

2009

Geographic and individual variation in carotenoid coloration in golden-crowned kinglets (*Regulus satrapa*)

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**GEOGRAPHIC AND INDIVIDUAL VARIATION IN CAROTENOID
COLORATION IN GOLDEN-CROWNED KINGLETS (*REGULUS SATRAPA*)**

by

Celia Kwok See Chui

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada
2009

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Geographic and individual variation in carotenoid coloration in golden-crowned kinglets
(*Regulus satrapa*)

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows: in all cases, the key ideas and data collection, analysis, and interpretation were primarily performed by the author. Chapters 2 and 3 were co-authored with my advisor, Dr. Stéphanie Doucet, who supported my research financially, provided feedback on ideas, assisted with statistical analyses, and imparted editorial suggestions during the writing process of both manuscripts. Chapter 3 was also a collaborative effort with Dr. Kevin McGraw, who provided advice on methods and data interpretation, and contributed feedback on this manuscript.

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Thesis Chapter	Publication title/full citation	Publication status*
<i>Chapter 2</i>	<i>Chui, C. K. S. & Doucet, S. M. (2009) A test of ecological and sexual selection hypotheses for geographical variation in coloration and morphology of golden-crowned kinglets (Regulus satrapa). Journal of Biogeography.</i>	<i>Published online: 17 June 2009</i>

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ABSTRACT

Phenotypic variation arises through natural selection for local adaptation, sexual selection for conspicuous visual communication, and genetic drift. Large-scale variation is often demonstrated via clinal gradients, and small-scale variation is commonly exhibited by quality-indicating traits. My goal was to investigate geographic and individual phenotypic variation in golden-crowned kinglets, with particular focus on carotenoid-based ornaments. Through a museum study, I found that kinglet body size and coloration weakly followed well-established ecogeographic rules. However, sexual dichromatism was reduced in colder climates, providing support for a poorly recognized environmentally-induced cline. In a separate study, I captured migrating kinglets to determine how carotenoid content mediates inter- and intrasexual crown colour variation. I found that crown coloration was associated with migration timing, and females displayed additional condition-dependence of this trait. Overall, small-scale variation in crown coloration is dependent on pigment differences and individual quality, while large-scale variation is likely governed by differences in selection pressures.

ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Stéphanie Doucet for giving me the opportunity to conduct field work in Costa Rica, yet accepting me into her lab nonetheless when I chose to spend my summers in Windsor. Her dedication to excellence and extraordinary attention to detail have improved my own skills as a researcher, and her guidance over the past two years has been taken to heart. To my committee members, Drs. Daniel Mennill and Melania Cristescu, thank you for providing valuable input on my thesis project and for accepting v.2.0 when the original plans slightly collapsed.

Thanks to the following sources for funding my thesis and conference presentations: NSERC, Society of Canadian Ornithologists, American Ornithologists' Union, Government of Ontario, and the University of Windsor.

I am grateful to Janet Hinshaw (University of Michigan Museum of Zoology), Jeremiah Trimble (Harvard Museum of Comparative Zoology), and the museum curators and curatorial assistants for allowing their ornithology collections to be open and accessible for research. Thanks to Allison Mistakidis, David Bradley, Karen Cogliati, and Melissa Abdellah for additional museum assistance for Chapter 2.

To Phil Roberts, thank you for approving my field project and introducing me to the Holiday Beach Migration Observatory. I would especially like to thank Bob Hall-Brooks, Carl Pascoe, and Rachel Powless for imparting their wisdom about bird migration and banding. They volunteered many extra hours and days to ensure that I could collect enough data for Chapter 3. Field work was the most fun and rewarding aspect of my research, and I feel privileged to have been a part of this organization.

I am indebted to Dr. Kevin McGraw for inviting me to visit his lab at Arizona State University for carotenoid extraction and HPLC training. His collaboration was paramount to the completion of Chapter 3. Thanks to Matt Toomey and Mike Butler from the McGraw lab for assistance with HPLC protocols. Many people at the University of Windsor provided the equipment, consumables, and advice that enabled me to conduct the lab work for Chapter 3: Drs. Barb Zielinski and Yogesh Katare, Dr. Aaron Fisk and Sandra Ellis, Dr. Trevor Pitcher, Dr. Steve Loeb, Lara Jerant, Jane Sylvester, Bob Hodge, and Rodica Leu and Ingrid Churchill.

To the past and present members of the Doucet lab, thank you for proofreading my work and listening to my practice presentations – I am slowly overcoming my fear of public speaking. To Jessica Cuthbert, Allison Mistakidis, Karen Cogliati, Daniel Hanley, Melissa Abdellah, Katie Rieveley, Michelle Bondy, Jillian Faraci, Fabio Castelli, and Summer Ross. To all of the friends that I've made in the Biology Department, thank you very much for making my time here enjoyable, from Biobeer to the DNACes to my first hockey game. Special thanks to David Bradley, Karan Odom, Nicole Barker, Kyle Swiston, and Jess and Daniel for the birding outings that continue to confirm my passion.

I would like to thank my parents for cultivating my love for birds since I was a child, and giving me the freedom to pursue my own happiness. Finally, to Chanson Brumme: thank you for sharing my love of birds, when you knew nothing before you met me. Thank you for discussing statistics, reading my papers, and providing free accommodation when I visited the HMCZ. More importantly, thank you for your unconditional support... I wouldn't have survived the past two years without you.

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CHAPTER 1

GENERAL INTRODUCTION

PHENOTYPIC TRAIT VARIATION

Phenotypic variation within a species has been recognized for centuries; indeed, Darwin's observations of variable traits inspired his theories of natural and sexual selection (Darwin, 1859, 1871). Even today, ecologists and evolutionary biologists continue to ask: what are the proximate and ultimate mechanisms behind phenotypic differences? Investigating patterns of phenotypic variation enables us to explore the evolutionary processes that can lead to population divergence, reproductive isolation, and speciation (Zink & Remsen, 1986; Coyne & Orr, 2004). My MSc thesis focuses on phenotypic trait variation in a widespread North American passerine species, the golden-crowned kinglet (*Regulus satrapa*). I investigated various factors that can influence geographic variation in plumage coloration and morphology (Chapter 2), and individual variation in carotenoid-based crown coloration (Chapter 3).

The role of natural selection on morphology and coloration

Intraspecific trait variation can result from selection for adaptations to different environments. Major factors that exert natural selection pressures include food availability, presence of competitors, physical habitat characteristics, and predation risk (reviewed in Endler, 1986). One of the most famous examples of natural selection based on food availability is the rapid evolution of the beaks of Darwin's medium ground finch (*Geospiza fortis*). After a severe drought in 1977, natural selection had favoured an increase in body size and bill dimensions: only larger birds (with larger beaks) were able to crack open the large, hard seeds that were still available (Boag & Grant, 1981). Another prominent example of natural selection is the effect of predation pressure and substrate composition on the ornamental coloration of wild guppies (*Poecilia reticulata*).

Male guppies inhabiting streams with dangerous predators normally exhibit fewer, smaller, and less colourful spots. When transplanted to streams with low predation, males evolved more conspicuous coloration within 10-15 generations (Endler, 1980). In addition, guppies evolved larger colour patches when living on coarse vs. fine gravel, which enabled more effective background matching (Endler, 1980, 1983). These findings demonstrate that natural selection favours crypsis in the face of high predation risk.

The role of sexual selection on ornaments and sexual dimorphism

Continuing with guppies, females prefer males with larger and more colourful carotenoid-based spots (Houde, 1997); hence, phenotypic traits involved in signalling are often under opposing forces of natural and sexual selection. Mate choice and intrasexual competition are the two driving forces of sexual selection and favour conspicuous traits that can effectively communicate information to conspecifics in order to enhance reproductive success. Signal detectability is affected by physical features such as the light environment (Théry, 2006); therefore, habitat differences can lead to variation in sexually selected traits (Schluter & Price, 1993). Population divergence can also result from differences in the intensity of sexual selection, which is influenced by population density, operational sex ratio (OSR), mating system, rates of extra-pair paternity (EPP), parental investment, and life history (reviewed in Andersson, 1994). For example, populations with male-biased OSR, high levels of EPP, or low male parental care would be predicted to have stronger sexual selection for elaborate male ornaments (e.g., Møller & Birkhead, 1994; Dunn *et al.*, 2001). In species where females invest more time at the nest than males, sexual selection favours conspicuous male traits and natural selection favours cryptic female traits to avoid nest predation (Wallace, 1889), resulting in sexual

dimorphism and dichromatism (reviewed in Badyaev & Hill, 2003). Investigating intraspecific variation in sexual dimorphism and dichromatism can reveal valuable information about the various selection pressures that are operating in different populations (e.g., Badyaev, 1997).

The role of genetic drift?

In theory, genetic drift can play an essential role in the genotypic and phenotypic divergence of allopatric populations. Theory predicts that small, isolated populations with low genetic diversity should be more susceptible to genetic drift and neutral mutations (Wright, 1931). However, there is currently little empirical evidence that genetic drift **alone** can effect variation in morphology or coloration (e.g., Clegg *et al.*, 2002; Yeh, 2004; but see Jordan & Snell, 2008). My MSc thesis focuses primarily on the potential contributions of natural and sexual selection.

Different scales of phenotypic variation

Intraspecific variation can be studied on multiple scales: geographic (i.e., large-scale) variation involves examining phenotypic differences between populations or subspecies. In contrast, individual (i.e., small-scale) variation focuses more on differences within a population. The complement of both types of studies provides a holistic view of how phenotypic variation can arise within a single species.

Geographic variation and ecogeographic rules

Geographic variation has often been studied in terms of ecogeographic rules – empirical patterns of biological traits that parallel variation in geographic or environmental

variables. Three commonly studied rules involving phenotypic traits are Bergmann's rule, Allen's rule, and Gloger's rule (Millien *et al.*, 2006). The first two rules describe variation in morphology with respect to temperature: body size tends to be larger (Bergmann's rule, Bergmann, 1847; see also James, 1970) and appendage length shorter (Allen's rule, Allen, 1877) in animals living in colder climates. Gloger's rule concerns melanin coloration and states that animals in warm, humid environments tend to be darker than those in cool, arid habitats (Gloger, 1833). All three patterns were originally thought to serve thermoregulation purposes; however, other hypotheses have since been proposed. For example, although a single mechanism cannot account for Bergmann's rule in all animals studied (Blackburn *et al.*, 1999), fasting endurance may be a viable option for both endo- and ectotherms (Ashton, 2002). Among the multiple potential explanations for Gloger's rule (Burt & Ichida, 2004), there has been a recent interest in the resistance of melanized feathers to bacterial degradation (Shawkey & Hill, 2004).

Aside from conventional ecogeographic rules, Badyaev (1997) observed an altitudinal pattern of variation in sexual dimorphism and dichromatism in a comparative analysis of cardueline finches. He attributed this variation to the need for bi-parental investment in harsher ecological conditions (see also Badyaev & Ghalambor, 2001), which would reduce sexual selection for elaborate male ornamentation (Andersson, 1994), as well as intensify natural selection for crypsis at the nest. Badyaev's (1997) findings open up a new avenue for investigating large-scale variation in sexually selected traits, such as carotenoid coloration.

Individual variation in signals of quality

Traits that honestly signal an individual's phenotypic and/or genotypic quality are of particular interest when studying variation. These quality indicators are important for mate choice and intrasexual competition (Andersson, 1994) and tend to be highly variable within a population (Dale, 2006). Secondary sexual characters such as weapons (e.g., antlers in red deer [*Cervus elaphus*], Clutton-Brock *et al.*, 1982) and ornaments (e.g., carotenoid coloration in threespine sticklebacks [*Gasterosteus aculeatus*], Milinski & Bakker, 1990; elongated tails in barn swallows [*Hirundo rustica*], Møller, 1994) are classic examples of condition-dependent traits. Mechanisms of variation in carotenoid-based plumage coloration are discussed in the following section.

PLUMAGE COLORATION

Carotenoid coloration

Pigments and colours

Carotenoid pigments are largely responsible for the red, orange, and yellow colours seen in a wide variety of animals, ranging from fish to reptiles and birds. Carotenoids cannot be synthesized endogenously; instead, these pigments are derived from plants and proceed up the food chain. Lutein and zeaxanthin are abundant in many plants and insects, especially larvae (Goodwin, 1980, 1984). These yellow hydroxycarotenoids are deposited unmodified to produce the golden yellow plumage of many species (McGraw, 2006). Alternatively, they can be transformed into other common yellow pigments, such as 3'-dehydrolutein and canary xanthophylls A and B (Stradi, 1998).

Along with β -carotene and β -cryptoxanthin, lutein and zeaxanthin are also the proposed precursors to many red ketocarotenoids found in feathers (Stradi, 1998; McGraw, 2006). The pigment composition of red or orange plumage can be much more complex than that of yellow plumage. For example, 11 carotenoids were isolated in male red-collared widowbirds (*Euplectes ardens*, Andersson *et al.*, 2007), and a suite of 13 carotenoids is responsible for the colourful patches of male house finches (*Carpodacus mexicanus*, Inouye *et al.*, 2001). Carotenoid coloration can vary depending on the types, amounts, and relative proportions of pigments present. Studies have found that birds can adopt two general strategies to become more colourful: 1) deposit as many pigments as possible (Saks *et al.*, 2003a; McGraw & Gregory, 2004), or 2) selectively deposit more colourful red pigments (e.g., Inouye *et al.*, 2001; McGraw *et al.*, 2001).

In species that exhibit sexual dichromatism in carotenoid coloration, this can be due to the ability of only one sex (usually the male) to metabolize dietary carotenoids (McGraw, 2006). Alternatively, the sexes may differ in their pathways of carotenoid conversion, such that males can metabolize ingested pigments into red ketocarotenoids, while females instead modify the same pigments into derived yellow xanthophylls (e.g., Hudon, 1991; Stradi *et al.*, 1998). The proximate factor(s) controlling dichromatism is not well understood. Hypotheses include sex differences in the enzymatic machinery necessary for carotenoid conversion (McGraw, 2006), or differences in hormone levels that control biochemical pathways during moult (Kimball, 2006).

Condition-dependence and trait variation

Because carotenoids cannot be synthesized, Endler (1980) originally formulated the 'carotenoid limitation' hypothesis, stating that only individuals with superior foraging ability can obtain these valuable pigments (see Grether *et al.*, 1999; Hill *et al.*, 2002). Other steps in the biochemical pathway, including absorption, transport, and metabolism (reviewed in McGraw, 2006), may also be condition-dependent (Olson & Owens, 1998). For example, food-deprived house finches were less efficient at metabolizing dietary yellow carotenoids into red pigments (Hill, 2000), and carotenoid absorption and/or transport were nutritionally sensitive in American goldfinches (*Carduelis tristis*, McGraw *et al.*, 2005). Furthermore, feather growth rate (as a measure of nutritional condition, Grubb, 1989) was significantly correlated with carotenoid plumage coloration in house finches (Hill & Montgomerie, 1994) and great tits (*Parus major*, Senar *et al.*, 2003).

Based on the well-known functions of carotenoids in humans, Lozano (1994) first proposed the idea that ornamental carotenoid coloration may also be an honest indicator of immune health and parasite resistance (see also Hamilton & Zuk, 1982). More colourful birds are expected to be of higher quality, since they harbour sufficient carotenoids both to serve immune demands and for sexual signalling (e.g., Saks *et al.*, 2003b; Aguilera & Amat, 2007). For example, infection with endoparasites resulted in duller plumage at the next moult in house finches (Brawner *et al.*, 2000) and European greenfinches (*Carduelis chloris*; Hōrak *et al.*, 2004). In the latter species, there is also evidence that plumage coloration predicts future resistance to infection (Lindström & Lundström, 2000; Hill & Farmer, 2005). In contrast, studies on the effects of feather mites have found conflicting results, suggesting that these organisms may not exert a strong adverse effect on individual condition (reviewed in Hill, 2006a).

Furthermore, variation in carotenoid coloration in males can advertise direct benefits available to females. For example, more colourful males may be socially dominant or provide superior courtship feeding, fertilization insurance, paternal care, or nest defence (reviewed in Griffith & Pryke, 2006). Altogether, carotenoid-based coloration can be influenced by multiple environmental and genetic regulators, and variation in these colourful ornaments is subject to strong sexual selection pressures, especially mate choice.

Melanin and structural coloration

There are two major classes of melanin pigments: eumelanins are responsible for dark brown, grey, and black colours; and pheomelanins produce colours ranging from yellow to reddish brown (Prota, 1992). Unlike carotenoids, melanins are synthesized endogenously via melanocytes in the epidermis of birds and mammals (Duval *et al.*, 2002). Structural coloration can be divided into iridescent colours; non-iridescent blue, green, and violet colours; and white reflected from unpigmented feathers. This type of coloration is produced by the scattering of light due to nanometre-scale variations in the arrangement of keratin, air vacuoles, and/or melanin granules within feather microstructure (Prum, 2006). Some colours are produced by a combination of these mechanisms. Certain green colours are produced when yellow carotenoids are deposited on top of a melanin layer, as seen in the olive-green plumage of many warbler species (Fox & Vevers, 1960). Other green colours result from the combination of structural blue coloration and yellow carotenoids (Fox, 1976). Recently, feather microstructure was found to enhance carotenoid coloration: carotenoid pigments absorb incoherently

scattered light from the underlying white structural tissue and reflect yellow light, resulting in more saturated, colourful plumage (Shawkey & Hill, 2005).

TECHNIQUES FOR MEASURING COLOUR VARIATION

Reflectance spectrometry and colour analysis

Prior to the advent of more sophisticated methods of colour quantification, researchers assessed animal and plant coloration through arbitrary rankings and comparing the patch of interest to photographs or colour swatches (reviewed in Montgomerie, 2006). The introduction of reflectance spectrometers in the 1990s has allowed for a more objective quantification of colour (Andersson & Prager, 2006). We can now measure colour at all wavelengths visible to birds, including both ultraviolet (UV) and visible spectra (300-700 nm, Cuthill, 2006). Reflectance spectrometry involves shining full-spectrum light onto a colour patch, and collecting the light reflected back. Measurements are taken in reference to a white standard and a dark signal, and data acquisition software enables colour to be visualized as spectrographs. While various means of colour analysis are available (reviewed in Montgomerie, 2006), researchers often calculate the HSB tri-stimulus colour variables (hue, saturation, brightness) using colour analysis software (e.g., Montgomerie, 2008). We can thus investigate colour variation by comparing these variables between individuals or populations. In terms of pigment-based coloration, pigment concentration is generally inversely related to brightness, and positively correlated with hue and saturation (Andersson & Prager, 2006). If pigment concentration is a condition-dependent trait, hue should be the most reliable colorimetric variable to assess honest signalling (Andersson & Prager, 2006).

Carotenoid extraction and high-performance liquid chromatography

Biochemical analysis of carotenoids in plumage using advanced chromatographic techniques is a relatively new but prolific field of research (reviewed in Table 5.1 in McGraw, 2006). In the 1990s, Stradi et al. (1995) pioneered the use of modern pigment extraction methods and high-performance liquid chromatography (HPLC) for feather carotenoid analyses. Carotenoids are extracted from feathers by means of thermochemical (Hudon & Brush, 1992) or mechanical procedures (Stradi *et al.*, 1995), and the coloured solution is injected into the HPLC system. Pigments are separated via differences in polarity, where polar carotenoids (xanthophylls) elute more quickly than non-polar compounds (carotenes). A photodiode array detector records the UV and visible spectra and the results are then visualized as chromatograms with each compound as an individual peak. We can identify carotenoids by comparing their retention times, peak wavelength(s), and peak shapes with internal or external reference standards, or with published data for known carotenoids (e.g., Stradi *et al.*, 1995; Stradi, 1998; Inouye *et al.*, 2001; Andersson *et al.*, 2007). We can then quantify the total and relative amounts of each pigment by measuring the areas under the peaks. Pigment concentrations can be calculated by measuring the absorbance of each pigment/sample by spectrophotometry or by plotting HPLC peak area on a calibration curve generated with a reference standard.

STUDY SYSTEM

Many years ago, a boy found on the doorstep the body of a tiny feathered gem. ... He picked it up and was entranced with the delicate beauty of its soft olive colors and with its crown of brilliant orange and gold, which glowed like a ball of fire.
— Arthur Cleveland Bent, *Life Histories of North American Thrushes, Kinglets, and their Allies* (1949)

Golden-crowned kinglets

The golden-crowned kinglet belongs to the Family Regulidae (Order Passeriformes) and is one of the smallest bird species in North America. In the 1950s, Robert and Carlyn Galati's extensive observations on this species' breeding biology revealed that kinglets are serially monogamous and exhibit bi-parental care: females are responsible for incubating eggs and brooding nestlings, males participate in nest/territory defence and mate-feeding, and both sexes contribute to nest-building and chick-feeding (Galati & Galati, 1985; Galati, 1991). This type of mating and breeding system suggests that one or both sexes may be choosy regarding their mates (Andersson, 1994), and golden-crowned kinglets may be an ideal study species for the investigation of an ornamental trait present in both sexes.

