The Condor 114(2):407–411
© The Cooper Ornithological Society 2012

JUVENAL PLUMAGE POLYMORPHISM IN YELLOW WARBLERS IS NOT ASSOCIATED WITH SEX

Vanya G. Rohwer[1,3], Frances Bonier[1], and Paul R. Martin[1,2]

[1]*Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada*
[2]*Yanayacu Biological Station, Napo, Ecuador*

Abstract. Polymorphisms of the juvenal plumage in birds have received little study in comparison to other plumages. We investigate a polymorphism in the juvenal plumage of the Yellow Warbler (*Setophaga petechia*) in southeastern Ontario, Canada. Yellow Warblers in juvenal plumage show either yellow or white plumage on their lower ventral surface of the neck, breast, and belly, and these differences have been speculated to be associated with sex. We tested the hypothesis that juvenal plumage color varies with sex by using blood samples to molecularly sex 10 yellow and 14 white nestlings. We found no relationship between plumage color and sex (yellow nestlings: 70% male, 30% female; white nestlings: 64% male, 36% female). Using the same 24 nestlings that we sexed, we measured feather color with a spectrometer and found that white and yellow morphs differ significantly in reflectance spectra of red and yellow chroma. Of 66 nestlings whose plumage color we visually inspected in the wild, 73% were white and 27% were yellow, showing that white nestlings are more common than yellow in our study population. We propose alternative explanations for the possible significance of yellow and white polymorphism in the juvenal plumage of the Yellow Warbler.

Key words: *carotenoids,* Setophaga petechia*, lutein, nestling, plumage polymorphism, Yellow Warbler.*

El Polimorfismo del Plumaje Juvenil de *Setophaga petechia* No Está Asociado con el Sexo

Resumen. Los polimorfismos del plumaje juvenil de las aves han sido poco estudiados con relación a otros plumajes. Investigamos un polimorfismo en el plumaje juvenil de *Setophaga petechia* en el sudeste de Ontario, Canadá. Los individuos de *D. petechia* en plumaje juvenil muestran o plumaje amarillo o blanco en la superficie ventral baja del cuello, el pecho y el abdomen, y se ha especulado que estas diferencias están asociadas al sexo. Evaluamos la hipótesis de que el color del plumaje juvenil varía con el sexo mediante el uso de muestras de sangre para determinar el sexo de 10 pichones amarillos y 14 blancos. No encontramos una relación entre el color del plumaje y el sexo (pichones amarillos: 70% machos, 30% hembras; pichones blancos: 64% machos, 36% hembras). Usando los mismos 24 pichones a quienes determinamos el sexo, medimos el color de las plumas con un espectrómetro y encontramos que los morfotipos blanco y amarillo difieren significativamente en el espectro de reflectancia cromático del rojo y amarillo. De los 66 pichones a quienes inspeccionamos visualmente el color del plumaje en el campo, 73% fueron blancos y 27% amarillos, mostrando que los pichones blancos son más comunes que los amarillos en nuestra población de estudio. Proponemos explicaciones alternativas sobre el posible significado del polimorfismo amarillo y blanco en el plumaje juvenil de *S. petechia*.

INTRODUCTION

Color polymorphisms are widely observed in nature and occur in a diversity of animal taxa including invertebrates, reptiles, fishes, mammals, and birds (Gray and McKinnon 2006, and citations within). Color polymorphism arises when multiple color morphs occur among individuals of the same age within a single interbreeding population and the least common morph occurs in abundances higher than predicted by random mutations (Huxley 1955, Galeotti et al. 2003). Among birds, plumage polymorphisms are found in approximately 3.5% of extant species (Galeotti et al. 2003). Some orders appear highly polymorphic (e.g., Strigiformes and Cuculiformes), while others have proportionately many fewer color polymorphisms (e.g., Piciformes and Passeriformes) (Galeotti et al. 2003). Polymorphic plumages vary widely from discrete morphs, such as the light and dark morphs of herons (Mock 1980), to morphs that vary continuously in color, such as those of the Common Buzzard (*Buteo buteo*; Ulfstrand 1977).

