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Source: *The Auk*, 134(1) : 22-33

Published By: American Ornithological Society

URL: <https://doi.org/10.1642/AUK-16-63.1>

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RESEARCH ARTICLE

## Diet explains red flight feathers in Yellow-shafted Flickers in eastern North America

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Submitted March 23, 2016; Accepted July 28, 2016; Published October 12, 2016

### ABSTRACT

Yellow-shafted Flickers (*Colaptes auratus auratus* subspecies group of the Northern Flicker) occasionally have orange to red flight feathers in eastern North America far from the hybrid zone with the Red-shafted Flicker (*C. a. cafer* subspecies group). Blocks of feathers of anomalous color tend to show bilateral symmetry and may differ from one year to the next in the same bird. It has been suggested that hybridization with *cafer* must be the source of the red color and that selection for that color could explain the high incidence of red or orange shafts in eastern flickers in some localities. Complex, though largely unproven, physiological mechanisms have been hypothesized to explain the variegated look. We evaluated a simpler, dietary explanation for the variation: that the pigment rhodoxanthin acquired exogenously at the time of feather molt comes to rest alongside the carotenoids normally present in these feathers. An exogenous source of rhodoxanthin exists in the berries of nonnative bush honeysuckles (*Lonicera* spp.) now naturalized in eastern North America and the American Midwest. We confirm the presence of rhodoxanthin and a probable metabolite, rather than the 4-keto-carotenoids found in the Red-shafted Flicker, in the red flight feathers of 2 Yellow-shafted Flickers from eastern North America. In these individuals, dietary rhodoxanthin appeared to interfere with the production of lutein, the main carotenoid in *auratus*. A fairly abrupt appearance of red color in earlier-molted primaries (usually p4 or p5) followed by its gradual fading in subsequent primaries in a large series of aberrantly colored flickers from eastern North America and the American Midwest supports a dietary explanation. We use data on the timing of replacement of primaries in the Northern Flicker at Manomet in Plymouth, eastern Massachusetts, to infer that these aberrantly colored Yellow-shafted Flickers on average acquired the unusual red pigment in early August.

**Keywords:** *Colaptes auratus*, Northern Flicker, rhodoxanthin, carotenoids, bush honeysuckles, *Lonicera* spp., diet

### La diète explique la présence de plumes de vol rouges chez le pic doré dans l'est de l'Amérique du Nord

#### RÉSUMÉ

Le pic doré (groupe de sous-espèces *Colaptes auratus auratus*) a parfois des rémiges et rectrices de couleur orangée à rouge dans l'est de l'Amérique du nord loin de la zone de contact avec le pic rosé (groupe de sous-espèces *C. a. cafer*). Les blocs de plumes aux couleurs inusitées souvent sont latéralement symétriques et peuvent changer d'une année à l'autre chez le même individu. Il a été suggéré que l'hybridation avec *cafer* doit être la source de la couleur rouge et qu'une sélection pour cette couleur pourrait expliquer l'incidence élevée de rachis orangés à rouges chez les pics de l'est de certaines localités. Des mécanismes physiologiques compliqués, mais sans trop de fondement, ont été proposés pour expliquer les patrons variés. Nous évaluons une explication plus simple reliée à la diète pour la couleur inusitée: que la rhodoxanthine acquise de la diète pendant la période de mue se retrouve juxtaposée aux caroténoïdes qui se trouvent normalement dans ces plumes. Une source externe de rhodoxanthine existe dans les baies de deux espèces exotiques de chèvrefeuilles (*Lonicera* spp.) maintenant naturalisées dans l'est de l'Amérique du nord et le Midwest américain. Nous confirmons la présence de rhodoxanthine et d'un métabolite probable, plutôt que les céto-4-caroténoïdes du pic rosé, dans les rémiges rouges de 2 pics dorés de l'est de l'Amérique du nord. Chez ces oiseaux, la rhodoxanthine externe semble interférer avec la production de la lutéine, le principal caroténoïde chez *auratus*. L'apparence plutôt abrupte de la couleur rouge dans les rémiges primaires (habituellement p4 ou p5) qui sont remplacées d'abord, suivi par sa disparition graduelle dans les primaires subséquentes dans une série de peaux de pics dorés de couleur aberrante de l'est de l'Amérique du nord et du Midwest américain supportent une explication reliée à la diète. Nous utilisons une base de données sur la mue des primaires des pics dorés à Manomet à Plymouth,

Massachusetts pour inférer que les pics dorés aux plumes de couleur aberrante en moyenne acquièrent le pigment rouge inusité en début août.

*Mots-clés:* *Colaptes auratus*, rhodoxanthine, caroténoïdes, chèvrefeuilles, *Lonicera* spp., diète

## INTRODUCTION

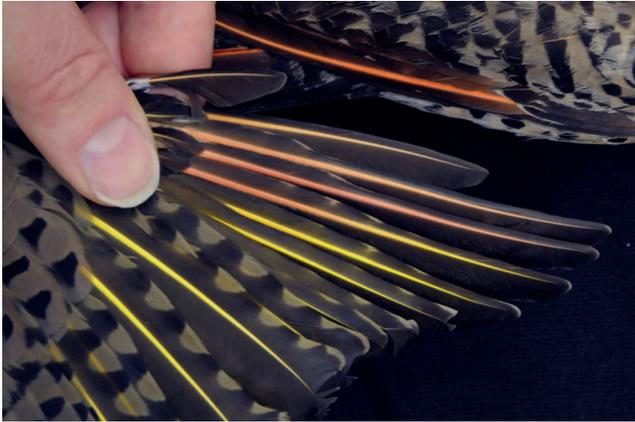
The Yellow-shafted (*auratus* group) and Red-shafted (*cafer* group) forms—actually subspecies groups—of the Northern Flicker (*Colaptes auratus*) differ conspicuously in the coloration of rachides and undersides of flight feathers (Short 1965, Wiebe and Moore 2008), as suggested by their names. The difference in color is under genetic control (Test 1969, Moore and Buchanan 1985), the result of the deposition in *cafer* of 4-keto derivatives of the carotenoid pigments found in *auratus* (Hudon et al. 2015).

In spite of the striking color difference, *auratus* and *cafer* hybridize broadly where their ranges come together across the North American Great Plains and along the eastern edge of the Canadian Rocky Mountains (Short 1965, Moore 1987), and the 2 forms are considered conspecific (AOU 1983, 1998). The hybridization results in a broad cline in shaft and vane color of flight feathers from bright yellow in the east to salmon pink across the hybrid zone to the west (Short 1965, Moore 1987). However, the fact that nearly a third of Yellow-shafted Flickers in eastern North America, far from the hybrid zone, have orange or red color on some flight feathers has been considered an “enigma” (Short 1965, Ingold and Weise 1985). Many hypotheses have been suggested to explain the anomaly. Short (1965) believed that the red color must have originated from western *cafer* populations and also invoked the possibility of active selection for the red color to explain the high incidence of red or orange shaft colors in some localities in the east. Test (1969) noted that the red color of variants was more “coppery” in tone than that of *cafer* and hybrids, and thought this unlikely, but could not provide an alternative explanation. Johnson (1969) intimated that perhaps some of the color differences between the 2 forms have deep genetic roots and could occasionally show up in the wrong form.