One of the most distinctive features of golden-crowned kinglets is their crown coloration. Aside from ruby-crowned kinglets (*Regulus calendula*), the Regulidae possesses a conserved pattern of sexual dichromatism: males have an orange crown-patch bordered by yellow feathers, and females have a uniformly yellow crown (Martens & Päckert, 2006). In both sexes the colourful crown is accented by black lateral crown stripes. Male kinglets normally keep the crown-patch hidden; however, they will expose these central orange feathers and completely displace the yellow during courtship and in the presence of intruders (Martens & Päckert, 2006). Although not as visually striking, female kinglets will also erect their crown feathers when agitated (Blake, 1968). These behaviours suggest that crown coloration in both sexes play a role in sexual selection.

Research on the crown coloration of the Regulidae previously consisted only of pigment analyses on goldcrest (*Regulus regulus*) feathers (Stradi, 1998). Despite the close phylogenetic relationship between goldcrests and golden-crowned kinglets (Päckert

et al., 2003), the basic carotenoid profiles of these two species may not be the same due to differences in habitat and diet. Golden-crowned kinglets are primarily insectivorous conifer specialists that feed opportunistically on a wide assortment of arthropods, especially caterpillars and other larvae (Galati & Galati, 1985; Heinrich & Bell, 1995). It is unknown whether individuals select exceptionally nutritious and/or carotenoid-rich items during the moulting and migratory periods. There is evidence that kinglets may consume small amounts of fruit matter during migration (Parrish, 1997), which could provide additional carotenoids for ornamental coloration.

There are five subspecies of golden-crowned kinglets throughout Canada and the United States, with additional isolated populations in Mexico and Guatemala (Ingold & Galati, 1997). While some populations are resident, golden-crowned kinglets are generally short-distance migrants. Previous descriptions of trait variation were limited to subspecific differences in morphology and plumage coloration (reviewed in Ingold & Galati, 1997). Qualitative accounts illustrated male crown coloration as ranging from ‘cadmium orange’ in the Pacific northwest (*R. s. olivaceous*) to ‘flame scarlet’ in the tropics (*R. s. clarus*), and the female crown in general as varying from ‘wax yellow’ to ‘yellow chrome’ (Jenks, 1936). Overall, evidence of crown colour variation in golden-crowned kinglets, combined with the wide-ranging distribution of this species and knowledge of the sexually selected nature of carotenoid coloration, provided an exciting research opportunity to investigate geographic and individual variation in more detail.

Geographic and individual variation in golden-crowned kinglets

The overall goal of my thesis was to characterize both large- and small-scale variation in golden-crowned kinglet crown coloration, and to investigate possible proximate factors

governing the observed variation. In Chapter 2, I investigated geographic variation in coloration and morphology in relation to the ecogeographic rules and Badyaev's (1997) hypothesis regarding variation in sexual dimorphism and dichromatism. In Chapter 3, I investigated the condition-dependent and pigmentary bases of individual variation in crown coloration in a migrating population of kinglets. Together, these studies provide a more comprehensive view of how plumage colour can vary within a species, which also has implications for the evolution of sexually selected traits and preferences. Both data chapters were written in preparation for submission to scientific journals: Chapter 2 has been published in the *Journal of Biogeography*, and Chapter 3 is currently in preparation for submission to *Functional Ecology*.

REFERENCES

- Aguilera, E. & Amat, J. A. (2007) Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (*Carduelis chloris*). *Naturwissenschaften*, **94**, 895-902.
- Allen, J. A. (1877) The influence of physical conditions in the genesis of species. *Radical Review*, **1**, 108-140.
- Andersson, M. B. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Andersson, S. & Prager, M. (2006) Quantifying colors. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 41-89. Harvard University Press, Cambridge, MA.
- Andersson, S., Prager, M. & Johansson, E. I. A. (2007) Carotenoid content and reflectance of yellow and red nuptial plumages in widowbirds (*Euplectes* spp.). *Functional Ecology*, **21**, 272-281.
- Ashton, K. G. (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography*, **11**, 505-523.
- Badyaev, A. V. (1997) Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behavioral Ecology*, **8**, 675-690.
- Badyaev, A. V. & Ghalambor, C. K. (2001) Evolution of life histories along elevational gradients: trade-off between parental care and fecundity. *Ecology*, **82**, 2948-2960.
- Badyaev, A. V. & Hill, G. E. (2003) Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 27-49.
- Bergmann, C. (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **3**, 595-708.

- Blackburn, T. M., Gaston, K. J. & Loder, N. (1999) Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions*, **5**, 165-174.
- Blake, C. H. (1968) Crown display of golden-crowned kinglet. *The Auk*, **85**, 506.
- Boag, P. T. & Grant, P. R. (1981) Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galápagos. *Science*, **214**, 82-85.
- Brawner, W. R., Hill, G. E. & Sunderman, C. A. (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *The Auk*, **117**, 952-963.
- Burt, E. H. & Ichida, J. M. (2004) Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. *The Condor*, **106**, 681-686.
- Clegg, S. M., Degnan, S. M., Moritz, C., Estoup, A., Kikkawa, J. & Owens, I. P. F. (2002) Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. *Evolution*, **56**, 2090-2099.
- Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. (1982) *Red Deer: Behavior and Ecology of Two Sexes*. University of Chicago Press, Chicago, IL.
- Coyne, J. A. & Orr, H. A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA.
- Cuthill, I. C. (2006) Color perception. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 3-40. Harvard University Press, Cambridge.
- Dale, J. (2006) Intraspecific variation in coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 36-86. Harvard University Press, Cambridge.
- Darwin, C. (1859) *On the Origin of Species by Means of Natural Selection*. J. Murray, London, UK.
- Darwin, C. (1871) *The Descent of Man, and Selection in Relation to Sex*. J. Murray, London, UK.
- Dunn, P. O., Whittingham, L. A. & Pitcher, T. E. (2001) Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution*, **55**, 161-175.
- Duval, C., Smit, N. P. M., Kolb, A. M., Régnier, M., Pavel, S. & Schmidt, R. (2002) Keratinocytes control the pheo/eumelanin ratio in cultured normal human melanocytes. *Pigment Cell Research*, **15**, 440-446.
- Endler, J. A. (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76-91.
- Endler, J. A. (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes*, **9**, 173-190.
- Endler, J. A. (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
- Fox, D. L. (1976) *Animal Biochromes and Structural Colours*. University of California Press, Berkeley, CA.
- Fox, H. M. & Vevers, G. (1960) *The Nature of Animal Colours*. Macmillan, New York, NY.
- Galati, B. & Galati, C. (1985) Breeding of the golden-crowned kinglet in northern Minnesota. *Journal of Field Ornithology*, **56**, 28-40.
- Galati, R. (1991) *Golden-crowned Kinglets: Treetop Nesters of the North Woods*. Iowa State University Press, Ames, IA.

- Gloger, C. (1833) *Das Abändern der Vögel durch Einfluss des Klimas*. A. Schulz, Breslau, Germany.
- Goodwin, T. W. (1980) *The Biochemistry of the Carotenoids. Vol. I: Plants*. Chapman and Hall, London, UK.
- Goodwin, T. W. (1984) *The Biochemistry of the Carotenoids. Vol. II: Animals*. Chapman and Hall, London, UK.
- Grether, G. F., Hudon, J. & Millie, D. F. (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 1317-1322.
- Griffith, S. C. & Pryke, S. R. (2006) Benefits to females of assessing color displays. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 233-. Harvard University Press, Cambridge, MA.
- Grubb, T. C. (1989) Ptilochronology: feather growth bars as indicators of nutritional status. *The Auk*, **106**, 314-320.
- Hamilton, W. D. & Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384-387.
- Heinrich, B. & Bell, R. (1995) Winter food of a small insectivorous bird, the golden-crowned kinglet. *Wilson Bulletin*, **107**, 558-561.
- Hill, G. E. (2000) Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology*, **31**, 559-566.
- Hill, G. E. (2006) Environmental regulation of ornamental coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 507-560. Harvard University Press, Cambridge, MA.
- Hill, G. E. & Farmer, K. L. (2005) Carotenoid-based plumage coloration predicts resistance to a novel parasite in the house finch. *Naturwissenschaften*, **92**, 30-34.
- Hill, G. E., Inouye, C. Y. & Montgomerie, R. (2002) Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society of London - Series B: Biological Sciences*, **269**, 1119-1124.
- Hill, G. E. & Montgomerie, R. (1994) Plumage colour signals nutritional condition in the house finch. *Proceedings of the Royal Society of London B: Biological Sciences*, **258**, 47-52.
- Hörak, P., Saks, L., Karu, U., Ots, I., Surai, P. F. & McGraw, K. J. (2004) How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology*, **73**, 935-947.
- Houde, A. E. (1997) *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, Princeton, NJ.
- Hudon, J. (1991) Unusual carotenoid use by the western tanager (*Piranga ludoviciana*) and its evolutionary implications. *Canadian Journal of Zoology*, **69**, 2311-2320.
- Hudon, J. & Brush, A. H. (1992) Identification of carotenoid pigments in birds. *Methods in Enzymology*, **213**, 312-321.
- Ingold, J. L. & Galati, R. (1997) Golden-crowned kinglet (*Regulus satrapa*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/301> (last accessed 23 January 2009).
- Inouye, C. Y., Hill, G. E., Stradi, R. D. & Montgomerie, R. (2001) Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *The Auk*, **118**, 900-915.

- James, F. C. (1970) Geographic size variation in birds and its relationship to climate. *Ecology*, **51**, 365-390.
- Jenks, R. (1936) A new race of golden-crowned kinglet from Arizona. *The Condor*, **38**, 239-244.
- Jordan, M. A. & Snell, H. L. (2008) Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex). *Molecular Ecology*, **17**, 1224-1237.
- Kimball, R. T. (2006) Hormonal control of coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 431-468. Harvard University Press, Cambridge, MA.
- Lindström, K. & Lundström, J. (2000) Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. *Behavioral Ecology and Sociobiology*, **48**, 44-51.
- Martens, J. & Päckert, M. (2006) Family Regulidae (kinglets and firecrests). *Handbook of the Birds of the World* (ed. by J. del Hoyo, A. Elliot and D. Christie), 330-349. Lynx Edicions, Barcelona, Spain.
- McGraw, K. J. (2006) Mechanics of carotenoid-based coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 177-242. Harvard University Press, Cambridge, MA.
- McGraw, K. J. & Gregory, A. J. (2004) Carotenoid pigments in male American goldfinches: what is the optimal biochemical strategy for becoming colourful? *Biological Journal of the Linnean Society*, **83**, 273-280.
- McGraw, K. J., Hill, G. E. & Parker, R. S. (2005) The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. *Animal Behaviour*, **69**, 653-660.
- McGraw, K. J., Hill, G. E., Stradi, R. & Parker, R. S. (2001) The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiological and Biochemical Zoology*, **74**, 843-852.
- Milinski, M. & Bakker, T. C. M. (1990) Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344**, 330-333.
- Millien, V., Lyons, S. K., Olson, L., Smith, F. A., Wilson, A. B. & Yom-Tov, Y. (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters*, **9**, 853-869.
- Møller, A. P. (1994) *Sexual Selection and the Barn Swallow*. Oxford University Press, Oxford, UK.
- Møller, A. P. & Birkhead, T. R. (1994) The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution*, **48**, 1089-1100.
- Montgomerie, R. (2006) Analyzing colors. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 90-147. Harvard University Press, Cambridge, MA.
- Montgomerie, R. (2008) CLR, version 1.04. Queen's University, Kingston, ON. Available at: <http://post.queensu.ca/~mont/color/analyze.html> (last accessed 27 May 2008).
- Olson, V. A. & Owens, I. P. F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution*, **13**, 510-514.

- Päckert, M., Martens, J., Kosuch, J., Nazarenko, A. A. & Veith, M. (2003) Phylogenetic signal in the song of crests and kinglets (Aves: *Regulus*). *Evolution*, **57**, 616-629.
- Parrish, J. D. (1997) Patterns of frugivory and energetic condition in Nearctic landbirds during autumn migration. *The Condor*, **99**, 681-697.
- Prota, G. (1992) *Melanins and Melanogenesis*. Academic Press, New York, NY.
- Prum, R. O. (2006) Anatomy, physics, and evolution of structural colors. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 295-353. Harvard University Press, Cambridge, MA.
- Saks, L., McGraw, K. J. & Hörak, P. (2003a) How feather colour reflects its carotenoid content. *Functional Ecology*, **17**, 555-561.
- Saks, L., Ots, I. & Hörak, P. (2003b) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia*, **134**, 301-307.
- Schluter, D. & Price, T. (1993) Honesty, perception and population divergence in sexually selected traits. *Proceedings of the Royal Society B: Biological Sciences*, **253**, 117-122.
- Senar, J. C., Figuerola, J. & Domènech, J. (2003) Plumage coloration and nutritional condition in the great tit *Parus major*: the roles of carotenoids and melanins differ. *Naturwissenschaften*, **90**, 234-237.
- Shawkey, M. D. & Hill, G. E. (2004) Feathers at a fine scale. *The Auk*, **121**, 652-655.
- Shawkey, M. D. & Hill, G. E. (2005) Carotenoids need structural colours to shine. *Biology Letters*, **1**, 121-124.
- Stradi, R. (1998) *The Colour of Flight: Carotenoids in Bird Plumage*. Solei Gruppo Editoriale Informatico, Milan, Italy.
- Stradi, R., Celentano, G. & Nava, D. (1995) Separation and identification of carotenoids in bird's plumage by high-performance liquid chromatography--diode-array detection. *Journal of Chromatography*, **670**, 337-348.
- Stradi, R., Hudon, J., Celentano, G. & Pini, E. (1998) Carotenoids in bird plumage: the complement of yellow and red pigments in true woodpeckers (Picinae). *Comparative Biochemistry and Physiology Part B*, **120**, 223-230.
- Théry, M. (2006) Effects of light environment on color communication. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 148-173. Harvard University Press, Cambridge, MA.
- Wallace, A. R. (1889) *Darwinism*. Macmillan, London, UK.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97-159.
- Yeh, P. J. (2004) Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution*, **58**, 166-174.
- Zink, R. M. & Remsen, J. V. (1986) Evolutionary processes and patterns of geographic variation in birds. *Current Ornithology*, **4**, 1-69.

CHAPTER 2

A TEST OF ECOLOGICAL AND SEXUAL SELECTION HYPOTHESES FOR GEOGRAPHICAL VARIATION IN COLORATION AND MORPHOLOGY OF GOLDEN-CROWNED KINGLETS (*REGULUS SATRAPA*) *

* This chapter was the outcome of joint research with my advisor, Dr. Stéphanie Doucet

SYNOPSIS

Aim: To date, studies on geographical variation have investigated Bergmann's rule extensively, yet Gloger's rule remains infrequently tested, and climatic predictors of variation in carotenoid coloration have not yet been studied. In addition, hypotheses based on sexual selection, which predict that sexual dimorphism should vary with population density and climatic conditions, have received little attention. Our goals were to characterize geographical variation in the coloration and morphology of golden-crowned kinglets, *Regulus satrapa* (Passeriformes, Regulidae), and to investigate possible ecological and sexual selection correlates of this variation.

Location: The entire species range of golden-crowned kinglets, comprising North and Central America.

Methods: We collected data from 511 museum specimens, dating from 1847 to 2006, encompassing all five subspecies of golden-crowned kinglets. We used reflectance spectrometry to quantify crown and mantle coloration, and measured wing-chord and tarsus length to approximate body size. We obtained geographical and climatic data from online databases, and population density estimates from the literature.

Results: There were significant subspecific and gender differences in crown coloration and morphology: male kinglets were generally larger and more colourful. Our data revealed mixed support for Bergmann's rule: tarsus length decreased with increasing latitude, while patterns of variation in wing-chord and tarsus length showed conflicting results with temperature. Mantle coloration exhibited an opposite trend to that predicted by Gloger's rule: upperparts became lighter with increasing relative humidity. Crown coloration was negatively correlated with actual evapotranspiration, suggesting that primary productivity levels are not directly linked to carotenoid abundance. Sexual

dimorphism and dichromatism generally increased with greater population density, lower latitudes and elevations, and warmer temperatures, supporting a previously observed pattern of variation in sexual dimorphism.

Main conclusions: Geographical variation in golden-crowned kinglets yielded mixed support for Bergmann's rule and contradicted Gloger's rule, suggesting that other mechanisms may be operating. Allen's rule is likely to be a stronger factor influencing tarsus length. Differences in the degree of sexual dimorphism and dichromatism in varying climatic conditions suggest that the intensity of sexual selection differs between habitats. Further studies on geographical variation in sexual dimorphism in various taxa may reveal a previously unrecognized ecogeographical rule.

INTRODUCTION

In many species, variation in ecological and social selection pressures leads to phenotypic divergence between populations. Such geographical variation in phenotypic traits may arise through genetic differences that have accumulated through natural selection (e.g., Boag & Grant, 1981; Endler, 1991; Reznick *et al.*, 1997), sexual selection (West-Eberhard, 1983; Endler & Houde, 1995) or genetic drift (reviewed in Coyne & Orr, 2004). In contrast, trait differences could result from phenotypic plasticity in response to differing environments (Price *et al.*, 2003). Understanding patterns of geographical variation can provide insights into the extent of reproductive isolation between populations (Zink & Remsen, 1986). The purpose of our study was to characterize the geographical variation in coloration and morphology in a wide-ranging passerine species, and to evaluate ecological and sexual selection hypotheses that may explain the observed variation.

Sexually selected traits, such as body size and ornamental coloration, are particularly interesting traits in which to investigate geographical variation because they are governed by both natural and sexual selection and contribute importantly to individual fitness (Andersson, 1994). Studies on geographical variation in body size have commonly focused on testing Bergmann's rule (reviewed in Blackburn *et al.*, 1999; Millien *et al.*, 2006; Gaston *et al.*, 2008). This ecogeographical rule, which states that body size should increase in colder climates, was originally proposed to explain interspecific body size variation in homeotherms (Bergmann, 1847). Bergmann's rule has since been expanded to encompass both inter- and intraspecific variation in body size, and studies often use latitude and elevation as proxies for variation in temperature (reviewed in Blackburn *et al.*, 1999; see also Ashton, 2002b; Guillaumet *et al.*, 2008). Bergmann's rule has been generally, but not universally, supported in birds and mammals (Ashton *et al.*, 2000; Ashton, 2002b; Meiri & Dayan, 2003). By contrast, several other taxonomic groups do not seem to adhere to Bergmann's rule (e.g., Ashton, 2002a; Ashton & Feldman, 2003).

Research on geographical variation in melanin coloration has often addressed Gloger's ecogeographical rule, which states that animal coloration should be darker (increased pigment deposition) with increasing humidity (Gloger, 1833; Millien *et al.*, 2006; Gaston *et al.*, 2008). Less attention has been devoted to rigorously testing Gloger's rule; studies to date have been mostly limited to human-based visual assessments of colour in mammals (e.g., Sutton & Patterson, 2000; Stoner *et al.*, 2003; Lai *et al.*, 2008) and birds (e.g., Zink & Remsen, 1986; Aldrich & James, 1991). However, the evidence thus far provides strong support for this coloration trend; for example, of 52 North

American bird species assessed, 94% were in concordance with Gloger's rule (Zink & Remsen, 1986).

While no general ecogeographical rules have been proposed for non-melanic colour variation, the condition-dependence (Hill, 2006a) and sexually-selected nature (Hill, 2006b) of carotenoid coloration suggest that these ornaments would be particularly variable (Dale, 2006). In turn, different environmental and social selection pressures between populations may result in geographical variation. Seminal studies on interpopulational variation in carotenoid coloration originally focused on the orange spots of guppies (*Poecilia reticulata*, e.g., Endler, 1980, 1983). Subsequently, researchers have investigated variation in reptiles (e.g., Kwiatkowski & Sullivan, 2002; Macedonia *et al.*, 2002) and birds (e.g., Hill, 1993; Norris *et al.*, 2007); however, research on this subject continues to lag behind work on other ecogeographical rules.

To better understand the role of sexual selection, geographical variation should be studied in terms of sexual dichromatism and dimorphism rather than absolute changes in the more ornamented gender. While a few studies have investigated differences between the genders (e.g., Macedonia *et al.*, 2002; Howes & Lougheed, 2007), research in this area is still lacking, especially in birds. One geographical pattern in sexual dimorphism that is currently not well-recognized is based on sexual selection. Badyaev (1997) proposed that elevational variation in sexual dimorphism results from the need for greater bi-parental investment in colder climates and increased mating opportunities in warmer climates, leading to a reduced intensity of sexual selection on males at higher elevations. Recent work on cardueline finches supports this hypothesis: both sexual dichromatism and male song elaboration are reduced for species living at higher elevations (Snell-Rood & Badyaev, 2008).

Finally, many studies of geographical variation have been limited to a restricted portion of a species' range (but see Aldrich & James, 1991; Howes & Lougheed, 2007; Norris *et al.*, 2007). Here, we use a comprehensive museum study to characterize geographical variation in sexual dichromatism and dimorphism in golden-crowned kinglets (*Regulus satrapa* Lichtenstein, 1823) on a continental scale. Golden-crowned kinglets occur from the west to east coasts of North America, and from Alaska south to Mexico, with additional isolated populations in southern Mexico and Guatemala (Martens & Päckert, 2006). To date, only qualitative accounts of subspecific variation in morphology and coloration exist for this species (Jenks, 1936; Martens & Päckert, 2006). Although both male and female kinglets have olive-grey upperparts, females have a uniformly yellow crown, whereas males have an orange crown-patch bordered by yellow feathers. The orange feathers are usually concealed; however, males expose this crown-patch by ptiloerection during courtship and antagonistic interactions (Martens & Päckert, 2006), suggesting that it functions as a sexual ornament.

