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Hormonal, Behavioral, and Life-History Traits Exhibit Correlated Shifts in Relation to Population Establishment in a Novel Environment

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ABSTRACT: Climate change, habitat alteration, range expansions, and biological invasions are all predicted to require rapid shifts in multiple traits including behavior and life history, both for initial population establishment and subsequent adaptation. Hormonal mechanisms likely play a key role in facilitating or constraining plastic and genetic responses for suites of traits, but few studies have evaluated their role in shaping contemporary adaptation or diversification. We examined multiple phenotypic adjustments and associated hormonal changes following a recent (early 1980s) colonization event, in which a temperate-breeding songbird, the dark-eyed junco (*Junco hyemalis*), became established in the Mediterranean climate of San Diego, California. The milder climate has led to an extended breeding season and year-round residency, and we document shifts in multiple sexually selected behaviors and plumage traits. Testosterone titers in San Diego were elevated for longer but with a lower peak value compared to a nearby native-range population, and correlations between testosterone and related traits were similar within and among populations. A common garden study indicated that changes in testosterone likely represent plastic responses to the less seasonal environment of the city, providing the context against which subsequent genetic changes in morphology likely occurred. We argue that correlated shifts in multiple traits, organized by underlying physiology, may be a generally important element of many successful adjustments to changing environments.

Keywords: adaptation, testosterone, social behavior, urbanization, climate change, *Junco*, plasticity.

Introduction

In an age of accelerated environmental change (Crutzen 2002), understanding mechanisms that facilitate or con-

strain the successful adaptation of organisms and populations has become a major objective in biology (Hoffmann and Sgro 2011; Hansen et al. 2012). When new environments differ strongly from those previously encountered, shifts in optimal life-history and behavioral strategies are predicted to require rapid coordinated divergence for multiple traits (Sih et al. 2011; Tuomainen and Candolin 2011). Striking changes in life history and behavior—whether plastic, genetic, or both—have been documented across taxa in association with changing climate (Møller et al. 2010; Knudsen et al. 2011), habitat alteration (Shochat et al. 2006), biological invasions (Davis 2009), and range expansions (Martin and Fitzgerald 2005; Phillips et al. 2010), yet few studies have considered the proximate organismal mechanisms that underlie contemporary phenotypic divergence for suites of traits (Partecke et al. 2005, 2006; Denver et al. 2009; Kitano et al. 2010; Liebl and Martin 2012).

Endocrine mechanisms are known to play a central role in mediating life-history trade-offs and trait correlations (Finch and Rose 1995; Ketterson and Nolan 1999; Adkins-Regan 2005), and they modulate both plastic and genetic responses to novel selective landscapes (Martins et al. 2007; van Oers et al. 2011; Atwell et al. 2012). As a mechanistic link between the genetic and environmental factors that determine the phenotypic potential of individuals and populations facing environmental change, hormonal systems are predicted to play a key role in facilitating or constraining adaptive processes (Sinervo and Svensson 1998; Ketterson and Nolan 1999; Hau 2007; Adkins-Regan 2008). Importantly, hormone-mediated phenotypic changes could proceed via phenotypic plasticity, genetic evolution, or both, for example, wherein initial plastic responses may facilitate estab-

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lishment or persistence, with subsequent genetic changes following via accommodation or assimilation (West-Eberhard 2003; Price 2006; Crispo 2007).

One classic life-history trade-off involves behavioral allocation to mating effort versus parental effort. Within and among animal populations, some males invest more in mating effort and others in raising offspring (Magrath and Komdeur 2003). In birds, such differences covary with behavioral and morphological characters, including social ornaments, extra-pair mating behavior, and nest attendance (Magrath and Komdeur 2003). Experimental and correlational studies, including those in our study species, the dark-eyed junco (*Junco hyemalis*), indicate that testosterone modulates the trade-off between mating and parental effort within animal populations (fig. 1), with high testosterone levels corresponding to increased investment in mate attraction (e.g., aggressive behavior and sexual traits) and reduced parental effort (e.g., feeding and defense of offspring; Ketterson and Nolan 1999; Ketterson et al. 2001, 2009; Adkins-Regan 2005). Testosterone also mediates trade-offs between investment in reproduction and in viability (Sinervo et al. 2000; Ketterson et al. 2001; Adkins-Regan 2005).

In addition to mediating trade-offs within species, testosterone profiles differ between species and between geographically distant populations in ways that are associated with life-history and behavioral differences (Goymann et al. 2004, 2006; Garamszegi et al. 2008; Hau et al. 2010; Horton et al. 2010). This suggests that altered expression of testosterone (and other hormones) might play a key role in regulating rapid shifts in behavior and life history in response to environmental change (Denver et al. 2009; Ketterson et al. 2009). The key proposition we assess in this study is, if new or altered environments generate conditions that alter testosterone profiles, then hormonal changes may result in correlated shifts in multiple traits, potentially providing a better match to the novel conditions (Agrawal and Stinchcombe 2009). In this way, variation along major axes of life-history variation (i.e., parental care vs. mate attraction or reproduction vs. survival) can be facilitated by rapid modulations in hormonal profiles. Alternatively, hormone-phenotype correlations could constrain adaptive processes for suites of traits or previously identified hormone-phenotype correlations could vary independently within or among recently diverged populations (Ketterson et al. 2009). By investigating divergence in hormonal profiles and shifts in related behavioral traits within and among two recently diverged populations, our study aims to evaluate the degree to which a hormonally mediated suite of characters has diverged in relatively integrated versus independent ways following colonization of a novel environment.

In this study, we evaluated multiple phenotypic adjust-

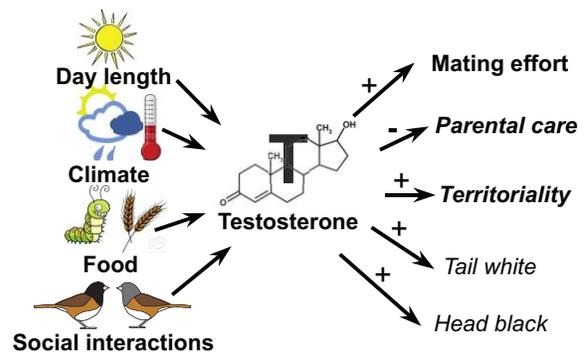


Figure 1: Environmentally sensitive hormones can have pleiotropic effects on suites of traits, including positive (plus signs) and negative (minus sign) modulation of social behavior and plumage, as documented in prior research in songbirds and other vertebrates, including prior experimental (boldface) and correlational (italics) results from our study species, dark-eyed juncos, as reviewed in Ketterson et al. (2009). Such hormonal signals may shape multitrait responses when populations encounter novel or changing environments.

ments and hormonal changes in testosterone associated with a recent colonization event where dark-eyed juncos (typically breeding in montane forests) established a breeding population in San Diego, California—a novel, climatically mild, urban environment. An extended breeding phenology and the sedentary life history of the recently established San Diego junco population (Yeh and Price 2004) predicts a shift in emphasis away from mating effort toward parental care (Magrath and Komdeur 2003; Reale et al. 2010). To test this prediction, we compared the mating and parental behaviors and two socially selected plumage ornaments of the males of the colonist population to the males of an ancestral range population. In order to ask how changes in testosterone may be connected to phenotypic divergence, we characterized seasonal profiles and peak levels of systemic plasma testosterone immediately upon capture and after physiologically induced enhancement (through injection of gonadotropin-releasing hormone [GnRH] or GnRH challenge; see “Methods”). We compared testosterone profiles between the colonizing and ancestral range populations to ask whether between-population shifts in life history and behavior were predicted by changes in testosterone, as would be predicted based on within-population patterns of covariation between testosterone, behavior, and life history. For example, if higher testosterone individuals are less parental within a population, would a population that exhibited less parental behavior also have lower testosterone on average? In a subsequent common garden study, we asked whether the hormonal and plumage differences observed in the field represented plastic versus genetic or early developmental differences. We interpret our findings in the context of the

inferred genetic evolution of socially selected plumage traits documented in our study system and in light of the potential role of phenotypic plasticity in facilitating population divergence.