Given the extreme diversity of plumage polymorphisms observed in birds, much research has been directed toward explaining the evolution, maintenance, and function of these polymorphisms within species (Galeotti et al. 2003, Roulin 2004, and citations within). Most of the literature focuses on polymorphisms of adult or immature plumages (for reviews see Galeotti et al. 2003, Roulin 2004). By contrast, comparatively few studies have examined the basis and function of polymorphisms in the juvenal plumage of nestlings and fledglings (but

Manuscript received 11 June 2011; accepted 23 November 2011.
[3]E-mail: 6vgr@queensu.ca

The Condor, Vol. 114, Number 2, pages 407–411. ISSN 0010-5422, electronic ISSN 1938-5422. © 2012 by The Cooper Ornithological Society. All rights reserved. Please direct all requests for permission to photocopy or reproduce article content through the University of California Press's Rights and Permissions website, http://www.ucpressjournals.com/reprintInfo.asp. DOI: 10.1525/cond.2012.110086

see Munro et al. 1968, Chaniot 1970, Cooke and Ryder 1971, Johnsen et al. 2003, Tschirren et al. 2005, Fitze and Tschirren 2006, Isaksson et al. 2006). This publication bias might result from polymorphisms of juvenal plumage being fewer and/or limited knowledge of juvenal plumages because they are retained for relatively short periods (Howell et al. 2003).

Recently, several studies have investigated variation in the carotenoid-based plumages of nestling Blue (*Cyanistes caeruleus*) and Great (*Parus major*) tits. The variation in these species' juvenal plumage is thought to be due to prenatal maternal and post-natal early environmental/parental effects (Johnsen et al. 2003, Isaksson et al. 2006), but no selective advantages for the differences have been found to date (Tschirren et al. 2005, Fitze and Tschirren 2006). Only one study, of the Blue Tit, has found juvenal color to be associated with sex (Johnsen et al. 2003). Here we examine a distinct yellow and white polymorphism in the juvenal plumage of the Yellow Warbler in southeastern Ontario, Canada.

The polymorphism of the juvenal plumage of the Yellow Warbler was first described by Southern (1961), who found both white and yellow nestlings in a single nest in Wisconsin (see Fig. 1 for representative yellow and white morphs). Yellow Warblers retain their juvenal plumage for only a brief period. Shortly after fledging, young replace their juvenal plumage with basic I or first basic plumage, which they retain through their first winter (Pyle 1997). Basic I plumage color differs by sex, the body plumage of most males being brighter yellow than that of females (Hobson et al. 2000). In addition, Hobson et al. (2000) found that the ratio of yellow to dull brown on the outer rectrices (acquired during pre-juvenal molt; Lowther et al. 1999) was greater in most males than in females. Although Southern (1961) did not speculate on the basis or function of the color polymorphism of nestling Yellow Warblers, others have posited that the yellow and white morphs indicate sex, like the differences in basic I plumage (Lowther et al. 1999, Hobson et al. 2000). Yet we know of no studies that have tested this idea. In this study, we test the hypothesis that the color of the juvenal plumage varies with sex. If color were associated with sex, we predicted that yellow should correspond to males, white to females, following a general pattern of brighter yellow in males' basic and alternate plumages (Lowther et al. 1999, Hobson et al. 2000).

METHODS

VISUAL INSPECTION OF NESTLINGS, AND BLOOD AND FEATHER COLLECTION

During June and July 2010, we visually inspected 66 nestlings from a total of 21 nests at the Queen's University Biological Station in southeastern Ontario, Canada (44° 34′ N, 76° 19′ W, 135 m elevation). We inspected nestlings visually 6–9 days after hatching when juvenal feathers had erupted from their sheaths. We examined feathers from the upper breast, flank, and belly and categorized nestlings' color as either yellow or white. While there is variation within these two color morphs (e.g., pale yellow, dirty white; see Fig. 1), nestlings can be reliably classified into distinct yellow or white morphs. We collected feather samples from 60 nestlings by plucking 5–8 breast feathers from each individual, targeting feathers that were the most developed within the breast region. We used a subset of these samples to measure feather color with a spectrometer to quantify differences in plumage color. We collected blood samples from 59 of the same nestlings from which we had collected feathers and used a subset of these samples to genetically sex individuals whose feather color we had scored. After bachial venipuncture, we collected blood samples (~75 µL) into heparinized microcapillary tubes. All feather and blood samples were collected under the appropriate Canadian Wildlife Service permit (CA 0199).

MEASURING COLOR

To measure feather color in a subsample of 24 nestlings we used Ocean Optics (Dunedin, FL) equipment: a USB4000 UV-vis spectrometer, a PX-2 pulsed xenon lamp, a fiber-optic cable and probe (P400-2-UV-VIS), and SpectraSuite software. To measure feather color, we stacked all 5–8 breast feathers on top of each other, placed them on black velvet, and maintained them perpendicular to the probe. Feathers from a single nestling were all similar to each other in color, so stacking the fluffy, downlike plumes provided a more accurate measure of color because the black velvet was less likely to reduce reflectance measures. We recalibrated light and dark standards prior to measuring the color of all feather samples.