The goals of our study were to characterize geographical variation in sexual dichromatism and dimorphism in golden-crowned kinglets, and to investigate possible mechanisms driving variation in morphology and colour. If kinglet body size follows Bergmann's rule, we predict that birds would be larger with decreasing temperature and therefore with increasing latitude and elevation (James, 1970). If kinglet mantle coloration follows Gloger's rule, we predict that birds would exhibit darker pigmentation with increasing humidity (Gloger, 1833). If carotenoid coloration is influenced by access to environmental carotenoids (Grether *et al.*, 1999), we predict that crown colour would correlate with variation in primary productivity, which may be partly determined by the abundance and types of food available in the environment (Hector *et al.*, 1999).

If sexual selection pressures differ between kinglet populations, then the extent of sexual dimorphism may also vary geographically (Badyaev, 1997; Kwiatkowski & Sullivan, 2002). We predict that the intensity of sexual selection would be greater in areas with higher population density, which would allow individuals to be choosier about mates, as well as increase intrasexual competition (Kokko & Rankin, 2006). If the intensity of sexual selection for larger and more ornamented males is greater when male parental investment requirements are lower (Badyaev, 1997), we predict that the degree of sexual dichromatism and dimorphism would be greater in warmer climates and at lower latitudes and elevations.

MATERIALS AND METHODS

We collected data from 511 golden-crowned kinglet specimens from the University of Michigan Museum of Zoology and the Harvard Museum of Comparative Zoology. Specimens dated from 1847 to 2006. Collection locations ranged from Alaska south to Guatemala and east to Newfoundland, with most specimens originating from the continental United States and the west coast of Canada. Age and gender were determined from information on the specimen vouchers and confirmed by plumage; our analyses included only individuals in adult plumage. Of these specimens, 332 were males and 179 were females. All five recognized subspecies were represented in this dataset: 322 individuals of *R. s. satrapa*, 144 of *R. s. olivaceous*, 31 of *R. s. apache* (and previously *R. s. amoenus*), eight of *R. s. clarus* and six of *R. s. aztecus*. We used dial calipers to measure bill length and tarsus length to the nearest 0.05 mm and a wing rule to measure wing-chord to the nearest 0.5 mm for each kinglet; we could not record some measurements from specimens in poor condition.

Reflectance spectrometry

To quantify kinglet plumage coloration, we measured reflectance using an Ocean Optics USB4000 reflectance spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). A single fibre-optic probe transmitted full-spectrum light from the lamp and transferred light reflected from the specimen back to the spectrometer. The probe was mounted with a rubber sheath that extended *c.* 5 mm past the tip to maintain a fixed distance to the specimen and exclude external light. We measured reflectance by placing the probe perpendicular to the feather surface and taking five readings for each of two body regions per individual, each of which comprised an average of 30 spectra collected sequentially by OOIBase32 software (Ocean Optics, Dunedin, FL, USA): the crown (orange in males, yellow in females) and the mantle (olive-grey in both genders). We chose these regions because the sexually dimorphic crown colour of *Regulus* species results from carotenoid pigmentation (Stradi, 1998) and may be under differential sexual selection, whereas the sexually monomorphic mantle colour probably results from a combination of melanin and carotenoid pigments (Fox & Vevers, 1960) and is unlikely to be a target of sexual selection in this species. We also measured the outer yellow feathers of male crowns to compare these with the female's yellow crown, although most of our analyses focus on the conspicuously dichromatic crown centre. All spectral measurements were expressed as the percentage of reflected light relative to a Spectralon white standard (Ocean Optics, Dunedin, FL, USA).

We restricted our analyses to wavelengths of 300-700 nm, which comprise the extent of the avian visual spectrum (Cuthill, 2006). We used the software program CLR (Montgomerie, 2008) to calculate three colorimetric variables summarizing variation in reflectance data: saturation, hue and brightness (increased brightness usually corresponds

to lighter pigmentation) (Montgomerie, 2006). We calculated saturation as the difference between the maximum and the minimum brightness, divided by the total brightness; hue as the wavelength at which the spectrum reaches half of its maximum reflectance; and brightness as the average reflectance between 300 and 700 nm.

Geographical data

We recorded collection locations from specimen vouchers and searched online databases to determine their latitude, longitude and elevation. Geographical coordinates were obtained from Global Gazetteer v2.1 (Falling Rain Genomics, 2006), the U.S. Board of Geographic Names (U.S. Geological Survey, 2009) and the Canadian Geographical Names Data Base (Natural Resources Canada, 2007). We determined whether the specimens were collected from the breeding, wintering or year-round range (Ingold & Galati, 1997); analyses are based on the entire dataset unless otherwise noted. For some analyses, we were interested in comparing colour and morphology across populations. To that end, we mapped out the specimen collection locations and grouped the kinglets into 20 geographical clusters based on observed cohesion of these plotted points to ecoregions defined by the U.S. Department of Agriculture, Forest Service (Bailey & Cushwa, 1981; Bailey, 1994). We were able to obtain average estimates of population density of golden-crowned kinglets in undisturbed habitats for nine of our ecoregion clusters (Ingold & Galati, 1997). Following Kwiatkowski & Sullivan (2002), we used these data in our analyses as an index of the intensity of sexual selection within each breeding population.

Climate data

We collected climate data for each specimen's collection location from the nearest climate station available using average measurements from all available years (a minimum of 30 years). We obtained mean dry-bulb temperature and relative humidity from Weatherbase (Canty and Associates, 2009), the Comparative Climatic Data Publication (National Climatic Data Center, 2007) and the National Climate Data and Information Archive (Environment Canada, 2009). We calculated wet-bulb temperature using dry-bulb temperature, relative humidity and elevation. We collected actual evapotranspiration data from published records (Thorntwaite Associates, 1964a, b) and used these as a measure of primary productivity (Rosenzweig, 1968). We restricted some analyses involving climate to the breeding and moulting periods because morphological development of nestlings and feather growth occur during those months (Ricklefs, 1968; Ingold & Galati, 1997); thus, actual evapotranspiration and relative humidity data were average values for May through September. We used mean January and July temperatures to test the effects of seasonality on the species' adherence to Bergmann's rule.

Statistical analyses

All analyses were conducted using JMP 6.0.0 (SAS Institute, Cary, NC, USA). We used generalized linear models to analyse subspecies and gender differences in morphology and coloration, and to determine the effects of geographical and climatic variables on kinglet morphology and coloration. We controlled for the possible effects of collection year on specimen coloration by including these data as covariates in our analyses (Doucet & Hill, 2009). Interestingly, there was little evidence of colour deterioration of the

museum specimens despite a 159-year span in collection dates (see Results). We log-transformed elevation data because of significant skewing by the high-elevation kinglets. We calculated sexual dimorphism and dichromatism scores as the ratio of mean female measurements to mean male measurements, with a score of one representing no gender differences. We used Pearson correlations to test for associations between dimorphism, dichromatism, population density, and geographical and climatic variables.

RESULTS

The climatic and geographical variables that we used in our analyses were generally highly correlated with each other (Table 2.1). Thus, to investigate Bergmann's rule, we conducted analyses with temperature separately from analyses with latitude and elevation.

Subspecific and gender differences in coloration and morphology

In contrast to females, male crown reflectance spectra were shifted towards longer wavelengths and were much brighter (Fig. 2.1a). Mantle reflectance spectra were very similar between the genders, although males were slightly lighter in colour (Fig. 2.1b). Both gender and subspecies explained variation in crown saturation (Table 2.2, Fig. 2.2b). Gender also explained variation in crown brightness and hue, whereas variation in mantle saturation and hue was not associated with gender or subspecies (Table 2.2, Fig. 2.2). In similar analyses comparing the yellow crown feathers of males vs. females, we found that females were significantly brighter ($P < 0.0001$), males were significantly more saturated ($P = 0.001$), and crown hue did not differ between the genders ($P = 0.64$).

Variation in tarsus length and wing-chord was also explained by gender and subspecies (Table 2.3). Variation in bill length was significantly predicted by subspecies,

though we did find a non-significant trend with gender as well (Table 2.3). In general, males were larger than females, with longer tarsi and wing-chords (Fig. 2.3). *R. s. satrapa*, which is described as being the largest subspecies overall (Martens & Päckert, 2006), had the longest wing, while the smaller *R. s. olivaceous* had the shortest wing (Fig. 2.3c). In subsequent analyses, we used wing-chord and tarsus length as proxies for body size in golden-crowned kinglets.

Does Bergmann's rule explain geographical variation in body size?

We restricted the following analyses to specimens collected within the species' breeding or year-round ranges (Ingold & Galati, 1997) because kinglets collected in their wintering range are unlikely to have developed morphologically at those locations. Bergmann's rule can be investigated intraspecifically by comparing mean differences in body size across subspecies or races, or by examining continuous variation in size within a species (James, 1970). Based on the median latitude of subspecies ranges (Martens & Päckert, 2006), we would expect kinglet body size trends as follows: *olivaceous* > *satrapa* > *apache* > *aztecus* > *clarus*. Based on the mean breeding temperatures of these ranges, we would expect quite different trends: *olivaceous* > *aztecus* > *clarus* > *apache* > *satrapa*. Subspecific variation in size did not follow either of these trends (Fig. 2.3).

To test Bergmann's rule in more detail, we investigated the effects of temperature variation on kinglet body size. We found that both January and July wet-bulb temperatures significantly predicted variation in body size; however, trends occurred in opposite directions (Table 2.4). As winters became colder, wing-chord increased whereas tarsus length decreased. However, wing-chord increased and tarsus length decreased as summers became warmer, which contradicts winter trends. We also investigated

Bergmann's rule with respect to the effects of latitude and elevation after controlling for longitude (Table 2.4). Tarsus length decreased with increasing latitude, while variation in wing-chord was not associated with latitude (Table 2.4). Neither morphological measurement was correlated with elevation (Table 2.4).

Does Gloger's rule explain geographical variation in mantle coloration?

To test Gloger's rule, we investigated the relationship between average relative humidity and golden-crowned kinglet mantle coloration. Mantle brightness was significantly correlated with relative humidity (model: $R^2 = 0.054$, $F = 4.7$, d.f. = 3,249, $P = 0.003$; relative humidity: $P = 0.02$): as relative humidity increased, mantle coloration became lighter, in contrast to the predictions of Gloger's rule.

Does primary productivity explain geographical variation in crown coloration?

We investigated the relationship between primary productivity and kinglet coloration (Table 2.5). Actual evapotranspiration was negatively correlated with crown hue and saturation (Table 2.5). As actual evapotranspiration increased, crown colour became yellower and less saturated.

Geographical variation in sexual dichromatism and dimorphism

First, to examine whether variation in sexual selection pressures affected coloration and body size in similar ways, we investigated whether sexual dichromatism varied in parallel with sexual size dimorphism. Crown brightness dichromatism was negatively associated with tarsus length dimorphism (Fig. 2.4a; $r = 0.50$, $n = 20$, $P = 0.02$). Dichromatism was not correlated with dimorphism for any other trait (all $P > 0.17$).

We compared populations in different ecoregion clusters to examine whether population density, as an index of the intensity of sexual selection, influenced variation in dichromatism and dimorphism. Dimorphism and dichromatism increase when scores diverge away from 1.0 in either direction, and decrease when scores converge towards 1.0. Dichromatism in crown brightness tended to increase with population density (Fig. 2.4b; $r = 0.61$, $n = 9$, $P = 0.08$). No other measure of dimorphism or dichromatism was significantly associated with population density (all $P > 0.38$).

To examine whether the intensity of sexual selection varies with environmental conditions, we investigated whether sexual dimorphism decreases with colder climates for ecoregion clusters within the kinglet breeding and year-round ranges. Dichromatism in crown hue decreased with increasing latitude (Fig. 2.4c; $r = 0.50$, $n = 16$, $P = 0.047$), and dichromatism in mantle brightness and saturation decreased as mean July wet-bulb temperatures decreased (Fig. 2.4d-e; brightness: $r = 0.56$, $n = 16$, $P = 0.03$; saturation: $r = 0.62$, $n = 16$, $P = 0.01$). Wing-chord dimorphism tended to decrease with increasing elevation ($r = 0.42$, $n = 16$, $P = 0.10$) and mantle hue dichromatism tended to decrease with decreasing July wet-bulb temperatures ($r = 0.49$, $n = 16$, $P = 0.06$). In all cases, both genders contributed to the changes in dimorphism: females became larger and more colourful while males became smaller and less colourful in colder climates.

DISCUSSION

Our study investigated ecological and sexual selection correlates of geographical variation in golden-crowned kinglets on a continental scale. Firstly, we found that while morphological differences are apparent across subspecies, variation in crown coloration is not as striking as originally proposed (Jenks, 1936) and intrapopulation variation is

substantial. While we did not find strong support for Bergmann's and Gloger's ecogeographical rules, we did find that the degree of sexual dimorphism and dichromatism decreased in less hospitable environments, suggesting that climatic conditions can affect the intensity of sexual selection.

Kinglet body size correlations with climatic variables produced contradictory results between wing-chord and tarsus length, and between winter and summer temperatures. Body size was not correlated with elevation, and tarsus length actually decreased with increasing latitude, in contradiction to Bergmann's rule. The variable migration patterns of golden-crowned kinglets (Ingold & Galati, 1997) may confound wing-chord trends; more northerly migrants tend to have longer wings in general (Mayr, 1970). In fact, migratory species tend not to adhere to Bergmann's rule (Zink & Remsen, 1986); however, there are resident populations of kinglets throughout North and Central America (Martens & Päckert, 2006). Interestingly, geographical variation in the body size of the ruby-crowned kinglet (*Regulus calendula*), as measured by wing-chord, also contradicted Bergmann's rule (Browning, 1979). It is possible that group huddling in kinglets overwintering in colder areas can offset the thermoregulatory disadvantage of small body size (Heinrich, 2003).

Tarsus length may not be a good proxy for body size because of the importance of preventing heat loss through bare parts in birds, independent of body size (Burt, 1986). In addition to the reduced surface area, shorter tarsi can be tucked more easily into the body feathers for warmth (Hill *et al.*, 1980). Because we originally focused on sexually selected traits such as body size and coloration, one ecogeographical rule that we did not consider *a priori* was Allen's rule, which states that animals should have shorter appendages in colder climates (Allen, 1877; Millien *et al.*, 2006; Gaston *et al.*, 2008).

Our results show that geographical variation in kinglet tarsus length indeed supports Allen's rule, while variation in wing-chord does not (as might be expected because wing-chord is determined by the length of the primary feathers rather than the vascularised limb).

Golden-crowned kinglets exhibited lighter plumage with increasing humidity, which does not support Gloger's rule and is in contrast to the results obtained in most bird studies (Zink & Remsen, 1986), including an earlier analysis of the congeneric ruby-crowned kinglets (Browning, 1979). Other factors, such as genetic drift, may be more important in explaining variation in mantle coloration. In addition, the scale of a study may influence whether patterns of variation adhere to ecogeographical rules. For example, comparing two populations in highly contrasting environments (e.g., the arid south-western US vs. the humid Pacific Northwest, Zink & Remsen, 1986; Burt & Ichida, 2004) might reveal more striking differences than our correlative study of continent-wide variation.

As habitat productivity levels increased, kinglet crown coloration became yellower and less saturated, contrary to our predictions. While there is evidence that primary productivity is correlated with terrestrial plant biodiversity (e.g., Hector *et al.*, 1999) there is limited evidence of a direct link between carotenoid availability and primary terrestrial productivity (but see Teramura & Sullivan, 1994). In addition, studies on avian diets in the wild are lacking and many sources of carotenoids remain unknown (McGraw, 2006). Alternatively, variation in carotenoid availability may be masked by variation in natural and sexual selection on male and female plumage traits, as suggested by our interpopulation analyses.

Sexual dimorphism and dichromatism generally did not vary in parallel, which suggests that different forms of sexual selection may favour different characters (Kodric-Brown & Brown, 1984). However, the negative correlation between crown brightness and tarsus length dimorphism suggests that restrictions on energy allocation may mediate a trade-off between investment in more colourful plumage ornaments or larger body size (Andersson, 1994).

We found increasing male-biased dichromatism in crown brightness with increasing population density, where male crown coloration became less pigmented relative to that in females. This may imply that male golden-crowned kinglets in high density populations experience greater competition for resources, such as high quality territories and food (Hairston *et al.*, 1960); the stress of competition may lead to poorer individual condition and decreased deposition of pigments into feathers (Hill, 2006a). The lack of associations between population density and other coloration and morphological variables could be due to our small sample size of populations with known densities. Not much is known about the effects of absolute population density on the intensity of sexual selection (Kokko & Rankin, 2006). Some studies have focused on the effects of density-dependent gender ratio biases (e.g., Clutton-Brock *et al.*, 1997); unfortunately, such data are lacking for this species (Ingold & Galati, 1997).

Sexual size dimorphism and dichromatism in golden-crowned kinglets decreased in colder climates, which supports Badyaev's (1997) observed pattern of variation in cardueline finches. In serially-monogamous golden-crowned kinglets, males are involved in nest defence and mate- and chick-feeding (Ingold & Galati, 1997). Although it is unknown whether male investment varies between habitats/populations (Ingold & Galati, 1997), the relatively high male investment indicates that a breeding pair's reproductive

success would undoubtedly be compromised by the lack of bi-parental care.

Interestingly, not only did male golden-crowned kinglets converge towards a more cryptic ‘female’ appearance, as might be expected if males were required to spend more time at the nest (Johnson, 1991), but females also converged towards a more conspicuous ‘male’ phenotype in colder climates, suggesting that increases in male investment might result in mutual mate choice (Kokko & Johnstone, 2002). Such geographical variation in sexual ornaments may have implications for the extent of gene flow and reproductive isolation between populations (Zink & Remsen, 1986).

Our study focused on large-scale intraspecific variation in coloration and morphology in golden-crowned kinglets, a common, yet understudied, passerine species. In contrast to many studies concerning general ecogeographical rules in birds, geographical variation in golden-crowned kinglets does not support Gloger’s rule and provides mixed results for Bergmann’s rule, suggesting that other mechanisms aside from adaptations to climatic variation may explain the observed geographical differences. Interestingly, geographical variation in sexual dimorphism and dichromatism in golden-crowned kinglets appears to support the hypothesis that sexual selection is reduced in colder climates (Badyaev, 1997). Further research on both inter- and intraspecific trends in other taxa may well uncover an ecogeographical trend in sexual dimorphism.

ACKNOWLEDGEMENTS

We thank D. Mindell and J. Hinshaw at University of Michigan Museum of Zoology, and S. Edwards and J. Trimble at the Harvard Museum of Comparative Zoology for access to their collections. Thanks to A. Mistakidis, K. Cogliati, and D. Bradley for museum assistance. We are grateful to R. Montgomerie for providing access to his CLR colour

analysis program. We appreciate comments on the manuscript provided by J. Ingold, E. Burt Jr, and the Doucet Lab. This research was supported by the Natural Sciences and Engineering Research Council of Canada (CGS-M to C.K.S.C., Discovery and Research Tools and Instrumentation grants to S.M.D.) and the Taverner Award from the Society of Canadian Ornithologists (C.K.S.C.).

REFERENCES

- Aldrich, J. W. & James, F. C. (1991) Ecogeographic variation in the American robin (*Turdus migratorius*). *The Auk*, **108**, 230-249.
- Allen, J. A. (1877) The influence of physical conditions in the genesis of species. *Radical Review*, **1**, 108-140.
- Andersson, M. B. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Ashton, K. G. (2002a) Do amphibians follow Bergmann's rule? *Canadian Journal of Zoology*, **80**, 708-716.
- Ashton, K. G. (2002b) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography*, **11**, 505-523.
- Ashton, K. G. & Feldman, C. R. (2003) Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution*, **57**, 1151-1163.
- Ashton, K. G., Tracy, M. C. & de Queiroz, A. (2000) Is Bergmann's rule valid for mammals? *The American Naturalist*, **156**, 390-415.
- Badyaev, A. V. (1997) Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behavioral Ecology*, **8**, 675-690.
- Bailey, R. G. (1994) *Ecoregions of the United States (map)*. USDA Forest Service, Washington, DC.
- Bailey, R. G. & Cushwa, C. T. (1981) *Ecoregions of North America (map)*. U.S. Fish and Wildlife Service, Washington, DC.
- Bergmann, C. (1847) Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **3**, 595-708.
- Blackburn, T. M., Gaston, K. J. & Loder, N. (1999) Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions*, **5**, 165-174.
- Boag, P. T. & Grant, P. R. (1981) Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galápagos. *Science*, **214**, 82-85.
- Browning, M. R. (1979) A review of geographic variation in continental populations of the Ruby-crowned Kinglet. *Nemouria*, **21**, 1-9.
- Burt, E. H. (1986) Color and energy balance. *Ornithological Monographs*, **38**, 38-57.
- Burt, E. H. & Ichida, J. M. (2004) Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. *The Condor*, **106**, 681-686.
- Canty and Associates (2009) Weatherbase. Canty and Associates, Great Falls, VA. Available at: <http://www.weatherbase.com> (last accessed 5 June 2008).