Methods

Study System

The dark-eyed junco, *Junco hyemalis*, is a small (~17 g) passerine bird that breeds in temperate forests throughout much of North America, including the mountains of Southern California (*J. hyemalis thurberi* race; Miller 1941; Nolan et al. 2002; Unitt 2005; fig. A1; figs. A1, A2 are available in the appendix). By the best account, a small population of juncos became established in coastal suburban San Diego in the early 1980s, likely originating from the native range in the nearby mountains (~60 km east), as a result of overwintering birds remaining to breed (Rasner et al. 2004; Yeh 2004; fig. A1). Since then, an isolated breeding population of ~70 pairs has persisted to the present, existing as a biogeographic island on the campus of the University of California–San Diego (UCSD), with limited subsequent immigration (Yeh and Price 2004). Both molecular and quantitative genetic data suggest that initial populations were sufficiently large and phenotypic distances great enough that founder effects are generally unlikely to explain phenotypic divergence in this system (Rasner et al. 2004; Yeh 2004; Atwell et al. 2012; Whittaker et al. 2012). The colonization event thus provides a unique opportunity to examine mechanisms associated with contemporary adaptation to a novel environment.

The Mediterranean climate and suburban habitat of coastal San Diego and the UCSD campus differ strikingly from the climate and habitat experienced by native montane forest-breeding populations (fig. A1; Yeh and Price 2004). Likely in response to the milder climate and available food and water resources, females in San Diego lay up to 4 clutches per year, compared with 1–2 clutches in the native range. Further, the San Diego population is resident (sedentary), remaining on or near their territories year-round, in contrast to the altitudinal migrations and winter flocking observed among native-range juncos (Yeh and Price 2004; Unitt 2005). Demographic studies indicate that the extension of the breeding season has been essential to population persistence (Yeh and Price 2004).

General Field Methods

We studied Southern California junco (*J. h. thurberi*) populations from San Diego (colonist; elevation 30 m; lat. 32°52'N, long. 117°10'W) and Mount Laguna (native range; elevation 1,700 m; lat. 32°52'N, long. 116°25'W).

The populations first came under study in 1998 (Yeh 2004; Yeh and Price 2004); here, we consider prebreeding and breeding activities from February through July in two consecutive years, 2006 and 2007, and a common garden study conducted during 2007–2009. Most birds in our study populations (>85%) were individually marked with color bands. Each year, we mapped territories and monitored nesting (San Diego: $n \approx 50$ pairs; Mount Laguna: $n \approx 30$ pairs) and collected blood samples (<100 μ L) from adults and nestlings for microsatellite analyses of paternity and hormone assays. Additional details and maps of the study populations can be found in figure A1 (or see Yeh 2004; Yeh and Price 2004). We detected no differences in nest density, which should be proportional to territory size, between the two populations, as estimated by comparing nearest neighboring nest distances for a random subset of 15 nests per population (San Diego: 75.8 ± 8.5 m; Mount Laguna: 88.7 ± 11.5 m; $t_{28} = 0.91$, $P = .37$).

Plumage Ornamentation

Tail White. We quantified the tail white plumage of each captured male using the same method described in prior research of this system (Yeh 2004). Briefly, we photographed each tail feather and then used Scion Image software (www.scioncorp.com) to quantify the percentage of white on each feather and, finally, the tail as a whole (sum). The feather plumage trait referred to as tail white is known to be socially selected in juncos, with more white improving mating success and conferring high dominance status (Ralph et al. 1979; Hill et al. 1999; fig. A2). Tail white also varies with age in juncos, as first-year males have less white than older males (Nolan et al. 2002; Yeh 2004), a difference we controlled for across both populations in subsequent analyses (generalized linear model [GLM], age: $F_{1,187} = 3.63$, $P = .03$).

Head Black. Distinctive plumage traits of the Oregon subspecies of dark-eyed juncos include a dark hood, which is black in males and gray in females, as well as a brown patch of feathers on the upper back (Miller 1941; Nolan et al. 2002; fig. A2). The hood color darkens with age (e.g., older males have darker and more homogenous black plumage) and is considered a socially selected trait: experimentally darkening the hood is enough to reverse previously established dominance relationships, with darkened birds becoming more dominant (Holberton et al. 1989). We developed a measure to characterize natural variation in the amount of black plumage on the head feathers of males. In some birds, the entire hood is completely black (i.e., 100% homogenous); whereas in others, only the most proximal portion of the top of the head is homogeneously dark ($\approx 0\%$ homogenous), with brown

feathers extending variable distances from the back plumage across the head toward the culmen. Using calipers, we first measured the length of the entire hood from the culmen (i.e., the proximal dorsal terminus of the bill) to the dorsal base of the hood along the midline of the head to the nearest millimeter. We then measured the length of the homogenous black feathers along the same line. Each male's "head black" score was calculated as the proportional length of homogenous black head feathers relative to the total hood length. As with the tail white trait, we found age differences in the head black score in both populations, with first-year birds having less homogeneous black compared to older birds (GLM, age: $F_{1,187} = 19.87$, $P < .001$), and we controlled for this effect in subsequent analyses of head black.

Parental Behavior

During the first year of our field study (2006), a single observer (J. W. Atwell) collected parental behavior data during 1-h focal watches in the field. During the second year of the study (2007), an observer scored 4-h video recordings. The observer of the video recordings was blind to each nest's population. Parental behavior data were collected between the hours of 0700 and 1300, when nestlings were 3–6 days old (San Diego: April 1–June 19; Mount Laguna: May 19–June 28). Brood size did not differ between populations (2006: $t_{18} = 1.6$, $P = .1$; 2007: $t_{38} = 0.2$, $P = .8$), and thus we report only on feeding rates as visits/hour in the results (visits/hour and visits/nestling/hour were highly correlated; all $r > 0.67$, $P < .001$). We compared mean feeding rates between populations using separate GLM regressions for each year. We controlled for the effects of nestling age, male age, brood size, and date (linear) in our analyses of parental care (table A2; tables A1–A6 are available in the appendix).