Characteristically, the orange to red color in eastern *auratus* flickers appears in a subset of flight feathers, usually adjacent primaries, secondaries, and/or rectrices in a generally bilaterally symmetrical fashion (Short 1965, Test 1969, Ingold and Weise 1985). Interestingly, in single individuals, affected feathers may differ from one year to the next (Ingold and Weise 1985). To explain the variable expression Ingold and Weise (1985) proposed elaborate, though largely unproven, controls of carotenoid deposition in the Northern Flicker, e.g., genes turned on and off during the molt process that can influence all growing flight feathers but are imperfect in timing, genes always on but exerting their action only on certain flight feathers, and similar mechanisms.

The possibility that the unusual color is dietary in origin has not been given serious consideration in this context. A dietary origin is warranted on the basis of a growing list of birds in eastern North America that occasionally show orange feathers where yellow ones are the norm (Mulvihill et al. 1992, Flinn et al. 2007, Hudon et al. 2013). In Cedar Waxwings (*Bombycilla cedrorum*) and Baltimore Orioles (*Icterus galbula*), the aberrant color results from the incorporation of a carotenoid of deep red hue, rhodoxanthin, acquired exogenously (Hudon and Brush 1989, Brush 1990, Mulvihill et al. 1992, Witmer 1996, Hudon et al. 2013). Known sources of rhodoxanthin are few in nature, especially in forms that birds can ingest (see Hudon 1991). One recognized source is the berries of Tatarian (*Lonicera tatarica*) and Morrow’s (*L. morrowii*) honeysuckles and their hybrid, Bell’s honeysuckle (*L. × bella*), bush honeysuckles native to southern Russia, western and central Asia (*L. tatarica*), and Japan (*L. morrowii*), introduced as early as the 1700s and now naturalized and widely distributed in eastern North America and the American Midwest (Edminster 1950, Jackson 1974, Witmer 1996). Although ants and their pupae form the bulk of the diet of Northern Flickers, flickers also ingest fruit in the fall when they are replacing their flight feathers during the prebasic or preformative molts (Beal 1911, Test 1969). However, it is unclear whether honeysuckle berries are available when flickers molt their flight feathers (J. Craves personal observation).

We evaluate here the possibility that rhodoxanthin acquired exogenously, not the 4-keto-carotenoids found in *cafer*, is responsible for red color in flight feathers of Yellow-shafted Flickers in eastern North America. We accomplish this assessment initially by characterizing the carotenoid pigments present in the red feathers of 2 Yellow-shafted Flickers from eastern North America and the spectral properties of the feathers and extracts. Secondly, we document the distribution of red color in an additional 39 flickers with unevenly colored (referred to as “aberrant” from here on) primaries from eastern North America and the American Midwest held in various museums. In particular, we were interested in assessing whether the distribution of red color in these birds is consistent with changes we would expect from the ingestion of an exogenous pigment of deep red hue. This includes looking for an abrupt color change in feathers that were growing when the exogenous pigment was ingested. Test (1969) previously showed that carotenoids added to the diet of Northern Flickers can be deposited in growing feathers almost immediately, peaking within a few



**FIGURE 1.** Coloration of the shafts of the outer primaries of MCLA 16 compared to those of a Red-shafted Flicker from Alberta (RAM Z66.64.13; at the top).

hours. In addition, we expected that if the birds ingested the pigment only once that their flight feathers would become progressively less red as they are replaced until the pigment is cleared from the body. As a corollary, the number of red feathers should broadly correlate with the amount of pigment ingested, as measured from the redness of the reddest feather. Finally, we use data on timing of replacement of primaries in flickers at Manomet, in Plymouth, Massachusetts, to infer approximately when the aberrant flickers deposited the red pigment to narrow down possible sources of the red pigment.

## METHODS

### Material

We characterized the carotenoids in 2 Yellow-shafted Flickers that showed no indication of intermediate characteristics due to hybridization with the Red-shafted Flicker except for the presence of red feathers in the wings and tail. The first bird, collected in West Stockbridge, Berkshire County, Massachusetts, on September 9, 1995 (Massachusetts College of Liberal Arts #16 [hereafter MCLA 16]; Figure 1), was a hatch-year (HY) male still actively growing its last 3 primaries (p8–p10) and the innermost rectrix (r1). Red tones are evident on 6 primaries, p4–p9 (p10 still in pin), identical on both sides, as well as on rectrices r2–r5, with r5 still growing and r1 in pin. P6 was the reddest primary with a shaft matching Munsell chip 7.5R 6/10 (from the Munsell Books of Color, Glossy Collection, Macbeth Division, Kollmorgen Instruments Corporation, New Windsor, New York, USA), similar to Smithe's (1975) Peach Red [color #94], which is actually redder than the shafts of Red-shafted Flickers in collections at the Royal Alberta Museum (RAM; Figure 1), which vary in hue from 10R to 1.25YR (Hudon et al. 2015); p4 had red hints only on its proximal fourth, whereas p5

turned red fairly abruptly about half-way from yellow distally; p7, p8, and p9 were of relatively uniform colors but of progressively less red coloration (Figure 1). On the tail, r2 was the reddest rectrix (shaft 10R 6/10; vane 2.5YR 4/8; compared to Munsell hues of 1.25YR – 2.5YR and 10R – 2.5YR, respectively, for Red-shafted Flickers), and r3 to r5 were of relatively uniform orange color but with progressively less red, r5 being the yellowest.

The second was a second-year (SY) female found dead on April 28, 2008, in Bryn Mawr, Montgomery County, Pennsylvania, and prepared as a study skin and spread wing (Academy of Natural Sciences of Drexel University #192905 [hereafter ANSP 192905]). This bird is similar to MCLA 16, except that it has fewer and slightly different primaries with red tints: p3–p7. P5 was the reddest with a shaft matching Munsell chip 2.5YR 7/8, closest to Smithe's Salmon Color [#106]. On the tail, only r2–r4 had red tints (right r2 missing); the shaft in r3, the reddest, matched Munsell chip 7.5YR 7/8, approaching Smithe's Salmon Color [#6] (vane 7.5YR 5/6); left r4 had only hints of red (largely yellow-orange, almost yellow), while right r4 was entirely Spectrum Yellow [#55], like r1 and r5.

We compared the carotenoids and spectral characteristics of feathers in these birds to those of Red-shafted Flickers and hybrid Yellow-shafted × Red-shafted flickers from Alberta held at the Royal Alberta Museum. We note that the type of color aberration scrutinized here has not yet been documented in Alberta.