- Clutton-Brock, T. H., Rose, K. E. & Guinness, F. E. (1997) Density-related changes in sexual selection in red deer. *Proceedings of the Royal Society B: Biological Sciences*, **264**, 1509-1516.
- Coyne, J. A. & Orr, H. A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA.
- Cuthill, I. C. (2006) Color perception. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 3-40. Harvard University Press, Cambridge.
- Dale, J. (2006) Intraspecific variation in coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 36-86. Harvard University Press, Cambridge.
- Doucet, S. M. & Hill, G. E. (2009) Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long-tailed manakins *Chiroxiphia linearis*. *Journal of Avian Biology*, **40**, 146-156.
- Endler, J. A. (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76-91.
- Endler, J. A. (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes*, **9**, 173-190.
- Endler, J. A. (1991) Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, **31**, 587-608.
- Endler, J. A. & Houde, A. E. (1995) Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456-468.
- Environment Canada (2009) National climate data and information archive. Environment Canada, Gatineau, QC. Available at: <http://www.climate.weatheroffice.ec.gc.ca> (last accessed 5 June 2008).
- Falling Rain Genomics (2006) Global gazetteer version 2.1. Falling Rain Genomics, Palo Alto, CA. Available at: <http://www.fallingrain.com/world> (last accessed 5 June 2008).
- Fox, H. M. & Vevers, G. (1960) *The Nature of Animal Colours*. Macmillan, New York, NY.
- Gaston, K. J., Chown, S. L. & Evans, K. L. (2008) Ecogeographical rules: elements of a synthesis. *Journal of Biogeography*, **35**, 483-500.
- Gloger, C. (1833) *Das Abändern der Vögel durch Einfluss des Klimas*. A. Schulz, Breslau, Germany.
- Grether, G. F., Hudon, J. & Millie, D. F. (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 1317-1322.
- Guillaumet, A., Ferdy, J.-B., Desmarais, E., Godelle, B. & Crochet, P.-A. (2008) Testing Bergmann's rule in the presence of potentially confounding factors: a case study with three species of *Galerida* larks in Morocco. *Journal of Biogeography*, **35**, 579-591.
- Hairston, N. G., Smith, F. E. & Slobodkin, L. B. (1960) Community structure, population control, and competition. *The American Naturalist*, **94**, 421-425.
- Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P. W., Loreau, M., Minns, A., Mulder, C. P. H., O'Donovan, G., Otway, S. J., Pereira, J. S., Prinz, A., Read, D. J., Scherer-Lorenzen, M., Schulze, E.-D., Siamantziouras, A.-S. D.,

- Spehn, E. M., Terry, A. C., Troumbis, A. Y., Woodward, F. I., Yachi, S. & Lawton, J. H. (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123-1127.
- Heinrich, B. (2003) Overwintering of golden-crowned kinglets during winter. *The Wilson Bulletin*, **115**, 113-114.
- Hill, G. E. (1993) Geographic variation in the carotenoid plumage pigmentation of male house finches (*Carpodacus mexicanus*). *Biological Journal of the Linnean Society*, **49**, 63-86.
- Hill, G. E. (2006a) Environmental regulation of ornamental coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 507-560. Harvard University Press, Cambridge, MA.
- Hill, G. E. (2006b) Female mate choice for ornamental coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 137-200. Harvard University Press, Cambridge, MA.
- Hill, R. W., Beaver, D. L. & Veghte, J. H. (1980) Body surface temperatures and thermoregulation in the black-capped chickadee (*Parus atricapillus*). *Physiological Zoology*, **53**, 305-321.
- Howes, B. J. & Lougheed, S. C. (2007) Male body size varies with latitude in a temperate lizard. *Canadian Journal of Zoology*, **85**, 626-633.
- Ingold, J. L. & Galati, R. (1997) Golden-crowned kinglet (*Regulus satrapa*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/301> (last accessed 23 January 2009).
- James, F. C. (1970) Geographic size variation in birds and its relationship to climate. *Ecology*, **51**, 365-390.
- Jenks, R. (1936) A new race of golden-crowned kinglet from Arizona. *The Condor*, **38**, 239-244.
- Johnson, S. G. (1991) Effects of predation, parasites, and phylogeny on the evolution of bright coloration in North American male passerines. *Evolutionary Ecology*, **5**, 52-62.
- Kodric-Brown, A. & Brown, J. H. (1984) Truth in advertising: the kinds of traits favored by sexual selection. *The American Naturalist*, **124**, 309-323.
- Kokko, H. & Johnstone, R. A. (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **357**, 319-330.
- Kokko, H. & Rankin, D. J. (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **361**, 319-334.
- Kwiatkowski, M. A. & Sullivan, B. K. (2002) Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=ater). *Evolution*, **56**, 2039-2051.
- Lai, Y.-C., Shiroishi, T., Moriwaki, K., Motokawa, M. & Yu, H.-T. (2008) Variation in coat color in house mice throughout Asia. *Journal of Zoology*, **274**, 270-276.
- Macedonia, J. M., Brandt, Y. & Clark, D. L. (2002) Sexual dichromatism and differential conspicuousness in two populations of the common collared lizard (*Crotaphytus*

- collaris*) from Utah and New Mexico, USA. *Biological Journal of the Linnean Society*, **77**, 67-85.
- Martens, J. & Päckert, M. (2006) Family Regulidae (kinglets and firecrests). *Handbook of the Birds of the World* (ed. by J. del Hoyo, A. Elliot and D. Christie), 330-349. Lynx Edicions, Barcelona, Spain.
- Mayr, E. (1970) *Populations, species, and evolution: an abridgment of animal species and evolution*. Belknap Press of Harvard University Press, Cambridge, MA.
- McGraw, K. J. (2006) Mechanics of carotenoid-based coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 177-242. Harvard University Press, Cambridge, MA.
- Meiri, S. & Dayan, T. (2003) On the validity of Bergmann's rule. *Journal of Biogeography*, **30**, 331-351.
- Millien, V., Lyons, S. K., Olson, L., Smith, F. A., Wilson, A. B. & Yom-Tov, Y. (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters*, **9**, 853-869.
- Montgomerie, R. (2006) Analyzing colors. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 90-147. Harvard University Press, Cambridge, MA.
- Montgomerie, R. (2008) CLR, version 1.04. Queen's University, Kingston, ON. Available at: <http://post.queensu.ca/~mont/color/analyze.html> (last accessed 27 May 2008).
- National Climatic Data Center (2007) Comparative climatic data for the United States through 2007. National Climatic Data Center, Asheville, NC. Available at: <http://www.ncdc.noaa.gov/oa/ncdc.html> (last accessed 5 June 2008).
- Natural Resources Canada (2007) Canadian geographical names data base. Natural Resources Canada, Ottawa, ON. Available at: http://geonames.nrcan.gc.ca/index_e.php (last accessed 5 June 2008).
- Norris, D. R., Marra, P. P., Kyser, T. K., Ratcliffe, L. M. & Montgomerie, R. (2007) Continent-wide variation in feather colour of a migratory songbird in relation to body condition and moulting locality. *Biology Letters*, **3**, 16-19.
- Price, T. D., Qvarnström, A. & Irwin, D. E. (2003) The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1433-1440.
- Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, **275**, 1934-1937.
- Ricklefs, R. E. (1968) Patterns of growth in birds. *The Ibis*, **110**, 419-451.
- Rosenzweig, M. L. (1968) Net primary productivity of terrestrial communities: prediction from climatological data. *The American Naturalist*, **102**, 67-74.
- Snell-Rood, E. C. & Badyaev, A. V. (2008) Ecological gradient of sexual selection: elevation and song elaboration in finches. *Oecologia*, **157**, 545-551.
- Stoner, C. J., Bininda-Emonds, O. R. P. & Caro, T. (2003) The adaptive significance of coloration in lagomorphs. *Biological Journal of the Linnean Society*, **79**, 309-328.
- Stradi, R. (1998) *The Colour of Flight: Carotenoids in Bird Plumage*. Solei Gruppo Editoriale Informatico, Milan, Italy.
- Sutton, D. A. & Patterson, B. D. (2000) Geographic variation of the western chipmunks *Tamias senex* and *T. siskiyou*, with two new subspecies from California. *Journal of Mammalogy*, **81**, 299-316.

- Teramura, A. H. & Sullivan, J. H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research*, **39**, 463-473.
- Thornthwaite Associates (1964a) Average climatic water balance data of the continents. VI. North America (excluding United States). *Publications in Climatology*, **17**, 235-414.
- Thornthwaite Associates (1964b) Average climatic water balance data of the continents. VII. United States. *Publications in Climatology*, **17**, 419-615.
- U.S. Geological Survey (2009) U.S. board on geographic names. U.S. Geological Survey, Reston, VA. Available at: <http://geonames.usgs.gov> (last accessed 5 June 2008).
- West-Eberhard, M. J. (1983) Sexual selection, social competition, and speciation. *The Quarterly Review of Biology*, **58**, 155-183.
- Zink, R. M. & Remsen, J. V. (1986) Evolutionary processes and patterns of geographic variation in birds. *Current Ornithology*, **4**, 1-69.

Table 2.1. Pearson correlation coefficients between climatic and geographical variables used in analyses of Bergmann’s rule, Gloger’s rule and productivity on golden-crowned kinglet (*Regulus satrapa*) coloration and morphology.

	Jan wet-bulb temperature	Jul wet-bulb temperature	Latitude	Longitude	Elevation	Actual evapotranspiration	Relative humidity
Jan wet-bulb temperature	–	0.17**	-0.61***	-0.31***	-0.12*	-0.08	0.18**
Jul wet-bulb temperature		–	-0.34***	0.61***	-0.23***	0.71***	0.22***
Latitude			–	-0.37***	-0.33***	-0.17**	0.03
Longitude				–	-0.25***	0.68***	0.20***
Elevation ^a					–	-0.39***	-0.40***
Actual evapotranspiration ^b						–	0.34***
Relative humidity ^b							–

^a log-transformed data, ^b average values for breeding and moulting season (May-Sep)

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

Table 2.2. Gender and subspecific differences in crown and mantle coloration in golden-crowned kinglets (*Regulus satrapa*).

Dependent variables	Model effects	<i>F</i>	d.f.	<i>P</i>
Crown brightness	Whole model ($R^2 = 0.10$)	9.4	6, 498	<0.0001
	Gender	46.5	1, 503	<0.0001
	Subspecies	1.5	4, 500	0.20
	Year collected	0.8	1, 503	0.38
Crown saturation	Whole model ($R^2 = 0.71$)	200.0	6, 498	<0.0001
	Gender	1190.2	1, 503	<0.0001
	Subspecies	3.0	4, 500	0.02
	Year collected	1.1	1, 503	0.29
Crown hue	Whole model ($R^2 = 0.92$)	985.0	6, 498	<0.0001
	Gender	5855.6	1, 503	<0.0001
	Subspecies	2.3	4, 500	0.06
	Year collected	1.6	1, 503	0.21
Mantle brightness	Whole model ($R^2 = 0.042$)	3.6	6, 498	0.002
	Gender	5.8	1, 503	0.02
	Subspecies	1.8	4, 500	0.13
	Year collected	4.7	1, 503	0.03
Mantle saturation	Whole model ($R^2 = 0.021$)	1.8	6, 498	0.10
	Gender	3.4	1, 503	0.07
	Subspecies	1.7	4, 500	0.14
	Year collected	0.9	1, 503	0.33
Mantle hue	Whole model ($R^2 = 0.0079$)	0.7	6, 498	0.68
	Gender	1.0	1, 503	0.32
	Subspecies	0.8	4, 500	0.56
	Year collected	0.2	1, 503	0.67

Table 2.3. Gender and subspecific differences in morphological characters in golden-crowned kinglets (*Regulus satrapa*).

Dependent variables	Model effects	<i>F</i>	d.f.	<i>P</i>
Bill length	Whole model ($R^2 = 0.38$)	49.2	6, 485	<0.0001
	Gender	3.6	1, 490	0.06
	Subspecies	57.6	4, 487	<0.0001
	Year collected	1.8	1, 490	0.18
Tarsus length	Whole model ($R^2 = 0.15$)	14.5	6, 493	<0.0001
	Gender	27.4	1, 498	<0.0001
	Subspecies	15.4	4, 495	<0.0001
	Year collected	11.0	1, 498	0.001
Wing-chord	Whole model ($R^2 = 0.50$)	82.8	6, 497	<0.0001
	Gender	175.4	1, 502	<0.0001
	Subspecies	55.9	4, 499	<0.0001
	Year collected	28.4	1, 502	<0.0001

Table 2.4. The effects of a) winter and summer temperatures and b) latitude and elevation (controlling for longitude) on the body size of golden-crowned kinglets (*Regulus satrapa*).

Dependent variables	Model effects	<i>F</i>	β'	d.f.	<i>P</i>
Analyses with temperature					
Tarsus length	Whole model ($R^2 = 0.083$)	4.78		4, 211	0.001
	Gender	3.71	-0.13	1, 214	0.06
	Year collected	0.01	-0.01	1, 214	0.92
	Jan. wet-bulb temperature	8.54	0.20	1, 214	0.004
	Jul. wet-bulb temperature	7.66	-0.20	1, 214	0.006
Wing-chord	Whole model ($R^2 = 0.33$)	26.47		4, 212	<0.0001
	Gender	43.65	-0.37	1, 215	<0.0001
	Year collected	9.84	-0.19	1, 215	0.002
	Jan. wet-bulb temperature	13.30	-0.21	1, 215	0.0003
	Jul. wet-bulb temperature	21.47	0.28	1, 215	<0.0001
Analyses with latitude and elevation					
Tarsus length	Whole model ($R^2 = 0.13$)	7.1		5, 235	<0.0001
	Gender	7.3	-0.17	1, 239	0.007
	Year collected	1.3	-0.08	1, 239	0.26
	Latitude	5.3	-0.19	1, 239	0.02
	Log (Elevation)	0.05	0.02	1, 239	0.82
	Longitude	27.8	-0.37	1, 239	<0.0001
Wing-chord	Whole model ($R^2 = 0.48$)	44.2		5, 237	<0.0001
	Gender	67.9	-0.39	1, 241	<0.0001
	Year collected	11.4	-0.17	1, 241	0.0008
	Latitude	0.02	-0.008	1, 241	0.90
	Log (Elevation)	2.3	0.09	1, 241	0.13
	Longitude	89.5	0.50	1, 241	<0.0001

Table 2.5. The effects of primary productivity during breeding and moulting seasons on the crown coloration of golden-crowned kinglets (*Regulus satrapa*). Actual evapotranspiration data are average values for May-Sep.

Dependent variables	Model effects	<i>F</i>	β'	d.f.	<i>P</i>
Crown brightness	Whole model ($R^2 = 0.069$)	6.7		3, 271	0.0002
	Gender	18.1	-0.25	1, 273	<0.0001
	Year collected	1.4	0.07	1, 273	0.24
	Actual evapotranspiration	0.1	-0.02	1, 273	0.72
Crown saturation	Whole model ($R^2 = 0.69$)	197.7		3, 271	<0.0001
	Gender	581.2	-0.82	1, 273	<0.0001
	Year collected	0.2	-0.02	1, 273	0.64
	Actual evapotranspiration	7.9	-0.10	1, 273	0.005
Crown hue	Whole model ($R^2 = 0.92$)	994.9		3, 271	<0.0001
	Gender	2964.9	-0.96	1, 273	<0.0001
	Year collected	1.9	-0.02	1, 273	0.17
	Actual evapotranspiration	9.4	-0.05	1, 273	0.002

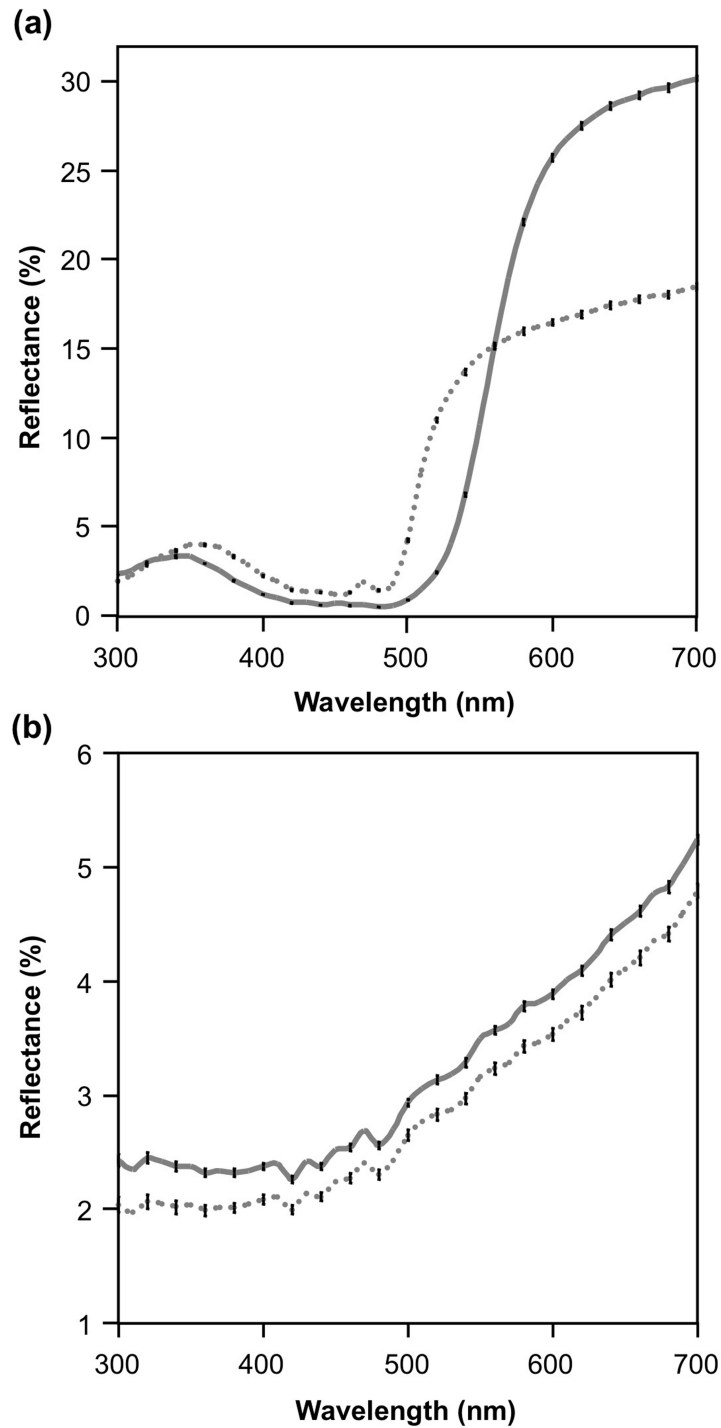


Figure 2.1. Mean reflectance spectra for the a) crown and b) mantle coloration of male (solid line, $n = 332$) and female (dotted line, $n = 179$) golden-crowned kinglets (*Regulus satrapa*). Vertical lines indicate standard error bars. Note variation in y-axis scale.