Extra-Pair Behavior

We determined rates of extra-pair paternity by genotyping adults and nestlings at seven assumed neutral microsatellite markers, using methods similar to those reported elsewhere (Price et al. 2008). Nestlings were designated as either within-pair or extra-pair offspring. We extracted total genomic DNA from blood using phenol-chloroform protocols. We amplified the following loci using fluorescently labeled primers (Operon, Ebersberg, Germany; Applied Biosystems [ABI], Foster City, CA) in multiplexed polymerase chain reactions using Qiagen (Venlo, Netherlands) multiplex kits and manufacturer-supplied protocols in 10- μ L reactions: GF01b; Dpu01 and Dpu16; and four primer sets designed for dark-eyed juncos, JH_Ju05, JH_A03, JH_MM4.1, and JH_MM4.2. The resulting prod-

uct was then diluted (1 : 20) and mixed with a molecular size standard (GeneScan-500 LIZ; ABI), and fragment size was measured with the ABI 3730 DNA Analyzer and the GeneMapper 4.0 software. Each individual was genotyped a minimum of two times to confirm allele size and whether an individual was heterozygote or homozygote. If more than two loci failed to amplify, the individual was excluded from analysis.

We successfully assigned paternity using the software Cervus 3.0 (Field Genetics, London) to a total of 353 nestlings from 129 broods (2006: Mount Laguna = 42 nestlings in 14 broods, San Diego = 88 nestlings in 33 broods; 2007: Mount Laguna = 88 nestlings in 26 broods, San Diego = 141 nestlings in 56 broods). In San Diego, several pairs had multiple broods, and occasionally mates changed between broods, so the 32 broods in 2006 were produced by 25 males and 24 females, while the 56 broods in 2007 were produced by 38 males and 38 females. Mother-offspring pairs with one or zero mismatches were retained in the analysis. In the simulation used to generate the probability distribution for evaluating candidate fathers, we used the sampled number of candidate fathers, a 0.90 proportion of candidates sampled, and an error rate of 0.05.

Within each population, all genotyped males were evaluated as potential sires of all nestlings, assuming known mothers. We were able to assign sires (both within-pair and extra-pair) to 91%–95% of offspring in San Diego (including 23 of 31 extra-pair young [EPY] in 2006 and 21 of 29 EPY in 2007) and 80%–85% of offspring in Mount Laguna (8 of 15 EPY in 2006, 15 of 30 EPY in 2007). The difference between the sites is likely due to more complete sampling of the population in San Diego. We compared the proportion of within-pair and extra-pair young between populations for each year separately and with years pooled using χ^2 analysis.

Territorial Aggression

In this study, we quantified male territorial aggression within each population for a subset of individual males ($n = 27$ per population) during the early breeding season, in order to evaluate covariation of this behavior with testosterone. These trials were conducted within each population during a 38-day period corresponding roughly to 2 weeks before and 2 weeks after the mean first egg dates in 2007 (Mount Laguna: April 11–May 18; San Diego: March 11–April 17), when territorial activity and the seasonal peak of testosterone are predicted to be highest in this species (Ketterson and Nolan 1999; Ketterson et al. 2001).

We used a 15-min simulated territorial intrusion (STI) to measure territorial aggression, following previously es-

established methods for juncos (McGlothlin et al. 2007). The STI protocol included a lure bird in a small cage, a small speaker, and a song playback introduced near the center of the focal male's territory. We used two lure birds and six playback tapes per population, which were randomly assigned to each trial. We erected mist nets (which were left closed during the behavioral trial) above the lure bird and speaker. We recorded the following three behavioral variables during each 15-min trial: (1) time spent within 5 m of the lure, (2) number of flyovers, and (3) number of long-range songs (trills) sung. Following the trial, the mist net was opened, and each male was captured; blood samples were then collected in order to assess testosterone levels.

We used principal component analysis to reduce correlated behavioral variables into a single score for each male (table A1). We calculated separate principal components within each population, as our focus here was on measuring within-population covariation between testosterone and aggression, and each population showed different patterns of trait loadings (table A1), perhaps due to differential timidity or shyness in response to the experimental equipment (cage, net, speaker, etc.), as was documented in prior studies (Atwell et al. 2012). Population comparisons of male territorial aggression presented in figure 2C are from a previously published song playback study in this system that did not include a cage or net (Newman et al. 2006).

Sampling for Testosterone

We collected blood samples across the breeding season in both populations during 2006 (Mount Laguna: March 4–July 12; San Diego: February 3–July 15) and 2007 (Mount Laguna: March 7–July 10; San Diego: February 6–July 2), in order to measure circulating plasma testosterone concentrations. In order to capture males, we used both passive mist nets and walk-in traps baited with seed, as well as STIs (lure bird + song playbacks; see above). To evaluate seasonal profiles of testosterone, several males were sampled multiple times across the season, with a minimum of 2 weeks between samples (Mount Laguna: $n = 88$ males, 212 captures; San Diego: $n = 95$ males, 296 captures). For analyses of covariation between testosterone and plumage traits (see below), these repeated measures were controlled for as random effects of individuals in a generalized linear mixed model (GLMM). A subset of blood samples collected following the standardized STI protocol described above ($n = 27$ males/population; Mount Laguna: April 11–May 18; San Diego: March 11–April 17) were used to compare peak testosterone levels and to analyze covariation between testosterone and social behaviors including parental care and aggression, and this

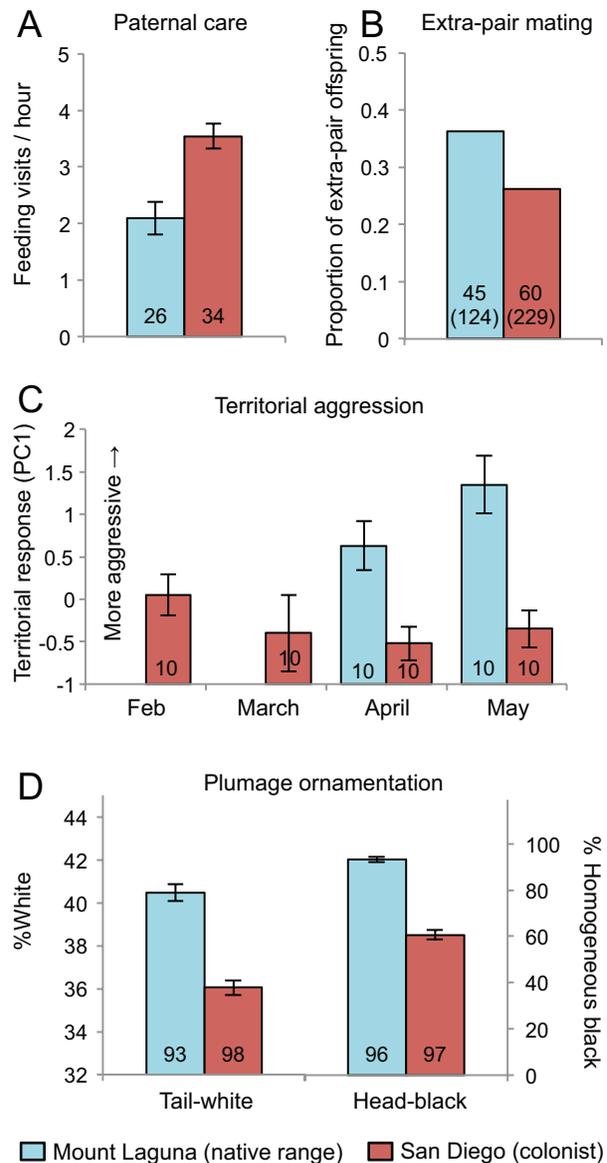


Figure 2: Changes in social behaviors and plumage ornamentation in San Diego (colonist; red bars) versus Mount Laguna (native range; blue bars) junco populations. San Diego birds exhibited increased frequency of male visits to feed nestlings (A); reduced extra-pair paternity (B); reduced territorial aggression in response to territorial song playbacks (C; from Newman et al. 2006); and reduced elaboration of two socially selected plumage ornaments, tail white and head black (D). Sample sizes are indicated inside the base of each bar. Error bars represent mean \pm SE.

smaller data set contained no repeated sampling of individual males.

