX 4. SUPPLEMENTAL MATERIAL

Figure 4.S1. Annual proportions of camera days with white (red) and brown (gray) mismatch occurrences (in %) plotted against anomalies in the number of snowdays each season in Canada, Colorado and New England. Study area-specific anomaly in the number of snow days was calculated for each year as the difference between the mean number of snow days during each season and the mean number of snow days during 1980-2009 at all camera sites.

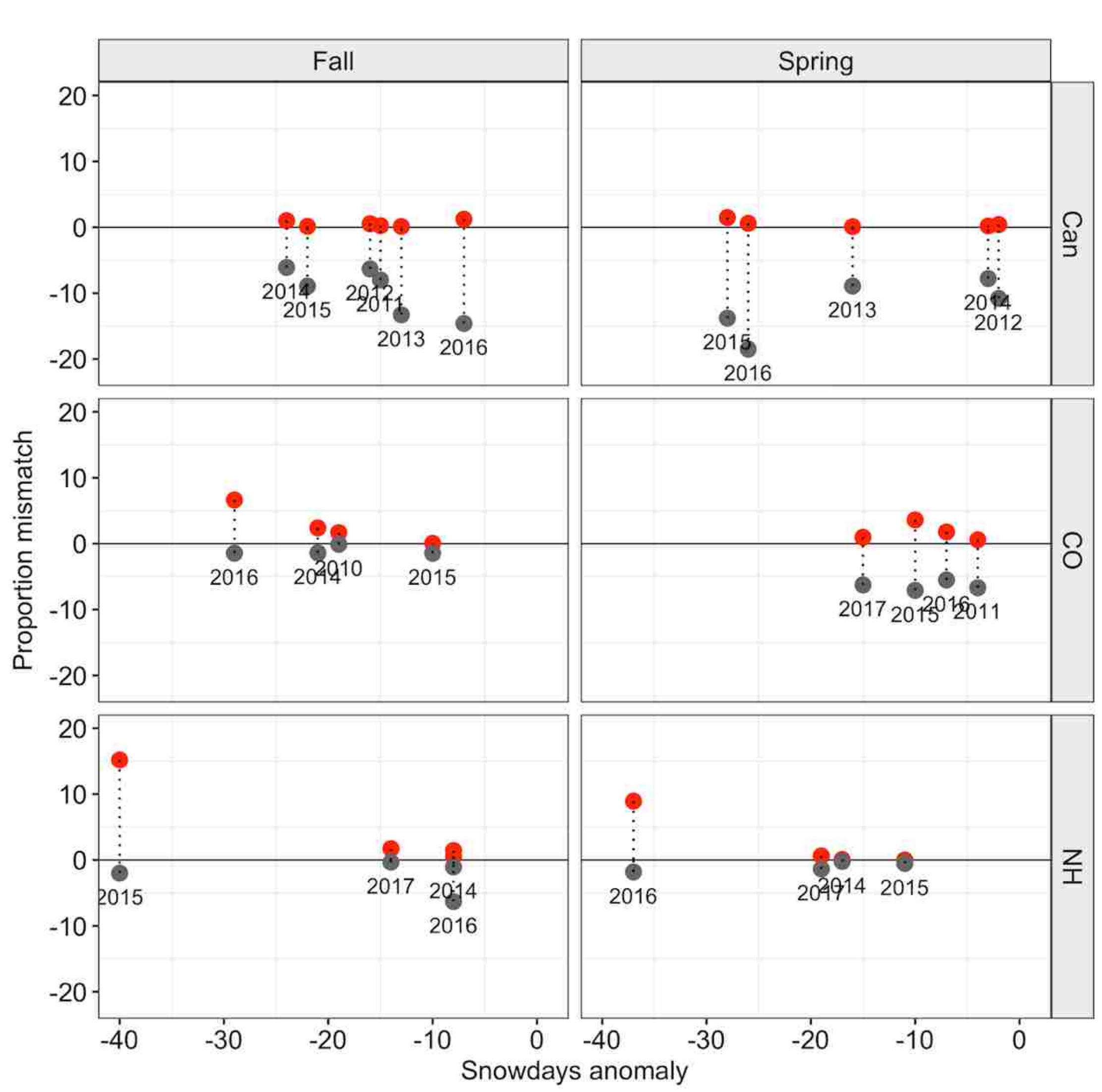


Figure 4.S2. Duration of snow season (days; falls = bottom; spring = top) in (a), and seasonal minimum and maximum temperatures in (b) spring and (c) fall in each area during the years of monitoring and for the 30-year mean (black points; 1980-2009). Annotation near the bottom show standard deviation in the depicted climate variable calculated for the years of monitoring.

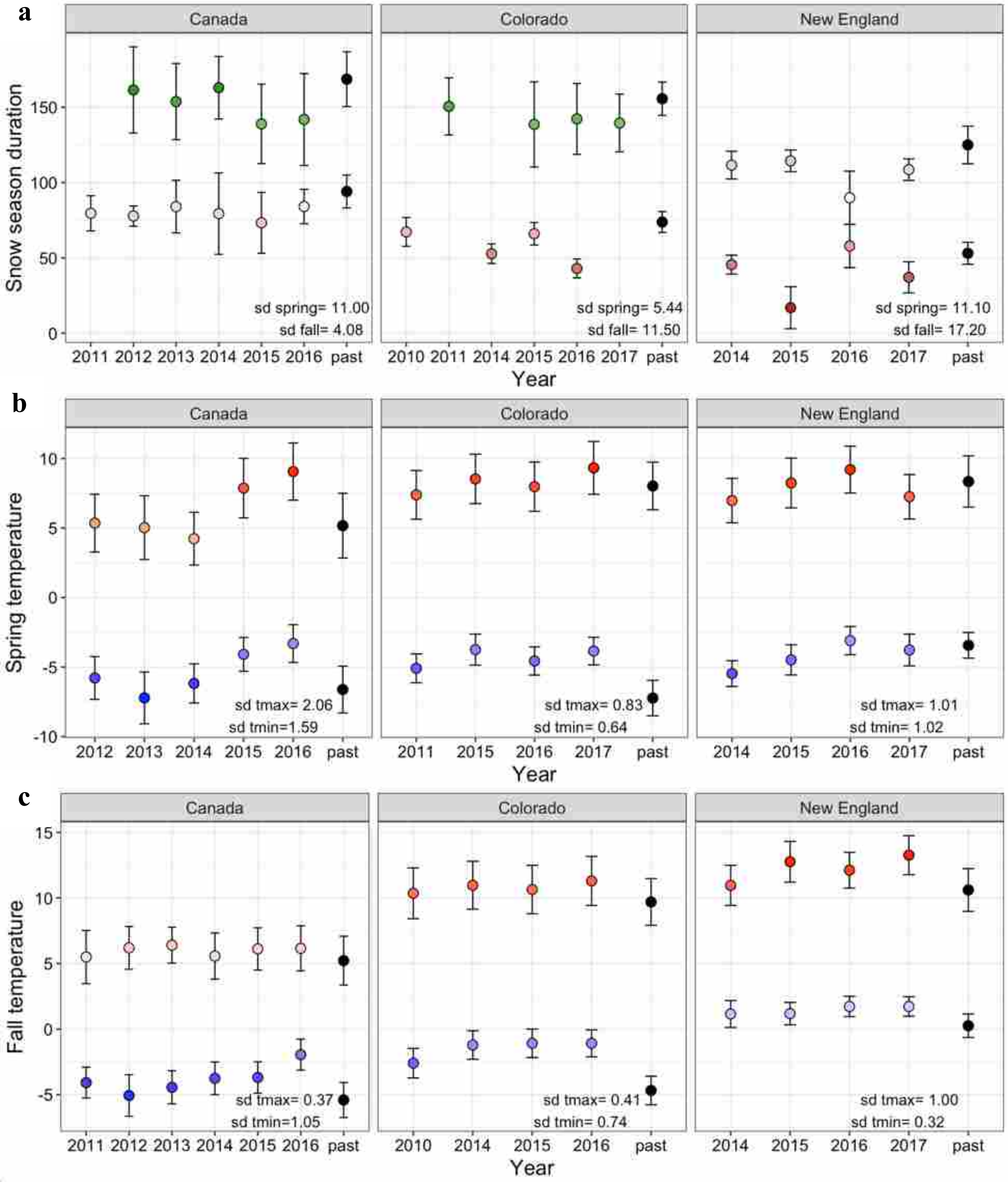


Figure 4.S3. Estimated annual fall molt initiation (left) and completion (right) dates in the studied hare populations in Canada, Colorado and New England. Points show mean date estimates and are colored by the annual duration of fall snow season (in days). Horizontal lines show 95% credible intervals (overlapping CRIs identify same dates).

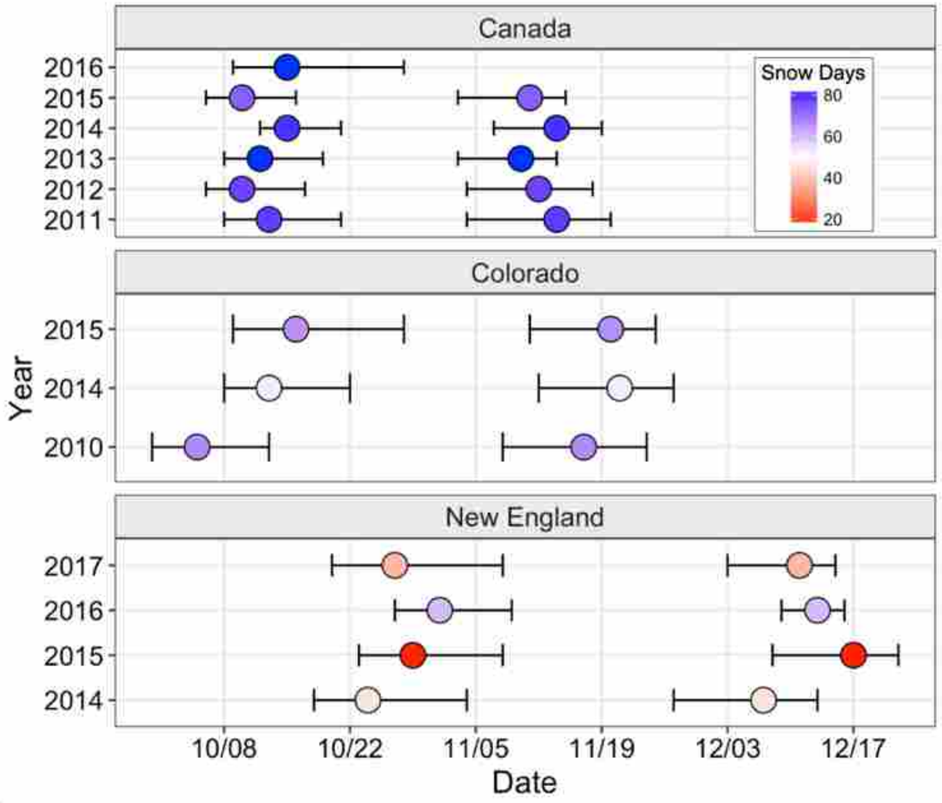


Figure 4.S4. Mean annual number of days with snow on the ground at camera trap sites in the falls (bottom) and spring (top) 1980-2017. The colored points depict seasons of molt phenology monitoring. Vertical lines show standard deviation. Dashed lines show linear regression slopes for each study area. Number in the bottom show estimated snow days reduction in days between 1980-2017.

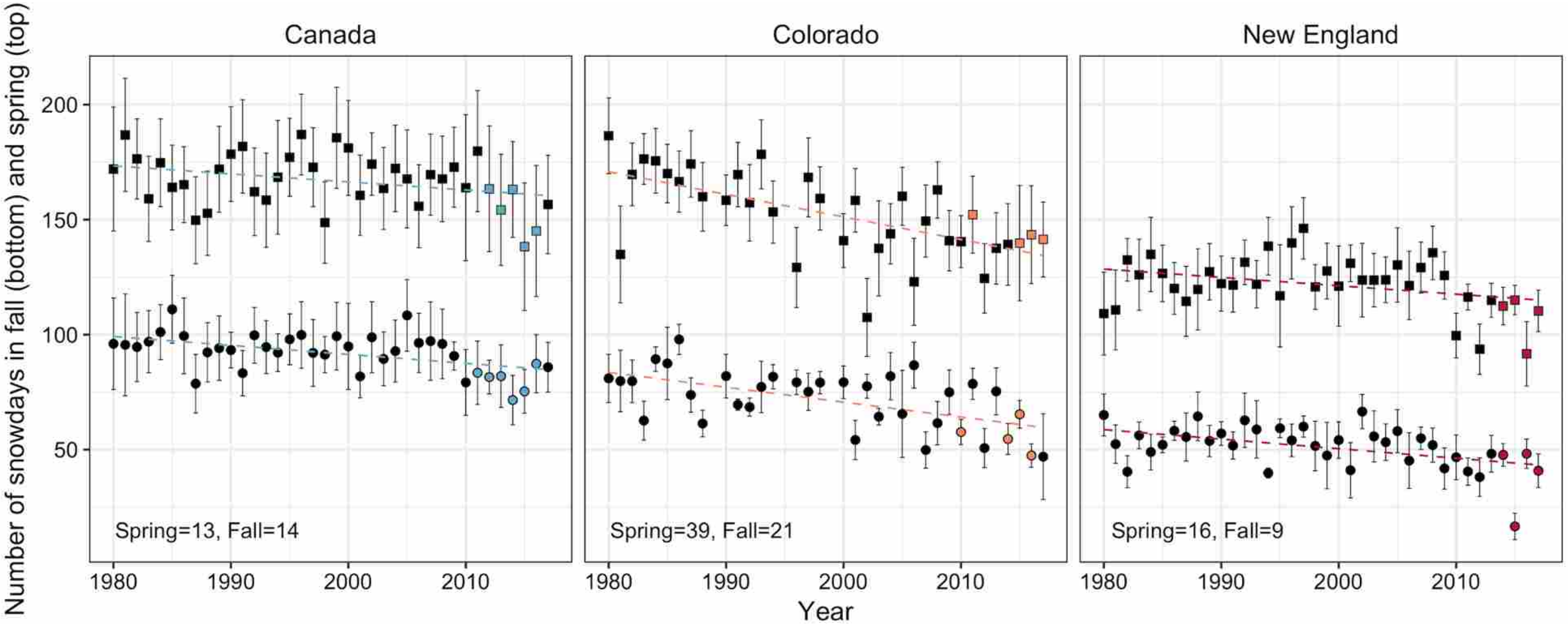


Table 4.S1. Pearson's correlation coefficients between annual minimum (tmin) or maximum (tmax) temperature and seasonal snow duration during the course of the study (2010-2017) and the 30-year period (1980-2009). The coefficients are calculated across all camera sites.