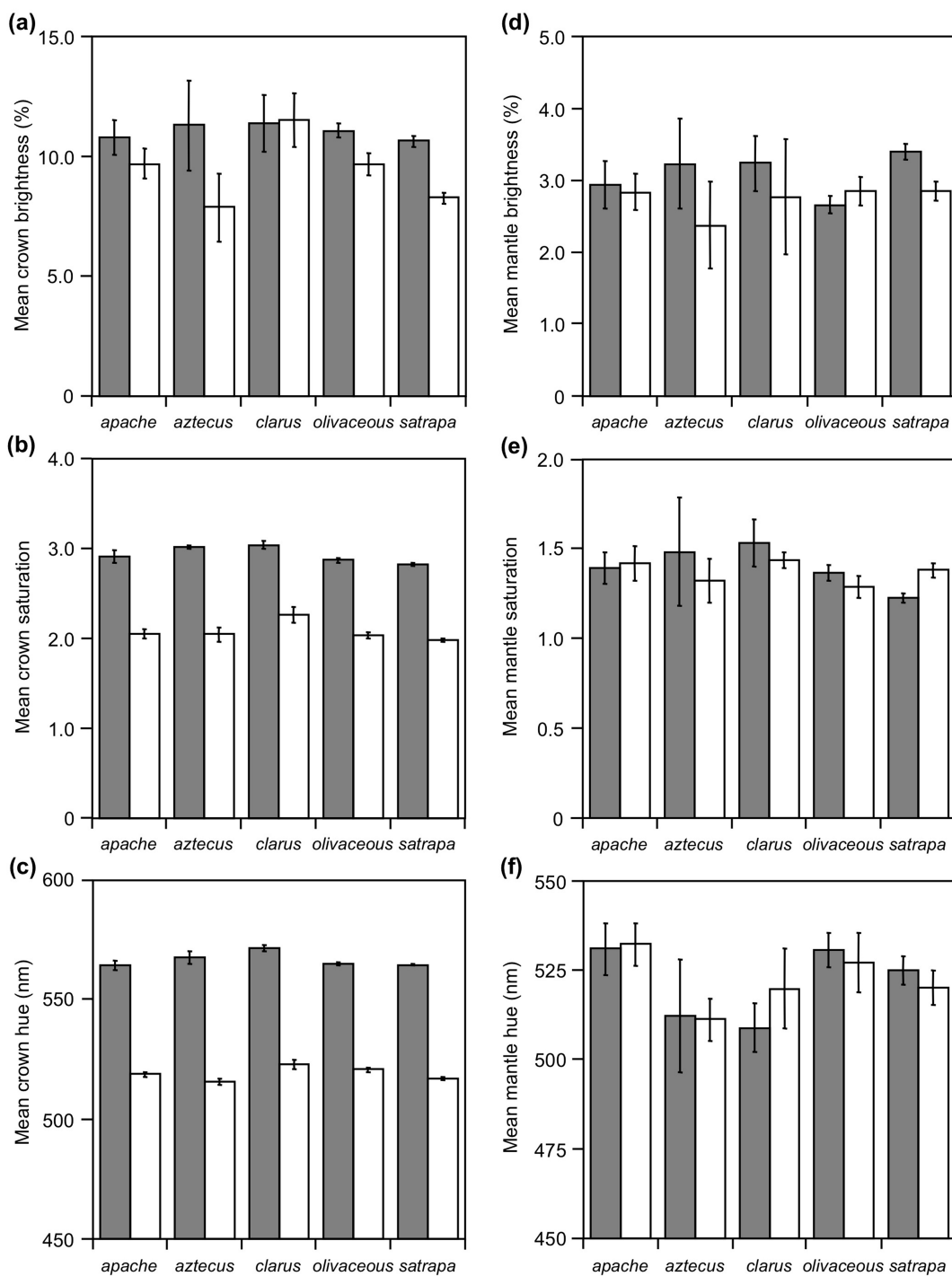


Figure 2.2. Subspecies differences in golden-crowned kinglets (*Regulus satrapa*) for a) crown brightness, b) crown saturation, c) crown hue, d) mantle brightness, e) mantle saturation, and f) mantle hue stratified by gender (males: dark bars, females: light bars). Sample sizes for subspecies: *R. s. apache* (males, $n = 18$; females, $n = 13$), *R. s. aztecus* (males, $n = 3$; females, $n = 3$), *R. s. clarus* (males, $n = 5$; females, $n = 3$), *R. s. olivaceous* (males, $n = 96$; females, $n = 48$), *R. s. satrapa* (males, $n = 210$; females, $n = 112$). Vertical bars indicate standard errors.

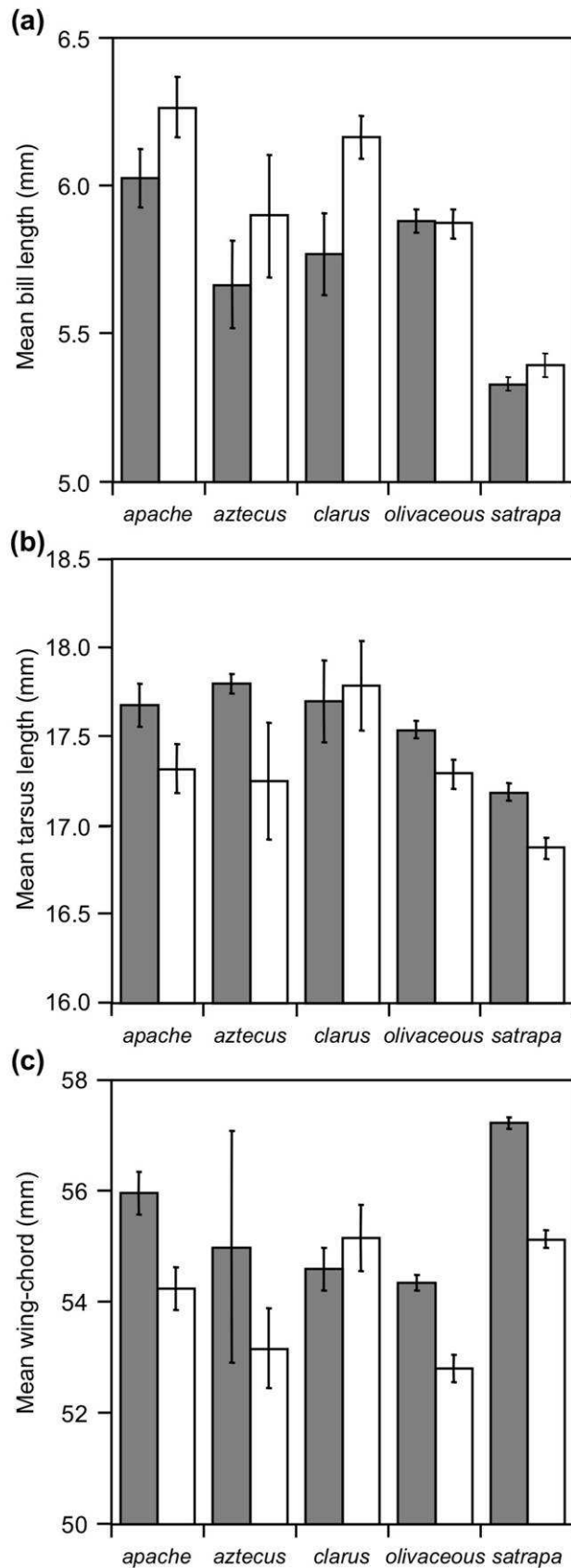


Figure 2.3. Subspecies differences in golden-crowned kinglets (*Regulus satrapa*) for a) bill length, b) tarsus length, and c) wing-chord stratified by gender (males: dark bars, females: light bars). Sample sizes for subspecies: *R. s. apache* (males, $n = 18$; females, $n = 13$), *R. s. aztecus* (males, $n = 3$; females, $n = 3$), *R. s. clarus* (males, $n = 5$; females, $n = 3$), *R. s. olivaceous* (males, $n = 96$; females, $n = 48$), *R. s. satrapa* (males, $n = 209$; females, $n = 112$). Sample sizes for *R. s. olivaceous* and *R. s. satrapa* vary slightly for each panel due to some specimens in poor condition. Vertical bars indicate standard errors.

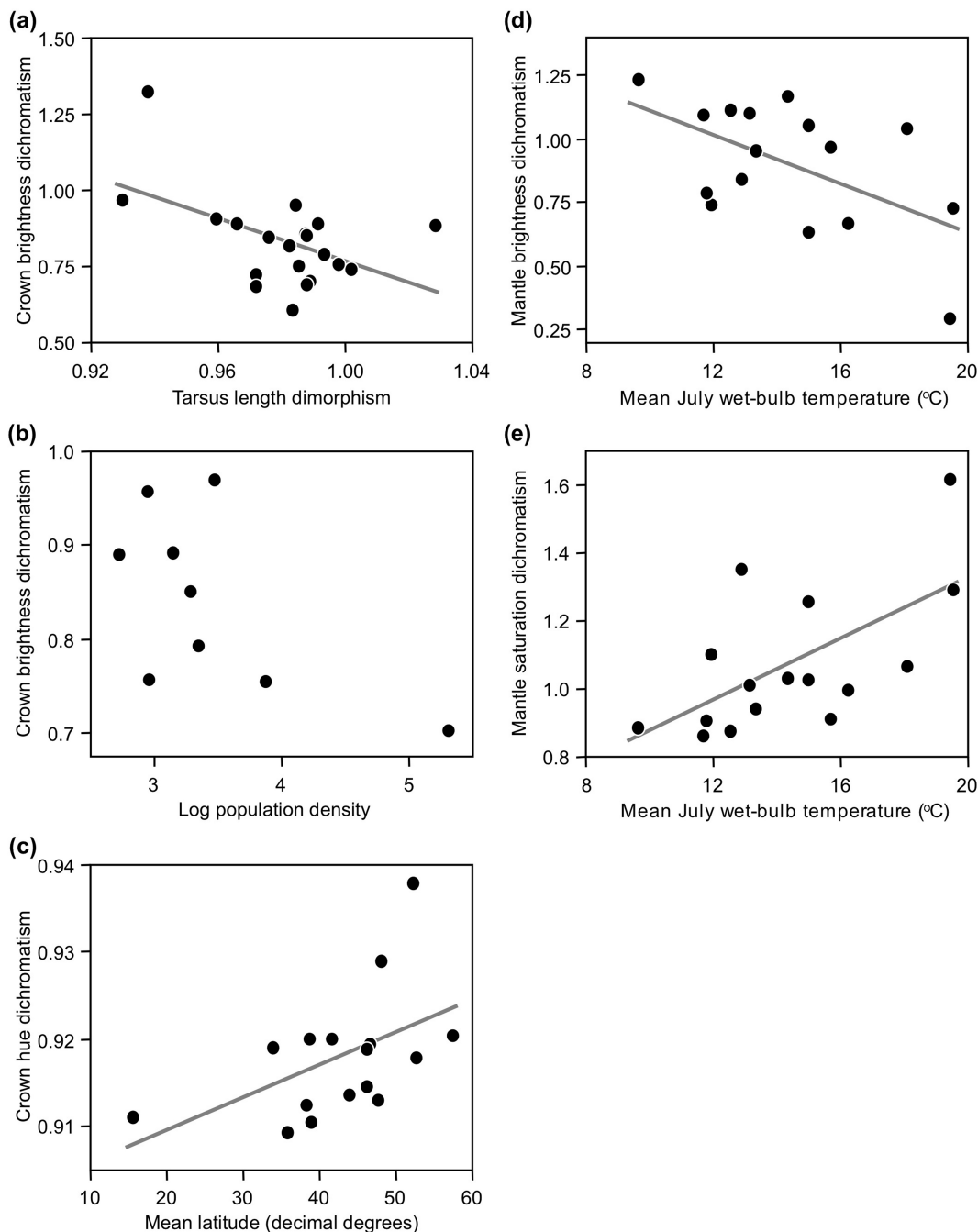


Figure 2.4. Sexual dimorphism data for golden-crowned kinglets (*Regulus satrapa*) based on population means defined by ecoregion clusters (see Materials and Methods for details). a) The relationship between tarsus length dimorphism and crown brightness dichromatism ($n = 20$ populations); b) the effects of golden-crowned kinglet population density (number of males or breeding pairs per 40 ha; Ingold & Galati, 1997) on crown brightness dichromatism ($n = 9$ populations); c) the effects of latitude on crown hue dichromatism; and the effects of July wet-bulb temperature on dichromatism in d) mantle brightness and e) mantle saturation ($n = 16$ populations for panels c-e). Sexual dimorphism scores are calculated as the ratio of mean female values to mean male values.

CHAPTER 3

MIGRATION TIMING, INDIVIDUAL CONDITION AND CAROTENOID CONTENT MEDIATE CROWN COLOUR VARIATION IN GOLDEN- CROWNED KINGLETS (*REGULUS SATRAPA*) *

* This chapter was the outcome of joint research with my advisor, Dr. Stéphanie Doucet, and Dr. Kevin McGraw from Arizona State University

SYNOPSIS

1. Carotenoid-based ornaments are important for mate choice in many species because they are often honest indicators of quality. Carotenoid coloration can be dependent on pigment access and utilization, nutritional condition and parasitic infection. Trait variation is therefore expected to be high within a population.
2. Migration is one of the most stressful periods of the avian annual cycle and differences in individual condition (and ornamental coloration) may be especially apparent at this time. Developing colourful plumage in the fall may be particularly important for species lacking prealternate moult.
3. The purpose of this study was to investigate pigmentary and condition-dependent bases of carotenoid colour variation in a small, migratory passerine species. Golden-crowned kinglets (*Regulus satrapa*, Family Regulidae) exhibit sexually dichromatic crown coloration, and their crown displays have been proposed to function in sexual selection.
4. We collected data from 186 kinglets during fall migration in south-western Ontario. We recorded arrival date, body condition, fat and pectoral muscle scores, wing mites and feather growth rate as measures of condition. We quantified crown coloration using reflectance spectrometry and analysed feather carotenoids using high-performance liquid chromatography.
5. Yellow crown feathers of female kinglets contained only yellow hydroxycarotenoids, whereas orange feathers of males harboured a suite of eight carotenoid pigments. Males with more colourful (redder) crowns contained greater amounts of red and orange ketocarotenoids in their feathers, especially canthaxanthin.

6. Crown hue in both sexes and crown saturation in males were predicted by condition variables. More colourful kinglets of both sexes were earlier migrants through our study site. Females with deeper yellow crowns were in better condition with fewer feather mites. However, male kinglets with more saturated coloration possessed smaller pectoral muscles.
7. This is the first study to identify plumage carotenoids in this family in North America. We determined the pigmentary basis for both inter- and intrasexual colour variation in golden-crowned kinglets. Our results also suggest that fall migration timing may be important, and provide further support for the condition-dependence of carotenoid coloration and the signalling function of female ornaments.

INTRODUCTION

Carotenoid coloration has been identified as a condition-dependent trait important in visual signalling and sexual selection in a variety of taxa ranging from fishes (e.g., Milinski & Bakker, 1990; Evans & Norris, 1996; Houde, 1997; Amundsen & Forsgren, 2001; Maan *et al.*, 2006) to reptiles (Kwiatkowski & Sullivan, 2002) and birds (reviewed in Hill, 2006b; Senar, 2006). Proximate factors that mediate the expression of plumage carotenoids in birds include dietary pigment access, immunocompetence and nutritional condition (reviewed in Hill, 2006a; McGraw, 2006). Furthermore, the detrimental effects of endoparasites on individual health and plumage coloration are well-documented (reviewed in Hill, 2006a); however, the effects of ectosymbionts such as feather mites remain equivocal (reviewed in Proctor & Owens, 2000). Overall, the dependence of carotenoid coloration on multiple genetic and environmental factors enables these

ornaments to be particularly variable, which may enhance their ability to honestly reveal individual quality.

From a biochemical standpoint, the expression of carotenoid coloration also depends on the types and concentrations of pigments present (Hill *et al.*, 2002). Some species adopt a ‘more-is-better’ strategy to produce more colourful plumage compared to conspecifics. For example, greenfinches (*Carduelis chloris*) and American goldfinches (*Carduelis tristis*) produce deep yellow plumage by depositing high concentrations of canary xanthophylls (lemon yellow pigments derived from dietary lutein and zeaxanthin, Saks *et al.*, 2003a; McGraw & Gregory, 2004). Other species have the capacity to metabolize dietary yellow pigments into carotenoids that exhibit redder hues and can selectively deposit these red pigments to grow more colourful orange or red plumage (reviewed in McGraw, 2006). In house finches (*Carpodacus mexicanus*), for example, intraspecific variation in carotenoid coloration primarily results from differing proportions of red and yellow pigments, rather than their absolute quantities (Inouye *et al.*, 2001). In the present study, we investigated the pigment composition and possible condition-dependence of a carotenoid-based trait in a small, migratory passerine species, the golden-crowned kinglet (*Regulus satrapa* Lichtenstein, 1823).

Migration is a stressful and energetically demanding activity: amongst many other physiological changes and costs, sustained migratory flights require individuals to deplete their fat reserves, metabolize muscle protein, and lose body water, thus necessitating stopovers to refuel (Berthold, 2001). It has also been shown that strenuous exercise is associated with reduced immune response (Råberg *et al.*, 1998), which may, in turn, affect an individual’s ability to deposit fat (Merilä & Svensson, 1995). Differences in individual health may therefore be especially apparent during migration, providing a

unique opportunity to investigate the condition-dependence of plumage ornaments.

Unlike the multitude of studies on the benefits of early spring migration (see e.g., Lozano *et al.*, 1996; Hasselquist, 1998; Kokko, 1999; Forstmeier, 2002), the importance of early fall migration has rarely been investigated. Possible benefits include avoidance of adverse weather conditions and migratory predators (Berthold, 2001) and securing high-quality winter territories (Mills, 2005). If plumage traits are honest indicators of quality, we may expect early fall migrants to have more colourful plumage. This may be especially important for species that undergo only one moult per year (i.e., their fall plumage will also be their breeding plumage next year), if ornamental coloration is an important criterion for mate choice.

Research on fall migration and plumage colour is limited: a study on migrating blue tits (*Parus caeruleus*) found that birds with larger fat reserves exhibited brighter carotenoid coloration, which may indicate a link between nutritional condition at moult and subsequent migratory success (Svensson & Merilä, 1996). Research on American redstarts (*Setophaga ruticilla*) previously suggested that males breeding late in the season undergo costly moult-migration overlap (e.g., Merilä, 1997; Pérez-Tris *et al.*, 2001) and deposit fewer carotenoids into their plumage, which may affect sexual selection the following year (Norris *et al.*, 2004b). However, a recent study countered that these are actually high-quality individuals that adventitiously lost and re-grew feathers (with reduced chroma) during the overwintering period, and are not facing reproductive trade-offs (Reudink *et al.*, 2008). Furthermore, geographic colour variation in redstarts was attributed to differences in carotenoid availability at their moulting localities rather than individual condition (Norris *et al.*, 2007).

We studied golden-crowned kinglets during fall migration in south-western Ontario, Canada. One unique aspect of our study site is the migration bottleneck that exists as a result of the local topography and the Great Lakes, which could potentially funnel migrating kinglets from various breeding areas in Canada (U.S. Geological Survey, 2006). Golden-crowned kinglets are distributed throughout North America and are generally short-distance migrants (Martens & Päckert, 2006). With the exception of the ruby-crowned kinglet (*Regulus calendula*), the other five Regulidae species exhibit a conserved pattern of sexual dichromatism: females have a uniformly yellow crown, whereas males have an orange crown patch bordered by yellow feathers (Martens & Päckert, 2006). While these orange feathers are normally concealed, male kinglets will expose them during courtship and antagonistic interactions (Martens & Päckert, 2006), implicating a possible role in sexual selection. There has been little research on plumage colour in this family aside from carotenoid pigment analyses on goldcrest (*Regulus regulus*) crown feathers (Stradi, 1998). Recently, we performed quantitative analyses of large-scale geographic variation in golden-crowned kinglet coloration (Chapter 2). We found that the degree of sexual dichromatism varied with climate, which may be associated with reduced sexual selection pressures for ornamental traits in harsher climates (Badyaev, 1997). We also found that crown colour variation within a population can be almost as extensive as the overall variation seen throughout the species range.

The purpose of our study was to investigate individual variation in carotenoid coloration in migrating golden-crowned kinglets. We sought to characterize the carotenoid profile of kinglet crown feathers and to quantify the pigmentary basis for individual variation in colour. We predicted that if variation in crown coloration is dependent on carotenoid content, kinglets with redder crowns should deposit greater

concentrations of red pigments into their feathers, whereas birds with yellower plumage hues should deposit proportionately more yellow pigments. We then sought to investigate the potential for condition-dependence of crown coloration in both male and female kinglets. We predicted that if carotenoid coloration is an honest indicator of quality, then crown colour should be associated with various measures of condition, including migration arrival date, body condition, fat reserves, pectoral muscle size, prevalence of feather mites and feather growth rate.

MATERIALS AND METHODS

We conducted field work at the Holiday Beach Migration Observatory (HBMO) in Amherstburg, Ontario (42° 02' 24" N, 83° 02' 24" W), an important stopover site for many migratory raptor and passerine species (IBA Canada, 2004). We began monitoring the area for migrants on 13 September 2008 with a set-up of mist-nets and continuous playback of golden-crowned kinglet songs and calls (Cornell Lab of Ornithology, Ithaca, NY, USA). However, because we encountered most kinglets at their expected peak migration times (based on previous HBMO data), it was unclear whether audio playback was especially attractive. We captured 186 golden-crowned kinglets between 0700 and 1530 (EST) between 4 October and 9 November. Each bird was assigned a unique band number as issued by the Bird Banding Laboratory. We sexed birds by crown coloration and aged them as hatching year (HY) or after hatching year (AHY) by assessing skull pneumatization and age-specific plumage characters (Pyle, 1997). Of the 186 kinglets captured, 16 (8.6%) were AHY females, 36 (19.4%) were AHY males, 54 (29.0%) were HY females and 80 (43.0%) were HY males.

Condition variables

For each kinglet, we recorded six variables that are putatively linked to body condition: migration arrival date, residual body mass, fat and pectoral muscle scores, wing mite infestation and feather growth rate. We noted arrival date with the prediction that earlier migrants would be in better condition (e.g., Kokko, 1999; Ninni *et al.*, 2004). We assumed this to be the date of capture because the Holiday Beach Conservation Area is only 9.0 km² (IBA Canada, 2004) and migrating flocks of kinglets are easily heard, yet we rarely recaptured individuals ($n = 3$). We used an electronic balance to measure body mass to the nearest 0.1 g, a wing rule to measure unflattened wing-chord to the nearest 0.5 mm, and digital callipers to measure tarsus length to the nearest 0.1 mm. We then calculated body condition values using the residuals from a linear regression ($r = 0.20$, $n = 185$, $P = 0.005$) of body mass over tarsus (Brown, 1996; Schulte-Hostedde *et al.*, 2005).