Furthermore, we examined the distribution of red color on study skins of additional Yellow-shafted Flickers with aberrant red feathers from eastern Canada (Ontario and Quebec) held at the Canadian Museum of Nature and the Royal Ontario Museum, and digital images of more flickers, primarily spread wings or specimens mounted with a spread wing, held in various museums in eastern North America and the American Midwest (Appendix Table 1). For each aberrant flicker, we recorded the affected flight feathers, and assessed the fraction of each feather with reddish tones. For the study skins from eastern Canada we also determined the coloration of the shaft at the base (where it is widest) of all primaries of aberrant color, under natural daylight, by matching to the closest color chip in the Munsell Book of Colors. Color chips vary in hue increments of 1.25 for colors of high chroma like those in the flicker, spanning 80 pages of color chips. In order to estimate and test for correlations we converted the Munsell Hue notations, ranging from the most yellow (2.5Y) to most red (7.5R), to numeric values. Intervals between hue notations in the Munsell color scheme represent equal amounts of change in hue.

### Spectrophotometry

We acquired reflectance spectra for the shafts of primary feathers of MCLA 16, ANSP 192905, and several Alberta

specimens (RAM Z82.27.118, Z82.33.73, Z83.44.01, and Z03.01.05) at their widest at the base of the feather in situ over the range of 350 to 800 nm using an Ocean Optics USB2000 spectrophotometer (Ocean Optics, Dunedin, Florida, USA) fitted with a R400-7 Y-shaped probe (Ocean Optics) shining light from a Mikropack DH-2000-Bal deuterium tungsten halogen lamp (Ocean Optics). The probe was placed 2.2 mm directly above the shaft of interest using a RPH-1 reflection probe holder (Ocean Optics). The spectrophotometer was operated with the OOIBase32 2.0.1.4 software using the following settings: integration time: 10 msec; spectra averaged: 100; boxcar smoothing: 5. Reflectance was compared to that of 99% Spectralon white standard (Ocean Optics) “light” control, while turning the light source off served as the “dark” control.

### Biochemistry

We sampled vane sections roughly 1 cm<sup>2</sup> in area from both normally and aberrantly colored feathers of the 2 aberrant flickers from the eastern USA, as well as Red-shafted Flickers from western Alberta (RAM specimens Z93.13.08 and Z96.18.06). To rule out environmental contaminants like adventitious coloration or the application of colored material, we washed the material first with a dilute aqueous solution of dishwashing detergent (Sunlight, Phoenix Canada, Toronto, Ontario, Canada) and, once dried, with petroleum spirit prior to extraction. We weighed the vane samples with a Denver Instruments Pinnacle Series PI-225D (Denver Instruments, Arvada, Colorado, USA) precision analytical balance.

We extracted feather carotenoids using either methanol overnight in the dark or warm pyridine over a hot plate following Hudon et al. (2013) in sealed 1-dram borosilicate glass vials flushed with N<sub>2</sub> gas. The extracted carotenoids were transferred into methyl-*tert*-butyl ether (MTBE) with deionized water. We replaced the deionized water twice to remove as much of the methanol (or pyridine) as possible, and transferred the MTBE epiphase with the carotenoids to a clean vial. The ether extract was evaporated over a stream of N<sub>2</sub> gas and the pigments redissolved in a known volume of hexanes (OmniSolv, EM Science, Gibbstown, New Jersey, USA), using anhydrous sodium sulfate powder to remove any trace of water. We extracted rhodoxanthin from the berries of local bushes of *L. tatarica*, and the 4-keto-carotenoids in the Red-shafted Flicker from RAM specimens Z93.13.08 and Z96.18.06.

We acquired absorption spectra of the pigment extracts using the Ocean Optics spectrophotometer fitted with a CUV-FL-DA cuvette holder (Ocean Optics). Pigment concentration was determined in mg carotenoid per gram of feather tissue using the formula ( $A_{\text{peak}} \times \text{volume of extract [mL]} \times 10$ ) / ( $E_{1\text{cm}}^{1\%} \times \text{feather mass (g)}$ ), where  $A_{\text{peak}}$  is the absorption at the extract's maximum, and  $E_{1\text{cm}}^{1\%}$  is the

extinction coefficient of a generic carotenoid in hexane, taken to be 2,500 (Britton 1985). We initially identified the carotenoids in the red sections of flicker feathers on the basis of color, relative mobility ( $R_f$ ) and comparison to known standards after analytical thin-layer chromatography (TLC) on flexible sheets of silica gel (PE SIL G; Whatman Ltd, Maistone, Kent, England) and aluminum oxide (60 neutral; EM Science, Cherry Hill, New Jersey, USA) using mixtures of hexane and acetone (2:1 and 3:1, respectively) (Hudon et al. 2013).

We quantified individual carotenoids by high-performance liquid chromatography (HPLC) using a Waters instrument (Waters, Milford, Massachusetts, USA) equipped with 2 Waters 501 pumps, a 712 WISP autoinjector, a System Interface Module, and a Lambda Max 481 UV detector. We separated the carotenoid pigments isocratically using a Phenomenex Luna normal-phase column (150 mm × 4.6 mm i.d., 3 μM; Phenomenex, Torrance, California, USA) assisted with a hexane:acetone (86:14) mobile phase flowing at 1 mL min<sup>-1</sup> (Panfili et al. 2004, Prum et al. 2012). Carotenoids were detected at 450 nm. We determined the areas under the curve of the different pigments using the Millennium 2010 Chromatography software (v2.15.01; Waters, Milford, Massachusetts, USA). On this system, rhodoxanthin from *L. tatarica* separated into 3 large peaks at 7.8, 9.2, and 10.7 min roughly in 1:2:1 proportions, representing common stereoisomers of the retro-carotenoid (Englert and Vecchi 1982), and a few minor peaks. By comparison the 5 principal yellow carotenoids of the Yellow-shafted Flicker, β-carotene, β-cryptoxanthin, 3'-dehydro-lutein, lutein, and zeaxanthin (Hudon et al. 2015), eluted at 2.0, 5.5, 13.7, 21.2, and 22.6 min, respectively. We confirmed the identification of 3'-dehydro-lutein through reduction of its carbonyl group (C=O) with sodium borohydride in methanol, yielding lutein (Andrewes et al. 1974). The 4 principal red 4-keto-carotenoids of the Red-shafted Flicker, canthaxanthin, adonirubin, astaxanthin, and α-doradexanthin (Hudon et al. 2015), eluted at 4.9, 7.4, 11.9, and 14.8 min, respectively.