	Fall			**Spring**		
Time Period	tmin x tmax	tmin x snow	tmax x snow	tmin x tmax	tmin x snow	tmax x snow
2010-2017	0.77	-0.68	-0.73	0.78	-0.62	-0.66
1980-2009	0.66	-0.85	-0.83	0.61	-0.74	-0.64

Table 4.S2. Effect of geospatial and long-term climate covariates on snowshoe hare molt phenology. Mean effect sizes and 95% credible intervals (CRI) estimates for slopes for univariate models including data from all years and populations combined. Betas indicate effect of latitude, elevation, duration of snow season, mean seasonal minimum and maximum temperature during 1980-2009 on the probability of brown ($\beta2_{brown}$) and white coat color ($\beta2_{white}$). Asterisks indicate CRIs not overlapping 0. Values reflect standardized data.

Covariate	**Fall $\beta2_{brown}$**	**Fall $\beta2_{white}$**	**Spring $\beta2_{brown}$**	**Spring $\beta2_{white}$**
Latitude	-0.624 (-1.474, 0.165)	0.566 (-0.136, 1.293)	0.689* (0.376, 1.012)	-1.127* (-1.500, -0.764)
Elevation	-1.952* (-2.739, -1.224)	2.165* (1.450, 3.033)	-1.325* (-1.631, -1.039)	1.812* (1.513, 2.140)
Snow	0.752* (0.077, 1.494)	0.446* (0.214, 1.123)	-0.809* (-1.143, -0.492)	0.382* (0.015, 0.746)
tmax	1.693* (1.046, 2.409)	-1.855* (-2.479, -1.288)	0.776* (0.440, 1.123)	-0.546* (-0.938, -0.165)
tmin	2.182* (1.542, 2.976)	-2.370* (-2.894, -1.909)	1.280* (0.998, 1.579)	-1.665* (-2.029, -1.325)

Table 4.S3. Effect of annually varying climate covariates on snowshoe hare molt phenology. Betas are the slopes of reaction norms β2 (=mean effect size of annually varying climate covariate) and their 95% credible intervals (CRI) on probability of being in brown ($\beta2_{brown}$) and white coat color ($\beta2_{white}$). Results are based on univariate models using standardized data. Asterisks indicate CRIs not overlapping zero.

Covariate	**Fall $\beta2_{brown}$**	**Fall $\beta2_{white}$**	**Spring $\beta2_{brown}$**	**Spring $\beta2_{white}$**
Snow annual	1.036* (0.475, 1.618)	1.466* (1.009, 1.929)	-1.627* (-1.969, -1.303)	1.965* (1.637, 2.320)
tmax annual	1.718* (1.063, 2.489)	-2.070* (-2.850, -1.432)	1.587* (1.208, 2.003)	-0.831* (-1.239, -0.434)
tmin annual	2.122* (1.427, 2.944)	-2.344* (-2.943 -1.845)	1.273* (0.921, 1.655)	-1.090* (-1.480, -0.718)

APPENDIX 1

WINTER COAT COLOR POLYMORPHISMS IDENTIFY GLOBAL HOTSPOTS FOR EVOLUTIONARY RESCUE FROM CLIMATE CHANGE

L. Scott Mills[1,2*]†, Eugenia V. Bragina[2]†, Alexander V. Kumar[2,3], Marketa Zimova[2,3], Diana J. R. Lafferty[2,3], Jennifer Feltner[2,3], Brandon M. Davis[2,3], Klaus Hackländer[2,4], Paulo C. Alves[3,5,6], Jeffrey. M. Good[7], José Melo-Ferreira[5,6], Andreas Dietz[8], Alexei V. Abramov[9], Natalia Lopatina[10], Kairsten Fay[2]

[1]Wildlife Biology Program and Office of the Vice President for Research and Creative Scholarship, University of Montana, Missoula, MT 59812, USA.

[2]Fisheries, Wildlife, and Conservation Biology Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA.

[3]Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA.

[4]Institute of Wildlife Biology and Game Management, BOKU - University of Natural Resources and Life Sciences, Vienna, Austria.

[5]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.

[6]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 4169-007 Porto, Portugal.

[7]Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

[8]German Aerospace Center, Earth Observation Center, German Remote Sensing Data Center, Oberpfaffenhofen, Wessling 82234, Germany.

[9]Zoological Institute, Russian Academy of Science, Saint Petersburg 199034, Russia.

[10]Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, 630091, Russia.

†Shared first authorship

ABSTRACT

Maintenance of biodiversity in a rapidly changing climate will depend on the efficacy of evolutionary rescue, whereby population declines due to abrupt environmental change are reversed by shifts in genetically-driven adaptive traits. However, a lack of traits known to be under direct selection by anthropogenic climate change has limited the incorporation of evolutionary processes into global conservation efforts. In 21 vertebrate species, some individuals undergo a seasonal color molt from summer brown to winter white as camouflage against snow, while other individuals remain brown. Seasonal snow duration is decreasing globally, and fitness is lower for winter white animals on snowless backgrounds. Based on 2,713 georeferenced samples of known winter coat color – from 8 species across trophic levels – we identify environmentally driven clinal gradients in winter coat color, including polymorphic zones where winter brown and white morphs co-occur. These polymorphic zones, underrepresented by existing global protected area networks, indicate hotspots for evolutionary rescue in a changing climate.

One Sentence Summary: Multi-species spatial analysis of winter coat color polymorphisms indicate regions of enhanced evolutionary potential to respond to changes in snow duration.

MAIN TEXT

The significance of evolution in fostering the persistence of species facing rapid environmental change is a fundamental tenet of biology that underlies the modern field of conservation biology (*1-3*). Despite the central role of evolution for maintaining biodiversity, criteria to facilitate adaptation by wild species remain largely absent from conservation planning (*4, 5*). This is a particularly acute omission in a rapidly changing climate (*6, 7*) where evolutionary rescue may reverse population declines via adaptive evolutionary change in phenotypes (*8,9*).

As a first step to demonstrate how evolutionary rescue might enter conservation planning for climate change, we describe a fitness-relevant trait that exhibits clines of locally adapted morphs shaped directly by climate. At least 21 bird and mammal species undergo photoperiod-induced seasonal coat color molts from brown to white in some portions of their range to maintain crypsis against seasonal snow presence or absence (Table 1). This seasonal phenological trait is confronting decreased seasonal snow cover duration, one of the most persistent and widespread signals of climate change (*10,11*). Field studies show that winter white animals mismatched against snowless ground suffer a high fitness costs due to increased predator-caused mortality, which in the absence of evolutionary shifts would result in substantial population declines (*12*). In fact, coat color mismatch against decreased snow duration may have already contributed to range contractions for several species (*13-16*).

Although the seasonal brown-white-brown color trait is a classic polyphenism — whereby multiple morphs are produced by a single individual (*17*) — individuals in some populations molt to brown winter coats, thereby not undergoing the circannual color change. This intra-specific variation results in monomorphic winter white and brown populations, but also in polymorphic populations that include sympatric winter white and brown color morphs. Importantly, this phenotypic variation is genetically determined: latitudinal transplants, common garden, and breeding experiments with several seasonal color molting species have consistently showed minimal plasticity in the expression of winter phenotype and instead suggested a simple genetic basis involving one or a few major loci [e.g. (*18-22*)].

The enhanced standing genetic and phenotypic variation fostered by genetically based polymorphisms have long been linked to individual fitness and scope for evolution to rescue populations from abrupt environmental change (*23, 24*). Specifically, color polymorphisms have served as powerful models demonstrating evolution in nature, including iconic examples of evolutionary response to anthropogenic stressors (*25-28*). For the seasonal coat color trait, selection is expected to act on all winter color morphs based on local snow duration, but evolutionary rescue to changing climate should be enhanced by polymorphic regions where both brown and white winter morphs co-occur.

Here we use a hierarchical approach across organismal scales (individual, population, species) to spatially map geographic clines in winter coat color against local climate variables (*29*). We collated georeferenced descriptions of winter coat color from 2,713 specimens spanning 60 countries across species ranges, with data sources including published accounts and specimens at 26 museums globally (Table S1). From these georeferenced winter color morph samples, we built predictive models of winter color phenotypes across geographic ranges for 8 mammal species that span trophic levels: 4 hare species and 4 carnivore species (3 weasels and Arctic fox).

The response variable for our global generalized mixed model was the probability of an individual having a winter white coat, with species as a random effect and fixed effects including climate and landscape-level covariates (Table S2). As expected for a trait under selection for crypsis against snow or bare ground, the most important covariates emerging from the global model were snow cover duration and two climate variables affecting snow seasonality and transience. The probability of being white in winter (as opposed to brown) increased positively with snow duration and with seasonality (ranges of mean monthly temperatures; BIO2), and negatively with isothermality (BIO3; an index of snow transience).

Using the 3 environmental covariates identified in the best-fitting model, we created for each of the 8 species a predictive range-wide map that assigned to each pixel a probability of an individual being white in winter (Fig. 1, Figs S1-S8). Based on five-fold cross validation, models fit georeferenced winter color morph data well (*29*). Across species, clinal gradients in winter color follow expected environmental gradients based on snow duration and ephemerality: winter white morphs were more likely in regions with more persistent snowpack that tended to be more northern, higher elevation, and less maritime (Fig. 1). These results suggest that strong natural selection for camouflage against varying snow duration underlies phenotypic variation in winter color morphs across environmental gradients.

To identify hotspots that foster evolutionary rescue, we converted the continuous probabilities of individuals being winter white (versus brown) into polymorphic zones, using both a narrow (40%<P[winter white]<60%) and broad (20%<P[winter white]<80%) criteria. Depending on the species and criteria, polymorphic zones comprised 1% to 57% of a species range (Table S4). The species with the most widespread polymorphic zones (for Narrow/Broad criteria) are arctic fox (10%/57%), white-tailed jackrabbit (13%/43%), and long-tailed weasel (9%/33%). Mountain hares have the most restricted polymorphic zone (1%/2%).

Given that the clinal gradient of winter color represents fine-tuned adaptation to local snow conditions, how must winter phenotypes shift to adaptively track projected reductions in snow duration? Based on our model, we plotted the current probabilities of being white in winter against snow duration for 4 species (Table S6) to characterize 'optimal' winter coat color as shaped by past selection (Fig. 2). Depending on the species and snow duration, a plausible reduction of 30-50 days of seasonal snow cover during this century (*30*) would require many winter white populations to become polymorphic and polymorphic populations to become winter brown to maintain optimal winter coat colors.

Next, we combined the polymorphic zones of the 8 species to identify regions with multi-species polymorphic zones (Fig. 3). Although under the broad criteria two or more species shared putative polymorphic zones across much of the northern hemisphere (Fig. 3A, B), narrow criteria multi-species polymorphic zones were limited to a few regions in North America (Fig. 3C) and Great Britain (Fig. 3D).

Polymorphic zones within and across these 8 species ranges identify regions that currently hold disproportionately high potential to initiate evolutionary rescue from camouflage mismatch in this fitness relevant trait impacted by climate change. In addition to being hotspots for *in situ* evolutionary rescue, these areas may also facilitate gene flow of adaptive alleles to monomorphic populations (*31, 32*).

While protected areas cover 13% of the world's terrestrial area (*33*), multi-species polymorphic zones are poorly represented by existing protected areas (Table S5). Even under our broad criteria, only 5% of multi-species polymorphic zones occur in the most strict protected areas described by IUCN [categories I and II; (*34*)]; all 6 IUCN categories of protected areas combined embrace only 10% of multi-species polymorphic zones (for the narrowly defined polymorphic zones: 4% fall in strict and 7% in all; Table S5).

The broad geographic ranges of color molting species, and their roles as flagships and strongly interacting predators and prey, amplify the value of understanding how climate mediated evolution may foster their persistence in the face of climate change. Failed adaptation by these species could have indirect impacts that reverberate through their ecosystems. Further, because the co-distributed species that make up the multi-species polymorphic zones represent both predators (e.g., weasels, Arctic fox) and prey (e.g., hares), differential molt responses in different species could exacerbate fitness costs and create cascading co-evolutionary outcomes.

Mismatch in seasonal coat color provides a visual metaphor for how climate change may affect biodiversity, and regions of sympatric winter color polymorphisms identify multi-species hotspots for evolutionary rescue in the face of reduced snow duration. Our framework to identify zones of enhanced potential to initiate evolutionary rescue from climate change could be applied to polymorphisms in other morphological or physiological traits affected by climate change. Identification of hotspots for evolutionary rescue provides novel opportunities to integrate evolutionary processes to conservation planning in a changing climate.

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Table 1. The 21 vertebrate species known to exhibit seasonal coat color molt. The first 8 species are those with sufficient sample sizes of georeferenced winter color phenotype to model range-wide distribution of color morphs. The other 14 species are those known to undergo seasonal coat color change in at least some populations. Species taxonomy follows the IUCN red list.