We scored the amount of fat using an 8-point scale (DeSante *et al.*, 2009), and the size of the pectoral muscles using a 5-point scale (modified from Barlein, 1995). We also recorded the time of day that each bird was captured because fat reserves can change throughout the day, especially in small birds (Gosler, 1996). We estimated the number of feather mites by eye on the outstretched right wing when backlit. There was a high prevalence ($n = 122$, 66% of birds captured) and variable number (range: 0-80) of mites on the flight feathers of golden-crowned kinglets, which suggests that wing mite infestation could be a good measure of individual condition. We did not observe any ticks or other ectoparasites that have been reported for this species (Ingold & Galati, 1997).

To calculate feather growth rate, we collected the outer right rectrix whenever possible, where growth bars are the most apparent. Each growth bar consists of one dark

and one light band representing a 24 h period of feather growth (Michener & Michener, 1938). We taped each rectrix to black construction paper and counted as many consecutive bands as possible by eye, marking the centre of each band. We used this approach because we wanted to optimize both the number of bands that we scored and the number of individuals we used in this analysis. On average, we were able to count 16.95 ± 2.25 days of growth. We then recorded the total length of the growth bar section, and calculated feather growth rate by dividing the total length by the number of growth bars. Feather growth rate has been shown to reveal nutritional condition at the time of moult (Grubb, 1989, 2006).

Reflectance spectrometry

We collected 10 central crown feathers from every kinglet (orange for males, yellow for females) and stacked and taped each individual's feathers on a piece of matte black construction paper. We measured crown plumage reflectance using an Ocean Optics USB4000 reflectance spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). A single fibre-optic probe transmitted full-spectrum light from the lamp and transferred light reflected from the specimen back to the spectrometer. The probe was mounted with a rubber sheath that extended *c.* 5 mm past the tip to maintain a fixed distance from the specimen and to exclude external light. We placed the probe at a 90° angle to the feather surface and took five readings, which comprised an average of 30 spectra collected sequentially by OOIBase32 software. All spectral measurements were expressed as the percentage of reflected light relative to a Spectralon white standard.

We restricted our analyses to wavelengths of 300-700 nm, which comprise the extent of the avian visual spectrum (Cuthill, 2006). We used the software program CLR

(Montgomerie, 2008) to calculate three colorimetric variables that summarize the variation in reflectance data: brightness, saturation and hue (Montgomerie, 2006).

Brightness was calculated as the average reflectance between 300 and 700 nm; saturation as the difference between the maximum and the minimum reflectance, divided by the total brightness; and hue as the wavelength at which the spectrum reached half of its maximum reflectance.

Carotenoid extraction and chromatography

We conducted high-performance liquid chromatography (HPLC) analyses on feathers from a subset of the kinglets captured. In male kinglets, the coloured barbs extend almost to the base of the crown feather. In females kinglets, however, the coloured portion comprises only about half of the feather; therefore, each individual female could not provide sufficient sample for carotenoid extraction. We thus restricted quantitative HPLC analyses to male crown coloration, and we pooled crown feathers from multiple females for a qualitative assessment of carotenoid content. Based on our spectrometric measurements of hue, we selected 10 males that showed the ‘yellowest’ crowns (designated ‘short-wavelength hues’), 10 that had ‘orange’ crowns (‘medium-wavelength hues’), and 10 that showed the ‘reddest’ crowns (‘long-wavelength hues’) to represent the range of wavelengths reflected in this population. Crown hue and saturation were highly positively correlated in male kinglets ($r = 0.80$, $n = 116$, $P < 0.0001$); therefore, we were also selecting males that generally represented the range of colour saturation.

We extracted feather carotenoids using a mechanical extraction procedure (McGraw *et al.*, 2003). Briefly, we trimmed off the coloured barbs and weighed each sample to the nearest 0.0001 g. We finely ground the feathers in methanol using 440C

stainless steel balls and a SPEX 8000D Mixer/Mill (SPEX CertiPrep, Metuchen, NJ, USA) at 60 Hz for 2 h to solubilize the pigments. We then centrifuged the solution for 5 min at 10,000 RPM, transferred the supernatant and evaporated the latter to dryness under a stream of nitrogen.

We resuspended the extracts in 200 μ l HPLC mobile phase (42:42:16, v/v/v, methanol:acetonitrile:dichloromethane), and injected 50 μ l of each sample into a Waters Alliance 2695 Separations Module (Waters Corporation, Milford, MA, USA) installed with a Waters YMC Carotenoid 5 μ m column (4.6 x 250 mm) with column heater set to 30 °C. Mobile phase was administered at a constant flow rate of 1.2 mL min⁻¹. Data were collected from 300-550 nm using a Waters Alliance 2998 Photodiode Array Detector and analysed with Waters Empower 2 Software. We identified feather carotenoids by comparing their retention times (RT), spectral shapes, and absorbance maxima (λ_{max}) to published data on known carotenoids (e.g., Stradi *et al.*, 1995; Stradi, 1998; Inouye *et al.*, 2001; Andersson *et al.*, 2007). To account for chromatographic noise, peaks below 0.01 absorbance units were not considered. Golden-crowned kinglet chromatograms revealed late-eluting esterified pigments; however, because we could not confidently identify the parent carotenoid for all of these peaks, we categorized them into broader groupings of red, orange, and yellow carotenoids (Stradi, 1998) after determining that 'free' pigments and their esters were positively correlated within each colour category (all $P < 0.02$). Esterified peaks were highly repeatable when aliquots of the same samples were run in tandem ($n = 3$, mean repeatability = 96.2%).

Because reference standards for other carotenoids were unavailable, we quantified pigment concentrations using a calibration curve generated from an external lutein

standard (see Toomey & McGraw, 2009). Carotenoid concentration in the standard (mg g^{-1}) was calculated with the following formula: $(A \times \text{volume of extract [mL]} \times 10) / E$, where A was the absorbance of the sample at λ_{max} (448 nm for xanthophylls) using a Beckman DU530 UV/Vis spectrophotometer, and E is the extinction coefficient at 1% per centimetre of the relevant carotenoid at λ_{max} (2550 for lutein, Britton, 1985). We calculated total carotenoid concentration in each kinglet sample by summing up the HPLC peak areas. However, because we did not measure absorbance by spectrophotometry and because we instituted the aforementioned peak exclusion threshold, these values may not perfectly represent actual total carotenoid content. Nevertheless, total carotenoid concentrations should be consistent for inter-sample comparisons.

Statistical analyses

Data analyses were conducted using JMP 6.0 (SAS Institute, Cary, NC, USA). Due to our uneven sampling of males and females, and of HY and AHY birds, we used a general linear model incorporating sex, age and sex-by-age interactions to investigate sex- and age-specific differences in coloration and condition. For our coloration vs. condition analyses, we constructed general linear multivariate models using our six measures of condition as predictor variables, and we assigned the three colorimetric variables as dependent variables. Since golden-crowned kinglets exhibit substantial crown colour dichromatism, we conducted coloration vs. condition analyses split by sex to investigate whether male and female ornaments signal different kinds of quality. For our HPLC analyses, one sample from the long-wavelength hue group failed and was thus excluded

from further analyses. We compared the concentrations and relative percentages of each pigment as well as carotenoid groups (red, orange, yellow) between three categories of male crown hue (long, medium, short-wavelength hues) using ANOVA and *post hoc* Tukey tests.

RESULTS

Carotenoid pigment analyses

The yellow crown feathers of female kinglets contain only yellow xanthophylls, the majority being lutein (RT 6.5 min, λ_{\max} 447 nm) and 3'-dehydrolutein (RT 5.1 min, λ_{\max} 446 nm), with lesser amounts of canary xanthophyll A (RT 4.6 min, λ_{\max} 444 nm) and zeaxanthin (RT 8.0 min, λ_{\max} 453 nm). In contrast, at least eight carotenoid pigments are responsible for producing the orange colour of male crown feathers, including red ketocarotenoids (canthaxanthin [RT 8.8 min, λ_{\max} 476 nm] and astaxanthin [RT 7.5 min, λ_{\max} 479 nm]), orange ketocarotenoids (α -doradoxanthin [RT 5.9 min, λ_{\max} 455, 474 nm] and small amounts of echinenone [RT 10.6 min, λ_{\max} 468 nm]) and yellow hydroxycarotenoids (lutein, zeaxanthin, 3'-dehydrolutein and low quantities of canary xanthophyll A).

Males with long-wavelength crown hues deposited greater amounts of both red and orange ketocarotenoids (ANOVA: both $F_{2,26} = 10.7$, $P = 0.0004$), contributing to more carotenoids in general ($F_{2,26} = 8.63$, $P = 0.001$), than individuals whose crown hues exhibited medium and short wavelengths (Fig. 3.1a). However, the relative percentage of red ketocarotenoids was not significantly higher in kinglets with long-wavelength crown hues ($F_{2,27} = 2.20$, $P = 0.13$). Rather, males with long-wavelength hues deposited higher

proportions of orange ketocarotenoids than those with short-wavelength hues ($F_{2,27} = 5.90$, $P = 0.008$), while the opposite was true for yellow hydroxycarotenoids ($F_{2,27} = 10.4$, $P = 0.0005$; Fig. 3.1b). Birds with medium and short-wavelength hues were not significantly different for any of the above analyses.

Looking at pigment variation in more detail, we found that feathers reflecting long-wavelength hues had higher concentrations of all identified red and orange ketocarotenoids than feathers exhibiting short wavelengths, and we found a similar pattern for long- vs. medium-wavelength hues except for α -doradoxanthin (Fig. 3.2a). Echinenone was the only ketocarotenoid that differed between medium and short-wavelength hues; the latter group did not possess echinenone at all (Fig. 3.2a). Comparisons of relative pigment proportions revealed alternative patterns of variation: differences in red and orange ketocarotenoids were no longer as prominent between hue categories, although canthaxanthin remained significantly higher in feathers with long- vs. short-wavelength hues ($F_{2,27} = 6.68$, $P = 0.004$; Fig. 3.2b).

Among the yellow xanthophylls, concentrations of lutein and 3'-dehydrolutein did not differ among birds as a function of crown hue; however, feathers displaying short-wavelength hues possessed significantly greater amounts of zeaxanthin than feathers with medium-wavelength hues ($F_{2,26} = 4.97$, $P = 0.01$; Fig. 3.2a). Interestingly, individuals with short-wavelength hues deposited less canary xanthophyll A (a lemon yellow colour, Stradi, 1998) than birds with either long- or medium-wavelength hues. Patterns of variation in pigment concentrations may be attributed to the intercorrelations between the various feather carotenoids (Table 3.1). Analyses of pigment percentages showed that male kinglets with short-wavelength crown hues deposited relatively more 3'-

dehydrolutein, lutein and zeaxanthin than individuals with long-wavelength hues, and birds with medium-wavelength hues had intermediate levels (Fig. 3.2b).

Correlations between condition variables and pigment concentrations showed that earlier migrants deposited more canthaxanthin ($r = -0.38$, $n = 29$, $P = 0.04$) and echinenone ($r = -0.43$, $n = 29$, $P = 0.02$), and also tended to possess more canary xanthophyll A ($r = -0.33$, $n = 29$, $P = 0.08$) and less zeaxanthin ($r = 0.34$, $n = 29$, $P = 0.07$). These results are consistent with the carotenoid content of feathers exhibiting long-wavelength hues, and confirm that crown pigmentation is associated with arrival date.

Sex- and age-specific differences in coloration, morphology and condition

Based on reflectance data, there was no difference in crown brightness between the sexes; however, male kinglets had significantly more saturated and ‘redder’ (longer-wavelength) crown coloration than females (Table 3.2; Fig. 3.3). Males also exhibited greater variation in these colorimetric variables (saturation: 2.43 ± 0.14 vs. 1.73 ± 0.08 ; hue: 569.07 ± 5.02 nm vs. 518.34 ± 2.91 nm). Age and sex-by-age interactions did not significantly influence crown coloration (Table 3.2). Among morphological variables, male kinglets were significantly larger than females in terms of wing-chord and tarsus length, and AHY individuals also tended to have longer tarsi (Table 3.2). After controlling for time of day, we found that AHY birds had slightly greater fat reserves than HY birds (model: $R^2 = 0.11$, $F_{4,179} = 5.34$, $P = 0.0004$; age: $F_{1,182} = 3.34$, $P = 0.07$), which suggests that AHY birds are in better condition. However, AHY individuals were also infested with significantly more wing mites (model: $R^2 = 0.14$, $F_{3,180} = 9.47$, $P < 0.0001$; age: $F_{1,182} = 20.1$, $P < 0.0001$).

Crown coloration vs. condition

Among female kinglets, only variation in crown hue was explained by differences in individual condition (Table 3.3). Females that migrated earlier and had fewer feather mites and higher body condition exhibited deeper yellow crown hues (Table 3.3). Among male kinglets, variation in both crown saturation and hue were predicted by condition variables (Table 3.4). Males that arrived earlier had more saturated and ‘redder’ crown coloration. Contrary to expectation, however, males that had smaller pectoral muscles also had more saturated crown coloration (Table 3.4). Intercorrelations among the condition variables do not appear to be responsible for these patterns (Table 3.5).

DISCUSSION

Our study found that crown coloration in golden-crowned kinglets is influenced by the amounts of carotenoid pigments deposited into feathers, as well as differences in migration timing and other measures of individual condition. We found that male kinglets produce more colourful (i.e., redder) crown coloration by depositing greater concentrations of carotenoids into their feathers, especially red and orange ketocarotenoids. This strategy is consistent with research conducted on house finches (Inouye *et al.*, 2001), albeit within a smaller range of natural hues. Although the relative proportions of ketocarotenoids revealed different patterns compared to their concentrations, levels of canthaxanthin were consistently higher for male kinglets with redder crowns, suggesting that canthaxanthin could be an important pigment for orange colour generation. For insectivores such as the Regulidae, red feather colorants are proposed to result from metabolic conversions of yellow pigments harboured by prey items (Stradi, 1998), as opposed to the direct ingestion of red ketocarotenoids by aquatic

birds (e.g., Fox, 1962). It would be interesting to determine whether increased levels of canthaxanthin in kinglets are due to greater access to the purported dietary precursor, β -carotene (better foraging skills or preferentially choosing prey items rich in this pigment). Alternatively, high-quality males may have more efficient absorption, circulation or metabolism of β -carotene. Furthermore, individuals may selectively incorporate canthaxanthin over other carotenoids into their crown feathers (McGraw, 2006).

Males with less colourful (i.e., yellower) crown feathers incorporated fewer carotenoids overall, as well as greater proportions of yellow hydroxycarotenoids. The transformation of dietary carotenoids into red pigments is thought to be energetically expensive (Hill, 1996; Olson & Owens, 1998; Hill, 2002), and research in house finches suggests that only individuals in good nutritional condition are capable of extensive carotenoid metabolism (Hill, 2000). Since total carotenoid content was similar for feathers with medium- and short-wavelength hues, perhaps the higher levels of zeaxanthin in the latter group are the result of inadequate carotenoid metabolism. Further research into the biochemical pathways of both plasma and feather carotenoids in this species would be fruitful for understanding why pigment composition varies among individual kinglets.

Interestingly, we found several differences in crown pigmentation between kinglets and the closely-related goldcrest. In the latter species, lutein and zeaxanthin produce the yellow coloration, and α -doradoxanthin, astaxanthin and adonirubin comprise the red pigments contributing to the orange crown coloration of males (Stradi, 1998). Thus, most notably, canthaxanthin was not isolated in goldcrest feathers, and we did not find adonirubin in golden-crowned kinglet feathers. As the proposed precursors of red

and orange ketocarotenoids are available in a variety of plant materials (Goodwin, 1980; McGraw, 2006), differences in vegetation and in the diet of arthropods between the habitats of kinglets and goldcrests may explain their dissimilar carotenoid profiles. In addition, we identified in golden-crowned kinglets several other carotenoids absent in the goldcrest: echinenone, canary xanthophyll A and 3'-dehydrolutein. Since echinenone is only present in small amounts and shares a very strong positive relationship with canthaxanthin (Table 5), this orange ketocarotenoid is likely present as the incomplete transformation of β -carotene (McGraw, 2006). The conversion of dietary lutein and zeaxanthin into canary xanthophylls and 3'-dehydrolutein, which result in less colourful (i.e., paler yellow) pigments, has previously been reported in other passerine species with red/orange plumage (e.g., Hudon, 1991; Inouye *et al.*, 2001; McGraw *et al.*, 2004; Andersson *et al.*, 2007; Hudon *et al.*, 2007).

Regarding the possible condition-dependence of carotenoid coloration in kinglets, arrival date was significantly associated with crown coloration in both sexes. While the advantages of early spring migration are well-established (e.g., Kokko, 1999; Ninni *et al.*, 2004), the importance of migration timing in the fall is more difficult to ascertain, especially for a species such as the golden-crowned kinglet, which does not hold winter territories (Ingold & Galati, 1997). Avoidance of migrating predatory birds, such as northern shrikes (Cade & Atkinson, 2002) and northern saw-whet owls (Rasmussen *et al.*, 2008), as well as inclement weather later in the season could be a promoting factor for early migration (Berthold, 2001; Martens & Päckert, 2006). High-quality individuals may be able to accumulate sufficient energy stores for earlier departure from the breeding grounds. In our study, earlier male migrants grew their tail feathers faster (Table 5) and

may thus be in better nutritional condition (Grubb, 2006). If crown coloration plays a role in competition and mate choice, more colourful males may be able to secure better territories and attract high-quality females earlier in the season, which could advance their breeding and thus enable earlier fall migration. Indeed, different periods of the avian annual cycle are likely to be inextricably linked (e.g., Marra *et al.*, 1998; Norris *et al.*, 2004a).

Contrary to our predictions for the condition-dependence of crown coloration, male kinglets that possessed smaller pectoral muscles had more colourful crown feathers. This result could be interpreted multiple ways: firstly, males that invest more energy into depositing carotenoids into their feathers during moult may have less energy to devote to increasing flight muscle mass prior to migration. Since golden-crowned kinglets do not have a prealternate moult before the breeding season (Ingold & Galati, 1997), it may benefit males to invest more into sexually selected ornamental coloration at this stage, even if this requires a trade-off with migratory ability. Secondly, pectoral muscles of migrating birds can vary in size relatively quickly, on the scale of days (Lindström *et al.*, 2000); therefore, pectoral score may not be an ideal measure of longer-term individual quality. Pectoral muscles are more likely to be depleted upon arrival at the stopover site after an extended migratory flight (Schwilch *et al.*, 2002), and a better measure of quality might be an individual's rate of rebuilding fat and protein reserves (Woodrey & Moore, 1997; Jones *et al.*, 2002). However, because we rarely recaptured kinglets on the same or subsequent days, we could not collect these measurements.

While the extent of variation in crown colour was greater in male kinglets, condition-dependence of this trait was surprisingly more apparent for females. Female kinglets with more colourful crown feathers were earlier migrants with higher body

condition and reduced wing mite infestation. As a measure of size-adjusted body mass, body condition index is commonly used to deduce an individual's current state of health (Schulte-Hostedde *et al.*, 2005); and body condition was significantly correlated with fat score in our study. Females that are better foragers may have greater access to nutrient- and/or carotenoid-rich food items, and would thus be able to deposit more fat as well as pigments. It is unknown whether the wing mites that we observed between the feather barbs in golden-crowned kinglets exist as parasitic or commensal symbionts (Proctor & Owens, 2000); however, a negative correlation between wing mites and colour suggests that wing mites may have a negative impact on females. Feather mites can be transmitted between individuals in close proximity, such as members of communal roosts, mates and offspring (Proctor & Owens, 2000). Assuming that mite infestation is relatively stable for each individual in the long term, it may be particularly unfavourable for females to harbour ectosymbionts in case of vertical transmission to their offspring in the nest.

The differences we documented between male and female kinglets may provide some insight into the intensity of sexual selection in this species. Studies on the quality-indicating functions of ornamental traits have traditionally focused on males (reviewed in Johnstone, 1995). However, evidence is now mounting that females can also possess condition-dependent characters (reviewed in Amundsen & Pärn, 2006) and our results provide further support for this idea. Golden-crowned kinglets are serially monogamous and both sexes are involved in breeding duties: females are solely responsible for incubating and brooding, males are involved in nest/territory defence and mate-feeding, and both sexes participate in nest-building and chick-feeding (Ingold & Galati, 1997). This type of breeding system would predict mutual mate choice (Andersson, 1994; Johnstone *et al.*, 1996). Our findings suggest that it may benefit male kinglets to select

females based on their condition-dependent crown coloration. As for female preferences, perhaps early-arriving, more colourful males exhibit some aspects of quality that our study could not identify, such as better parental care. Furthermore, song and behaviour may also be important sexually selected traits in this species (Ingold & Galati, 1997; Martens & Päckert, 2006).

Our study is the first to identify the feather carotenoid composition in a North American regolid species. We determined how carotenoid pigments contribute to sexual dichromatism and intrasexual colour variation in golden-crowned kinglets. Overall, our findings provide further support for the condition-dependent nature of carotenoid-based coloration and the quality-signalling potential of female ornamental traits. Our results also suggest that migration timing in the fall may have important fitness consequences. In conjunction with data on large-scale geographic colour variation in kinglets (Chapter 2), our study provides a more comprehensive and mechanistic view of how plumage coloration can vary intraspecifically. Because carotenoid coloration is an important trait used in sexual selection, especially mate choice (Hill, 2006b), future research on variation in carotenoid coloration should provide a thorough investigation of environmental and genetic factors that serve to maintain signal honesty.