The yellow plumage of nestling Yellow Warblers is likely the result of a single carotenoid, lutein (McGraw et al. 2003), although other carotenoids are present in the diet (Partali et al. 1987, McGraw et al. 2003). We used two measures of spectral purity in our analyses of nestling color: red chroma (S1R), which calculates the proportion of total reflectance in the range 605–700 nm (S1R = $R_{605-700}/R_{300-700}$), and yellow chroma (S1Y), which calculates the proportion of total reflectance in the range 550–625 nm (S1Y = $R_{550-625}/R_{300-700}$) (Endler 1990, Montgomerie 2006). These two color variables were highly correlated ($r^2 = 0.97$), but we did not collapse them into a single principal component of color so that these data may be used for future comparative studies (Montgomerie 2006).

MOLECULAR SEXING OF NESTLINGS

We used a published method (Griffiths et al. 1998) to molecularly sex the same 24 nestlings (10 yellow, 14 white) whose color we measured with a spectrometer. In short, we extracted DNA from blood samples by using a Qiagen DNEasy tissue kit (Valencia, CA), following the manufacturer's instructions for nucleated blood cells. We then amplified fragments of sex-specific CHD-Z (present in males and females) and CHD-W (present in females only) genes with primers P2 and P8 (Griffiths et al. 1998), then ran these amplified fragments through a 2% agarose gel.

Females are indicated by the presence of two visible bands on an agarose gel, representing fragments from both CHD-W and CHD-Z genes. Males are indicated by a single band, representing fragments from the CHD-Z gene. To confirm the accuracy of this method for sexing Yellow Warblers, we sampled two adult males and two adult females whose sex was determined by gonadal inspection postmortem by VGR. All four adults of known sex were accurately sexed with the genetic material. We analyzed all samples of nestlings and adults in duplicate with 100% agreement of sex identification.

STATISTICAL ANALYSES

To determine if reflectance spectra of yellow and white nestlings differed, we used a two-tailed *t*-test (all spectral data fit a normal distribution). To test the hypothesis that nestling color is associated with sex, we used a Fisher's exact test because some samples contained fewer than five individuals (Crawley 2005).

RESULTS

SPECTRAL DATA

For both red and yellow chroma, nestlings categorized as yellow had significantly different spectral profiles than did nestlings categorized as white (two-tailed *t*-test, $P < 0.001$, Fig. 1, 2). The spectrum for yellow nestlings showed a pronounced trough and reduced reflectance over 360–500 nm, where yellow and other carotenoid-based pigments are absorbed (Fig. 1B). In contrast, white nestlings showed a shallow trough and greater reflectance over 360–500 nm, with reflectance measures typically increasing with wavelength (Fig. 1B).

IS NESTLING COLOR RELATED TO SEX?

We found no relationship between nestling color and sex (Fisher's exact test, $P = 1.00$, Fig. 2). Of the 10 yellow nestlings we sexed, 7 were male and 3 were female, whereas of the 14 white nestlings we sexed, 9 were male and 5 were female. Of these 24 nestlings, 16 (67%) were males and 8 (33%) were females.

We visually inspected the plumage color of 66 nestlings (from 21 nests), and found that the white morph is more common than the yellow ($\chi^2 = 13.6$, $P = 0.0002$), with 48 (72.7%) scored as white and 18 (27.3%) scored as yellow. The distribution of yellow and white nestlings within and among nests varied (Table 1). Five nests contained both white and yellow nestlings, two contained only yellow nestlings, and 10 contained only white nestlings (excluding four nests where we could not reliably assess the color of all nestlings because some nestlings had already fledged or because clutch size may have been affected by parasitism by Brown-headed Cowbirds (*Molothrus ater*); see Table 1).

DISCUSSION

We found that the yellow and white polymorphism in the juvenal plumage of nestling and fledgling Yellow Warblers is not related to sex. We also found that the visually observed plumage polymorphism is reflected in quantitative differences in spectral properties of the plumage.

