

chemotaxis proteins, and translocase proteins. The network contains previously unknown interactions that suggest mechanisms connecting bacterial pathogenesis and chemotaxis. For instance, CheZ, a chemotaxis dephosphorylase that regulates cell motility, is linked to the surface receptor and virulence factors adhesin AidA and Flp pilus-associated FimT.

The new higher order protein associations detected by LAPP provide a framework for understanding the complex logical dependencies that relate proteins to one another in the cell. They may also be useful in modeling and engineering biological systems, generating biological hypotheses for experimentation, and investigating additional protein properties. It is likely that the logic relationships between proteins in the cell extend beyond ternary relationships to include much larger sets of proteins. We anticipate that the ideas underlying the logical analysis of phylogenetic profiles can be extended to the investigation of other kinds of genomic data, such as gene expression, nucleotide polymorphism, and phenotype data.

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Materials and Methods
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Reproductive Effort, Molting Latitude, and Feather Color in a Migratory Songbird

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Toward the end of the breeding season, migratory songbirds face crucial tradeoffs between the timing of reproduction, molt, and migration. Using stable hydrogen isotopes, we show that male American redstarts investing in high levels of reproduction late in the season adopt a unique strategy of combining molt and migration. Tail feathers molted during migration also reflect less orange-red light, indicating reduced carotenoid concentration. Thus, we show how reproduction in a migratory animal can influence both life history strategies (location of molt) and social signals (feather color) during subsequent periods of the annual cycle.

Each year, toward the end of the temperate breeding season, billions of songbirds face crucial energetic tradeoffs between the costs of reproduction, the replacement of feathers (molt), and the hazards of long-distance migration to the tropics (1). To date, our inability to track individual birds moving between their breeding and wintering grounds has made studying the interaction between these events virtually impossible. Using stable hydrogen isotopes and reflectance spectrometry, we investigate how reproduction affects both molting latitude and the color of molted feathers in an 8-g neotropical-nearctic migratory

songbird, the American redstart (*Setophaga ruticilla*).

Redstarts (Fig. 1A) are socially monogamous, single-brooded passerine birds that provide biparental care to young for 2 to 3 weeks after the young leave the nest (2). Individuals breed in the deciduous forests of temperate North America and winter in the Caribbean and Middle America. From 2001 to 2004, we sampled tail feathers from individually marked males at a breeding site in Ontario, Canada (44°34' N, 76°19' W). These males were known to have bred at the same location the previous year (3). In eastern North America, stable hydrogen isotope (δD) values in precipitation follow a strong latitudinal gradient where low (more negative) values correspond to higher latitudes (Fig. 1B) (4). δD signatures in precipitation are transferred through food webs to higher-order consumers, including birds (5). Because feathers are metabolically inert after growth, δD values sampled from feathers in a given breeding season indicate the molting latitude from the previous autumn.

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Fig. 1. (A) Adult male American redstart after complete autumn molt. [Photograph by Robert Royce] (B) Distribution of post-breeding molt locations determined from δD values of tail feathers ($n = 30$). Contour lines indicate expected δD values throughout eastern North America (4). The eastern portion of the breeding range is shaded light gray (2). The size of the circles represents the frequency distribution of molt locations: large (near breeding grounds), $n = 18$ individuals; medium, $n = 9$; small, $n = 1$. The arrow shows the most likely fall migration route based on band-recapture data (16).

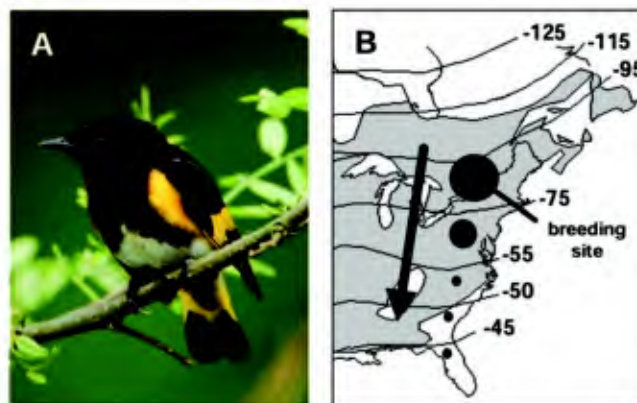
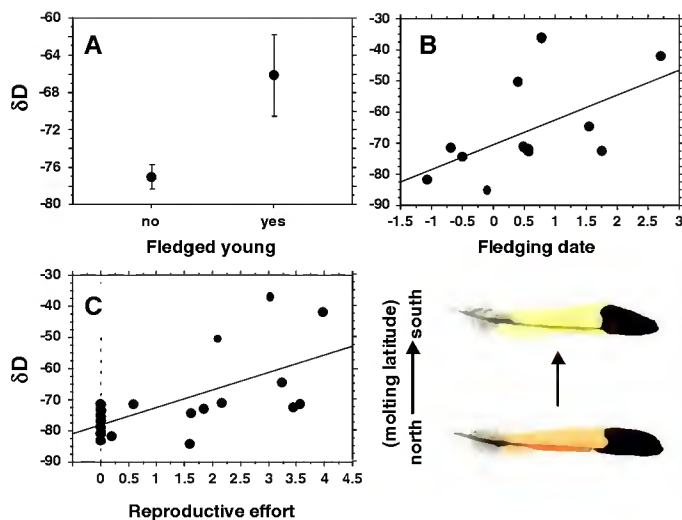