### Timing of Deposition of the Red Pigments

We can estimate when a flicker deposited red pigments in its primaries from the knowledge of the timing of flight feather replacement during the prebasic or preformative molt in the Northern Flicker. Test (1945) and Pyle (1997) provide general information on timing of molt in the Northern Flicker, but lack specifics as to individual feathers. Fortunately, the molt of individual flight feathers is routinely recorded by banders at a few migration monitoring stations in North America in the form of molt scores. This involves assigning a score from 0 (an old feather) to 5 (new feather fully grown with no trace of sheath at its base), with intermediate scores for conditions

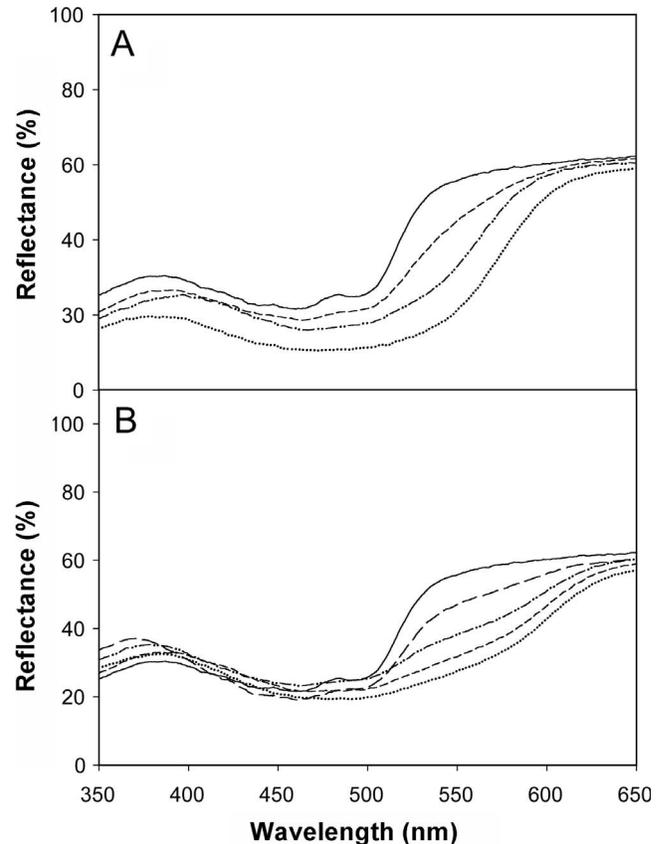
in between, to each flight feather on both wings and the tail (Ginn and Melville 1983). The scores of individual feathers in a tract (primaries, secondaries, and rectrices) are added to yield a molt score for that tract (Ginn and Melville 1983). Flickers typically replace their primaries centrifugally starting with the innermost primary (p1), staggering feather replacement so that at any time a few primaries are at different stages of growth (Test 1945).

We obtained data on primary molt scores of 134 Northern Flickers banded at Manomet in Plymouth, Massachusetts, between 1978 and 2014, including 105 birds actively molting one or more primaries on either wing. For the flickers with aberrantly colored feathers that we solicited from various museums (see above), we calculated the molt score corresponding to the score the birds would have had just before they started depositing red pigments, as if the red sections and subsequent new primaries had not grown yet. For earlier-molted primaries that show a transition from yellow to red we measured the distance from the tip of the feather to the point of transition to red (to the closest mm), as well as the total length of the primary. A molt score was calculated for all fully grown primaries with distal yellow and red basal sections using the formula—molt score =  $3.5 (l_y/l_t) + 1.0$ —where  $l_y$  is the length of the yellow (normally colored) section of a primary with red color, and  $l_t$  is the total length of the feather.

We developed this equation by extrapolating the score various lengths of primaries would elicit in a bander observing it in situ on a molting bird as a continuous variable. An earlier-molting primary with no trace of red got a score of 5. Primaries that were lost after the bird started depositing red pigments got a score of 0, unless they were preceded by a primary with a score of 3 (2.5 as a continuous variable) or more, in which case they received a score of 1 as they probably were missing when the bird started depositing red pigments based on the Manomet banding data. We then added the scores of individual primaries to get a total primary molt score. The molt scores for the 2 wings were highly correlated in specimens that had them ( $r = 0.99$ ,  $n = 18$ ). When information was available for only one wing (most spread wings), we doubled the score for the one wing to get a full primary molt score.

### Statistics

We conducted statistical tests using StatView for Windows 5.0 (SAS Institute 1998), except when we used a one-tailed  $t$ -test to assess the significance of the correlation between the proportion of feathers with evidence of red and the redness of the reddest feather. We used Sigma Plot 9.0 to fit linear (Newton and Rothery 2009) and nonlinear (sigmoid, logistic, and Gompertz) regressions to primary molt score data of flickers at Manomet as a function of day of year,



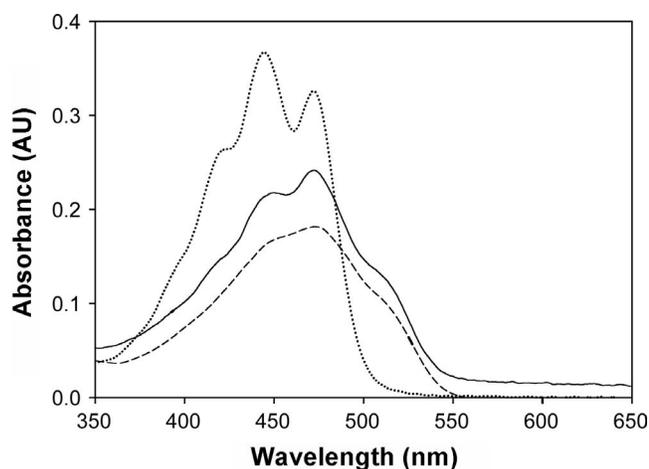
**FIGURE 2.** Reflectance spectra of shafts of primary feathers (A) of *auratus* and *cafer* (Z82.33.73 [solid line] and Z03.01.05 [dotted line], respectively) and 2 hybrids between them (Z82.27.118 and Z83.44.01), (B) 4 different primary feathers of MCLA 16 (non-solid lines), compared to *auratus* individual (Z82.33.73; solid line).

and calculate model-averaged parameter estimates and 95% confidence intervals. In the Results section, we report the values as means  $\pm$  SE, except where indicated otherwise.

## RESULTS

### Spectrophotometry

The shafts and vanes of flight feathers in the Northern Flicker owe their colors mainly to a broad peak of absorption in the blue to green part of the visible spectrum resulting in a dip in reflectance between 400 and 510 nm (Figure 2). The absorption spectra of the feathers we examined differed mainly in the position and steepness of a slope of increasing reflectance above 500 nm (Figure 2). Increased redness in the hybrids and the Red-shafted Flicker resulted mainly from a shift of the slope of increasing reflectance to longer wavelengths (Figure 2A). In contrast, in the aberrantly colored sections of both MCLA 16 and ANSP 192905 (not shown) redness resulted mainly from increased absorption centered  $\sim$ 570 nm



**FIGURE 3.** Absorption spectra in hexanes of carotenoids in feathers of different colors for MCLA 16: aberrant red vane of p6 (solid line), normal yellow vane of p3 (dotted line), and extract of the berries of *L. tatarica* (stippled line).

resulting in a shallowing of the slope of increasing reflectance (Figure 2B).

The carotenoid extract of the red vane sections of both MCLA 16 and ANSP 192905 (not shown) absorbed maximally at 472 nm compared to 445 nm for the yellow sections of the same individuals (Figure 3) and 468 nm for *cafer*. The absorption spectrum of the red sections also had a shoulder at ~520 nm, and approximated the spectrum of rhodoxanthin, for example from *L. tatarica* in hexanes (Figure 3).