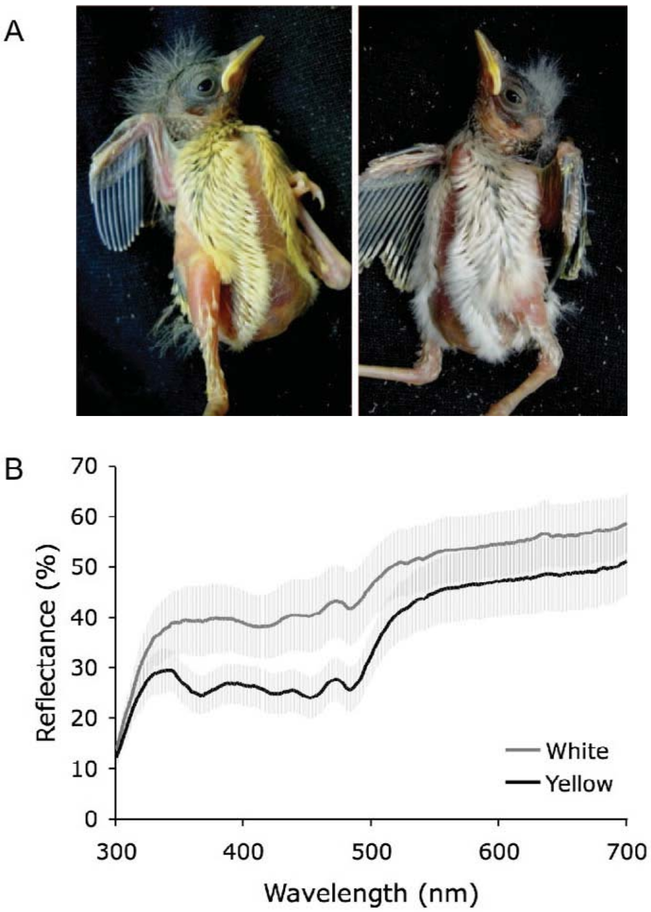

FIGURE 1. (A) Representative yellow and white nestling Yellow Warblers. Color morphs are visually distinct and differ significantly in reflectance of yellow and red chroma as measured with a spectrometer. (B) Mean reflectance spectra (background shading represents ± SD) from yellow (*n* = 10) and white (*n* = 14) color morphs.

Given that this polymorphism of the Yellow Warbler's juvenal plumage is not indicative of sex, why might this plumage be polymorphic? Possible alternative explanations include (1) signaling of nestlings' or fledglings' quality or condition, (2) indication of a broader polymorphism that corresponds to variation in the color of adults' alternate plumage and their reproductive strategies (Studds and Robertson 1985, Yezerinac and Weatherhead 1997), or (3) a character used for recognition of kin or individuals (Dale 2006). In all these cases, variation in juvenal plumage may allow parents or kin to identify individuals, which could be adaptive if resources or social interactions are allocated to offspring asymmetrically. In addition to these functional hypotheses, the proximate mechanism underlying the Yellow Warbler's yellow and white juvenal plumages may be environmental (e.g., diet variation or maternal effects) or genetic.