For each male, we sampled plasma soon after capture to measure initial testosterone levels, as well as 30 min after a GnRH-challenge injection, in order to assess each

individual male's ability to produce short-term increases in testosterone (post-GnRH testosterone) in response to a standardized physiological stimulus (pectoral injection of 1.25 μg of chicken gonadotropin-releasing hormone [GnRH-I American Peptide Stock 54-8-23] in 50 μL of 0.1 M phosphate-buffered saline). Details of this method are discussed elsewhere (Jawor et al. 2006; McGlothlin et al. 2007, 2008), but in brief, post-GnRH testosterone provides a repeatable measure of testosterone that varies seasonally (Jawor et al. 2006) and has been found to correlate with territorial aggression and parental behavior (McGlothlin et al. 2007), as well as with socially selected tail white plumage in juncos (McGlothlin et al. 2008). These short-term increases in testosterone in response to a GnRH challenge were also found to correlate with males' transient testosterone increases in response to an STI social challenge in juncos (McGlothlin et al. 2008), which likely represent an adaptation to mediate territorial and aggressive behaviors, while avoiding the costs of chronically elevated testosterone (Wingfield et al. 1990; but see Rosvall et al. 2012). Enzyme immunoassays (ADI-901-065, Assay Designs, Farmingdale, NY) were used to determine plasma concentrations of testosterone, and all samples were randomized across 96-well plates and assayed in duplicate, with a nine-point standard curve, and three standards included alongside unknowns on each plate to calculate and control for assay variation ($n = 48$ plates; intraplate variation = $9.35\% \pm 1.8\%$ [mean \pm SE]; interplate variation = 20.5%). We multiplied plasma values from each plate by a correction factor (intraplate standards mean/grand standards mean) to correct for interplate variation, following previously established methods (McGlothlin et al. 2007).

Analyses of Testosterone

In analyses of testosterone, hormone concentrations were natural log transformed to improve normality, and all initial models considered the linear effects of handling time (elapsed time between capture and initial blood sampling; all samples across season: mean = 14 min, range = 3–47 min; peak breeding subset post-STI samples: mean = 8 min, range 2–13 min), body mass, and date (Julian; linear or linear + quadratic), and male age (second year or after second year), as these variables (handling time, body mass, date, and age) may influence testosterone expression (Jawor et al. 2006; McGlothlin et al. 2007, 2008). Non-significant factors and covariates were removed from the models if doing so improved model fit, as estimated using AIC criteria.

Seasonal Profiles and Peak Testosterone between Populations

We used a locally weighted polynomial regression (20% fit) method to visualize differences in seasonal testosterone profiles between both Mount Laguna and San Diego populations across the breeding season. Data from 2006 and 2007 were pooled in these summaries, as seasonal profiles between years were qualitatively similar within each population.

We compared peak testosterone between populations during a 38-day period corresponding roughly to 2 weeks before and 2 weeks after the mean first egg dates in 2007 (Mount Laguna: April 11–May 18; San Diego: March 11–April 17), when territorial activity and the seasonal peak of testosterone are predicted to be highest in this species (Ketterson and Nolan 1999; Ketterson et al. 2001). Males for this analysis were captured following STI capture protocols ($n = 27$ per population; see above). Because handling time, date, or body mass did not significantly differ between populations and none was significantly associated with testosterone levels in this subset and did not improve model fit, we removed these variables from the GLM, ultimately comparing testosterone concentrations from the two time points ([1] immediately upon capture following the STI and [2] following a subsequent GnRH-challenge) using independent sample *t*-tests.

Testosterone-Phenotype Covariation within Populations

Correlations between testosterone and associated traits within each population (i.e., parental care, territorial aggression, and ornamental plumage) were tested using GLM (parental care, territorial aggression) and GLMM (tail white and head black plumage), in order to control for the variables predicted to influence testosterone levels, as described above. To test for a relationship between testosterone and territorial aggression and parental care, we used the testosterone measures collected immediately following the STI behavioral assay (described above). Separate univariate GLM for parental care and aggression were run, with each male's behaviors included as covariates and either initial or post-GnRH testosterone (sampled following the standardized STI protocol during peak breeding; see above) as response variables. To test for a relationship between testosterone and tail white or head black plumage, we used the entire seasonal testosterone data set, controlling for repeated sampling of individuals by including a random effect of individual in a GLMM, with the plumage traits as fixed predictors. Residuals from these models were used to visualize the relationship between testosterone and plumage traits. (For similar statistical approaches, see McGlothlin et al. 2007, 2008.) First, we completed these

analyses of testosterone-trait covariation separately for each population to test for significant associations within each population. Next, to test for significant differences in hormone-phenotype relationships between the two populations, we completed the same analyses with the populations pooled, including population and population \times trait interaction (i.e., behavior or plumage) as predictors.

Common Garden Study of Testosterone and Plumage

We captured 40 recently independent juveniles (day 25–40 posthatch, ~20 per sex per population) from each of the two study populations during July 2007 and transported them to separate but identical indoor aviaries at Indiana University. In some cases ($n = 12$ in Mount Laguna, $n = 19$ in San Diego), we knew the exact age of the captured juveniles because they were banded as nestlings (San Diego: 40.9 ± 4.3 days posthatch; Mount Laguna: 38.2 ± 1.7 days posthatch; $t_{29} = 0.59$, $P = .56$). Capture locations were distributed spatially throughout the study areas to avoid capturing closely related individuals (i.e., siblings), with juveniles collected over 30 days from more than 13 different locations and on 15 different dates within each study population. Based on the subset of captured juveniles that were banded as nestlings ($n = 31$ of 80) and thus had known parents, no siblings are included in the study.

In captivity, we housed all birds under identical conditions in mixed-sex (50 : 50 M : F) flocks of equivalent densities (1 bird/m²) in large (6.4 \times 3.2 \times 2.4-m) or small (2.5 \times 2.1 \times 2.4-m) aviary rooms of constant ambient temperature ($\approx 15^\circ\text{C}$). Photoperiod was updated every 2 weeks to simulate the native latitude (32°52'30"N for both populations). We measured testosterone (initial and GnRH-induced) profiles from February to August 2008 from a subset of males (San Diego: $n = 15$; Mount Laguna: $n = 13$) at ~2-week intervals, using blood sampling and assay protocols generally identical to the field methods described above. Peak levels were compared during the same seasonal period as in the field studies. Plumage ornaments (tail white and head black) were measured in October 2008, following the completion of the annual molt cycle. Because juncos do not reliably breed in captivity, we were unable to measure territorial aggression, parental care, or extra-pair paternity in the common garden.

Statistical and Animal Subject Notes

We conducted all statistical tests using SPSS Statistics software (ver. 19.0; IBM, Chicago). In the main text and figures, we report the statistical terms for the main effects of the traits under primary investigation and interpretation (i.e., population, testosterone, behavior, plumage), with

best-fit statistical models (i.e., GLM and GLMM tables) including test statistics for all included variables available in tables A2–A6. All reported P values are two-tailed. This research was conducted in compliance with best practices for animal research, including the approval of the Indiana University Animal Care and Use Committee (study 06-242) and with permits from the US Fish and Wildlife Service, the California Department of Fish and Game, and the US Forest Service.