FAMILY / Species	Origin of sampled specimens		
	Museums	Literature, citizen science, trapping records, etc.	TOTAL
LEPORIDAE			
Snowshoe hare (*Lepus americanus*)	335	132	**467**
White-tailed jackrabbit (*Lepus townsendii*)	130	14	**144**
Mountain hare (*Lepus timidus*)	149	74	**223**
Japanese hare (*Lepus brachyurus*)	8	54	**62**
MUSTELIDAE			
Short-tailed weasel/stoat/ermine (*Mustela erminea*)	623	32	**655**
Long-tailed weasel (*Mustela frenata*)	444	36	**480**
Least weasel (*Mustela nivalis*)	606	30	**636**
CANIDAE			
Arctic fox (*Vulpes lagopus*)	26	20	**46**
OVERALL SAMPLE SIZE:	**2,321**	**392**	**2,713**
OTHER KNOWN COLOR CHANGING SPECIES			
MURIDAE: Siberian [Djungarian] hamster (*Phodopus sungorus*); Collared lemming (*Dicrostonyx groenlandicus*); Wrangel Island collared lemming (*Dicrostonyx vinogradovi*); Palearctic collared lemming (*Dicrostonyx torquatus*); Ungava collared lemming (*Dicrostonyx hudsonius*); Richardson's collared lemming (*Dicrostonyx richardsoni*); Nelson's collared lemming (*Dicrostonyx nelsoni*); Ogilvie mountains collared lemming (*Dicrostonyx nunatakensis)*			
LEPORIDAE: Arctic hare (*Lepus arcticus*), Alaskan hare (*Lepus othus*)			
TETRAONIDAE: Rock ptarmigan (*Lagopus muta*); White-tailed ptarmigan (*Lagopus leucurus*); Willow ptarmigan (*Lagopus lagopus*)			

Figure 1. Clinal variation in winter color phenotypes for 6 mammal species. Colder colors (e.g., blue) indicate higher probability of winter white morphs (denoted by photo of a winter white snowshoe hare); warmer colors (e.g., orange) indicate higher probability of winter brown morphs (denoted by brown snowshoe hare); and greenish/yellow colors indicate polymorphic populations (See Figs S1-S8 for larger versions of these maps and for maps of Arctic fox and mountain hare). [Photo credits: L.S. Mills research archives].

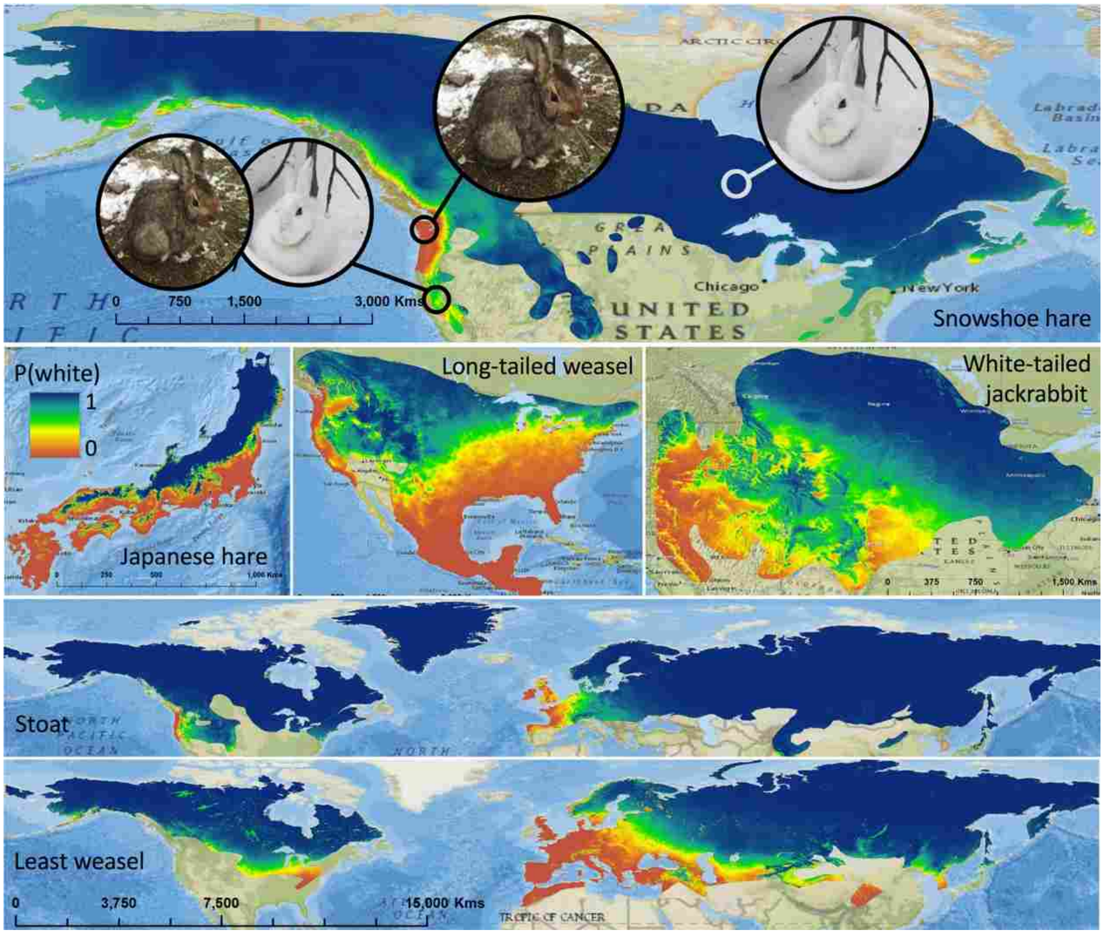


Figure 2. Change in probability of being winter white as snow duration changes for 4 molting species (Japanese hare: dark blue, white-tailed jackrabbit: light blue, least weasel: yellow, long-tailed weasel: red). The central colored area with both winter white and brown animals represents our broadly defined polymorphic zone (i.e., 20%<P[winter white]<80%).

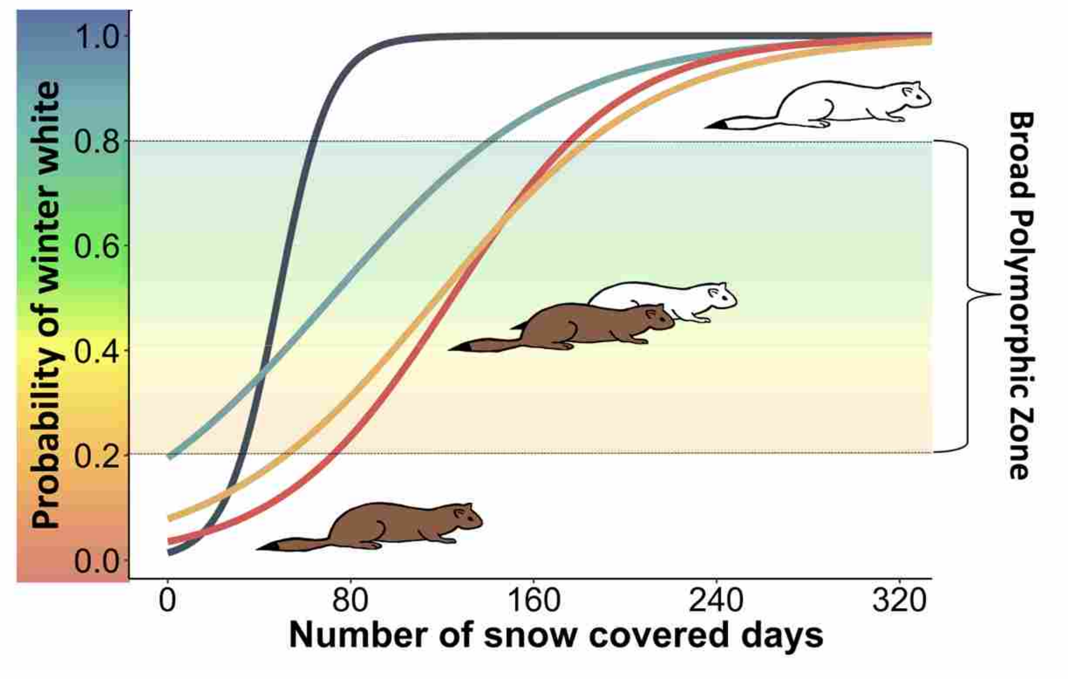
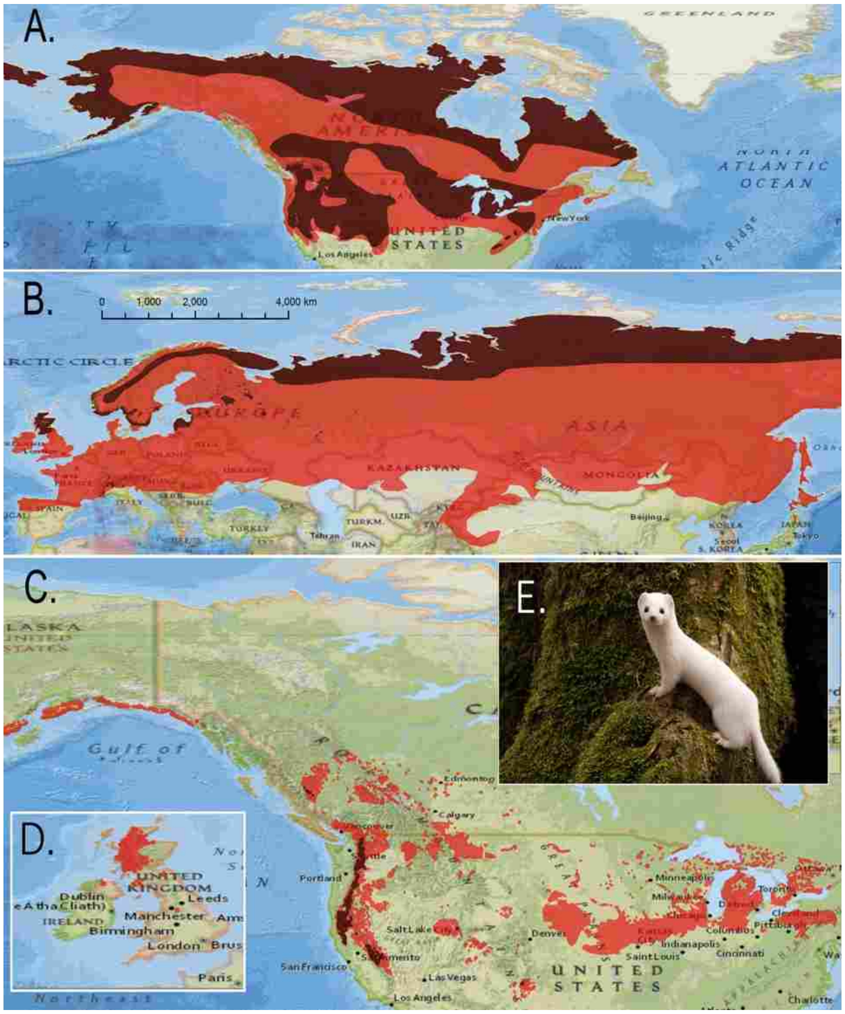


Figure 3. Regions with polymorphisms in winter coat color for multiple species. Panels indicate where polymorphic zones overlap for two (red) or three (brown) species, derived from predictive maps for 8 species (see Fig. 1). Polymorphic zones defined broadly as 20%<P[winter white]<80% in (A) North America and (B) Eurasia. (C) Polymorphic zones defined more narrowly as 40%<P[winter white]<60%; found only in North America and (D) Great Britain. (E) Example of camouflage mismatch [least weasel photo credit: Karol Zub]; in polymorphic zones as snow duration decreases, mismatched winter white morphs would be selected against in favor of the sympatric winter brown morphs.



APPENDIX 1.1-A

SUPPLEMENTARY MATERIALS FOR WINTER COAT COLOR POLYMORPHISMS IDENTIFY GLOBAL HOTSPOTS FOR EVOLUTIONARY RESCUE FROM CLIMATE CHANGE

This PDF file includes:

Materials and Methods
Tables S1 to S6

Other Supplementary Materials for this manuscript includes the following:

Figures S1 to S8
Database S1 as zipped archive: Geolocations of analyzed specimens

Materials and Methods

Experimental Design

We defined seasonally color molting species as those that -- in at least some part of their range -- exhibit fur that is predominantly brown (or greyish brown) in summer and that molt to fully or mostly white in the winter. This definition excludes species that: a) may be white, but not seasonally molting (e.g. polar bears *Ursus maritimus* and white Bengal tigers *Panthera tigris tigris* (*35*); b) have seasonal color molts in minor body parts (e.g. legs only); or c) undergo winter molts to non-white colors (e.g. the South American murine rodent *Calomys laucha* that molts from orange/brown in summer to dark gray during winter (*36*)). Anecdotal reports exist for other species that may undergo seasonal color molts, as we define it, in some parts of their range (e.g. European hares [*Lepus europeus*], Peary caribou [*Rangifer tarandus pearyi*]) but we were unable to confirm these and so they were not included.

Based on these criteria we identified 22 seasonally color molting species from 5 families (Table 1). We attempted to collate georeferenced descriptions of winter coat color for all species across their ranges. Sources included published and 'gray' literature, and observed museum specimens (Table S1 for Museum Locations). For the purpose of this study, we defined winter as between December 1 and March 31. We applied this temporally conservative designation to minimize the potential inclusion of false-positive polymorphic data points (i.e., winter white animals in spring transitioning to summer brown coats and thereby leading to false attribution of winter brown phenotype). Each winter coat color sample was designated as either white (including >60% white) or brown (including >60% brown/grey). We excluded data points far out of known species range, errors that occurred from factors such as mislabeled geolocations on museum tags or input errors, long-distance migrants, or the inclusion of specimens likely to have been translocated [common in hares] or kept domestically [common in Arctic foxes].

Although we attempted to collect data for all 22 species, only 8 species had at least 10 georeferenced individuals of both brown and white winter morphs (Table 1). Therefore, we limited our statistical analysis to these 8 species: Snowshoe hare *Lepus americanus*, White-tailed jackrabbit *L. townsendii*, Mountain hare *L. timidus,* Japanese hare *L. brachyurus*, Stoat *Mustela erminea*, Long-tailed weasel *M. frenata*, Least weasel *M. nivalis*, Arctic fox *Vulpes lagopus*. We used spatial data for species ranges from the IUCN database (http://www.iucnredlist.org).

To describe winter coat color phenotypes across species ranges from these samples, we built a generalized mixed model including 7 covariates a priori expected to affect winter coat color. Snow duration in winter determines duration of coat color mismatch, and in snowshoe hares has been shown to directly influence the color molt phenology (*37*). We derived snow cover duration as a covariate for each data point based on the Global SnowPack product of the German Aerospace Center (www.DLR.de/eoc; (*38*)) for all hydrological years (Sept. 1 – Aug. 31) between 2000/2001 and 2014/2015; these estimates are based on the Normalized Difference Snow Index (NDSI) and the Moderate Resolution Imaging Spectroradiometer (MODIS) operational snow cover products (MOD10A1 and MYD10A1 (*39*)). Gaps in the MOD10A1/MYD10A1 snow cover information caused by cloud cover or polar darkness have been interpolated according to the Global SnowPack processing scheme (*38*) to provide daily estimates of the global snow cover extent at 500 m spatial resolution.