### Biochemistry

The red carotenoids in the reddish sections of aberrant Yellow-shafted Flickers matched rhodoxanthin in number, color, and mobility on thin-layer chromatography (TLC). The 3 large peaks of rhodoxanthin in *L. tatarica* were observed on HPLC as well, but were present in different ratios (roughly 17:22:8) in the birds than in the berries. There was an additional peak at 7.3 min, not present in the yellow sections of the 2 aberrant flickers. We suspect that this is a metabolite of the third peak, as it was nearly as abundant as the peak at 10.7 min (third), now reduced by an equivalent amount compared to its abundance in the berries, such that the combined abundance of the peaks at 7.3 and 10.7 min added up broadly to the abundance of the first peak of rhodoxanthin.

Together the 3 main and the minor peaks of rhodoxanthin accounted for 54% and 38% of the absorption at 450 nm of extracts from the reddest sections of MCLA 16 and ANSP 192905, respectively. When including the peak at 7.3 min, this figure increased to 64% and 49% of absorption at 450 nm, respectively.

As a result of the deposition of rhodoxanthin (and the peak at 7.3 min) in the red sections of flicker feathers the

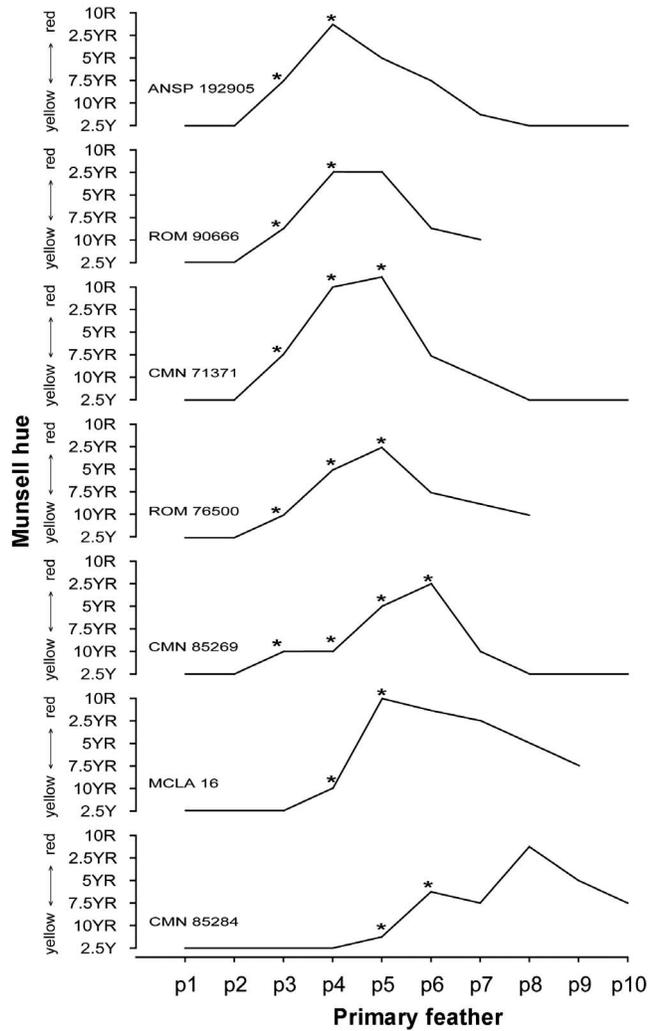
fraction of absorption at 450 nm assignable to the usual, yellow carotenoids in the Yellow-shafted Flicker decreased to 38% (MCLA 16) and 51% (ANSP 192905) from 100%. This decrease was most pronounced for lutein, the main yellow pigment in the Yellow-shafted Flicker, its absorption decreasing from 54% of absorption to 6% in MCLA 16 and from 50% to 17% in ANSP 192905. The decrease was not simply the result of the deposition of an additional pigment (rhodoxanthin) in the red sections, as amounts of lutein in the feathers also decreased in absolute terms, from  $170 \mu\text{g g}^{-1}$  feather to  $11 \mu\text{g g}^{-1}$  in MCLA 16, a 12-fold decrease, and from  $81 \mu\text{g g}^{-1}$  to  $20 \mu\text{g g}^{-1}$  in ANSP 192905 compared to its abundance in the corresponding yellow sections. A decrease by a third of absolute amounts of carotenoids in the red sections compared to the yellow sections can only account for a 3-fold reduction.

Interestingly, the absorption assignable to 3'-dehydro-lutein, a normal yellow carotenoid in the Yellow-shafted Flicker, increased in both proportion and absolute terms in the red sections, from 3% to 10% of absorption ( $9 \mu\text{g g}^{-1}$  to  $20 \mu\text{g g}^{-1}$ ) in MCLA 16 and from 3% to 11% ( $4.4 \mu\text{g g}^{-1}$  to  $12 \mu\text{g g}^{-1}$ ) in ANSP 192905, also in spite of a decrease by a third of total carotenoids in the red sections compared to the yellow sections.

### Timing of Pigment Deposition

**Feathers affected.** We recorded reddish tones on p5 to p7 of approximately three-quarters of the flicker specimens we examined. Flickers also had reddish tones on other primaries, though rarely on p2 and never on p1. The transition from bright yellow (Spectrum Yellow) to reddish tones usually occurred on p3 to p6, rarely on p2, p7, or p8. Redness usually appeared fairly abruptly (over a few mm), rising to a maximum in the second or third primary with an aberrant coloration, usually the first mostly red primary (Figure 4). The redness then usually progressively faded in subsequent primaries until the color returned to the typical bright yellow color (Figure 4). Occasionally, the redness rebounded but then subsided again afterward (for example CMN 85284 on Figure 4) or built slowly (CMN 85269). We observed a general trend for individuals with redder primaries, presumed to have ingested more rhodoxanthin, to show a greater number of feathers with red tones (Pearson's  $r = 0.59$ ,  $P = 0.005$ ,  $n = 18$ ; Figure 5). This is consistent with most birds having had a single "serving" of the pigment.

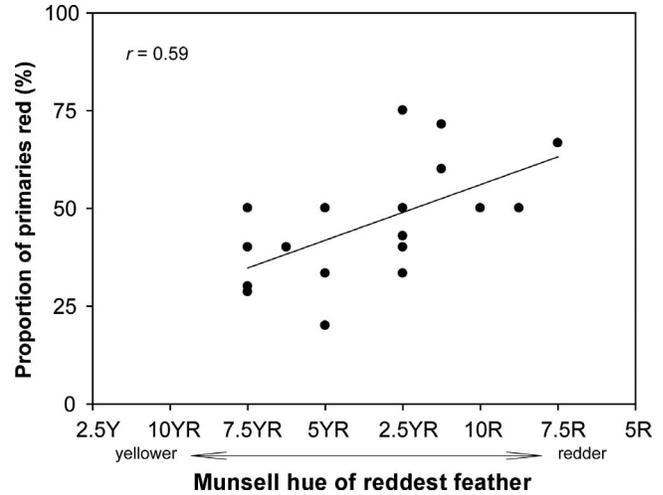
**Molt scores.** Assessed molt scores of aberrant flickers at the onset of deposition of red pigments ranged from 17.1 to 69.5, with a mean of  $44.6 \pm 1.8$  ( $n = 40$ ), corresponding broadly to the replacement of the second, eighth, and fifth primary, respectively. Molt scores averaged slightly higher in flickers from eastern Canada ( $\bar{x} = 47.1 \pm 3.0$ ,  $n = 18$ ) compared to those from the northeastern United States (Massachusetts, New York, Pennsylvania, and Maryland;  $\bar{x}$



**FIGURE 4.** Change in Munsell Hue of primary shafts (at their bases) of flickers from southern Canada and the eastern United States with more than 3 red-tinted primaries. Asterisks identify aberrant primaries that were yellow distally.