Results

Differences between Populations

Behavior. Rates of male provisioning of offspring were greater in San Diego than in the nearby native population in Mount Laguna (fig. 2A; 2006: $F_{1,19} = 5.2$, $P = .03$; 2007: $F_{1,2} = 10.64$, $P = .002$), indicating greater allocation to paternal effort. Rates of extra-pair paternity were lower in San Diego than in Mount Laguna (fig. 2B; 2006: $\chi_1^2 = 0.003$, $P = .96$; 2007: $\chi_1^2 = 6.84$, $P = .01$; years combined: $\chi_2^2 = 6.84$, $P = .04$). These observations are complemented by previously described responses to simulated social interactions, where males in San Diego responded less aggressively to territorial song playback than males in Mount Laguna (fig. 2C; Wilcoxon $T = 6$, $P < .05$; Newman et al. 2006).

Plumage. Head black was reduced in San Diego compared to head black in Mount Laguna (35% less; fig. 2D; $F_{1,192} = 137$, $P < .001$). Tail white was also lower in San Diego than in Mount Laguna by ~12% (fig. 2D; $F_{1,190} = 42.0$, $P < .001$), confirming a previously documented difference between the populations in tail white (Yeh 2004) and indicating that the difference between populations is stable over time. The plumage differences persisted in our captive common garden populations, with head black 29% less in the captive San Diego males than in the Mount Laguna males ($t_{25} = 2.34$, $P = .03$) and tail white 17% less in San Diego than in Mount Laguna ($t_{25} = 2.18$, $P = .04$).

Within-Population Correlates of Testosterone

Within both populations, one of the two measures of testosterone (initial or GnRH-induced) correlated negatively with rates of provisioning offspring and positively with aggressive behavior (fig. 3). With respect to parental effort, initial testosterone was negatively correlated with male feeding visits/hour when populations were pooled (fig. 3A, 3C; $F_{1,23} = 5.28$, $P = .031$). Comparing populations for initial testosterone, the population \times parental care interaction was nearly significant ($F_{1,23} = 3.73$, $P = .06$), as the relationship was notably stronger within the Mount

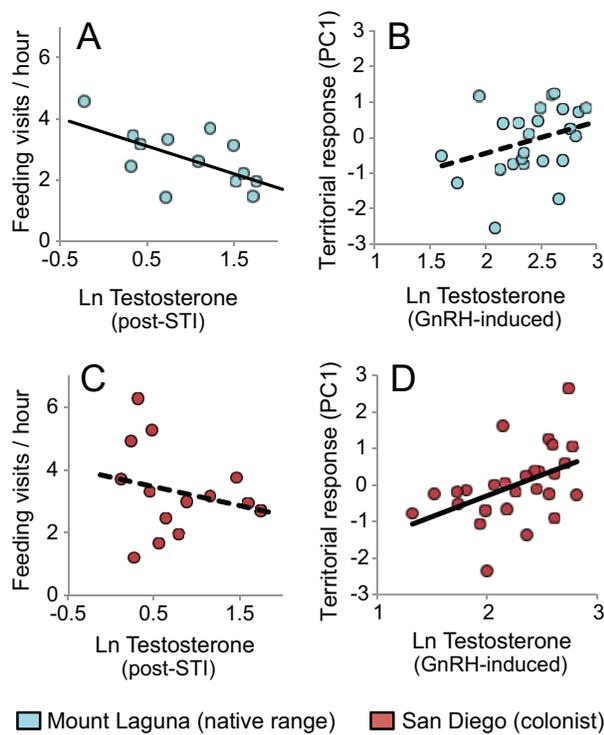


Figure 3: Covariation between testosterone and social behaviors within San Diego (colonist, red circles) and Mount Laguna (ancestral range, blue circles) populations. Individual testosterone levels generally correlated negatively with parental effort (Mount Laguna: $F_{1,12} = 10.9$, $P = .01$ [A]; San Diego: $F_{1,13} = 2.37$, $P = .15$ [C]) and positively with territorial aggression (Mount Laguna: $F_{1,26} = 2.86$, $P = .11$ [B]; San Diego: $F_{1,26} = 8.24$, $P = .01$ [D]) within both populations. Solid lines indicate significant covariation at $\alpha = 0.05$; dashed lines indicate nonsignificant trends ($P = .10$ – $.15$). GnRH = gonadotropin-releasing hormone; STI = simulated territorial intrusion.

Laguna population than the San Diego population (fig. 3A, 3C). GnRH-induced testosterone was not correlated with paternal behavior within either population ($P > .05$; table A3).

With regard to territorial aggression, GnRH-induced testosterone levels correlated positively with aggression scores across both populations (fig. 3B, 3D; populations pooled, $F_{1,48} = 9.58$, $P = .003$), with no significant population \times aggression interaction effect ($F_{1,48} = 0.23$, $P = .63$). The relationships between initial testosterone and aggressive behavior were qualitatively similar but nonsignificant when pooled, and within the San Diego population, initial testosterone was significantly and positively related to aggressive behavior (populations pooled, aggression: $F_{1,48} = 3.06$, $P = .08$; population \times aggression: $F_{1,48} = 1.72$, $P = .19$; populations separate, Mount Laguna: $F_{1,23} = 0.05$, $P = .81$; San Diego: $F_{1,23} = 9.22$, $P = .006$; table A4).

With respect to plumage, tail white was significantly but weakly correlated with GnRH-induced testosterone in only the San Diego population ($F_{1,123.7} = 4.53$, $P = .04$; fig. 4A). However, this relationship did not differ significantly between populations (populations pooled: interaction term: $F_{1,123.7} = 1.49$, $P = .2$; table A5). Initial testosterone was not correlated with tail white within either population or in populations pooled (all $P > .05$; table A5).

The other ornamental trait, head black, was positively correlated with GnRH-induced testosterone within both populations (fig. 4B, 4D; populations pooled, $F_{1,205.6} = 21.32$, $P < .001$), although this relationship was stronger in the native (Mount Laguna) population (population \times head black interaction term, $F_{1,205.1} = 8.01$, $P = .005$; fig. 4B, 4D). Initial testosterone was also significantly and positively correlated with head black within both populations (table A6).