In addition to snow cover duration, seasonal coat color is expected to be affected by transience or ephemerality in snow cover (e.g., how often snow cover appears then disappears as a function of variability in temperature and precipitation). As covariates for transience or ephemerality in snow cover we used the following variables from the Bioclim data set ((*40*) [http://www.worldclim.org]; see also (*41*)): BIO2 = Mean Diurnal Temperature Range (average monthly max temp - min temp); BIO3 = Isothermality in Diurnal Temperature (BIO2/Temperature Annual Range); BIO15 = Precipitation Seasonality (Coefficient of Variation). Although the Bioclim data set has a total of 19 climate variables plus monthly minimums and maximums, all others lacked reasonable *a priori* connection to winter coat color mismatch, and/or were correlated ($r > 0.65$) with the snow duration or transience covariates.

Finally, we included other landscape-level covariates that might be expected to affect the duration and/or transience of seasonal snow duration: altitude (highly correlated with topographic roughness), distance from a coast (indexing maritime vs continental climate conditions), and human density (accounting for potential urban heat islands affecting local snow duration and for particular phenotypes being more carefully recorded near urban areas; indexed from Global Human Influence Index, v2 (1995–2004): http://sedac.ciesin.columbia.edu/data/set/wildareas-v2-human-influence-index-geographic/data-download). Latitude was not included because it was highly correlated (r>0.65) with Isothermality (BIO3).

Statistical Analysis

We fit a generalized linear mixed model of brown (or mostly brown) and white (or mostly white) winter phenotypes to the global set of covariates, including species identity as a random effect, using the package 'lme4' (*42*) in Program R (version 3.3, R Development Core Team 2016). The model response variable was probability of an individual having a white coat color during winter. We standardized all covariates to a mean of 0 and a standard deviation (SD) of 1 to facilitate comparisons between models. Because we were interested in the main effects, we did not include interactions among covariates. A random effect for species identity was included to absorb a known cause of variation. We also included spatial correlation structure, but it did not improve the Akaike information criterion (AIC) so we proceeded without it.

We used three methods to evaluate the 'best' covariates in the global model for predicting global distribution of winter coat color phenotypes (Table S2). First, the P-values of coefficients for Snow Duration, Bio2, and Bio3 were <<0.001, while the others had P>0.05. Similarly, the odds ratios expressed on the real scale after one SD increase in each covariate also identified the same 3 covariates as having 95% confidence intervals not overlapping 1.0. Third, we ran all possible models (N=5,040) containing each covariate (without interactions) and summed the cumulative AIC support for the models containing each covariate; the same snow-related covariates had 100% AIC weight.

The 3 identified top covariates (snow duration and 2 covariates related to snow ephemerality) have intuitive connections to a trait presumed to be under selection to minimize camouflage mismatch against the presence or absence of snow. The probability of being white in winter (as opposed to brown) increased positively with longer snow duration and wider ranges of mean monthly temperatures (BIO2), and negatively with isothermality (BIO3, where lower values imply greater annual temperature oscillations compared to day-to-night oscillations).

Next, we developed species specific maps of predictive probabilities of an animal to be white using the 3 covariates identified in the global model: (snow duration, Bio2, and Bio3) using R package 'raster'. Based on the results of the species specific generalized linear mixed models, each cell of the map for each species was assigned a probability for an individual to be white (0-1) (Figures S1-S8).

To further explore model selection and evaluate model fit at the species level we performed a five-fold cross validation for each species-specific model (*43, 44*). Data were divided into 5 groups, 5 times; models were built with 4 groups, with the 5th group used as the ‘observed’ data. Each ‘observed’ sample was designated as either correctly or incorrectly classified by the predictive map (where predicted values <0.50 were considered ‘brown’ and >0.50 ‘white’). We first compared percentage correct classification from the full model having 7 covariates (Snow, BIO2, BIO3, BIO15, Alt, Dist to shore, Human footprint) to the reduced model with only the covariates identified as significant (Snow, BIO2, BIO3). The reduced model performed better than the full model for 7 species (and only 5% worse for the 8th species), providing further support for the 3-covariate model as a parsimonious alternative to the full model (Table S3).

The five-fold cross validation also provided a measure of fit of the actual winter phenotype samples to the predicted winter phenotypes derived from each species-specific model (Table S3). For 7 of 8 species, ≥ 80% of the winter coat color samples were correctly classified by their species-specific model (Arctic fox had a 70% accuracy).

To explicitly map polymorphic zones for each species, we converted the continuous probabilities of winter white into categorical polymorphic zones. Because no a priori categorical thresholds exist, we used both a broad (20%<P[winter white]<80%) and narrow (40%<P[winter white]<60%) criteria.

To analyze protected areas versus multi-species polymorphic zones (Table S5), we overlaid maps of multi-species zones with IUCN World Database on Protected Areas obtained from http://protectedplanet.net.

We plotted how winter color morph (brown to white) changes with snow duration (Fig. 2 in Main Text) for those species with at least 5% monomorphic range of each winter color morphs (i.e. ratio of predicted brown area / predicted white area >5%). Table S6 gives the ratio of predicted monomorphic brown area in a species geographic range divided by predicted monomorphic white area, where designated ‘brown’ and ‘white’ monomorphic areas are defined outside the narrow and broad polymorphic zone definitions (Table S4). For each species, we fit generalized linear models predicting the probability of being winter white for various snow cover levels. Each model used species-specific mean values of BIO2 and BIO3.

Table S1. Museums visited to collect georeferenced winter coat color samples for this project. (Full Dataset and metadata are in External Database).

MUSEUM NAME	LOCATION	CODE	# of SAMPLES	ACCESS DATE	PRIMARY COLLECTOR
North Carolina Museum of Natural Sciences	Raleigh, NC	NCS	13	Nov 2015	K. Fay
North Carolina State University Collections	Raleigh, NC	NCSM	13	Nov 2015	K. Fay
Smithsonian National Museum of Natural History	Washington, D.C	USNM	1080	Dec 2015	K. Fay, L. S. Mills, M. Zimova, E. Bragina, D. Lafferty, M. Jones
American Museum of Natural History	New York, NY	AMNH	204	May 2016	K. Fay
Yale Peabody Museum	New Haven, CT	YPM	35	May 2016	K. Fay
Harvard Museum of Comparative Zoology	Cambridge, MA	MCZ	279	May 2016	K. Fay
Denver Museum of Natural Sciences	Denver, CO	DMNS	77	Jun 2016	K. Fay
University of Colorado Museum of Natural Sciences	Boulder, CO	UCM	63	Jun 2016	K. Fay
Museum of Vertebrate Zoology	Berkeley, CA	MVZ	32	Mar 2016	J. Good
California Academy of Sciences	San Francisco, CA	CAS	42	Jun 2016	K. Fay
UW Burke Museum	Seattle, WA	UWBM	51	Jul 2016	K. Fay
Natural History Museum of Los Angeles	Los Angeles, CA	LACM	70	Jul 2016	J. Dines
University of Iowa Museum of Natural History	Iowa City, IA	UIMNH	16	Mar 2016	E. M. Fouts, C. Optiz
University of Wyoming Museum of Vertebrates	Laramie, WY	UWYMV	23	Jun 2016	B. Wommack, T. Berry
Central Michigan University Museum of Cultural and Natural History	Mount Pleasant, MI	CMU	22	Mar 2016	A. M. Riedel, K. Nicholson
Science Museum of Minnesota	St. Paul, MN	SMM	1	Mar 2016	D. Oehlenschlager
Slater Museum of Natural History	Tacoma, WA	PSM	12	2016	M. Jones

Hungarian Natural History Museum	Budapest, Hungary	HNHM, SMNH	39	2016	S. Lado
Senckenberg Natural History Museum (Naturmuseum Frankfurt),	Frankfurt, Germany	SMF	35	May 2016	S. Lado
Zoological Research Museum Alexander Koenig	Bonn, Germany	ZFMK	108	May 2016	S. Lado
Natural History Museum Vienna (Naturhistorisches Museum Wien),	Vienna, Austria	NMWf	131	May 2016	S. Lado
Natural History Museum Berlin (Museum fur Naturkunde)	Berlin, Germany	ZMB	95	Jun 2016	S. Lado
Swedish Museum of Natural History	Stockholm, Sweden	SMNH	309	June 2015	M. Zimova, J. Melo-Ferreira
Siberian Zoological Museum of the Institute of Animal Systematics and Ecology	Novosibirsk, Russia	SZM	9	July 2016	N. Lopatina
Zoological Institute of the Russian Academy of Sciences	Saint Petersburg, Russia	ZIN	43	June 2016	A. V. Abramov
Zoological Museum of Moscow University	Moscow, Russia	ZMMU	67	May 2015	E. Bragina

Table S2. Contributions of seven covariates (see above) used in the full all-species, all-covariate model to predict probability of having a white winter coat across the geographic range of eight species. The top covariates (in bold) were used in the species-specific predictive models discussed in text.

Covariate	Cumulative AIC weight of models containing covariate	Odds of being white after one standard deviation increase in covariate (CI)
Snow Cover Duration	**1.00**	**2.52 (2.08-3.07)**
Bio_2	**1.00**	**3.52 (2.81-4.43)**
Bio_3	**1.00**	**0.15 (0.11-0.20)**
Bio_15	0.65	0.87 (0.75-1.00)
Altitude	0.85	1.22 (0.99-1.52)
Distance to Shore	0.53	1.18 (0.95-1.47)
Human Footprint	0.28	0.98 (0.86-1.11)

Table S3. Performance of species-specific models predicting probability of being white in winter. On average across species, 86% of samples of known winter coat color were correctly assigned by the model as winter white or brown. Further, the reduced set of three covariates (See Table S2) performed better than the full set of 7 covariates for all but one species.

Species	% correctly classified points with full model	% correctly classified points with reduced model	Difference between full model and reduced model
Lepus americanus	86%	**(88%)**	-2%
Lepus townsendii	74%	**(80%)**	-4%
Lepus timidus	88%	**(83%)**	5%
Lepus brachyurus	87%	**(92%)**	-5%
Mustela erminea	88%	**(90%)**	-2%
Mustela frenata	84%	**(85%)**	-1%
Mustela nivalis	84%	**(86%)**	-2%
Vulpes lagopus	59%	**(70%)**	-11%

Table S4. Percent of each species range that occurs in putative polymorphic zones.

“Broad” polymorphic zone is defined by pixels with 20%<P[winter white]<80%, “Narrow” polymorphic zone as: 40%<P[winter white]<60%.

Species	Species Range Area (km^2)	Narrow Polymorphic Zone (km^2)	Broad Polymorphic Zone (km^2)	% Range in Narrow Polymorphic Zone	% Range in Broad Polymorphic Zone
Lepus americanus	9536004	150553	647560	1.6%	6.8%
Lepus townsendii	3453826	452320	1479383	13.1%	42.8%
Lepus timidus	20104174	155032	415106	0.8%	2.1%
Lepus brachyurus	286652	12232	41293	4.3%	14.4%
Mustela erminea	41516176	501821	1921866	1.2%	4.6%
Mustela frenata[1]	10800483	934493	3552130	8.7%	32.9%
Mustela nivalis	43721329	2446545	8264492	5.6%	18.9%
Vulpes lagopus	10804080	1128626	615978	10.4%	56.9%

[1] Species Range Area for *Mustela frenata* calculated only for the northern hemisphere. The entire species range area (including the southern hemisphere) is 12,271,542 km^2.

Table S5. Area and percentage of overlap between IUCN protected areas and multi-species (2 or more) polymorphic zones described using either a narrow (40-60%) or broad (20-80%) criteria. Our three groups of protected areas included IUCN categories I&II as strictly protected i.e. national parks and strict nature reserves, III&IV categories as less protected (natural monument, species management areas etc.) and V&VI as least protected (e.g. protected areas with sustainable use of natural resources (*45, 46*)).

Total area of multi-species 40-60% ACME zone (Narrow definition) =384,593 km^2

Protected Area category	**Area of overlap (km^2)**	**Percent overlap**
I&II	14,732	3.8%
III&IV	3,222	0.8%
V&VI	7,667	2.0%
Total	25,621	6.7%

Total area of multi-species 20-80% ACME zone (Broad definition) =39,366,162 km^2

Protected Area category	**Area of overlap (km^2)**	**Percent overlap**
I&II	1,822,761	4.6%
III&IV	1,175,164	3.0%
V&VI	1,043,315	2.7%
Total	4,041,241	10.3%

Table S6. Ratio of range predicted to be monomorphic winter brown divided by range predicted to by monomorphic winter white. Monomorphic white and brown areas are those outside the narrow and broad polymorphic zone definitions. Species with a highly skewed (<5%) ratio of brown / white were not included in Figure 2.

Species	brown/white Area (Narrow Polymorphic Zone)	brown/white Area (Broad Polymorphic Zone)
Vulpes lagopus	*2.9%*	*0.00%*
Lepus timidus	*0.2%*	*0.02%*
Mustela erminea	*2.0%*	*0.6%*
Lepus americanus	*1.5%*	*1.0%*
Mustela nivalis	24.7%	18.6%
Lepus townsendii	33.0%	21.3%
Lepus brachyurus	124.0%	122.6%
Mustela frenata	126.8%	140.0%

Author Contributions:

LSM conceived the idea, helped with analysis, and led the writing. EB, along with AVK, led the data analysis, contributed conceptually, and helped with writing. MZ helped with data analysis, figures, writing, and contributed conceptually. DJRL, JF, BMD, KH, PCA, JMG, and JMF contributed conceptually and with writing. AD, AVA, NL, and KF helped provide key input data. All authors read and approved the submitted manuscript.