= 40.8 ± 3.4, *n* = 13) and the American Midwest (Michigan, Wisconsin, and Illinois;  $\bar{x}$  = 45.2 ± 2.4, *n* = 9), but the differences were not statistically significant (Kruskal–Wallis Test *df* = 2, *H* = 3.507, *P* = 0.17).

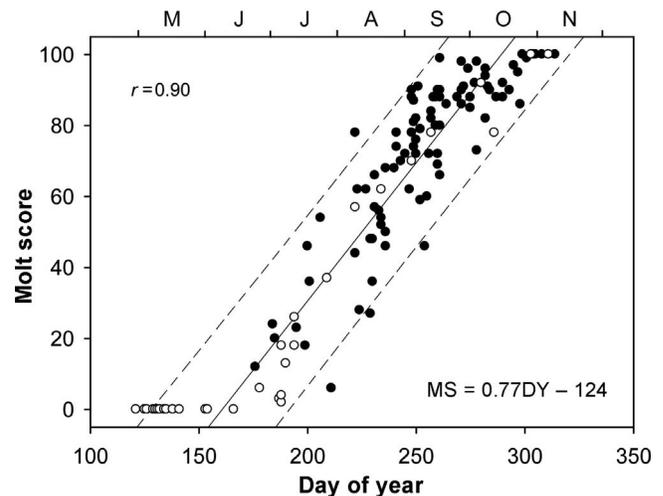
The primary molt score of Northern Flickers banded at Manomet varies as a function of day of year and can be approximated by the following linear regression: molt score = 0.77 × day of the year – 124 (*r* = 0.90; Figure 6). The replacement of primaries occurs slightly earlier, is faster and less variable in AHY/ASY birds (slope = 0.85, *y*<sub>0</sub> = –146; *r* = 0.96, *n* = 15) compared to HY/SY birds (slope = 0.71, *y*<sub>0</sub> = –108; *r* = 0.86, *n* = 87; Figure 6). Because it was not always possible to determine the age of the flickers we examined, we used the regression for birds of all ages to estimate when the birds would have been depositing red pigments. Nonlinear regressions produced even closer fits



**FIGURE 5.** Relationship between the proportion of molted primaries that are of aberrant colors and the redness of the shaft of the reddest primary in flickers from southern Canada and the eastern United States (*n* = 18).

(*r* = 0.96 for a 3-parameter logistic regression, for example), in part because primary score does not increase as fast at the beginning and end of the molt when few primaries are molted (Pimm 1973), but the choice of regression did not result in date projections that differed by more than 2 or 3 days in the middle of the feather molt from those of the linear regression for all birds.

When estimating the date of acquisition of the red pigment, variability in the timing of replacement of



**FIGURE 6.** Relationship between the molt score of Northern Flickers banded at Manomet between 1968 and 2014 and the day of year. ASY individuals are identified by hollow circles. The data points were fitted to a simple linear regression that was used subsequently to estimate when the aberrant flickers would have deposited the red pigment. Bird with finished molts (scores of 0 or 100) were excluded from the fitted model. We also show 95% confidence intervals (stippled lines).

primary feathers in the Northern Flicker created a far greater source of uncertainty than the choice of a particular regression. Indeed, a bird caught on August 10, and expected to have an average molt score of  $\sim 48$ , could be expected to have molt scores between  $\sim 24$  and  $\sim 71$  based on the 95% confidence interval calculated for the birds at Manomet. This is a difference in molt score of 48 (it is smaller for AHY/ASY [42]; greater for HY/SY [49]), corresponding to the replacement of a little over 4 or 5 primaries. We minimize the effect of this uncertainty by focusing on the average date for a large number of birds (41) from a wide range of localities in eastern North America and the American Midwest rather than on the individual dates. This results in dates from July 1 to September 7, and an average date of August 6, ranging from August 1 for the eastern U.S. to August 9 for southern Canada.

Using the linear regression to backdate dates of acquisition of the red pigment for birds that were still molting at the time of collection and had replaced fewer than 5 primaries since growing red feathers yielded dates of July 15 (CUMV uncatalogued), July 28 (ROM 90666), August 2 (CMN 85240), August 4 (UMMZ 155570), August 7 (ROM 72487), August 9 (UMMZ 126556), and August 27 (CMN 85241).

## DISCUSSION

Rhodoxanthin, not the 4-keto-carotenoids typically found in red feathers (Brush 1981, Stradi 1998, McGraw 2006), including those in the flight feathers of the Red-shafted Flicker (Hudon et al. 2015), underlies the red color of the primaries in 2 aberrantly colored Yellow-shafted Flickers from eastern North America. Rhodoxanthin's signature shoulder at  $\sim 570$  nm on a reflectance spectrum (see for example Figure 2B) was also evident on the aberrantly colored primaries of all flicker specimens on loan from southern Canada (data not shown).

The retro-carotenoid of deep red hue is not typically produced by birds from dietary carotenoids, but rather acquired exogenously from the diet (Hudon and Brush 1989, Hudon 1991; but see Hudon et al. 2007). The carotenoid has been documented in at least 2 species of songbirds with aberrant red colors in eastern North America (Hudon and Brush 1989, Hudon et al. 2013), and suspected in several additional species that have turned up with atypical red feathers in eastern North America and the American Midwest (Mulvihill et al. 1992, Brooks 1994, Craves 1999, Flinn et al. 2007, Hudon et al. 2013).

Nearly 12% of flickers banded at Manomet in eastern Massachusetts, and 36% of those considered "resident" (i.e. captured on 2 or more seasons over the years) have been recorded as having red flight feathers (T. L. Lloyd-Evans

personal observation). They were recorded as intergrades with Red-shafted Flicker, though introgression with that form likely explains only a small fraction of the observations, albeit a fraction that is not known at this time.