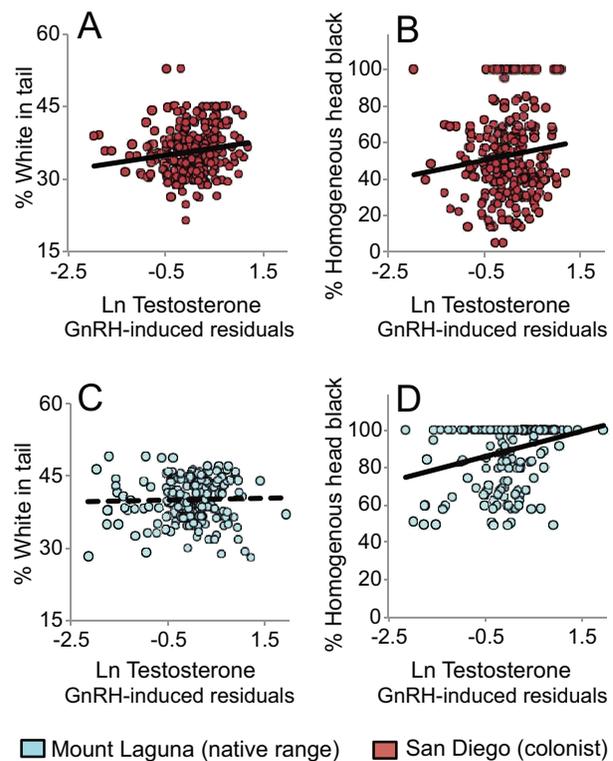


Figure 4: Covariation between testosterone and plumage within San Diego (colonist, red circles) and Mount Laguna (ancestral range, blue circles) populations, as measured from males sampled across the season (Mount Laguna: $n = 88$ males, 212 captures; San Diego: $n = 95$ males, 296 captures). A, Tail white (San Diego: $F_{1,123.7} = 4.53$, $P = .04$; Mount Laguna: $F_{1,77.3} = 0.19$, $P = .89$). B, Head black (San Diego: $F_{1,134.6} = 4.9$, $P = .03$; Mount Laguna: $F_{1,86.6} = 12.9$, $P = .001$). Solid lines indicate significant covariation ($\alpha = 0.05$); the dashed line indicates nonsignificant fit. Testosterone measures (residuals) are adjusted for repeated measures, body mass, date, year, and handling time. GnRH = gonadotropin-releasing hormone.

Differences in Testosterone between Populations

First, in association with the longer breeding season (fig. 5A, 5B), testosterone was elevated over a longer period in San Diego. The nonparametric curve fits indicate testosterone responses to GnRH challenge exceeded 5 ng/mL over 125 days in San Diego, compared to 75 days in Mount Laguna (fig. 5C, 5D). When we compared peak testosterone levels between populations by examining the 38 days surrounding the seasonal prebreeding peak period in each population (fig. 5C, 5D, dashed lines; $n = 27$ males per population), we found that testosterone levels over this period were lower in San Diego than in Mount Laguna (fig. 5E), both in response to simulated territorial intrusions (38% lower; $t_{1,52} = 1.82$, $P = .06$) and GnRH induction (27% lower; $t_{1,52} = 2.15$, $P = .03$).

In the common garden study, population differences in seasonal testosterone profiles did not persist (fig. 5F, 5G). We also found no difference in mean peak testosterone between populations in the common garden, when measured across a comparable seasonal period to the field study (fig. 5H; initial testosterone: $F_{1,30.7} = 0.14$, $P = .71$; GnRH-induced testosterone: $F_{1,30.7} = 0.21$, $P = .65$).

Discussion

To summarize, we found evidence for reduced extra-pair mating behavior (fig. 2B), reduced tail-white and head black plumage elaboration (fig. 2D), reduced territorial aggression (fig. 2C), and increased parental care (fig. 2A) in males of the San Diego (colonist) population when compared to the nearby ancestral range in Mount Laguna. We also found evidence of reduced peak testosterone in the colonist population (fig. 5E), which, when interpreted in light of earlier findings relating these traits to testosterone in juncos, suggests that the shifts in behavioral and morphological traits are linked to the altered testosterone profiles. Within our study populations, we found evidence for generally conserved patterns of trait covariation between testosterone and parental care, between testosterone and territorial aggression (fig. 3), and between testosterone and tail white and head black plumage ornamentation (fig. 4).

We predicted that (i) the longer breeding season in San Diego (fig. 5A, 5B) would be associated with prolonged seasonal production of testosterone and that (ii) the increased time devoted to paternal care would be associated with lower maximum levels of testosterone in San Diego when compared to the native range. Analyses of testosterone titers from free-living males support these predictions (fig. 5C–5E). We found evidence for prolonged seasonal testosterone expression profiles in the colonist population and reduced peak testosterone levels (fig. 5C–5E). Plumage differences persisted in the common garden

study, suggesting a genetic basis for the population differences in tail white and head black (see also Yeh 2004; Price et al. 2008). In contrast, seasonal testosterone profiles and peak testosterone levels converged in the common garden, suggesting plastic hormonal changes (fig. 5F, 5G).

Extensions of the breeding season in urban colonist populations have been observed in other species, for example, European blackbirds, *Turdus merula* (Partecke et al. 2004; Dominoni et al. 2013), which were also found to be more sedentary in the urban environment (Partecke and Gwinner 2007). Our finding of phenotypic plasticity underlying differences in breeding phenology and reproductive endocrine physiology is generally consistent with prior work in the European blackbird system, where seasonal profiles of reproductive hormones mostly, though not entirely, converged in a common garden (Partecke et al. 2004, 2005). In the blackbird system, experimental work suggests that anthropogenic light in the city may be the key environmental factor driving extended breeding phenology (Dominoni et al. 2013), which raises the possibility that artificial light (in addition to a milder climate and anthropogenic food and water sources) could be contributing to the extended breeding season and altered hormonal profiles in the San Diego junco population.

Prior experimental and correlational work in a Virginia population of juncos has shown that expression of the social behaviors in our study (extra-pair mating, aggression, and parental care) and one of the two plumage traits we studied (tail white) are associated with higher testosterone levels (fig. 1; Ketterson and Nolan 1999; Ketterson et al. 2001, 2009; McGlothlin et al. 2007, 2008). Taken together, our results imply that circulating hormones, behavior, and morphology covary between the two recently diverged populations in San Diego and Mount Laguna in ways that are consistent both within and between populations. Such a pattern could emerge if testosterone were simultaneously regulating several behavioral traits in our study—a possibility indicated by prior experimental (implant) studies in juncos (summarized in Ketterson et al. 2001)—or if altered testosterone had strong effects on a single trait, with resulting correlated responses for other traits. Similarly, other environmental or social influences could induce behavioral changes, with potential to feed back on hormone levels, perhaps further altering the suite of traits.

Entry into a novel environment must often present a host of adaptive challenges that lower the probability of persistence. However, these challenges can be mitigated if plastic changes result in correlated shifts in multiple traits that bring the population closer to the adaptive solution (Price et al. 2003; Reed et al. 2011). Here, we have shown how modulations of an integrating hormonal mechanism with multiple effects—levels of circulating testosterone—