APPENDIX 2

SNOW-MEDIATED PLASTICITY IN PHENOLOGY DOES NOT PREVENT CAMOUFLAGE MISMATCH

Alexander V. Kumar[1,2,3], Marketa Zimova[1], James R. Sparks[4], L. Scott Mills[2,5]

[1]Wildlife Biology Program, University of Montana, Missoula, MT 59812

[2]Department of Forestry and Environmental Resources, Program in Fisheries, Wildlife and Conservation Biology, North Carolina State University, Raleigh, NC 27695-7617

[3]Corresponding author; phone: 978 382 1342; email: avk36@cornell.edu

[4]Mssoula Field Office, Bureau of Land Management, Missoula, MT 59804

[5]Wildlife Biology Program and Office of the Vice President for Research and Creative Scholarship, University of Montana, Missoula, MT 59812

ABSTRACT

Global reduction in snow cover duration is one of the strongest outcomes of climate change. This loss of snow has severe negative consequences for seasonally color molting species, such as snowshoe hares (*Lepus americanus*) that rely on snow for crypsis. However, phenotypic plasticity may provide an avenue for hares to adapt to reduced snow duration. Plastic responses could occur in the color molt phenology or through hare behavior minimizing mismatch or its consequences. We quantified molt phenology of 200 wild snowshoe hares and measured microhabitat choice and local snow cover in Montana. Hares did not use behavioral plasticity to minimize coat color mismatch via background matching; instead they preferred snow free (brown) areas regardless of coat color, likely for thermoregulatory reasons. Furthermore, hares also did not adjust their behaviors to mitigate the costs of mismatch on survival by choosing denser vegetation for

resting areas. Importantly, we did find snow-mediated plasticity in both the initiation and the rate of the molt; greater snow cover was associated with whiter hares even though hares selected for non-snowy areas. However, the plasticity in average molt phenology in years of differing snow duration was insufficient to eliminate camouflage mismatch with white hares on brown snowless ground. We conclude that phenotypic plasticity is unlikely to facilitate adaptive rescue to camouflage mismatch under climate change.

INTRODUCTION

The detrimental effects of climate change on wild animal population persistence may be ameliorated by adaptation (Hoffmann and Sgró 2011). This adaptive rescue may occur through both evolutionary rescue via natural selection (Vander Wal et al. 2013) and ecological rescue via phenotypic plasticity (Ghalambor et al. 2007). Although both mechanisms are possible and can interact (Forsman 2015), phenotypic plasticity may provide the most rapid adaptive response in the face of climate change (Gienapp et al. 2008, Beever et al. 2017, Snell-Rood et al. 2018). Climate change induced plasticity in the timing of life history events has been demonstrated in various taxa including ground squirrels (Lane et al. 2012), flycatchers (Przybylo et al. 2000) and marmots (Ozgul et al. 2010). However, the ability of plasticity to sufficiently adjust life history events to maintain fitness under climate change is not well understood.

The effects of climate change on seasonal phenological processes can occur through abiotic drivers such as temperature (Both and Visser 2001, Kudo and Ida 2013) and precipitation (both rain (Penuelas et al. 2012) and snow (Lane et al. 2012)). Though snow is less studied as a driver of phenological processes, it is particularly relevant because a reduction in number of days with snow cover is one of the strongest signals of

climate change in the northern hemisphere (Brown and Mote 2009, Choi et al. 2010, Pederson et al. 2011). For the approximately 20 animal species worldwide that undergo circannual seasonal coat color molts from brown to white to match their background, reduced snow duration can increase camouflage mismatch, a striking and direct climate change stressor (Mills et al. 2013, 2018, Zimova et al. 2018). One such species that undergoes seasonal color molts is the snowshoe hare (*Lepus americanus*), (Grange 1932, Severaid 1945, Nagorsen 1983) an important prey species for a plethora of carnivores including the Canada lynx (Lynx canadensis), a threatened species in the USA.
Absent adaptive responses, color contrast (mismatch) resulting from white winter hares against a brown snowless background is predicted to increase substantially over the next century (Mills et al. 2013), with fitness consequences expected to lead to population declines (Zimova et al. 2016, Wilson et al. 2018). In fact, hares have experienced recent range contractions linked to reduced snow duration and mismatch related mortality (Burt et al. 2016, Diefenbach et al. 2016, Sultaire et al. 2016).

Plasticity in both molt phenology and in behaviors to minimize mismatch or its consequences may reduce or eliminate the fitness costs to hares of decreasing snow duration. The initiation of the seasonal color molt is thought to be largely driven by photoperiod (Lyman 1943) with some plasticity in the population molt rate in the spring modulated by snow or temperature in most color molting species (Zimova et al. 2018). The modulating effect of temperature on seasonal color change in several species has been well established in the lab (Rothschild 1942, Rust 1962) and the field (Watson 1963, Flux 1970, Zimova et al. 2014, Kumar 2015), albeit without clear proximate mechanisms.

The modifying effects of snow, however, are less clear (Grange 1932, Jackes and Watson 1975, Nagorsen 1983, Zimova et al. 2014).

Snow may affect the molt phenology directly or indirectly via its effect on ambient temperature. Indirectly, snow may provide a warmer more thermally stable subnivium space (Goodrich 1982, Pauli et al. 2013) or decrease temperature via its high albedo (Namias 1985, Choi et al. 2010). Direct effects of snow on the molt phenology may also occur. For example, light reflected off snow may influence production or suppression of melatonin through the same visual and neural pathway that regulates photoperiodic systems (Goldman 2001, Schwartz et al. 2001). Although snow has not been definitively linked to plasticity in color molt phenology, it is known to influence other phenological processes such as hibernation (Lane et al. 2012).

In addition to plasticity in phenology, animals may exhibit behavioral plasticity to cope with climate change (Beever et al. 2017, Zimova et al. 2018). For seasonally color-changing animals, plasticity in behaviors may reduce mismatch per se, or reduce the fitness consequences of mismatch. In a classic example of behavioral plasticity to minimize mismatch, molting ptarmigan preferentially forage in areas that maximize their crypsis during their color changing molts (Steen et al. 1992). In addition, the consequences of mismatch could be reduced through behaviors such as resting in dense vegetation where predator detection and capture rates are lower (Ivan and Shenk 2016). Snowshoe hares are capable of exhibiting behaviors to assess and reduce predation risk; for example, under a full moon hares increase their use of high cover areas (Gilbert and Boutin 1991, Gigliotti and Diefenbach 2018) or decrease their movement (Griffin et al. 2005). Although a general preference for dense cover is a hallmark of snowshoe hare biology (Adams 1959, Wolff 1980, Litvaitis et al. 1985), it is unclear if this preference increases when hares are mismatched (but see Zimova et al. 2014).

The central aim of this study is to examine the capacity of phenotypic plasticity to reduce mismatch or its subsequent survival costs on snowshoe hares under decreasing snow duration. First, we quantified plasticity in color molt phenology and the extent to which it is driven by snow cover. We predicted that molt phenology would track changes in snow cover during spring and fall. Second, we examined the potential of behavioral plasticity to reduce mismatch or its consequences. We tested for behavioral plasticity in background selection to see if hares select backgrounds that minimize mismatch. We also tested whether hares minimize the costs of mismatch by preferring denser (safer) habitats when they are mismatched. Finally, we evaluated whether any of these possible avenues of phenotypic plasticity are sufficient to reduce the expression of camouflage mismatch.

METHODS

Study areas

This research was conducted in two areas of the Upper Blackfoot region of western Montana, USA on land managed by the Bureau of Land Management (BLM); see Kumar et al. (2018) for details). Marcum Mountain (Lat. = 46.99°, Long. = -112.91°) and Chamberlain Creek (Lat. = 46.96°, Long. = -113.24°) are approximately 30 km apart at similar elevations (approximately 1450-1700 m.a.s.l.). Both areas have dominant tree species of western larch (approximately 25-50% of total stems) interspersed with Douglas fir (Pseudotsuga menziesii), subalpine fir (Abies lasiocarpa), lodgepole pine (Pinus contorta) and Engelmann spruce (Picea engelmannii) and an herbaceous understory. Likely predators of snowshoe hares at both areas include Canada lynx, bobcat

(*Lynx rufus),* cougar (*Puma concolor*), coyote (*Canis latrans),* red fox (Vulpes vulpes), American marten (*Martes americana*), long-tailed weasel (*Mustela frenata*), golden eagle (*Aquila chryseatos*), great horned owl (Bubo virginianus), barred owl (*Strix varia*), northern goshawk (*Accipiter gentilis*), and red-tailed hawk (*Buteo jamaicensis*).

Capture/handling

We live-trapped snowshoe hares during fall 2012 and summer and winter 2013 to 2014 using live-traps (51x18x18 cm, Tomahawk Live Trap Company, Tomahawk, WI) baited with alfalfa cubes and apples. We marked all hares >500g with a unique numbered ear tag and a VHF radio-collar (Wildlife Materials, Murphysboro, IL) weighing <40g. All capture and handling procedures were approved by the University of Montana Animal Care and Use Committee (permit no. AUP 021-13SMECS-050613).

Snowshoe hare molt phenology

Using VHF radiotelemetry, we tracked 49 individual snowshoe hares and obtained 280 locations during the fall brown-to-white and the spring white-to-brown molts. We used the VHF signal to visually detect hares during the day (usually at resting “forms”), which typically overlap the habitat used by foraging hares (Ferron and Ouellet 1992, Hodges 2000). We followed a standardized protocol of visual observation and photography to estimate hare percent white (Mills et al. 2013): (0%, 5%, 20%, 40%, 60%, 80%, 95%, and 100%). We considered a hare to be molting when it was >0% but <100% white.

Each time we located a hare we recorded associated resting site characteristics. We estimated snow cover at each hare location in 20% increments as the percent of the ground covered with snow (1 and 10 m circular radius around hare location). We computed color contrast (hare percent white – snow cover), and defined mismatch as occurring when the absolute value of color contrast ≥ 60% (Mills et al. 2013). We further differentiated mismatch between white hares found on brown snowless backgrounds (white hare mismatch) and brown hares found on snowy backgrounds (brown hare mismatch). We measured snow cover at both 1 and 10 m plot radii centered on the hare form to capture different scales with which crypsis may be perceived (Zimova et al. 2014). We recorded the temperature and wind speed at the hare form with a handheld weather meter (Kestrel 2000, Nielsen-Kellerman, Boothwyn, PA). We used a circular plot of 5 m radius centered on the hare form to quantify the total number of stems for all trees and shrubs.

In order to 1) test for the ability of hares to minimize mismatch or increase concealment and 2) unravel a causal (snow influencing the molt) relationship versus a correlative one (white hares preferring snow), we tested for resting site selection. We paired each hare location ("used") with an "available" location in a random direction and distance (weighted by hare home range size) from each hare location (see Appendix S1 for complete derivation and full methods). We recorded snow cover, temperature, wind speed and stem counts at each available point immediately after sampling the paired used point.

Statistical analyses

We used a consistent modeling framework to evaluate snowshoe hare molt phenology association with snow and hare resting site selection. We used mixed effects models using package "lme4" (Bates et al. 2015) in Program R (version 2.15.1, R Development Core Team 2012). We standardized all covariates to a mean of 0 and a standard deviation (SD) of 1 to facilitate comparisons between models; when covariates were strongly correlated (>0.7), we included only the covariate that best fit the data using a linear model (Dormann et al. 2013). Because inference changes when covariates change we only ran the full models, which included the response and all uncorrelated fixed and random effects. For comparison purposes, and to more explicitly consider model fit, we also model averaged using the full model and all combinations of the reduced models (Appendix S2) and found no major differences. To help interpret effect sizes we present effects in terms of how a one SD change in the predictor would affect the response.

Snowshoe hare molt phenology

We quantified the hare molt phenology during spring 2013, 2014 and fall 2013 at Marcum and spring 2014 and fall 2013 at Chamberlain (insufficient data prevented estimation of molt initiation and completion dates for some year/site combinations). To accommodate incomplete detection and high hare mortality, we used a Bayesian mixed effects change point analysis to estimate the mean population molt initiation and completion dates (Mills et al. 2013, Zimova et al. 2014). We used a repeated-measures model with individual hare as the random effect. The model was fit with Markov Chain Monte Carlo (MCMC) in OpenBugs with uninformative priors. All models were run with five chains of 100,000 iterations (discarding 10,000 burn-ins), with the Gelman-Rubin statistic indicating chain convergence ($\hat{R} \leq 1.1$). We calculated initiation and completion dates of the molt and the resultant 95% credible intervals for each site and season separately. We also estimated area-specific snow cover for all years and areas using the daily averages measured at all hare locations.

Snow association with molt phenology

We used a mixed effects modeling approach to evaluate whether snow cover was positively associated with hare percent white during the molt. We included only observations when hares were molting (i.e., 5-95% white). In addition, for this question only we were able to add data collected from 2009-2012 using the same protocols from nearby sites in Montana (Mills et al. 2013, Zimova et al. 2014), increasing sample size to 200 individual hares and 1,313 locations. We included a fixed effect for date to prevent attributing to snow cover any changes in the hare percent white that were actually due to season (e.g. photoperiod). Thus, all seasonal changes were absorbed by the date fixed effect leaving only daily variation in covariates to be explained by the model; though rigorous, this approach will produce smaller effect sizes. Because the fixed effect of date on percent white of the hare will have a positive slope in fall and negative slope in the spring we analyzed spring and fall separately. The full model consisted of percent white of the hare as the response, fixed effects for date and the standardized measurements of snow cover at 10 m, and random effects for individual hare, year and region.

Hare resting site selection

We tested for hare resting site selection by fitting a generalized linear model with a binomial distribution to examine the differences between locations used by hares and the paired available locations. The full model consisted of used versus available locations as the response and the standardized measurements of number of stems, temperature at the form, snow at 1 meter, wind speed at the form and all of their one-way interactions as fixed effects and a random effect for individual hare. We expressed the results as odds ratios on the real scale. We also tested for differences in resting site selection by subsetting the data for instances when hares were mismatched (color contrast ≥ 60%) compared to when they matched their background.

RESULTS

Snowshoe hare molt phenology

We found no significant differences (i.e. 95% credible intervals overlap) in either the estimated initiation or completion dates of snowshoe hare molts between the two study areas during the same season (Fig. 1a, Fig. 2a). Consequently, the molt duration for both study areas in fall 2013 and spring 2014 were similar: Marcum (36, 40 days) and Chamberlain (40, 39 days). Snow cover phenology was also similar between areas in fall (Fig. 1b) and spring (Fig. 2b).