Based on the timing of acquisition of rhodoxanthin in the aberrant flickers we examined, the pigment would need to be available in early August. Prime candidates as sources of rhodoxanthin are the berries of 2 nonnative bush honeysuckles, *L. morrowii* and *L. tatarica*, and their hybrid *L. × bella*, suggested as responsible for the red coloration in other birds with aberrant plumages (Brush 1990, Mulvihill et al. 1992, Witmer 1996, Hudon et al. 2013). The berries of another widely distributed and naturalized nonnative bush honeysuckle, the Amur honeysuckle (*L. maackii*), do not harbor rhodoxanthin (J. Hudon personal observation). Although by mid-August the berries of *L. morrowii* and *L. tatarica* have largely withered or been stripped from the bush in many states, they are usually available in early August (Mulvihill et al. 1992, Witmer 1996, J. A. Craves personal observation). The berries also persist well into August and September in parts of Canada (Hudon et al. 2013, J. Hudon personal observation), even possibly as late as mid-October locally farther south, for example around Ithaca in central New York (Witmer 1996). Late availability of honeysuckle berries in Canada could account for the fact that the birds from southern Canada had on average higher molt scores when depositing red pigments than those from the U.S., although an earlier, as yet undocumented, molt of flight feathers in Canadian birds could also explain this observation. We note that the molt score at the time of deposition of red pigments of specimens from New York at Cornell University Museum of Vertebrates ( $\bar{x} = 41.7 \pm 14.6$  SD,  $n = 9$ ) were not appreciably different from those in the other states.

Interestingly, honeysuckle berries are already available in eastern North America and the American Midwest by June (Mulvihill et al. 1992, Hudon et al. 2013), but apparently flickers do not ingest these berries when they first become available. This is consistent with the observation that flickers do not usually incorporate significant amounts of plant material, including fruits, in their diets until August (Test 1969).

An alternative source of rhodoxanthin, and the only other source we know of, the red fleshy arils of yew trees (*Taxus* spp.; Kuhn and Brockmann 1933), do not usually become widely available until September (Fordham 1967, Vance and Rudolf 2008, J. A. Craves personal observation). This genus is of interest if only because various ornamental cultivars of the yew came to be widely disseminated in human-modified habitats, in fact became the most popular narrow-leaved evergreen landscape plants of the second half of the 20th century in the northeastern and the upper

midwestern United States (Cochran 1992), and have been known to be eaten occasionally by birds (Fordham 1967, Martell 1974).

A dietary explanation, specifically the deposition of rhodoxanthin acquired exogenously, eliminates the need to evoke the fairly complex, and still largely unproven, controls of production/deposition of carotenoids hypothesized by Ingold and Weise (1985). More importantly, it nicely explains many unusual features of feather reddening in the Yellow-shafted Flicker, including why the birds usually show no other trait of the Red-shafted Flicker—because hybridization with the Red-shafted Flicker is not involved. A dietary explanation also explains why the aberration affects only some of the flight feathers or sections of feathers—the pigment shows up only in feather sections that were grown after the berries were ingested; why the red color might appear fairly abruptly, build up, then slowly decrease afterward—it is initiated with the ingestion of the pigment and fades as the pigment becomes incorporated into feathers and is cleared from the system; and also why birds with the most pronounced redness would have a greater number of red-colored primaries—they ingested larger amounts of rhodoxanthin. The latter correlation held even though some of the flickers were still molting or had run out of primaries to color, such that a complete record of changes in amounts of rhodoxanthin in the body was not always available. The timing of flight feather replacement explains why the color discrepancies would be largely symmetrical—because flight feathers on both wings usually molt at the same time, although disparities may occur (Test 1945); and why the feathers involved may be different from one molt to the next—the feathers affected are a function of when the berries are ingested in relation to the feather molt, which may differ from year to year.

The involvement of rhodoxanthin, not 4-keto-carotenoids, explains how the color in some individuals, like MCLA 16, could be redder in hue than in pure Red-shafted Flickers and hybrids, or more coppery in tone (Test 1969)—because rhodoxanthin absorbs at longer wavelengths than 4-keto-carotenoids and produces a deeper red color than 4-keto-carotenoids. Finally, a source of the pigment in bush honeysuckles would explain why the color variation is common in eastern North America and the American Midwest (Short 1965, Ingold and Weise 1985)—where the bush honeysuckles are now abundant, and how Yellow-shafted Flickers with aberrant red flight feathers could go as far back as 1889 and the early 1900s (Test 1969)—because the bush honeysuckles were first introduced to North America in the mid-1700s and 1800s (1752 for *L. tatarica* and 1875 for *L. morrowii*; Wyman 1949).

Ingold and Weise (1985) discounted a dietary explanation largely on the ground that feathers expected to be

replaced at about the same time based on the molt schedule published by Test (1945) did not always have the same color. But the patterns of reddened feathers in the male they captured on different years actually agreed rather well with Test's (1945) schedule of feather replacement in the Northern Flicker, except perhaps for a reddish r1 in year 1978. Discrepancies such as this one could arise as a result of an out-of-sequence replacement of a flight feather or ingestion of red pigments on multiple occasions.

### Biochemical Considerations

The presence of a novel, yet uncharacterized carotenoid in the feathers harboring rhodoxanthin and the change in proportions of the 3 main stereoisomers of rhodoxanthin in these feathers are consistent with the exogenous pigment being metabolized by the flicker. If confirmed, this would be the first report of the modification of exogenous rhodoxanthin by a bird after ingestion. However, we note that past attempts to separate rhodoxanthin chromatographically by HPLC were done using reverse phase media that yielded complex tracings with many peaks (for example Hudon et al. 2007, 2013), lacking the simple 1:2:1 pattern of stereoisomers generated by normal phases, where changes in proportions could easily be missed.

It is apparent that rhodoxanthin, or a metabolite, interferes with either the production or the deposition of lutein in flicker flight feathers to explain its great reduction in not just relative, but also absolute, terms in the feathers with rhodoxanthin. A clue as to what is happening in these feathers comes from the accumulation in both relative and absolute terms of 3'-dehydro-lutein, a yellow carotenoid normally present in the feathers of yellow color (Hudon et al. 2015). 3'-dehydro-lutein is thought to be an intermediate in the interconversion of  $\epsilon$ - and  $\beta$ -end-rings and the production of lutein from zeaxanthin (Matsuno et al. 1986, Katsuyama and Matsuno 1988, Nagao et al. 2015). It is plausible that rhodoxanthin, or a metabolite, interferes with the reduction of 3'-dehydro-lutein to produce lutein, resulting in an accumulation of the former and a decrease in lutein. We note that rhodoxanthin shares with 3'-dehydro-lutein a conjugated keto group at C-3' and could potentially bind, perhaps competitively, to the enzyme that reduces 3'-dehydro-lutein, preventing its transformation into lutein.

In the future it should be possible to assess the presence of exogenous rhodoxanthin in feathers spectrophotometrically without the need to extract and isolate the pigments, from the shoulder at  $\sim 570$  nm on reflectance spectra of feathers that contain it (see also Hudon et al. 2013). This is in contrast to the situation in the red head feathers of the Western Tanager (*Piranga ludoviciana*), a species where rhodoxanthin occurs naturally, where no such shoulder exists (Hudon 1991).