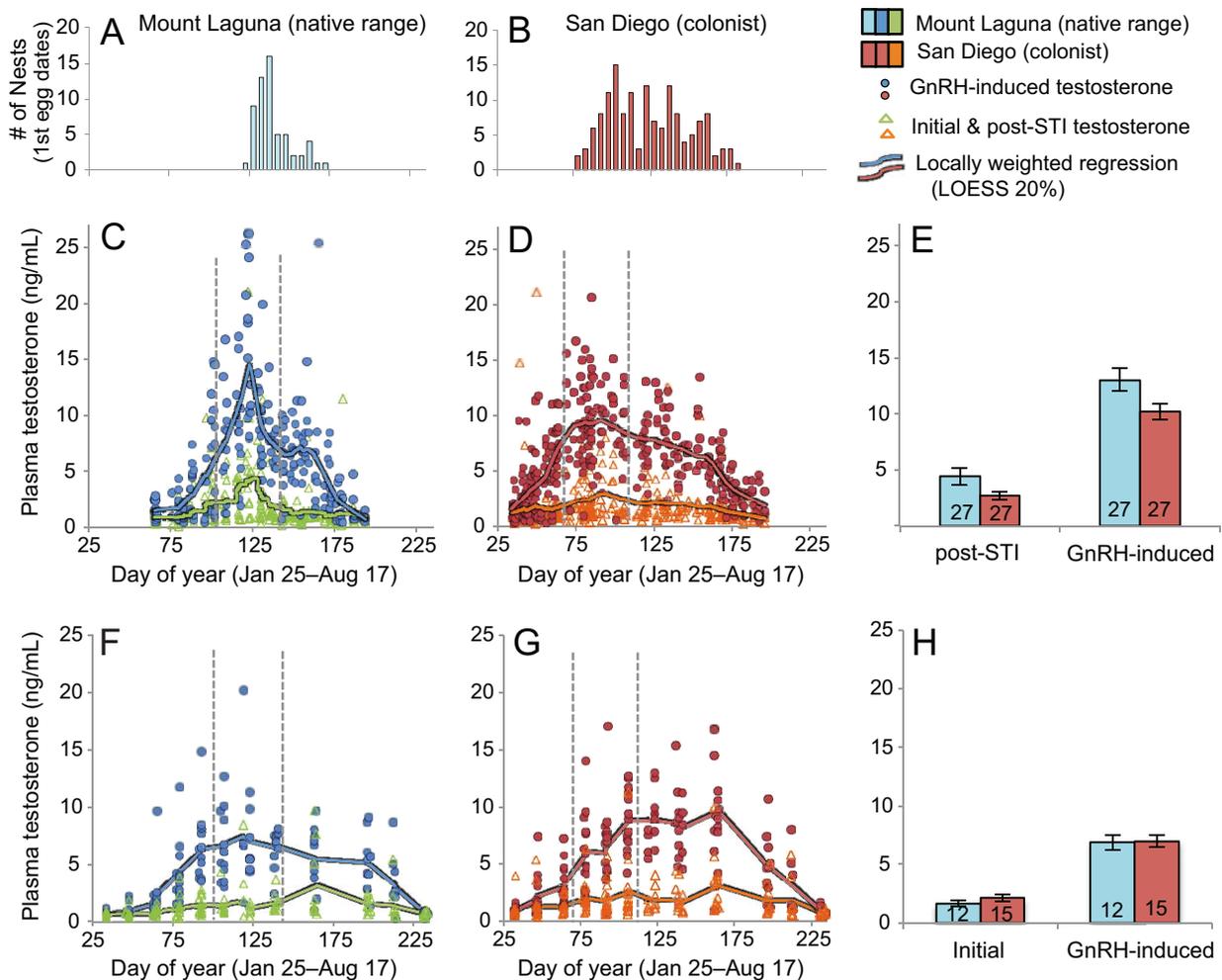


Figure 5: Seasonal profiles and peak testosterone levels in Mount Laguna (ancestral range) and San Diego (colonist) populations. *A, B*, Breeding phenology (frequency distribution of first-laid-egg dates) in each population (2006 and 2007 combined). *C–E*, Field results from Mount Laguna ($n = 88$ males, 212 captures; *C*) and San Diego ($n = 95$ males, 296 captures; *D*), including initial and post-simulated territorial intrusion (post-STI; triangles) and gonadotropin-releasing hormone (GnRH)-induced measures (circles), and a subset comparison of peak testosterone levels during the 38-day period of maximum production in each population (*E*; as indicated by the dashed lines in *C* and *D*, including post-STI and post-GnRH $n = 27$ males per population). *F–H*, as in *C–E*, but following 1 year of captivity in an indoor aviary common garden experiment after capture during early life (Mount Laguna: $n = 12$ males; San Diego: $n = 15$ males).

are associated with multiple shifts in the expression of behavioral and life-history traits in a manner that should result in phenotypically plastic change appropriate to novel conditions (Ketterson et al. 2009). Our results suggest a general mechanism by which suites of adaptively important traits could be adjusted in response to novel or changing environments. While our study is particular to one colonizing population and a single mediating hormone, the findings have the potential to promote a more general understanding of the physiological basis of successful introductions, invasions, or population persistence in the face of environmental change.

The first step in any invasion, establishment, or habitat

alteration will usually be a plastic response to the novel conditions (Baldwin 1896; Price et al. 2003). In the case under study, at least two environmental features may generate immediate alterations in testosterone profiles. First, birds respond to the milder climate and sustained food levels by raising additional broods each year (Yeh and Price 2004), and this results in testosterone remaining elevated over a longer period (fig. 5A–5D). Second, birds remain on or near their territories throughout the year and do not go through annual competition for mates and territories over a short time. In consequence, the reduction in social challenges and social instability could lead to a lower maximum peak in testosterone (fig. 5E; Wingfield et al. 1990, 2001). These

induced changes in testosterone may also be linked to changes in hormone-mediated behaviors, such as paternal care and the search for extra-pair copulations (Ketterson and Nolan 1999; McGlothlin et al. 2007), and plumage ornamentation (McGlothlin et al. 2008; Eikenaar et al. 2011; Lindsay et al. 2011). Modulations in plumage ornamentation could result from both the direct or indirect action of testosterone on feather development (Strasser and Schwabl 2004; Bokony et al. 2008; McGlothlin et al. 2008; Lindsay et al. 2011). However, correlates of testosterone with plumage ornamentation within populations are quite weak, suggesting that any plastic response in plumage to altered testosterone profiles in the novel environment will be small, which sets the stage for genetic evolution (see below). Further, it is important to consider that altered plumage could play a role in inducing hormonal changes (Safran et al. 2008) or that plumage may only be developmentally linked to hormone levels at other times of the year (Buchanan et al. 2003).

The common garden study of testosterone was conducted in a mild, captive environment. Here, the convergence between the Mount Laguna and San Diego populations suggests that both populations have the capacity to hasten the seasonal onset, prolong the duration, and modify peak levels of both initial and GnRH-induced testosterone. In fact, both the Mount Laguna and San Diego seasonal profiles of testosterone in the common garden more closely resemble those observed in the wild in the milder San Diego environment—with a longer duration of seasonal testosterone production and lower peak levels (fig. 5F–5H). It appears that in captivity, as in the milder environmental conditions of coastal San Diego, the birds are released from the ordinarily suppressive supplementary environmental cues—such as low temperatures, limited insect food availability, and variation in precipitation levels—found in the highly seasonal montane breeding habitat of the native range, perhaps via modulation of the gonadotropin-inhibitory hormones (Ukena et al. 2003). The captive testosterone profiles need to be interpreted cautiously, however, as absolute hormone values are often lower in captivity (fig. 5; also see Calisi and Bentley 2009; Fokidis et al. 2011), and divergence in another endocrine-behavior suite from the same study system—corticosterone and boldness—appears to be genetically based (Atwell et al. 2012), a finding that is also paralleled in the European blackbird system, where stress-induced corticosterone titers and personality traits remained divergent even under common captive environments (Partecke et al. 2006; Miranda et al. 2013).