However, we found significant inter-annual differences in the spring molt phenology between 2013 and 2014 in Marcum (Fig. 3a), roughly tracking annual differences in snow cover (Fig. 3). The 2013 molt initiated 20 days earlier (March 29 vs April 17) and completed 10 days earlier (May 17 vs. May 26). The advanced molt phenology in 2013 correlated with a shorter spring snow duration; snow melted earlier and faster in 2013 (Fig. 3b). High hare mortality prevented observations of the same individual for multiple fall or spring molts.

Although the molt phenology in both years followed snow cover phenology, the earlier melting year of 2013 resulted in increased camouflage mismatch. In 2013 we detected 13 occurrences of white hare mismatch (whites hares on snowless backgrounds) and one occurrence of brown hare mismatch (brown hares on snow) spread amongst 11 individuals (Fig. 4a). By contrast, in the later melting year of 2014, we detected only one occurrence of mismatch (white hare mismatch) (Fig. 4b).

Snow association with hare molt

We found a strong association of snow with snowshoe hare percent white even after controlling for date. An increase of one SD of percent snow cover (36% in fall and 44% in spring) was associated with an increase in percent white of hares by 3% (se=0.8%) in fall and 11% (se=1.3%) in spring.

Hare resting site selection

Overall, molting hares had strong preferences for resting sites with more total stems, less snow and colder temperatures during their spring and fall molts, even after controlling for other predictors. An increase in the number of stems by one SD (23 stems) increased the odds of hare use by 1.5 times (95% CI = 1.3-1.8). A decrease in percent snow cover by one SD (35%) increased the odds of hare use by 1.4 times (95% CI = 1.0-1.8). A decrease in temperature by one SD (8.0°C) increased the odds of hare use by 1.3 times (95% CI = 1-1.5). No other covariates were significant predictors of hare resting site selection.

These microhabitat choices were independent of individual mismatch suggesting a lack of behavioral plasticity to reduce mismatch or its fitness costs. Because molting hares consistently preferred snow-free (brown) resting sites, we found no evidence that white hares background match via preferentially choosing snowy areas to maintain crypsis. In fact, white hares tend to select resting sites with less snow cover even though brown ground increases their mismatch. Likewise, similar overall preferences for resting sites with denser cover (more stems) -- regardless of whether or not a hare was mismatched -- indicated that mismatched hares did not choose microhabitats that provided more protection from predation.

DISCUSSION

We found strong evidence that snow cover influenced both the initiation and the rate of the snowshoe hare molt in the wild, potentially facilitating plasticity to maintain crypsis under natural variation in snow duration. By directly modeling the effect of local snow cover on hare percent white we found that snow presence was associated with whiter hares, providing a plastic response to reduced snow duration. Furthermore, we found that average population phenology tracked snow cover in the spring. However, this snow-mediated plasticity did not prevent camouflage mismatch under decreased snow cover, as hares experienced higher camouflage mismatch during the year with shorter snow cover duration.

The snow effects on the molt phenology were found even after we controlled for date, effectively accounting for seasonal changes in snow on the coat color molt. Importantly, the positive association between snow and hare percent white did not arise from a behavioral preference for snow. To the contrary, snowshoe hares consistently preferred areas with less snow cover, consistent with previous field-based findings at other study sites (Zimova et al. 2014). Thus, associations between snow and the percent white of a hare arise from snow directly affecting hare molt phenology as opposed to behavioral plasticity of white hares preferring snowy areas.

Hare preference for resting sites with less snow may be driven by thermoregulatory constraints that override site selection against mismatch. Indeed, we found that the preference for areas with less snow was strongest when air temperatures were below freezing and absent above freezing. In addition to its effect on hare resting site selection, temperature may also have a weak influence on the hare color molt

(Watson 1963, Nagorsen 1983, Zimova et al. 2014, Kumar 2015). Field-based tests of the direct effects of temperature on coat color molt are not yet possible due to the absence of physiologically based connection between the trait and a relevant metric (e.g., daily mean, monthly maximum, number of days above freezing, etc.) or appropriate temporal window.

As with the absence of behavioral plasticity to reduce mismatch, hares also displayed no behavioral plasticity to avoid the negative costs of mismatch through concealment. Mismatched and matched hares displayed similar preferences for resting sites with dense stems. These results are consistent with Zimova et al. (2014), but contrast with Litvaitis (1991) who found that mismatched hares occupied areas with denser cover. These conflicting findings may be partially due to different methodologies; Litvaitis (1991) used baited traps to determine hare habitat use instead of directly tracking hares.

At the Marcum site where we could follow the spring molt across two years of very different snow duration, we documented significant differences in coat color phenology that corresponded to differences in snow duration (Fig. 3). The much shorter snow duration of 2013 at the Marcum site was corroborated by a nearby weather station (approx. 90 km away in Missoula) which recorded approximately 25% lower snow accumulation compared to 2014 (123 cm in 2013, 167 cm in 2014). In that early snow melt year of 2013, hares began their spring molt almost three weeks earlier and became brown about 10 days earlier than in 2014. Accordingly, the 2013 molt took about 50 days, 10 days longer than in 2014 and in other studies (Zimova et al. 2014). Thus, our findings imply that while photoperiod is the core modulator of the seasonal color molt, snow presence per se may facilitate plasticity in spring molt phenology, perhaps via the same neural pathways that perceive changes in photoperiod (Goldman 2001, Schwartz et al. 2001).

Might the plasticity in both the initiation and rate of the molt provide a means for adaptive rescue as hares confront rapidly declining snow duration? The observed difference in spring snow duration between 2013 and 2014 was approximately 20 days. This is less than the 29 to 35 day reduction in snow duration predicted in this area by mid-century under either the medium-low or high emissions scenario (Mills et al. 2013). Although population level mean molt phenology (initiation and rate of molt) shifted in the direction of the rapid 2013 snowmelt, we nevertheless detected white hare mismatch in 23% of our observations (Fig 4a), contrasted with just 2% of observations in 2014 (Fig. 4b). Moreover, analysis of raw data from Mills et al. (2013) revealed white hare mismatch in 15% of observations from the early melting year (2010) compared to just 1% of the observations in the later melting year (2011). It appears that snow-driven plasticity in molt phenology is insufficient to prevent increased mismatch in years of short snow duration possibly leading to future population declines (Zimova et al. 2016).

We found plasticity in phenology or behavior has limited potential to facilitate adaptive rescue to camouflage mismatch. Snowshoe hares did not adjust their behavior by seeking resting sites with matching backgrounds or more cover. In addition, despite snow-mediated phenotypic plasticity in the molt phenology, hares still experienced increased camouflage mismatch. Thus, although the snow-mediated plasticity in molt timing is substantial, it is not enough to prevent future camouflage mismatch and potential population declines in the face of earlier and faster snowmelt. For species lacking phenotypic plasticity to accommodate rapid environmental changes, persistence will likely depend on the scope and speed of evolutionary rescue through natural selection (Bay et al. 2017, Diamond et al. 2017, Manhard et al. 2017, Mills et al. 2018).

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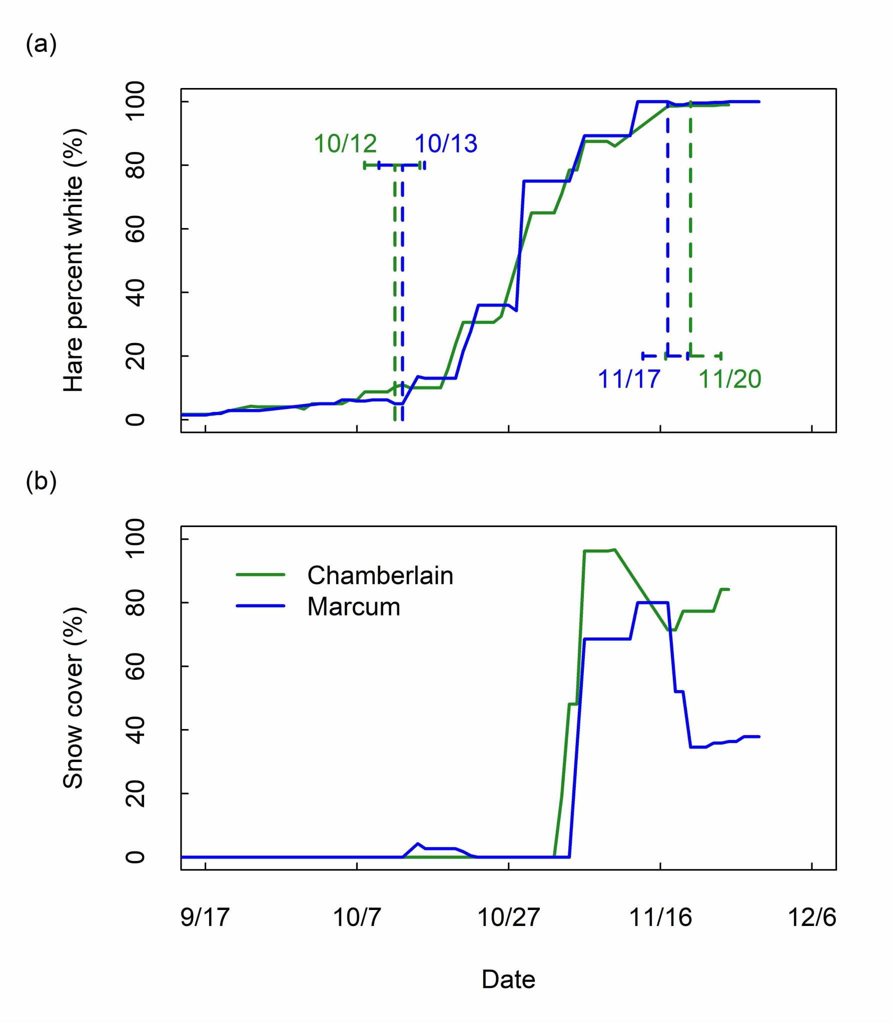

Fig. 1 Fall coat color phenology and snow cover in 2013 in two study areas in western Montana. (a) Averaged weekly observed coat color of 38 snowshoe hares at two different study areas. Dotted lines indicate mean molt initiation and completion dates and the 95% credible interval. (b) Averaged weekly snow cover in a 10 meter radius around each hare.

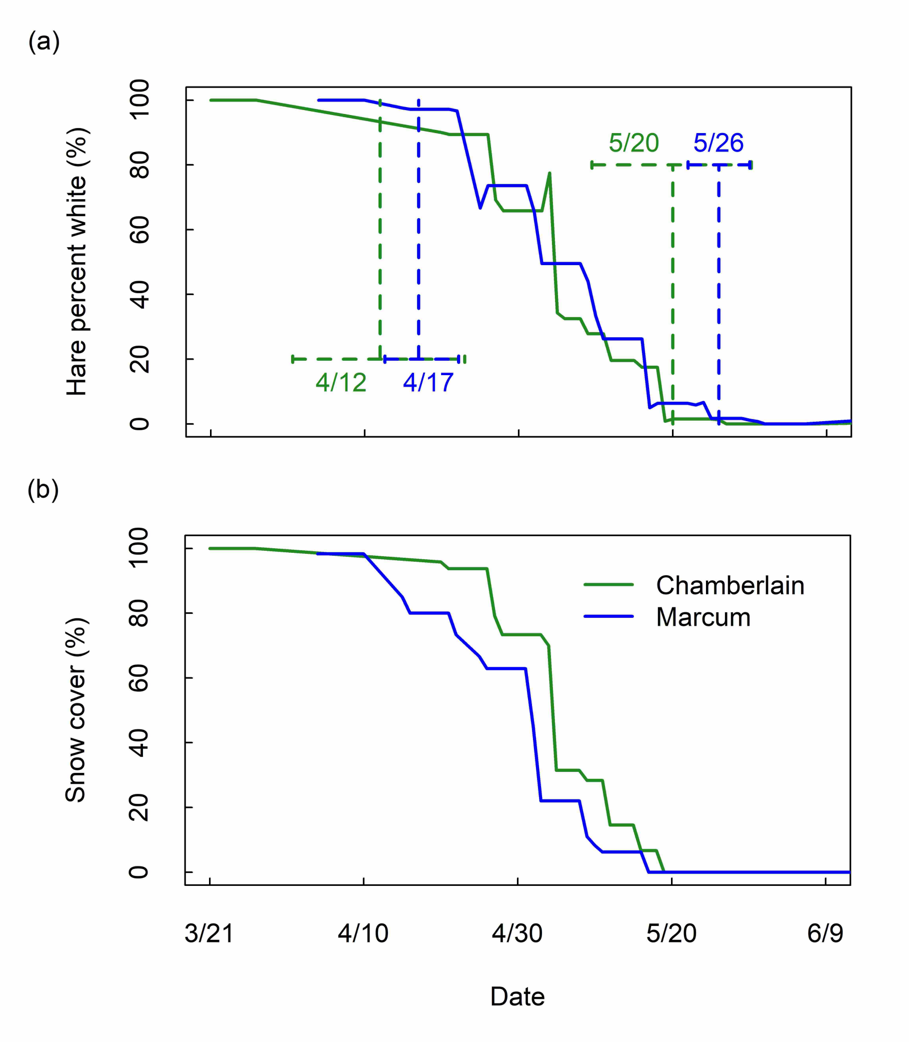

Fig. 2 Spring coat color phenology and snow cover in 2014 in two study areas in western Montana. (a) Averaged weekly observed coat color of 16 snowshoe hares at two different study areas. Dotted lines indicate mean molt initiation and completion dates and the 95% credible interval. (b) Averaged weekly snow cover in a 10 meter radius around each hare.