Fig. 2. Relation between molting latitude (δD) and reproduction of male American redstarts during the preceding breeding season. (A) Males that fledged young ($n = 12$) had lower mean ($\pm SE$) δD values than males that did not fledge young ($n = 9$, $t = -2.09$, $P = 0.05$). (B) Males that fledged young later in the season (fledging date standardized by year) tended to molt farther south, on average, than males that fledged young early in the season ($r^2 = 0.32$, $P = 0.05$, $n = 12$). (C) Males with high reproductive effort scores were more likely to molt south of the breeding grounds than males with low scores ($r^2 = 0.41$, $P = 0.002$, $n = 21$). Reproductive effort scores are a combination of the number of young fledged and fledging date (zeros = males that did not fledge young). (Lower right) Example of the color of a tail feather from a male that molted on the breeding grounds (bottom) versus a male that molted south of the breeding grounds (top).



Our analysis shows that 40% (12 out of 30) of males molted on staging areas up to 2000 km south of their breeding grounds [δD values for these birds were more positive than expected from the breeding ground signatures (Fig. 1B) (6)]. We then tested whether molting latitude was related to the amount and timing of parental care that adult males provided during the preceding breeding season. Males that successfully fledged young tended to molt tail feathers farther south than males that failed to fledge young (Fig. 2A). Among males that fledged young successfully, individuals that raised young late in the season tended to molt farther south than males that raised young early in the season (Fig. 2B). To look at these factors simultaneously, we developed an index of reproductive effort that took into account both the timing and the amount of parental care (7). Males with the highest reproductive effort scores were most likely to molt during migration, whereas males with scores of zero molted on the breeding grounds (Fig. 2C).

Using reflectance spectrometry (3), we also examined the influence of molt-migration on the signal quality of a male's feathers. Red chroma, a measure of the relative amount of light reflected in the red (575 to 700 nm) segment of the bird-visible spectrum (320 to 700 nm), was negatively correlated with δD ($r^2 = 0.31$, $P = 0.004$, $n = 24$). Thus, feathers molted farther south were less saturated with orange-red coloration than feathers molted on the breeding grounds (Fig. 2C), indicating that they also had lower carotenoid concentration (8).

Our findings show the importance of understanding how interactions among events that occur during different periods of the annual cycle can shape the ecology, behavior, and life history of migratory animals. Within a relatively short time period, migratory birds face tradeoffs between three of the most energetically demanding activities of the year. A short-lived migratory songbird providing parental care late in the breeding season is more likely to replace its feathers on staging areas during migration than on the breeding grounds after reproduction. Molt during migration may subsequently reduce survival during the following winter by delaying arrival in the tropics and limiting access to high-quality habitat. Winter habitat quality is known to influence physical condition and departure for spring migration, as well as the timing of arrival and reproduction on the temperate breeding grounds (9, 10).

Molt-migration can also influence feather color, a trait important for sexual selection during the following breeding season. Carotenoids in feathers are naturally occurring pigments that reflect immunocompetence (11), signal male quality, and affect female mate choice in many bird species (12). Physiological stress during molt can reduce carotenoid deposition in feathers (13), suggesting a mechanism by which redstarts that overlap molt and migration produce these poorer quality sexual signals.

Finally, studies using stable isotopes to measure both the geographic connectivity of populations between tropical and temperate regions (14) and breeding dis-

persal (15) assume that feathers provide an accurate chemical signature of the past year's breeding location. Our results suggest that this technique may miscalculate the numbers and locations of birds breeding at north-temperate latitudes and that future studies should consider molt ecology when using stable isotopes to track birds year-round.

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6. δD values from the tip and base of feathers were highly correlated ($r^2 = 0.66$, $P < 0.0001$, $n = 18$) with the slope ($1.2 \pm 0.2 SE$) and intercept ($14.6 \pm 11.7 SE$) of the geometric mean regression not significantly different from 1 and 0, respectively (t -test slope: $t = 1.03$, $P > 0.10$; intercept: $t = 1.24$, $P > 0.10$; $df = 32$), suggesting that retrices of each individual were generally molted at a single location. We present results from δD values of the feather base only. δD values were similar between years (analysis of variance: $F_{2,27} = 0.95$, $P = 0.40$), so data collected from 2002–2004 were pooled.
7. To calculate reproductive effort, we added the z scores (standardized by year) of fledging date (timing of breeding) and the number of young raised, then added 1.0 to each score to remove negative values, allowing individuals that did not fledge young to be assigned a score of zero. Thus, the highest scores for reproductive effort represented individuals that fledged many young late in the breeding season.
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