ACKNOWLEDGEMENTS

Feather collection was approved by the Canadian Wildlife Service Migratory Bird Regulations (Scientific Permit CA 0209) and the University of Windsor Animal Care Committee (Protocol 06-22). We are deeply indebted to B. Hall-Brooks, C. Pascoe, R. Powless, P. Roberts and other volunteer banders at the Holiday Beach Migration Observatory for their time and instruction on bird banding. Thanks to M. Toomey and M.

Butler for assistance with carotenoid extraction and HPLC protocols; and to Y. Katare, B. Zielinski, A. Fisk, S. Ellis, T. Pitcher, L. Jerant, S. Loeb, and J. Sylvester for logistical support in the laboratory. We thank R. Montgomerie for access to his CLR colour analysis program. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) CGS-M/PGS-M and E-Bird Canada award, the Society of Canadian Ornithologists Taverner Award and the Government of Ontario Textbook and Technology Grant (C.K.S.C.); NSERC Discovery and Research Tools and Instrumentation grants (S.M.D.); and the College of Liberal Arts and Sciences at Arizona State University and the National Science Foundation (K.J.M.).

REFERENCES

- Amundsen, T. & Forsgren, E. (2001) Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences*, **98**, 13155-13160.
- Amundsen, T. & Pärn, H. (2006) Female coloration: review of functional and nonfunctional hypotheses. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 280-345. Harvard University Press, Cambridge, MA.
- Andersson, M. B. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Andersson, S., Prager, M. & Johansson, E. I. A. (2007) Carotenoid content and reflectance of yellow and red nuptial plumages in widowbirds (*Euplectes* spp.). *Functional Ecology*, **21**, 272-281.
- Badyaev, A. V. (1997) Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behavioral Ecology*, **8**, 675-690.
- Barlein, F. (1995) Manual of Field Methods, revised edition. European-African Songbird Migration Network, Wilhelmshaven, Germany.
- Berthold, P. (2001) *Bird Migration: A General Survey*, 2nd edn. Oxford University Press, Oxford, UK.
- Britton, G. (1985) General carotenoid methods. *Methods in Enzymology: Steroids and Isoprenoids, Part B* (ed. by J. H. Law and H. C. Rilling), pp. 113-149. Academic Press, Orlando, FL.
- Brown, M. E. (1996) Assessing body condition in birds. *Current Ornithology*, **13**, 67-135.
- Cade, T. J. & Atkinson, E. C. (2002) Northern shrike (*Lanius excubitor*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/671> (last accessed 28 July 2009).

- Cuthill, I. C. (2006) Color perception. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 3-40. Harvard University Press, Cambridge.
- DeSante, D. F., Burton, K. M., Velez, P., Froehlich, D. & Kaschube, D. (2009) MAPS Manual: 2009 Protocol. Contribution no. 127 of the Institute for Bird Populations, Point Reyes Station, CA.
- Evans, M. R. & Norris, K. (1996) The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). *Behavioral Ecology*, **7**, 1-6.
- Forstmeier, W. (2002) Benefits of early arrival at breeding grounds vary between males. *Journal of Animal Ecology*, **71**, 1-9.
- Fox, D. L. (1962) Metabolic fractionation, storage and display of carotenoid pigments by flamingoes. *Comparative Biochemistry and Physiology*, **6**, 1-40.
- Goodwin, T. W. (1980) *The Biochemistry of the Carotenoids. Vol. I: Plants*. Chapman and Hall, London, UK.
- Gosler, A. G. (1996) Environmental and social determinants of winter fat storage in the great tit *Parus major*. *Journal of Animal Ecology*, **65**, 1-17.
- Grubb, T. C. (1989) Ptilochronology: feather growth bars as indicators of nutritional status. *The Auk*, **106**, 314-320.
- Grubb, T. C. (2006) *Ptilochronology: Feather Time and the Biology of Birds*. Oxford University Press, Oxford, UK.
- Hasselquist, D. (1998) Polygyny in great reed warblers: a long-term study of factors contributing to male fitness. *Ecology*, **79**, 2376-2390.
- Hill, G. E. (1996) Redness as a measure of the production cost of ornamental coloration. *Ethology Ecology & Evolution*, **8**, 157-175.
- Hill, G. E. (2000) Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology*, **31**, 559-566.
- Hill, G. E. (2002) *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. Oxford University Press, New York, NY.
- Hill, G. E. (2006a) Environmental regulation of ornamental coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 507-560. Harvard University Press, Cambridge, MA.
- Hill, G. E. (2006b) Female mate choice for ornamental coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 137-200. Harvard University Press, Cambridge, MA.
- Hill, G. E., Inouye, C. Y. & Montgomerie, R. (2002) Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society of London - Series B: Biological Sciences*, **269**, 1119-1124.
- Houde, A. E. (1997) *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, Princeton, NJ.
- Hudon, J. (1991) Unusual carotenoid use by the western tanager (*Piranga ludoviciana*) and its evolutionary implications. *Canadian Journal of Zoology*, **69**, 2311-2320.
- Hudon, J., Anciães, M., Bertacche, V. & Stradi, R. (2007) Plumage carotenoids of the pin-tailed manakin (*Ilicura militaris*): evidence for the endogenous production of rhodoxanthin from a colour variant. *Comparative Biochemistry and Physiology, Part B*, **147**, 402-411.

- IBA Canada (2004) Important Bird Areas of Canada: Holiday Beach/Big Creek Conservation Areas, Windsor, Ontario. IBA Canada, Ottawa, ON. Available at: <http://www.bsc-eoc.org/iba/site.jsp?siteID=ON034> (last accessed 19 March 2009).
- Ingold, J. L. & Galati, R. (1997) Golden-crowned kinglet (*Regulus satrapa*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/301> (last accessed 23 January 2009).
- Inouye, C. Y., Hill, G. E., Stradi, R. D. & Montgomerie, R. (2001) Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *The Auk*, **118**, 900-915.
- Johnstone, R. A. (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews*, **70**, 1-65.
- Johnstone, R. A., Reynolds, J. D. & Deutsch, J. C. (1996) Mutual mate choice and sex differences in choosiness. *Evolution*, **50**, 1382-1391.
- Jones, J., Francis, C. M., Drew, M., Fuller, S. & Ng, M. W. S. (2002) Age-related differences in body mass and rates of mass gain of passerines during autumn migratory stopover. *The Condor*, **104**, 49-58.
- Kokko, H. (1999) Competition for early arrival in migratory birds. *Journal of Animal Ecology*, **68**, 940-950.
- Kwiatkowski, M. A. & Sullivan, B. K. (2002) Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=ater). *Evolution*, **56**, 2039-2051.
- Lindström, A., Kvist, A., Piersma, T., Dekinga, A. & Dietz, M. W. (2000) Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *The Journal of Experimental Biology*, **203**, 913-919.
- Lozano, G. A., Perreault, S. & Lemon, R. E. (1996) Age, arrival date and reproductive success of male American redstarts *Setophaga ruticilla*. *Journal of Avian Biology*, **27**, 164-170.
- Maan, M. E., van der Spoel, M., Quesada Jimenez, P., van Alphen, J. J. M. & Seehausen, O. (2006) Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behavioral Ecology*, **17**, 691-699.
- Marra, P. P., Hobson, K. A. & Holmes, R. T. (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science*, **282**, 1884-1886.
- Martens, J. & Päckert, M. (2006) Family Regulidae (kinglets and firecrests). *Handbook of the Birds of the World* (ed. by J. del Hoyo, A. Elliot and D. Christie), 330-349. Lynx Edicions, Barcelona, Spain.
- McGraw, K. J. (2006) Mechanics of carotenoid-based coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 177-242. Harvard University Press, Cambridge, MA.
- McGraw, K. J. & Gregory, A. J. (2004) Carotenoid pigments in male American goldfinches: what is the optimal biochemical strategy for becoming colourful? *Biological Journal of the Linnean Society*, **83**, 273-280.
- McGraw, K. J., Hill, G. E. & Parker, R. S. (2003) Carotenoid pigments in a mutant cardinal: implications for the genetic and enzymatic control mechanisms of carotenoid metabolism in birds. *The Condor*, **105**, 587-592.

- McGraw, K. J., Wakamatsu, K., Clark, A. B. & Yasukawa, K. (2004) Red-winged blackbirds *Agelaius phoeniceus* use carotenoid and melanin pigments to color their epaulets. *Journal of Avian Biology*, **35**, 543-550.
- Merilä, J. (1997) Fat reserves and moult-migration overlap in goldcrests, *Regulus regulus* — A trade-off? *Annales Zoologici Fennici*, **34**, 229-234.
- Merilä, J. & Svensson, E. (1995) Fat reserves and health state in migrant goldcrest *Regulus regulus*. *Functional Ecology*, **9**, 842-848.
- Michener, H. & Michener, J. R. (1938) Bars in flight feathers. *The Condor*, **40**, 149-160.
- Milinski, M. & Bakker, T. C. M. (1990) Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344**, 330-333.
- Mills, A. M. (2005) Protogyny in autumn migration: do male birds "play chicken"? *The Auk*, **122**, 71-81.
- Montgomerie, R. (2006) Analyzing colors. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 90-147. Harvard University Press, Cambridge, MA.
- Montgomerie, R. (2008) CLR, version 1.04. Queen's University, Kingston, ON. Available at: <http://post.queensu.ca/~mont/color/analyze.html> (last accessed 27 May 2008).
- Ninni, P., de Lope, F., Saino, N., Haussy, C. & Møller, A. P. (2004) Antioxidants and condition-dependence of arrival date in a migratory passerine. *Oikos*, **105**, 55-64.
- Norris, D. R., Marra, P. P., Kyser, T. K., Ratcliffe, L. M. & Montgomerie, R. (2007) Continent-wide variation in feather colour of a migratory songbird in relation to body condition and moulting locality. *Biology Letters*, **3**, 16-19.
- Norris, D. R., Marra, P. P., Kyser, T. K., Sherry, T. W. & Ratcliffe, L. M. (2004a) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 59-64.
- Norris, D. R., Marra, P. P., Montgomerie, R., Kyser, T. K. & Ratcliffe, L. M. (2004b) Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science*, **306**, 2249-2250.
- Olson, V. A. & Owens, I. P. F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution*, **13**, 510-514.
- Pérez-Tris, J., de la Puente, J., Pinilla, J. & Bermejo, A. (2001) Body moult and autumn migration in the barn swallow *Hirundo rustica*: is there a cost of moulting late? *Annales Zoologici Fennici*, **38**, 139-148.
- Proctor, H. & Owens, I. (2000) Mites and birds: diversity, parasitism and coevolution. *Trends in Ecology & Evolution*, **15**, 358-364.
- Pyle, P. (1997) *Identification Guide to North American Birds. Part I: Columbidae to Ploceidae*. Slate Creek Press, Bolinas, CA.
- Råberg, L., Grahn, M., Hasselquist, D. & Svensson, E. (1998) On the adaptive significance of stress-induced immunosuppression. *Proceedings of the Royal Society of London B: Biological Sciences*, **265**, 1637-1641.
- Rasmussen, J. L., Sealy, S. G. & Cannings, R. J. (2008) Northern saw-whet owl (*Aegolius acadicus*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/042> (last accessed 28 July 2009).

- Reudink, M. W., Marra, P. P., Langin, K. M., Studds, C. E., Kyser, T. K. & Ratcliffe, L. M. (2008) Molt-migration in the American redstart (*Setophaga ruticilla*) revisited: explaining variation in feather δD signatures. *The Auk*, **125**, 744-748.
- Saks, L., McGraw, K. J. & Hõrak, P. (2003) How feather colour reflects its carotenoid content. *Functional Ecology*, **17**, 555-561.
- Schulte-Hostedde, A. I., Zinner, B., Millar, J. S. & Hickling, G. J. (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology*, **86**, 155-163.
- Schwilch, R., Grattarola, A., Spina, F. & Jenni, L. (2002) Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint. *The Journal of Experimental Biology*, **205**, 687-695.
- Senar, J. C. (2006) Color displays as intrasexual signals of aggression and dominance. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 87-136. Harvard University Press, Cambridge.
- Stradi, R. (1998) *The Colour of Flight: Carotenoids in Bird Plumage*. Solei Gruppo Editoriale Informatico, Milan, Italy.
- Stradi, R., Celentano, G. & Nava, D. (1995) Separation and identification of carotenoids in bird's plumage by high-performance liquid chromatography--diode-array detection. *Journal of Chromatography*, **670**, 337-348.
- Svensson, E. & Merilä, J. (1996) Molt and migratory condition in blue tits: a serological study. *The Condor*, **98**, 825-831.
- Toomey, M. B. & McGraw, K. J. (2009) Seasonal, sexual, and quality related variation in retinal carotenoid accumulation in the house finch (*Carpodacus mexicanus*). *Functional Ecology*, **23**, 321-329.
- U.S. Geological Survey (2006) Northern Prairie Wildlife Research Center: Migration of Birds – Routes of Migration. U.S. Geological Survey, Reston, VA. Available at: <http://www.npwrc.usgs.gov/resource/birds/migratio/routes.htm> (last accessed 16 April 2009).
- Woodrey, M. S. & Moore, F. R. (1997) Age-related differences in the stopover of fall landbird migrants on the coast of Alabama. *The Auk*, **114**, 695-707.

Table 3.1. Pearson correlation coefficients between carotenoid pigments identified in the crown feathers of male golden-crowned kinglets ($n = 29$).

Pigment	Astax.	Canthax.	α -doradex.	Echinenone	Can. xanth. A	3'-dehydrolut.	Lutein	Zeaxanthin
Astaxanthin	–	0.54 **	0.28	0.56 **	0.30	0.21	-0.07	0.16
Canthaxanthin		–	0.79 ****	0.85 ****	0.68 ****	0.39 *	0.20	0.06
α -doradexanthin			–	0.58 **	0.85 ****	0.69 ****	0.54 **	0.14
Echinenone				–	0.60 ***	0.16	-0.09	-0.19
Canary xanthophyll A					–	0.53 **	0.23	-0.12
3'-dehydrolutein						–	0.52 **	0.31
Lutein							–	0.55 **
Zeaxanthin								–

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Table 3.2. Sex and age differences in crown coloration and morphology in golden-crowned kinglets.

Dependent variable	Model effects	<i>F</i>	d.f.	<i>P</i>
Crown brightness	Whole model ($R^2 = 0.01$)	0.55	3, 180	0.65
	Age	0.03	1, 182	0.85
	Sex	1.02	1, 182	0.31
	Age*Sex	0.03	1, 182	0.87
Crown saturation	Whole model ($R^2 = 0.88$)	460.5	3, 180	< 0.0001
	Age	0.62	1, 182	0.43
	Sex	1021.2	1, 182	< 0.0001
	Age*Sex	0.0003	1, 182	0.96
Crown hue	Whole model ($R^2 = 0.97$)	1927.9	3, 180	< 0.0001
	Age	0.98	1, 182	0.32
	Sex	4201.9	1, 182	< 0.0001
	Age*Sex	1.49	1, 182	0.22
Wing-chord	Whole model ($R^2 = 0.37$)	34.6	3, 180	< 0.0001
	Age	2.14	1, 182	0.15
	Sex	68.6	1, 182	< 0.0001
	Age*Sex	0.32	1, 182	0.57
Tarsus length	Whole model ($R^2 = 0.08$)	5.41	3, 180	0.0014
	Age	3.45	1, 182	0.07
	Sex	4.51	1, 182	0.04
	Age*Sex	2.20	1, 182	0.14
Mass	Whole model ($R^2 = 0.04$)	2.49	3, 179	0.06
	Age	0.55	1, 182	0.46
	Sex	0.002	1, 182	0.96
	Age*Sex	5.51	1, 182	0.02

Table 3.3. Relationships between crown coloration and condition variables in female golden-crowned kinglets.

Dependent variable	Model effects	<i>F</i>	β'	d.f.	<i>P</i>
Crown brightness	Whole model ($R^2 = 0.10$)	0.78		8, 58	0.62
	Age	0	-0.0006	1, 65	1.00
	Time released	0.38	0.09	1, 65	0.54
	Arrival date	0.08	0.04	1, 65	0.78
	Body condition	1.02	0.16	1, 65	0.32
	Fat	0.49	0.11	1, 65	0.49
	Pectoral	0.71	-0.11	1, 65	0.40
	Wing mites	0.009	-0.01	1, 65	0.93
	Feather growth rate	0.72	-0.11	1, 65	0.40
Crown saturation	Whole model ($R^2 = 0.18$)	1.55		8, 58	0.16
	Age	0.009	0.01	1, 65	0.93
	Time released	3.26	-0.26	1, 65	0.08
	Arrival date	2.57	-0.21	1, 65	0.11
	Body condition	2.06	0.22	1, 65	0.16
	Fat	0.05	0.04	1, 65	0.82
	Pectoral	2.14	0.19	1, 65	0.15
	Wing mites	1.67	-0.18	1, 65	0.20
	Feather growth rate	2.64	-0.20	1, 65	0.11
Crown hue	Whole model ($R^2 = 0.25$)	2.38		8, 58	0.03
	Age	3.61	0.25	1, 65	0.06
	Time released	0.62	-0.11	1, 65	0.44
	Arrival date	4.15	-0.26	1, 65	0.05
	Body condition	4.49	0.31	1, 65	0.04
	Fat	1.53	-0.18	1, 65	0.22
	Pectoral	0.13	0.05	1, 65	0.72
	Wing mites	4.83	-0.29	1, 65	0.03
	Feather growth rate	0.88	-0.11	1, 65	0.35

Table 3.4. Relationships between crown coloration and condition variables in male golden-crowned kinglets.

Dependent variable	Model effects	<i>F</i>	β'	d.f.	<i>P</i>
Crown brightness	Whole model ($R^2 = 0.10$)	1.56		8, 107	0.14
	Age	0.17	-0.04	1, 114	0.68
	Time released	0.62	-0.08	1, 114	0.43
	Arrival date	1.73	0.13	1, 114	0.19
	Body condition	0.23	0.06	1, 114	0.63
	Fat	0.68	0.10	1, 114	0.41
	Pectoral	5.23	0.22	1, 114	0.02
	Wing mites	0.12	-0.04	1, 114	0.73
	Feather growth rate	0.01	0.01	1, 114	0.92
	Crown saturation	Whole model ($R^2 = 0.15$)	2.29		8, 107
Age		0.15	0.04	1, 114	0.70
Time released		0.71	-0.09	1, 114	0.40
Arrival date		9.27	-0.30	1, 114	0.003
Body condition		0.01	-0.01	1, 114	0.92
Fat		0.13	0.04	1, 114	0.72
Pectoral		5.94	-0.23	1, 114	0.02
Wing mites		0.49	0.07	1, 114	0.49
Feather growth rate		0.19	-0.04	1, 114	0.66
Crown hue		Whole model ($R^2 = 0.16$)	2.55		8, 107
	Age	0.03	-0.02	1, 114	0.86
	Time released	0.15	-0.04	1, 114	0.70
	Arrival date	14.4	-0.37	1, 114	0.0002
	Body condition	0.009	0.01	1, 114	0.93
	Fat	0.09	-0.04	1, 114	0.77
	Pectoral	1.57	-0.12	1, 114	0.21
	Wing mites	0.0001	0.001	1, 114	0.99
	Feather growth rate	0.02	0.01	1, 114	0.89

Table 3.5. Pearson correlation coefficients between condition variables used to investigate the condition-dependence of crown coloration in golden-crowned kinglets. Females shown in lower diagonal ($n = 70$), males in upper diagonal ($n = 116$).