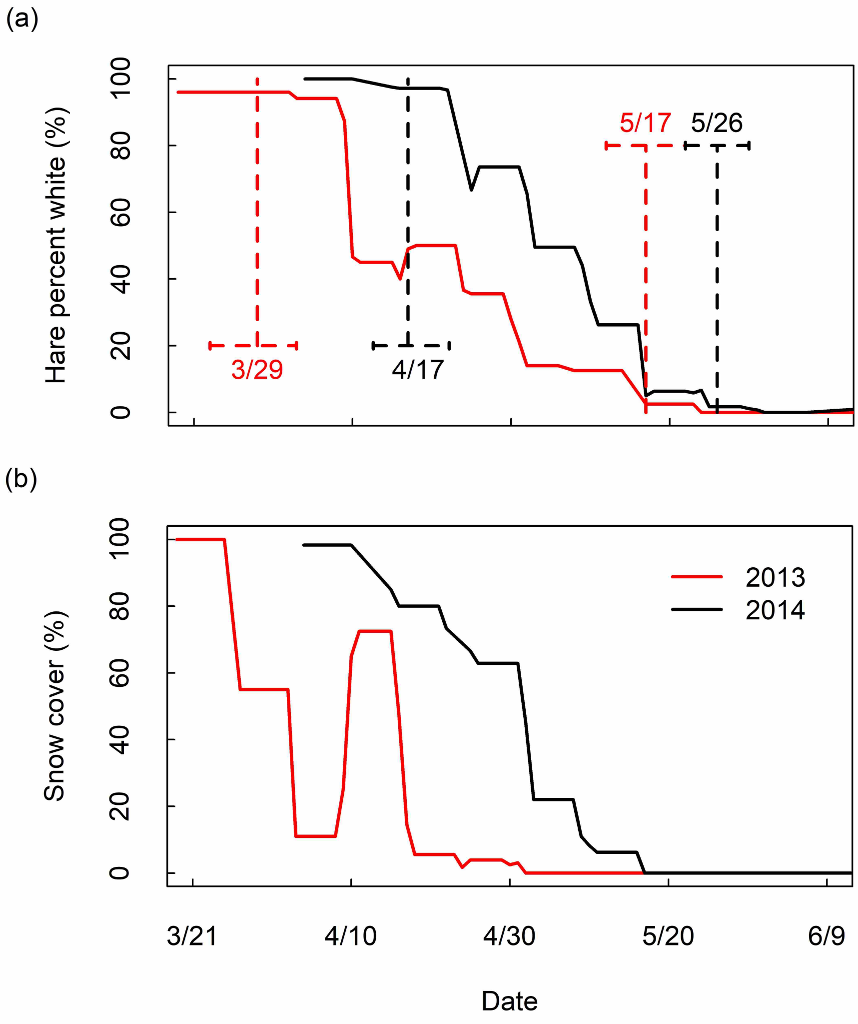

Fig. 3 Shifts in spring coat color phenology follow spring snow cover at the Marcum study site. (a) Averaged weekly observed coat color of 23 snowshoe hares. Dotted lines indicate mean molt initiation and completion dates and the 95% credible interval. (b) Averaged weekly snow cover in a 10 meter radius around each hare.

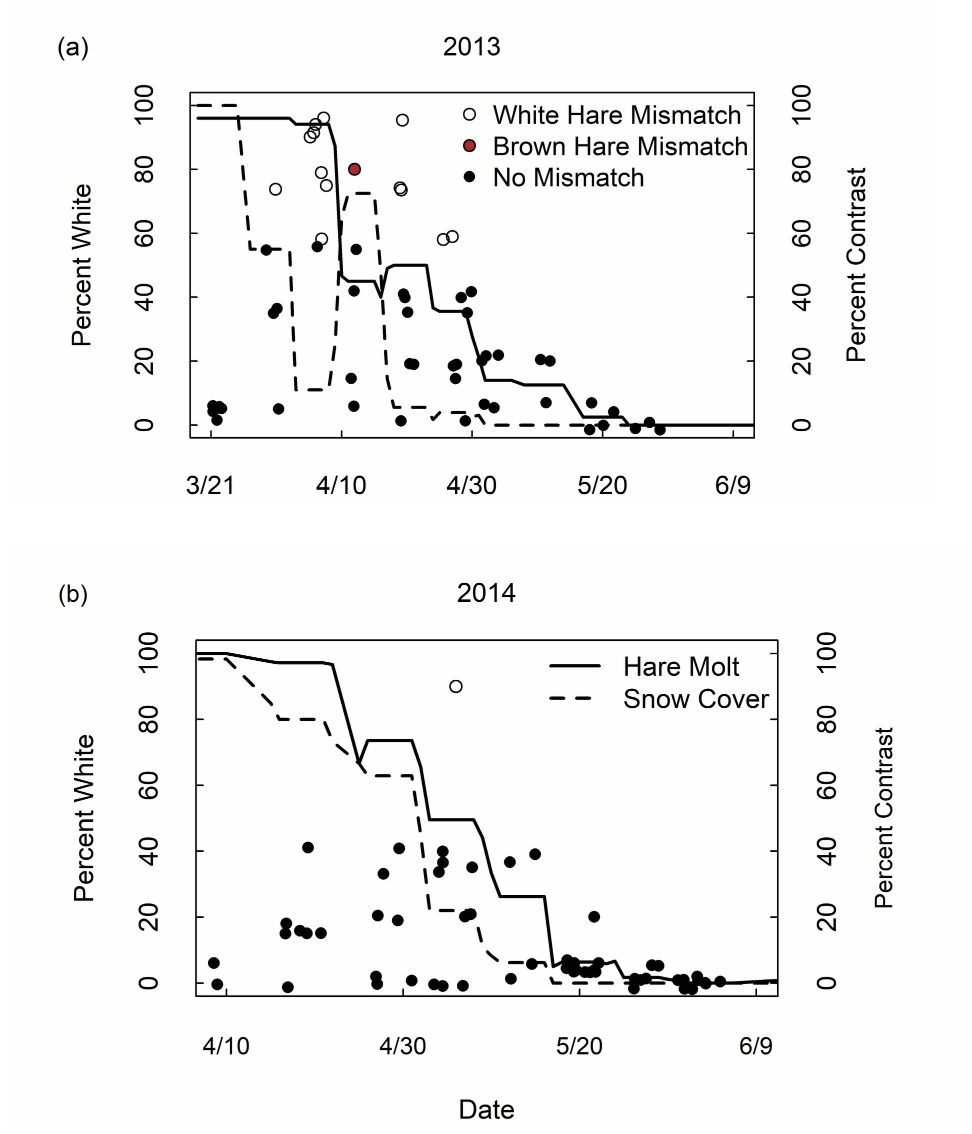

Fig. 4 Early snowmelt results in increased mismatch in western Montana hares. (a) Background contrast and resulting occurrence of mismatch from 56 observations of 15 individuals in 2013. White points indicate white hares mismatched on brown backgrounds, brown points indicate brown hares mismatched on white backgrounds and black points indicate no mismatch. Hare contrast = hare percent white - snow cover. Mismatch occurs when contrast ≥ 60%. (b) Background contrast and mismatch resulting from 57 observations of 8 individuals in 2014.

APPENDIX 3

NONINVASIVE MEASURES OF PHYSIOLOGICAL STRESS ARE CONFOUNDED BY EXPOSURE

Diana J. R. Lafferty[abcǂ*], Marketa Zimova[cǂ], Lindsay Clontz[a], Klaus Hackländer[d], L. Scott Mills[e]

[a] Department of Biology, Wildlife Ecology and Conservation Science Lab, Northern Michigan University, Marquette, MI 49855, USA

[b] Fisheries, Wildlife, and Conservation Biology Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA

[c] Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA

[d] Institute of Wildlife Biology and Game Management, University of Natural Resources and Life Sciences, Vienna, Gregor-Mendel-Str. 33, 1180 Vienna, Austria

[e] Office of Research and Creative Scholarship, University of Montana, Missoula, MT 59812, USA

[ǂ] These authors contributed equally to this work.

ABSTRACT

Measures of physiological stress can provide insight into how wildlife assimilate, cope with and respond to environmental change. Concentrations of stress hormone levels, particularly glucocorticoids and glucocorticoid metabolites are increasingly being used to index physiological stress in diverse taxa. Although feces is often abundant and can be collected noninvasively, exposure of feces to biotic and abiotic elements may influence fecal glucocorticoid metabolite (FGM) concentrations, leading to inaccurate conclusions of physiological responses of wildlife to environmental change. Using captive snowshoe hares (*Lepus americanus*) and simulated environmental conditions, our overarching goal was to learn how different realistic field conditions and temporal sampling constraints might influence FGM measurements. We quantified how fecal pellet age (i.e., exposure time of 0-6 days), summer temperature (warm [23.5°C], cool [7.3°C]), and precipitation (dry, wet) affected FGM concentrations. We found that fecal pellet age has a strong effect on FGM concentrations. Concentrations were lowest at the beginning and end of our exposure period (i.e. days 1 and 6) and highest in the middle. Next, we found that the effect of fecal pellet age on FGM concentrations varies across different environmental treatments with warm-dry and cool-wet conditions having the strongest effect on FGM concentrations over time. Given the confounding effects of exposure and environmental conditions, efforts should be made to preserve sampled pellets immediately following defecation. Whenever fresh fecal pellet collection is not an option, we strongly encourage researchers to develop a temporally consistent sampling protocol to ensure similar consistent exposure time and environmental conditions for all samples.

INTRODUCTION

Understanding wildlife responses to local and broad-scale environmental perturbations poses an enormous challenge for scientists, wildlife managers, and policy makers. The current need for reliable tools that can be used to measure and monitor wildlife responses to environmental stressors is exacerbated further by the ongoing global environmental change. Over the past decade, assessments of adrenocortical activity, particularly measures of glucocorticoids (stress hormones) and glucocorticoid metabolites, have been used increasingly to index the stress burden experienced by diverse taxa (Möstl and Palme 2002, Millspaugh and Washburn 2004, Palme et al. 2005, Sheriff et al. 2011a, Goymann 2012, Dantzer et al. 2014). In fact, fecal glucocorticoid metabolites (FGM) have been shown to provide an integrative measure of circulating glucocorticoids, representing the physiological state of the target animal over a well-defined period of time (Palme 2019). Feces is a particularly useful matrix for measuring stress hormone metabolite concentrations because it is relatively abundant and can be collected with minimal disturbance to study animals (Möstl and Palme 2002, Millspaugh and Washburn 2004, Palme 2019), thus eliminating the confounding effects of capture stress (Harper and Austad 2000, Millspaugh et al. 2001, Touma and Palme 2005).

While measures of FGM concentrations have great potential to contribute to integrative wildlife conservation and management (Madliger et al. 2016), important sampling caveats affect stress levels estimated from fecal samples (Möstl and Palme 2002, Millspaugh and Washburn 2004, Palme 2005, Dantzer et al. 2014). First, whether an increase, decrease or no changes in FGM concentration is detected within a target population often depends on the species being sampled as well as the technique used to measure FGM (Palme 2019). In addition, ambient temperature and humidity can affect bacterial metabolization that can result in possible increases or decreases in FGM concentrations (Terio et al. 2002, Washburn and Millspaugh 2002, Palme et al. 2013). For example, differences in microclimates across the range of American pika (*Ochotona princeps*) have been reported to influence FGM concentrations when feces are not immediately collected and preserved after defecation (Wilkening et al. 2016). Hot and dry environmental conditions were associated with decreased FGM concentrations in cheetah (*Acinonyx jubatus*; Terio et al. 2002), whereas precipitation seemed to cause a "washing-out effect" in which FGM leached from the feces of mountain hare (*Lepus* *timidus*, (Rehnus 2009). While the confounding effects of exposure time and abiotic environmental conditions can be mitigated in some situations by observing animals and collecting samples immediately after defecation (Creel et al. 1997, Creel et al. 2002) or possibly by sampling feces deposited in snow (Creel et al. 2009), observing defecation may be logistically challenging or perhaps impossible for a variety of taxa. Thus, understanding how environmental conditions affect FGM concentrations is critical for developing rigorous noninvasive sampling protocols for any wildlife study.

We use snowshoe hares (*Lepus americanus*), a key prey species negatively affected by climate change (Mills et al. 2013, Zimova et al. 2016), as an example to illustrate the variable influence of time and environmental conditions on FGM concentrations. Results from rigorous captive experiments and field-based studies demonstrate that an 11-oxoetiocholanolone enzyme immunoassay (EIA) can be used to reliably detect changes in snowshoe hare FGM concentrations over time (Sheriff et al. 2009a, Sheriff et al. 2009b, 2011b). Additionally, fresh snowshoe hare fecal pellets represent the physiological state of the individual 8-12 hours prior to defecation (Sheriff et al. 2009), giving a relative temporal frame of reference for measures of stress. However, to use FGM concentrations to measure population responses to environmental stressors, we must understand how FGM concentrations are influenced by feces' age and exposure to environmental conditions. Thus, using a captive collection of snowshoe hare and simulated environmental conditions, we examined the effect of realistic field conditions and temporal sampling constraints on FGM measurements. First, we tested how fecal pellet age (i.e., exposure time of 0-6 days), temperature (summer warm [23.5°C], summer cool [7.3°C]) and precipitation (dry, wet) affected FGM concentrations. Second, we evaluated at what fecal pellet age FGM levels begin to differ from FGM concentrations in control samples when exposed to different environmental treatments (precipitation and temperature combinations). Finally, we provide recommendations for field study design to achieve ecologically meaningful measures of FGM concentrations to infer stress levels within the target population.

MATERIALS AND METHODS

Animals and housing

Fifteen wild-caught adult snowshoe hares were used in this experiment. Animals were captured in Lolo National Forest in Montana, USA (2 females, 3 males) and Mt. Baker-Snoqualmie National Forest in Washington, USA (4 females, 6 males). Animals were transferred from the field to an environmental facility at North Carolina State University (NCSU), College of Veterinary Medicine (CVM). Environmental rooms at this facility replicated site-specific temperature and photoperiod from the capture location of the animals. Animals were housed individually in wire mesh enclosures (121.9 cm width × 60.9cm depth × 73.6cm height) including a wire mesh floor with a waste collection tray below the wire floor, lined with absorbent paper. Enclosures included an acrylic hide box (30.5cm width × 60.9cm depth × 73.6cm height) that functioned as a visual barrier between animals, as well as an edible grass mat and grass hut to hide in or sit atop. Water and premium natural adult rabbit food (Sherwood Pet Health, Logan, UT, USA) were available ad libitum. The NCSU Institutional Animal Care and Use Committee approved all capture, handling, transport, husbandry and sample collection procedures (protocol 14-069-0).