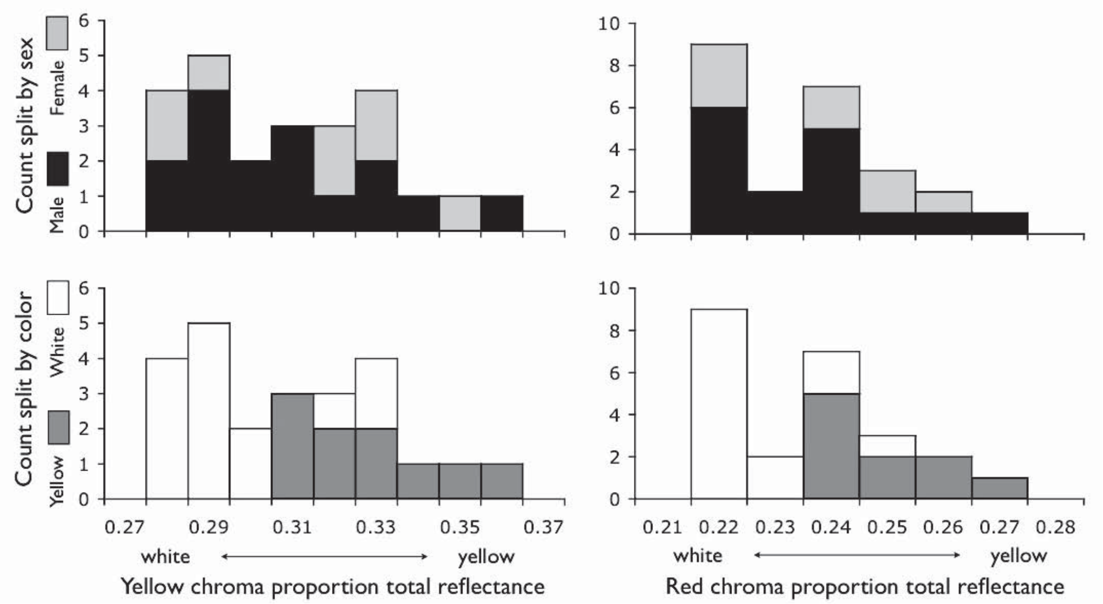

FIGURE 2. The relationships between spectral reflectance and nestling sex (top plots) and between spectral reflectance and plumage polymorphism (bottom plots). We found no relationship between reflectance and sex (two-tailed *t*-tests, $P > 0.65$ for both red and yellow chroma); both males and females vary similarly. We found strong differences in reflectance and plumage color (two tailed *t*-tests, $P < 0.001$ for both red and yellow chroma), indicating that visual differences in plumage color reflect quantitative differences in spectral properties of the plumage.

TABLE 1. Summary of nestling color and number of unhatched eggs for the 21 Yellow Warbler nests we examined. Each row represents a single nest.

Nest	White nestlings (*n*)	Yellow nestlings (*n*)	Eggs unhatched (*n*)
1	2	2	0
2	2	0	3
3[a]	0	1	0
4[a]	1	0	0
5	3	0	0
6	2	0	0
7[a]	4	0	0
8	4	1	0
9	4	0	0
10	3	0	0
11	3	0	2
12	0	3	2
13	1	3	0
14	5	0	0
15[b]	0	1	1
16	4	0	0
17	4	0	0
18	0	3	0
19	3	0	0
20	2	1	0
21	1	3	0

[a] At least one young fledged prior to assessment of nestling color, so we could not reliably assess the number of each morph in the nest.

[b] Nest parasitized by a Brown-headed Cowbird, so we could not reliably assess the number of each morph, as some Yellow Warbler eggs may have been ejected.

The dimorphic nature of yellow and white in the juvenal plumage is not typical of a trait that varies with quality, condition, or food because these factors usually vary continuously (e.g., Peig and Green 2009). In addition, in the five nests with both yellow and white nestlings, we observed no pattern of one color being associated with the most developed nestling (based on feather size), suggesting that plumage color is not related to the sequence in which the eggs are laid, and both white and yellow individuals fledged with equal success (total of 10 yellow and 10 white nestlings fledged from 5 nests).

If the yellow and white plumages of juvenal Yellow Warblers indicate different reproductive strategies of adults, then this plumage polymorphism may be adaptive if adults invest more in nestlings that signal potential for high reproductive output as future breeders. In the Yellow Warbler, alternative reproductive strategies have been suggested for males only, the strategies varying with the male's plumage characteristics (brightness of yellow and amount of rufous streaking on flanks; Studds and Robertson 1985, Yezerinac and Weatherhead 1997). However, these plumage characters appear to vary continuously (Yezerinac and Weatherhead 1997), unlike the discrete yellow and white juvenal plumages. We do not yet know if the morphs of the juvenal plumage correspond to adult breeding plumage, so this hypothesis remains intriguing but speculative.

The possibility that Yellow Warblers may use discrete color morphs for kin or individual recognition also remains untested. The dimorphic nature of the plumage polymorphism suggests that there is little variation in plumage characters that adults and juveniles may use to recognize individuals. Additionally, observations of Yellow Warblers raising Brown-headed Cowbirds suggest that adults may not use plumage characters to recognize individuals within their nests.

The evidence currently available is most consistent with the hypothesis that this polymorphism of the juvenal plumage reflects a broader polymorphism similar to the genetically based plumage polymorphism found in the White-throated Sparrow (*Zonotrichia albicollis*; Falls and Kopachena 2010). Future studies that track individuals from nestling to adulthood, experimentally manipulate environmental conditions, and investigate potential links between plumage coloration and nestling quality will help elucidate the causes and functions of polymorphism in the juvenal plumage of the Yellow Warbler.

LITERATURE CITED

Chaniot, G. E. Jr. 1970. Notes of color variation in downy Caspian Terns. Condor 72:460–465.

Cooke, F., and J. P. Ryder. 1971. The genetics of polymorphism in the Ross' Goose (*Anser rossii*). Evolution 25:483–490.

Crawley, M. J. 2005. Statistics: an introduction using R. Wiley, West Sussex, England.