	Arrival date	Body condition	Fat	Pectoral	Wing mites	Feather growth rate
Arrival date	–	0.17	0.19 *	0.05	-0.04	-0.25 **
Body condition	0.005	–	0.60 ***	0.16	-0.10	0.06
Fat	-0.01	0.58 ***	–	0.14	0.15	-0.05
Pectoral	0.11	-0.02	0.19	–	0.10	-0.18
Wing mites	-0.30 *	0.16	0.17	-0.11	–	0.03
Feather growth rate	-0.03	0.06	0.10	-0.05	0.14	–

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$

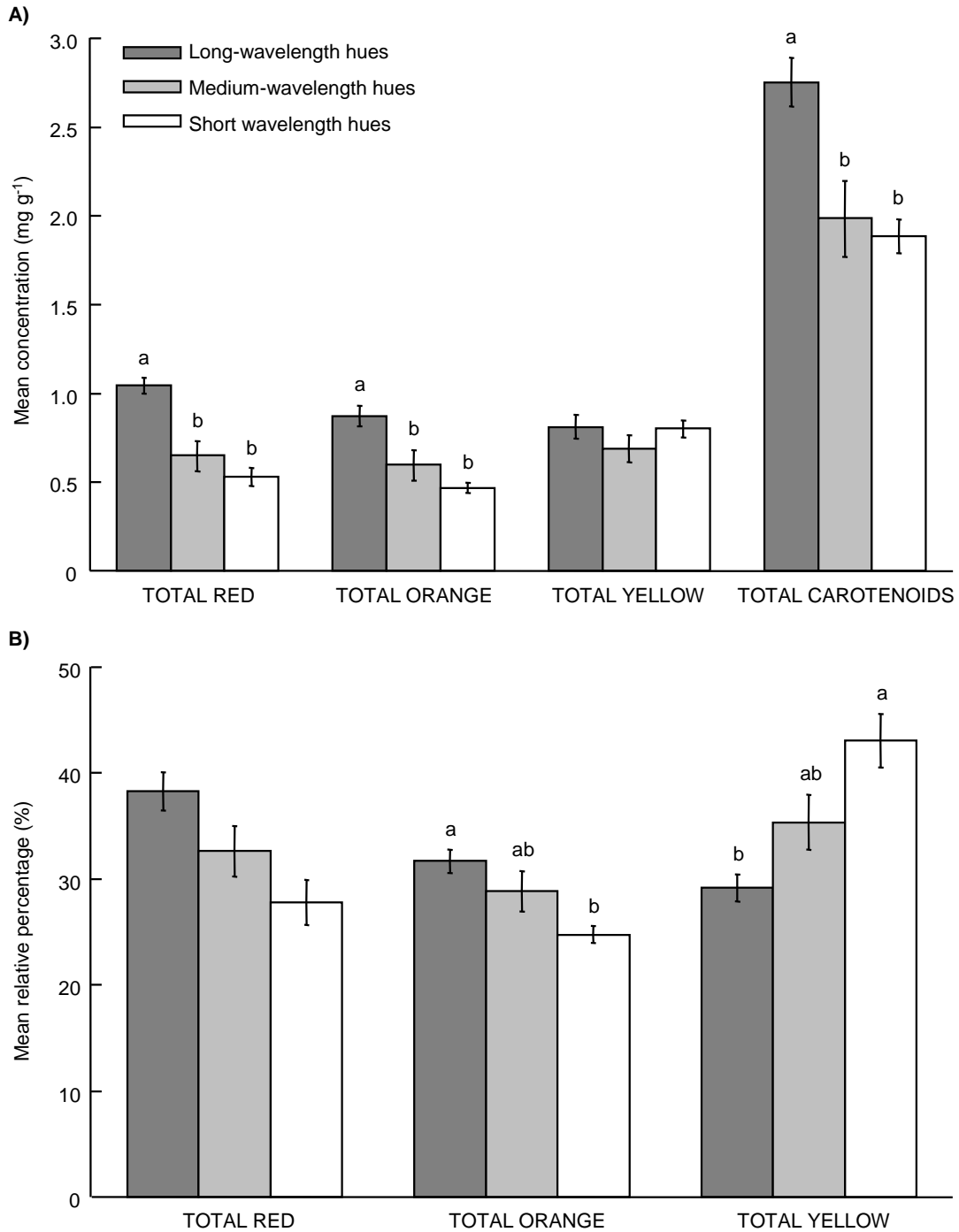


Figure 3.1. Comparisons of a) concentrations and b) relative percentages of carotenoid groups (mean \pm SE) in the feathers of male golden-crowned kinglets exhibiting different crown hues. Sample sizes are $n = 10$ for all categories except long-wavelength hue concentration ($n = 9$). Bars with different letters above indicate significant differences ($P < 0.05$) from ANOVA with *post hoc* Tukey tests.

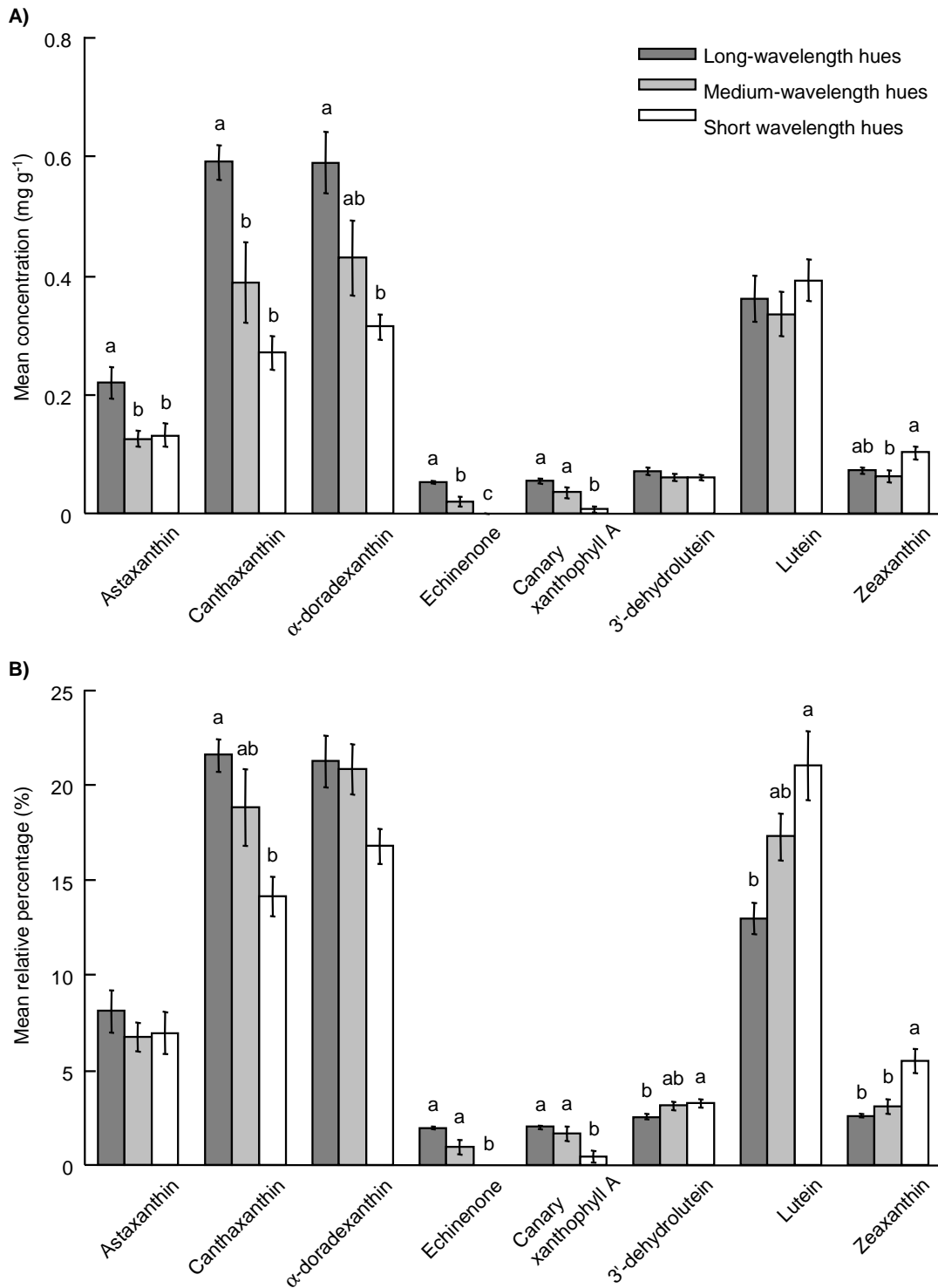


Figure 3.2. Comparisons of a) concentrations and b) relative percentages of all identified carotenoids (mean \pm SE) in the feathers of male golden-crowned kinglets exhibiting different crown hues. Sample sizes are $n = 10$ for all categories except long-wavelength hue concentration ($n = 9$). Bars with different letters above indicate significant differences ($P < 0.05$) from ANOVA with *post hoc* Tukey tests.

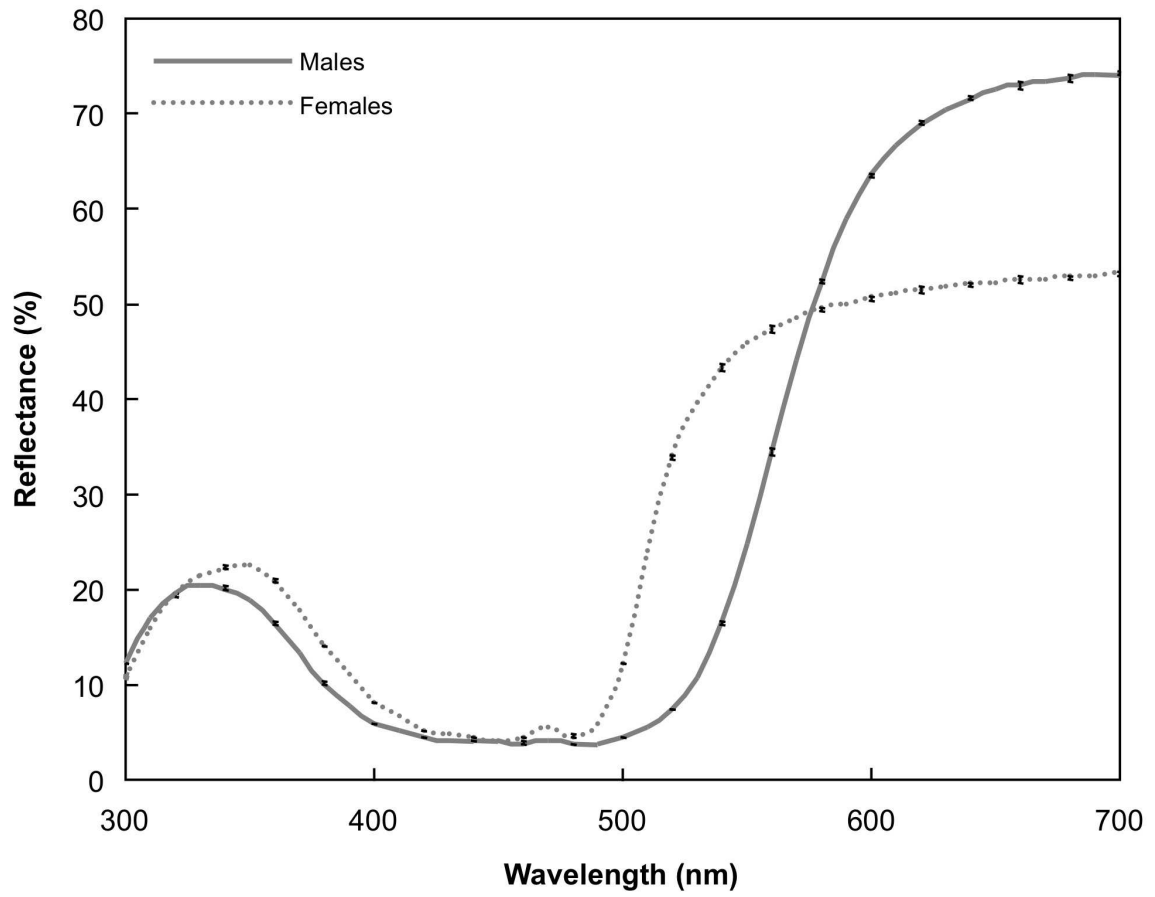


Figure 3.3. Reflectance spectra (mean \pm SE) for the crown coloration of male ($n = 116$) and female ($n = 70$) golden-crowned kinglets.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

THESIS SUMMARY AND DISCUSSION

Natural selection, sexual selection, and genetic drift are the primary processes that act on phenotypic traits to create variation within a species. On a large scale, natural selection for local adaptation has been demonstrated by the majority of vertebrate taxa that exhibit morphological and coloration clines with respect to geographic and climatic gradients (i.e., they follow ecogeographic rules, e.g., Zink & Remsen, 1986; Millien *et al.*, 2006). Sexual selection favours conspicuous signals that effectively communicate information about the bearer to potential mates; however, this is often counteracted by natural selection favouring crypsis against predators. Intraspecific variation in sexually selected traits can thus occur when the intensity of selection pressures differs between populations. On a smaller scale, phenotypic variation can arise among individuals when sexually selected traits are (at least partly) dependent on the environment, such as carotenoid coloration.

The goal of my thesis was to investigate geographic and individual phenotypic variation in golden-crowned kinglets (*Regulus satrapa*), with a particular focus on carotenoid plumage coloration. In Chapter 2, I investigated variation in morphology and coloration across kinglet subspecies as well as along environmental gradients. Golden-crowned kinglet subspecies exhibited some obvious differences in morphology and mantle coloration; however, carotenoid crown coloration was not as strikingly different as previously described (Jenks, 1936). In terms of ecological correlates, body size weakly followed temperature and latitude (Bergmann's rule), mantle coloration did not follow relative humidity (Gloger's rule), and carotenoid coloration was not associated with an index of primary productivity. Interestingly, sexual dimorphism and dichromatism varied with climatic conditions, which agree with Badyaev's (1997) observed pattern.

Intraspecific variation in crown and mantle coloration may be attributed to differences in the strength of natural and sexual selection between populations of kinglets inhabiting areas with varying climatic conditions.

In Chapter 3, I investigated proximate factors mediating individual variation in carotenoid coloration in a group of migrating golden-crowned kinglets. Carotenoid coloration has been shown to be an honest indicator of quality and an important trait used in mate choice (reviewed in Hill, 2006a, b; McGraw, 2006). We found that crown coloration in kinglets was dependent on migration timing, and females also displayed variation with respect to body condition and feather mite infestation. Feather pigment analyses showed that redder crown hues in males can be attributed to the deposition of greater amounts of carotenoids, especially derived red and orange ketocarotenoids. Thus, golden-crowned kinglets in better condition appear to adopt both strategies for becoming colourful (McGraw, 2006): by depositing more carotenoids and also selectively targeting pigments with redder hues. Our data also suggest that sexual dichromatism in this species results from the physiological capability of males to modify dietary carotenoids into red pigments, whereas females are limited to conversion to other yellow pigments.

The complement of these two studies provides a more comprehensive view of how phenotypic traits can vary within a single species. Chapter 2 suggests some ultimate factors to explain *why* coloration and morphology vary on a continental scale, and Chapter 3 demonstrates *how* and *why* carotenoid coloration varies among individuals, both inter- and intrasexually. Carotenoids are a popular topic of research because of the importance of this type of ornamental coloration in sexual selection (e.g., Olson & Owens, 1998). The findings of my thesis are relevant to other animals that exhibit ornamental carotenoid coloration, and these manuscripts contribute to the growing list of

literature in this field. Investigating variation in coloration and other phenotypic traits can reveal insights into the selection pressures that are operating in different populations, and such phenotypic divergence may have implications for reproductive isolation (Zink & Remsen, 1986; Coyne & Orr, 2004).

FUTURE DIRECTIONS

Although quite common throughout North America, golden-crowned kinglets often go unnoticed due to their tiny size, soft voice, and preference for the upper canopy. The Galatis' (1991) extensive efforts brought due attention to these diminutive birds. Until now, however, studies have largely neglected the conspicuous crown coloration of the Family Regulidae (but see Stradi, 1998). Because the central crown feathers play a major role in courtship and antagonistic interactions (Martens & Päckert, 2006), this study system has further research potential in the fields of animal communication and sexual selection.

Geographic variation

It would be fruitful to further investigate Badyaev's (1997) 'rule' for sexual dichromatism in golden-crowned kinglets and other species. He proposed multiple hypotheses to explain why sexual selection for ornamental male traits may be reduced in harsher climates, in association with increased bi-parental care: fewer opportunities for extra-pair paternity, increased cost of mate search due to a shorter breeding season, less time and energy to devote to moult, and increased predation pressure at the nest (Badyaev, 1997). Field observations of different kinglet populations will allow us to determine whether there are, in fact, changes in parental care in varying climatic conditions.

In addition, my observations of museum specimens were consistent with descriptions of subspecies differences in mantle coloration (Ingold & Galati, 1997). For example, birds from the Pacific Northwest (*R. s. olivaceous*) had greener mantles compared to the eastern nominate subspecies (*R. s. satrapa*), which were noticeably greyer. Although mantle coloration did not become darker, per se, with humidity (as predicted by Gloger's rule; see Chapter 2), natural selection for chromatic (rather than achromatic) background matching remains a possibility. Future research detailing geographic differences in habitat, vegetation, and predation pressures may reveal whether golden-crowned kinglet subspecies are indeed more cryptic in their own environment.

Individual variation

The expression of carotenoid coloration is influenced by factors other than those we examined in Chapter 3; therefore, further investigation into the condition-dependence of kinglet crown coloration should focus on individual differences in carotenoid access, immunocompetence, and endoparasite load (Hill, 2006a; McGraw, 2006). Gathering blood samples would be able to answer some of these questions. It would be ideal if we could identify the prey items of individual kinglets and quantify their carotenoid and nutritional contents. These data would indicate whether high-quality kinglets selectively ingest more carotenoid-rich foods. Furthermore, to determine if male crown coloration signals direct benefits such as better parental care, observations of variation in nest attendance and rate of chick-feeding would be informative (Griffith & Pryke, 2006).

The next logical step would be to show convincingly that crown coloration is evaluated for individual quality during mate choice and male-male competitions. In the 1970s and 1980s, Ellen Thaler conducted long-term aviary studies on goldcrests (*Regulus*

regulus) and firecrests (*Regulus ignicapilla*, Thaler-Kottek, 1990). She found that differences in head patterns and behavioural displays (e.g., posturing) served to maintain reproductive isolation between these two species (Löhrl & Thaler, 1992). Thaler also apparently maintained golden-crowned kinglets in aviaries (Ingold & Galati, 1997); however, any observations on reproductive behaviours have not been published. It would be extremely informative to determine through controlled experiments whether individuals with more colourful crown feathers are more successful at obtaining mates.

Alternatively, traits other than crown colour itself may be candidate signals for further investigation of sexual selection in this species. I noticed that our crown feather samples in Chapter 3 differed slightly in length among individuals (not measured). Since male kinglets display their orange crown-patch by ptiloerection, longer central feathers (as well as a wider tract for these feathers) would provide the appearance of a larger, more prominent crown. In addition, the black crown stripes bordering the carotenoid-based crown are a classic example of a possible amplifier (Hasson, 1991; Dale, 2006): these black stripes may aid in the assessment of (and maintain the signal honesty of) the trait of interest. Finally, crown displays often occur in conjunction with vocalizations (Ingold & Galati, 1997; Martens & Päckert, 2006). An integrative study of how mate choice decisions and the outcome of competitive interactions are influenced by variation in song traits as well as ornamental coloration would be invaluable for understanding sexual selection in kinglets and the Regulidae.

REFERENCES

- Badyaev, A. V. (1997) Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behavioral Ecology*, **8**, 675-690.
- Coyne, J. A. & Orr, H. A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA.

- Dale, J. (2006) Intraspecific variation in coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 36-86. Harvard University Press, Cambridge.
- Griffith, S. C. & Pryke, S. R. (2006) Benefits to females of assessing color displays. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 233-. Harvard University Press, Cambridge, MA.
- Hasson, O. (1991) Sexual displays as amplifiers: practical examples with an emphasis on feather decorations. *Behavioral Ecology*, **2**, 189-197.
- Hill, G. E. (2006a) Environmental regulation of ornamental coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 507-560. Harvard University Press, Cambridge, MA.
- Hill, G. E. (2006b) Female mate choice for ornamental coloration. *Bird Coloration: Function and Evolution* (ed. by G. E. Hill and K. J. McGraw), pp. 137-200. Harvard University Press, Cambridge, MA.
- Ingold, J. L. & Galati, R. (1997) Golden-crowned kinglet (*Regulus satrapa*). *The Birds of North America Online* (ed. by A. Poole). Cornell Lab of Ornithology, Ithaca, NY. Retrieved from The Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/301> (last accessed 23 January 2009).
- Jenks, R. (1936) A new race of golden-crowned kinglet from Arizona. *The Condor*, **38**, 239-244.
- Löhrl, H. & Thaler, E. (1992) Behavioural traits as an aid to solving taxonomic problems. *Bulletin of the British Ornithologists' Club, Centenary Supplement 112A*, 199-208.
- Martens, J. & Päckert, M. (2006) Family Regulidae (kinglets and firecrests). *Handbook of the Birds of the World* (ed. by J. del Hoyo, A. Elliot and D. Christie), 330-349. Lynx Edicions, Barcelona, Spain.
- McGraw, K. J. (2006) Mechanics of carotenoid-based coloration. *Bird Coloration: Mechanisms and Measurements* (ed. by G. E. Hill and K. J. McGraw), pp. 177-242. Harvard University Press, Cambridge, MA.
- Millien, V., Lyons, S. K., Olson, L., Smith, F. A., Wilson, A. B. & Yom-Tov, Y. (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters*, **9**, 853-869.
- Olson, V. A. & Owens, I. P. F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution*, **13**, 510-514.
- Stradi, R. (1998) *The Colour of Flight: Carotenoids in Bird Plumage*. Solei Gruppo Editoriale Informatico, Milan, Italy.
- Thaler-Kottek, E. (1990) *Die Goldhähnchen. Winter- und Sommergoldhähnchen Regulus regulus, Regulus ignicapillus*. A. Ziemsen, Wittenberg, Lutherstadt, Germany.
- Zink, R. M. & Remsen, J. V. (1986) Evolutionary processes and patterns of geographic variation in birds. *Current Ornithology*, **4**, 1-69.

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