Fecal sample collection

On evenings prior to pellet collection, we replaced the absorbent paper in the fecal tray beneath each enclosure between 2145 and 2200 hours to provide a clean substrate for sampling. Then for six consecutive days, between 0600 and 0610 hours, we collected all fecal pellets beneath each animal's enclosure, except pellets that were in contact with urine because urine may affect measured FGM concentrations (Palme 2005). We choose this eight-hour sampling frame to represent the time over which an animal may be held in a trap during field-based operations, which corresponds to the approximate lag time between stress-induced cortisol production and the expression of cortisol metabolites in snowshoe hares (Sheriff et al. 2009a). We placed each sample in an individually labeled bag and transferred it to a -20°C freezer within 5 minutes of collection. At the end of the collection period, we pooled each animal's pellets and gently mixed the pooled sample to avoid damaging the pellets, thereby creating a single large sample per individual. Each individual sample was then subsequently divided into 25 equal portions based on sample wet weight. The pooled sample was necessary to obtain sufficient volume to be divided into 25 subsamples for the temperature and precipitation treatments and to ensure we obtained sample representative of the average FGM concentration in pellet per individual as opposed to a random stress event, which could have unduly biased our FGM measurements.

Experimental treatments

To assess the effect of age (max 6 days), all but one subsamples (n=24) per individual were randomly assigned to one of 4 treatments for varying number of days. One subsample per individual was assigned to a pre-treatment control that was immediately returned to the -20°C freezer. Environmental treatments included: (1) warm-dry [WD], (2) warm-wet [WW], (3) cool-dry [CD], and (4) cool-wet [CW]. Warm and cool temperatures represented average summer temperature in Washington and Montana and were based on monthly 1981-2010 normal temperatures in July and August at the snowshoe hare capture sites (average summer warm=23.5°C; summer cool=7.3°C) (PRISM Climate Group). Similarly, summer precipitation was based on 1981-2010 normal precipitation data (PRISM Climate Group) during July and August (wet=1 mm; dry=0 mm). Accordingly, we applied 1 mm of precipitation daily at 0800 am via a handheld spray bottle each day 24 hrs. prior to sample collection for the WW and CW treatments. For the experiment, all but the control samples were placed in a 17.78 cm × 17.78 cm soak resistant paper bowl with approximately 40 pine (*Pinus* sp.) needles broken into thirds to cover the bottom of the bowl. Samples were then subjected to an assigned environmental treatment (WD, WW, CD, CW) for up to 6 days. We collected samples at the end of each day treatment period and immediately placed the samples in a -20°C freezer until FGM extractions.

Extraction and quantification of fecal glucocorticoid metabolites

We thoroughly homogenized each sample using a glass mortar and pestle and then dried the samples at 80°C for 48 hours. After drying, we removed a 0.15 g subsample and placed it in 8.0 ml glass centrifuge tube with 5.0 ml of 80% methanol. Next, we shook samples on a hand vortex for one minute and centrifuged each sample at 2,500 g for 15 minutes. Following centrifugation, 0.5 ml of supernatant was transferred to micro-centrifuge tubes and dried for 2 hours at 80°C. Fecal extracts were then shipped to the Department of Biomedical Sciences, Unit of Physiology, Pathophysiology and Experimental Endocrinology at the University of Veterinary Medicine in Vienna, Austria for steroid analysis using an enzyme immunoassay (EIA). Concentrations of FGM were quantified in re-dissolved fecal extracts using an 11-oxoetiocholanolone-EIA protocol developed by Palme and Möstl (1997) and validated for snowshoe hares by Sheriff et al. (2009).

Data analysis

Effects of environmental factors on exposed pellets

Prior to analysis, we assessed the data for outliers and tested for normality and equal variances using diagnostic plots. We subsequently log-transformed FGM data to meet the assumption of normality and confirmed it using diagnostic plots. To determine the effects of fecal pellet age, temperature and precipitation on the FGM concentration, we fitted linear mixed effects models using the package lme4 (Bates et al. 2014). We included the fixed effects of age (days 1 through 6 of treatment exposure), precipitation (wet, dry) and temperature (warm, cool), and the random effect of individual hare to account for among-individual differences in FGM concentrations. In addition to linear terms, we included a quadratic term to allow for a potential curvilinear response of FGM concentrations to fecal pellets' age.

Next, we tested the effect of pellets age on FGM concentrations to determine when FGM concentrations begin to vary within each environmental treatment relative to control FGM concentrations. We allocated the samples from the four different treatments into daily intervals (days 0 [i.e., control] through exposure day 6) and ran a simple mixed model for each treatment with a single fixed effect age coded as a factor and individual hares as a random effect. All statistical tests were conducted using R 3.3.2 (R Core Team 2016) and significance of fixed effects were assessed at $\alpha=0.05$ level.

RESULTS

We found that the age of feces affects FGM concentrations, and the age-FGM relationship has a curvilinear shape ($\beta_{Age}=0.395$, s.d.=0.085; $\beta^2_{Age}=-0.061$, s.d.=0.012;

Fig.1). FGM concentrations peaked on day 3 and were lowest on day 6. Temperature and precipitation had no significant effect on FGM concentrations during those days.

We found multiple significant differences between the FGM concentrations in the control samples and FGM concentrations exposed to the environmental treatments (Fig. 2). We did not detect a particular age at which FGM concentrations consistently began to differ from the control samples (i.e., day 0). Instead, we found substantial variation in the trend of FGM concentration over time across all experimental treatments. For example, in the CW treatment, FGM concentrations initially declined and then followed the curvilinear trajectory, whereas FGM concentrations in the CD treatment were the most consistent over the six-day exposure period.

DISCUSSION

Our results indicate that the length of time between snowshoe hare defecation (i.e., pellet age) and fecal sample collection can affect FGM measurements. Our data show an initial increase in FGM concentrations over the first two days followed by a decline in FGM concentrations over the subsequent four to six days (Fig. 1). The observed curvilinear FGM response to environmental exposure time underlines the importance of obtaining fresh fecal samples to mitigate the potential risks of sampling older feces that no longer accurately represent the physiological state of the studied animals.

Results from our controlled experiment also demonstrate that the effect of fecal pellet age varies across different environmental conditions. For example, the effect of fecal pellet age was strongest under warm dry and cool wet conditions, whereas FGM concentrations were least variable over the six-day exposure period represented by a cool and dry summer conditions. Results from earlier studies of diverse taxa have shown that increased temperatures sometimes result in higher FGM concentrations relative to control samples (Terio et al. 2002, Millspaugh et al. 2003, Wilkening et al. 2016). On the other hand, the effects of precipitation (or moisture) on FGM concentrations are variable, leading to increased or decreased FGM concentrations (Washburn and Millspaugh 2002, Stetz et al. 2013, Wilkening et al. 2016). Both of our experimental temperature treatments that included simulated precipitation resulted in initial FGM decreases, which may have been the result of a ‘washing-out’ effect in which our application of precipitation removed some FGMs from the sample. Furthermore, the effects of temperature and precipitation on the FGM concentrations may interact; for example, increased microbial activity in warmer temperatures may be offset by FGM degradation or potential washout when feces are exposed to precipitation. However, whether measures of FGM concentrations show an increase, decrease or no change, not only depends on the species being sampled but also the method used to measure FGM (Palme 2019). In conclusion, it is critical to reduce sample exposure to uncontrolled environmental conditions that may influence measurements of FGM concentrations.

Given the confounding effect of pellet exposure time on FGM measurements, efforts should be made to preserve sampled feces immediately following defecation. In the field, however, the difficulty of observing defecation by any free-ranging wildlife makes it challenging to collect fresh feces. If collection of fresh feces is not possible, researchers should consider conducting pilot studies to examine the effects of exposure time and environmental conditions on FGM concentrations to ensure ecologically meaningful interpretation of FGM measurements. Once researchers understand the effects of environmental conditions on FGM concentrations in their target taxa, rigorous field studies can be planned to minimize the potential impacts of abiotic factors on FGM concentrations (e.g., preferential sampling during cool dry conditions in the summer). In addition, researchers must apply a systematic sampling protocol to ensure all sampled feces are subjected to similar exposure times and environmental conditions prior to sample preservation. For example, if environmental conditions are stable, line transects or plots could be cleared of snowshoe hare pellets initially and re-checked for pellet samples within a specified timeframe (e.g., one day, two days). The opportunistic use of samples collected for purposes other than for measuring FGM concentrations (e.g., feces collection for genetic or occupancy studies) may not accurately reflect the physiological state of the animals sampled. Thereby, all samples that are not systematically collected within a standardized time frame after defecation should not be used to infer stress responses or should be used very cautiously when drawing conclusions about the stress response of the target population.

Noninvasive measures of physiological stress can be a powerful tool for evaluating wildlife responses to diverse environmental changes or for monitoring changes in stress among individuals in free-ranging populations. Our study demonstrates that rigorous study design and systematic sampling is essential for ensuring data are high quality and therefore useful for drawing meaningful ecological conclusions about wildlife stress. Here we recommend researchers to design field studies with respect to the potential effect of environmental conditions on FGM concentrations and develop standardized protocols for sample collection and storage prior to initiating field studies.

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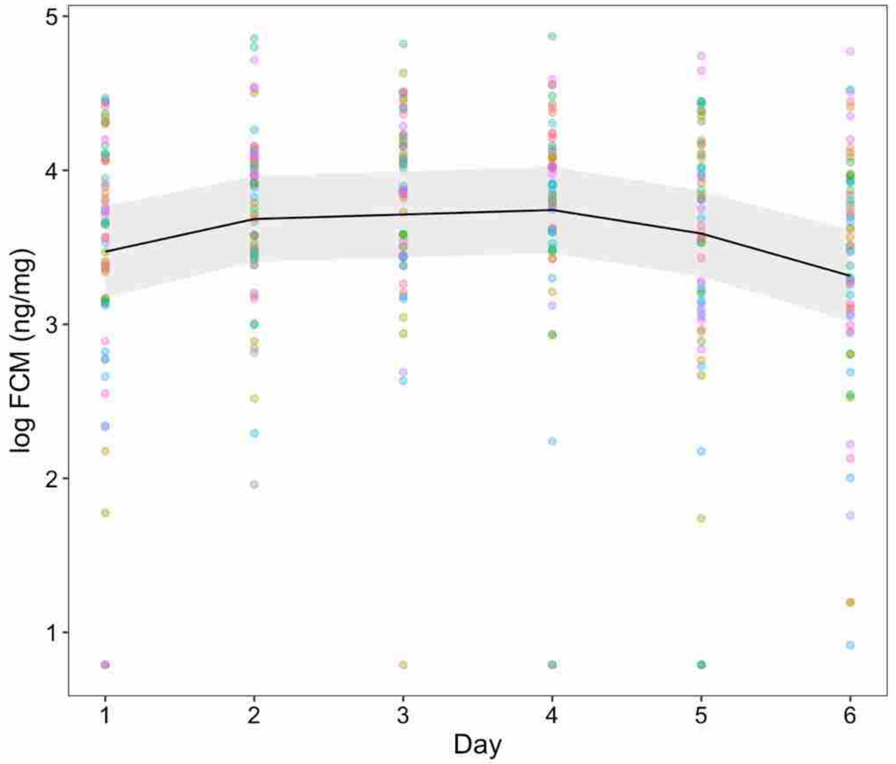

Figure 1. Predicted trend in FGM concentrations of snowshoe hare fecal pellets over time. Colored points show individual observations. Gray band depicts 95% confidence interval. FGM concentrations were log-transformed to meet the models' assumptions.

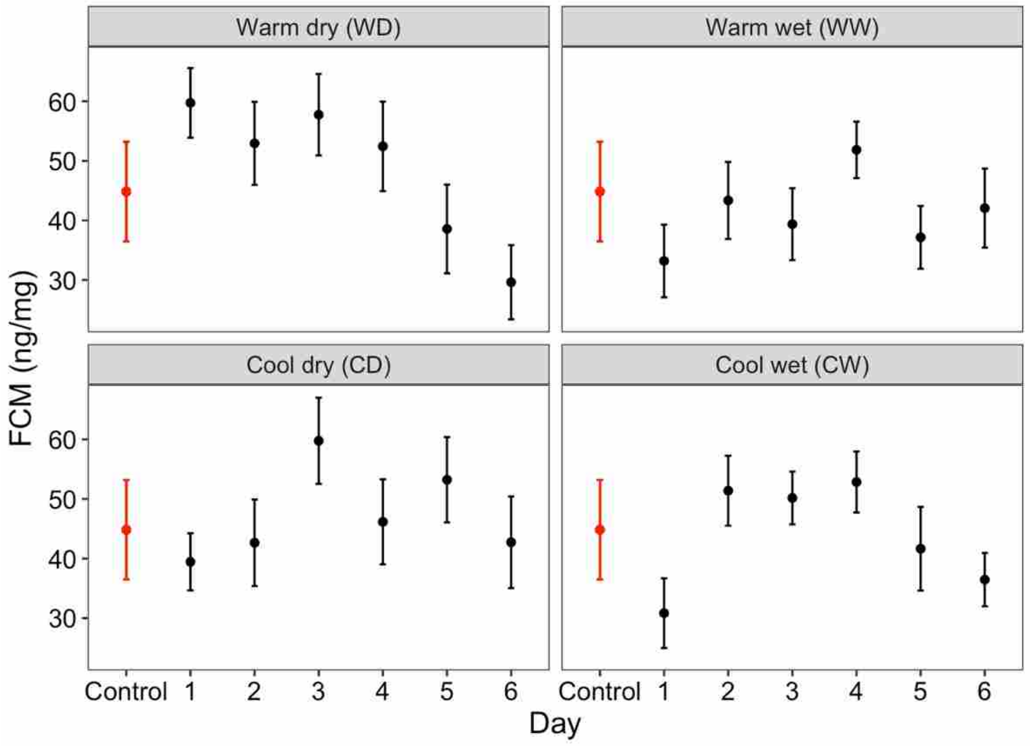

Figure 2. Observed FGM concentrations measured over time under different environmental treatments in snowshoe hares. Points show means and errors bars show standard errors across all individuals each day. Control FGM levels, shown in red, were quantified from samples collected immediately after defecation and are the same in each panel. FGM concentrations of pellets exposed to different environmental conditions are shown in black.