### Taxonomic Considerations

Our finding that diet largely explains the presence of red flight feathers in eastern flickers also has implications for the systematics of the Northern Flicker. Indeed, it is no longer possible to invoke the presence of red feathers in eastern flickers as evidence of extensive past or present introgression of Red-shafted genes into eastern Yellow-shafted populations, as intimated by Short (1965). Though the scale of hybridization between the 2 forms east of the Rockies constrains the splitting of the forms into distinct species, our findings are consistent with the notion that the 2 forms are genetically more distinct than previously understood, and perhaps approaching species status under the new comprehensive biologic species concept (Johnson et al. 1999).

Our findings also counter the notion expounded by Short (1965) that some populations of Yellow-shafted Flickers might be actively selecting for the red color to explain the prevalence and patchy distribution of aberrantly colored birds in the east. Such selection for red color would have been surprising given that flickers do not appear to discriminate on the basis of the color differences when pairing in the hybrid zone (Moore 1987, Flockhart and Wiebe 2009). Pockets of aberrantly colored flickers can be explained by the patchy distribution of the rhodoxanthin-bearing berries in the east.

### ACKNOWLEDGMENTS

We are grateful to Karen L. Wiebe for bringing MCLA 16 and ANSP 192905 to the attention of the lead author. We thank Lauren E. diBiccari for assistance extracting much of the data from raw molt cards of flickers at Manomet. We thank Michel Gosselin (Canadian Museum of Nature) and Mark Peck (Royal Ontario Museum) for lending museum specimens under their care; Charles M. Dardia (Cornell Museum of Vertebrates), Stephen P. Rogers (Carnegie Museum of Natural History), and Paula M. Holahan (University of Wisconsin Zoological Museum) for providing digital images of spread wings of aberrant flickers in their collections; Janet Hinshaw (University of Michigan Museum of Zoology) and Ben D. Marks (The Field Museum) for providing access to additional specimens; and Amanda D. Rodewald (Cornell University) for supplying the Amur honeysuckle berries.

**Funding statement:** This research was funded by the authors' institutions: Royal Alberta Museum, Academy of Natural Sciences of Drexel University, Manomet, Rouge River Bird Observatory, and Massachusetts College of Liberal Arts.

**Ethics statement:** This study was conducted on museum study skins; no ethics protocol was needed.

**Author contributions:** J.H., R.J.D. and N.H.R. conceived the idea; J.H. developed and designed the methods; J.H., J.A.C. and T.L.L.E. performed the experiments and collected the data; J.H. and D.P.S. analyzed the data; J.H. largely wrote the paper with assistance from J.A.C.; N.H.R., T.L.L.E. and D.P.S. contributed significant materials and resources.

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**APPENDIX TABLE 1.** List of aberrant flickers examined. The specimens with spread wings are identified with W (under Specimen nature), otherwise they were traditional study skins (S). Measurements were derived from digital images (D) or from birds in the hand (H) (under Evidence). Institutions that lent specimens or submitted digital images are MCLA: Massachusetts College of Liberal Arts; ANSP: The Academy of Natural Sciences of Drexel University; CMNH: Carnegie Museum of Natural History; CUMV: Cornell (University) Museum of Vertebrates; FMNH: The Field Museum (of Natural History); UWZM: University of Wisconsin Zoological Museum; UMMZ: University of Michigan Museum of Zoology; ROM: Royal Ontario Museum; CMN: Canadian Museum of Nature.

Institution	Catalog number	Sex	Specimen nature	Evidence	Date	Location
Eastern US						
MCLA	16	M	W	H	Sep 9, 1995	West Stockbridge, Berkshire Co., MA
ANSP	192905	F	W	H	Apr 28, 2008	Bryn Mawr, Montgomery Co., PA
ANSP	195253	-	W	D	Nov 2, 2013	Philadelphia, Philadelphia Co., PA
CMNH	S-9366	F	W	D	Oct 30, 1972	Laurel, Prince Georges Co., MD
CUMV	52453	-	W	D	Jun 2006	Tompkins Co., NY
CUMV	51711	-	W	D	no date	Rochester, Monroe Co., NY
CUMV	51229	M	W	D	no date	no locality
CUMV	50207	M	W	D	no date	Ithaca, Tompkins Co., NY
CUMV	49326	-	W	D	no date	no locality
CUMV	uncataloged	M	W	D	Jul 26, 2014	Ithaca, Tompkins Co., NY
CUMV	43638	F	W	D	Sep 21, 1994	Ithaca, Tompkins Co., NY
CUMV	48476	M	W	D	Jun 27, 1996	Ithaca, Tompkins Co., NY
CUMV	48697	M	W	D	no date	Cortland Co., NY
American Midwest						
FMNH	313492	F	W	H	Apr 2, 1992	Chicago, Cook Co., IL
FMNH	458909	F	W	H	Apr 15, 2007	Roselle, Du Page Co., IL
FMNH	463212	M	W	H	Oct 2, 2008	Chicago, Cook Co., IL
UWZM	20294	F	W	D	Apr 28, 1983	Madison, Dane Co., WI
UMMZ	113328	M	W	H	Sep 1944	Grosse Pointe, Wayne Co., MI
UMMZ	238193	M	W	H	Apr 23, 2003	Big Rapids, Mecosta Co., MI
UMMZ	126556	F	S	H	Sep 23, 1947	Burt Lake, Cheboygan Co., MI
UMMZ	155570	F	S	H	Aug 31, 1960	Midland, Midland Co., MI
CMNH	S-13576	F?	W	D	Jul 2, 1978	"Oakland at Romance", MI
Southern Canada						
ROM	102354	M	W	D	Oct 1, 2005	Simcoe Co., ON
ROM	91603	M	S	H	May 31, 1961	Toronto, ON
ROM	72487	M	S	H	Aug 25, 1945	Toronto, ON
ROM	90597	M	S	H	Jun 26, 1960	Toronto, ON
ROM	90666	M	S	H	Sep 11, 1959	Toronto, ON
ROM	121933	F	S	H	Jun 16, 1974	near Brooklin, Ontario Co., ON
ROM	76500	M	W	H	Sep 11, 1949	Whitby, Ontario Co., ON
ROM	91370	-	W	H	~Jul 1, 1958	Huyck's Point, Prince Edward Co., ON
ROM	75142	-	W	H	Jun 27, 1956	Toronto, ON
CMN	107510	-	W	D	no date	no locality
CMN	85240	F?	S	H	Aug 18, 1983	Shirleys Bay, Ottawa, ON
CMN	63375	M	S	H	May 2, 1975	Kingston, Frontenac Co., ON
CMN	93905	M	S	H	Aug 26, 1988	Lac de l'Argile, Papineau Co., QC
CMN	85241	M?	S	H	Sep 7, 1983	Bells Corners, Ottawa, ON
CMN	71371	M	S	H	May 20, 1981	Black Rapids, Ottawa, ON
CMN	85250	F	S	H	Sept 1983	Ottawa, ON
CMN	85269	M	S	H	Apr 26, 1985	Ottawa, ON
CMN	85284	M	S	H	Jul 7, 1986	Kingston, Frontenac Co., ON
CMN	93906	M	S	H	"May 29, 1988"	Hull, QC