Dale, J. 2006. Intraspecific variation in coloration, p. 36–86. *In* G. E. Hill and K. J. McGraw [eds.], Bird coloration II: function and evolution. Harvard University Press, Cambridge, MA.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41:315–352.

Falls, J. B., and J. G. Kopachena [online]. 2010. White-throated Sparrow (*Zonotrichia albicollis*), no. 128. *In* A. Poole [ed.], The birds of North America online. Cornell Lab of Ornithology, Ithaca, NY. <http://bna.birds.cornell.edu/bna/species/128>.

Fitze, P. S., and B. Tschirren. 2006. No evidence for survival selection on carotenoid-based nestling coloration in Great Tits (*Parus major*). Journal of Evolutionary Biology 19:618–624.

Galeotti, P., D. Rubolini, P. O. Dunn, and M. Fasola. 2003. Colour polymorphism in birds: causes and functions. Journal of Evolutionary Biology 16:635–646.

Gray, S. M., and J. S. McKinnon. 2006. Linking color polymorphism maintenance and speciation. Trends in Ecology and Evolution 22:71–79.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Hobson, K. A., H. L. Gibbs, H. den Haan, S. van Wilgenburg, and R. J. G. Dawson. 2000. Sexing hatch-year Yellow Warblers using plumage characteristics. North American Bird Bander 25:8–12.

Howell, S. N. G., C. Corben, P. Pyle, and D. I. Rogers. 2003. The first basic problem: a review of molt and plumage homologies. Condor 105:635–653.

Huxley, J. 1955. Morphism in birds. Acta International Ornithological Congress 11:309–328.

Isaksson, C., T. Uller, and S. Andersson. 2006. Parental effects on carotenoid-based plumage coloration in nestling Great Tits, *Parus major*. Behavioural Ecology and Sociobiology 60:556–562.

Johnsen, A., K. Delhey, S. Andersson, and B. Kempenaers. 2003. Plumage colour in nestling Blue Tits: sexual dichromatism, condition dependence and genetic effects. Proceedings of the Royal Society of London B 270:1263–1270.

Lowther, P. E., C. Celada, N. K. Klein, C. C. Rimmer, and D. A. Spector. 1999. Yellow Warbler (*Dendroica petechia*), no. 454. *In* A. Poole and F. Gill [eds.], The birds of North America. Birds of North America, Inc., Philadelphia.

McGraw, K. J., M. D. Beebee, G. E. Hill, and R. S. Parker. 2003. Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. Comparative Biochemistry and Physiology B 135:689–696.

Mock, D. W. 1980. White–dark polymorphism in herons. Proceedings of the First Welder Wildlife Foundation Symposium 1:145–161.

Munro, R. E., L. T. Smith, and J. J. Kupa. 1968. The genetic basis of color differences observed in the Mute Swan (*Cygnus olor*). Auk 85:504–505.

Montgomerie, R. 2006. Analyzing colors, p 40–147. *In* G. E. Hill and K. J. McGraw [eds.], Bird coloration I: mechanisms and measurements. Harvard University Press, Cambridge, MA.

Partali, V., S. Liaaen-Jensen, and T. Slagsvold. 1987. Carotenoids in food chain studies—II. The food chain of *Parus* spp. monitored by carotenoid analysis. Comparative Biochemistry and Physiology B 87:885–888.

Peig, J., and A. J. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891.

Pyle, P. 1997. Identification guide to North American birds, part 1: Columbidae to Ploceidae. Slate Creek Press, Bolinas, CA.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biological Reviews 79:815–848.

Southern, W. E. 1961. Dichromatism in juvenal Yellow Warblers. Auk 78:440–441.

Studds, M. V., and R. J. Robertson. 1985. Sexual selection and variation in reproductive strategy in male Yellow Warblers (*Dendroica petechia*). Behavioural Ecology and Sociobiology 17:101–109.

Tschirren, B., P. S. Fitze, and H. Richner. 2005. Carotenoid-based nestling colouration and parental favouritism in the Great Tit. Oecologia 143:477–482.

Ulfstrand, S. 1977. Plumage and size variation in Swedish Common Buzzards *Buteo buteo* L. (Aves, Accipitriformes). Zoologica Scripta 6:69–75.

Yezerinac, S. M., and P. J. Weatherhead. 1997. Extra-pair mating, male plumage coloration and sexual selection in Yellow Warblers (*Dendroica petechia*). Proceedings of the Royal Society of London B 264:527–532.