Following initial establishment of a population and any associated plastic changes, a second step is selection in the context of both environmental differences and the altered distribution of phenotypes (Price et al. 2003, 2008). In our

study, several lines of evidence imply that genetic evolution has driven the between-population differences in plumage pattern. These include the common garden studies we report here (differences in plumage persist even when testosterone is similar), measurements of direct selection on tail white in the field (Price et al. 2008), and molecular and quantitative genetic assessments that eliminate founder effects and genetic drift as sufficient cause (Rasner et al. 2004; Yeh 2004). Selection is expected when the match between the novel environment and induced phenotypic changes is not perfect (Price et al. 2003). Possible mismatches between the plastic response and the adaptive solution, which could have led to selection and genetic change, have previously been indicated in this system (Price et al. 2008). For example, as a consequence of the extended breeding season and the raising up to four broods in San Diego, young birds hatching late in the season are entering a social environment, composed of birds hatched earlier in the year, not often experienced in the history of the subspecies (Price et al. 2008), as birds breeding in the native mountain range are limited to a single brood. This social environment has apparently altered selection on the plumage pattern, favoring reduced expression (Yeh 2004; Price et al. 2008).

Just as adaptive phenotypic plasticity is most effective when multiple traits are modified in the appropriate direction, rapid evolutionary change is facilitated when genetic correlations promote the evolution of traits toward their joint optima (Lande 1979; Agrawal and Stinchcombe 2009). Because it is at the phenotypic level that testosterone organizes correlations between behavioral and plumage traits, genetic variation in testosterone levels should facilitate adaptive evolution in the same way that environmental variation facilitates adaptive plastic responses. However, we have little evidence that testosterone has genetically evolved between populations in our study and no direct evidence that genetic correlations have been important in facilitating the evolution of plumage. While our studies presented here have focused on sampling only male behaviors and testosterone levels, females show similar levels of divergence in tail white and head black plumage traits, and changes in female reproductive and social behaviors have played a foundational role in facilitating population establishment in this system (Yeh and Price 2004). An important avenue for future research is directly addressing hormonal and behavioral shifts in females in response to environmental change.

One of this study's major findings is that within-population correlations related to testosterone align with the between-population patterns. A generally unresolved question in evolutionary biology is why correlations within and between populations should match (Schluter 1996; Agrawal and Stinchcombe 2009). One possibility is that within-pop-

ulation correlations constrain the direction of between-population changes, which then need not match the adaptive solution (Schluter 1996). However, we suggest the simpler explanation that trade-offs between mate attraction and parental care operate similarly within and among populations (i.e., there are “ridges” in the adaptive surface that lead to correlational selection; Brodie et al. 1995; McGlothlin et al. 2005). On the temperate end of the population continuum, a short breeding season in a seasonal environment places emphasis on mate acquisition (a fast pace of life), whereas at the other end, long-term residency in a Mediterranean or tropical environment places emphasis on higher levels of parental care (a slow pace of life; Hau et al. 2010; Reale et al. 2010). Thus, entry into novel climates may often induce changes in integrating hormonal mechanisms that provide an approximate fit to the new environment.

In conclusion, successful colonization or persistence in novel or changing environments often requires changes in multiple traits. Phenotypically plastic responses may enable persistence, as originally suggested by Baldwin (1896), and such plastic adjustments are often regulated by integrating hormonal mechanisms that underlie trait correlations and trade-offs. Adaptation to a novel environment by selection is also accelerated if traits are associated in a way that facilitates the appropriate correlated responses. We have shown here how between-population and within-population covariation relate to changes in an underlying hormone, testosterone, and how changes in testosterone could result in adaptive shifts in related traits in behavior and life history, as observed in a contemporary colonization. Many successful invasions, range expansions, or responses to habitat alteration or climate change may exhibit similar features, although both the specific traits involved and their organizers will vary according to the species and the environment.

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Literature Cited

- Adkins-Regan, E. 2005. Hormones and animal social behavior. Princeton University Press, Princeton, NJ.
- . 2008. Do hormonal control systems produce evolutionary inertia? *Philosophical Transactions of the Royal Society B: Biological Sciences* 363:1599–1609.
- Agrawal, A. F., and J. R. Stinchcombe. 2009. How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences* 276:1183–1191.
- Atwell, J. W., G. C. Cardoso, D. J. Whittaker, S. Campbell-Nelson, K. W. Robertson, and E. D. Ketterson. 2012. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology* 23:960–969.
- Baldwin, J. M. 1896. A new factor in evolution. *American Naturalist* 30:441–445, 536–553.
- Balphy, M. H., D. F. Balphy, and H. C. Romesburg. 1979. Social status signaling in winter flocking birds: an examination of a current hypothesis. *Auk* 96:78–93.
- Bokony, V., L. Z. Garamszegi, K. Hirschenhauser, and A. Liker. 2008. Testosterone and melanin-based black plumage coloration: a comparative study. *Behavioral Ecology and Sociobiology* 62:1229–1238.
- Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* 10:313–318.
- Buchanan, K. L., M. R. Evans, and A. R. Goldsmith. 2003. Testosterone, dominance signalling and immunosuppression in the house sparrow, *Passer domesticus*. *Behavioral Ecology and Sociobiology* 55:50–59.
- Calisi, R. M., and G. E. Bentley. 2009. Lab and field experiments: are they the same animal? *Hormones and Behavior* 56:1–10.
- Crispo, E. 2007. The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* 61:2469–2479.
- Crutzen, P. J. 2002. Geology of mankind. *Nature* 415:23.
- Davis, M. A. 2009. *Invasion biology*. Oxford University Press, New York.
- Denver, R. J., P. M. Hopkins, S. D. McCormick, C. R. Propper, L. Riddiford, S. A. Sower, and J. C. Wingfield. 2009. Comparative endocrinology in the 21st century. *Integrative and Comparative Biology* 49:339–348.
- Dominoni, D., M. Quetting, and J. Partecke. 2013. Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society B: Biological Sciences* 280:20123017.
- Eikenaar, C., M. Whitham, J. Komdeur, M. van der Velde, and I. T. Moore. 2011. Testosterone, plumage colouration and extra-pair paternity in male North-American barn swallows. *PLoS ONE* 6: e23288.
- Finch, C. E., and M. R. Rose. 1995. Hormones and the physiological architecture of life-history evolution. *Quarterly Review of Biology* 70:1–52.
- Fokidis, H. B., L. Hurley, C. Rogowski, K. Sweazea, and P. Deviche. 2011. Effects of captivity and body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in curve-billed thrashers. *Physiological and Biochemical Zoology* 84:595–606.
- Garamszegi, L. Z., K. Hirschenhauser, V. Bókony, M. Eens, S. Hurrez-Boussès, A. P. Møller, R. F. Oliveira, and J. C. Wingfield. 2008. Latitudinal distribution, migration, and testosterone levels in birds. *American Naturalist* 172:533–546.
- Goymann, W., D. Geue, I. Schwabl, H. Flinks, D. Schmidl, H.

- Schwabl, and E. Gwinner. 2006. Testosterone and corticosterone during the breeding cycle of equatorial and European stonechats (*Saxicola torquata axillaris* and *S. t. rubicola*). *Hormones and Behavior* 50:779–785.
- Goymann, W., I. T. Moore, A. Scheuerlein, K. Hirschenhauser, A. Grafen, and J. C. Wingfield. 2004. Testosterone in tropical birds: effects of environmental and social factors. *American Naturalist* 164:327–334.
- Hansen, M. M., I. Olivieri, D. M. Waller, E. E. Nielsen, and the GeM Working Group. 2012. Monitoring adaptive genetic responses to environmental change. *Molecular Ecology* 21:1311–1329.
- Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* 29:133–144.
- Hau, M., R. E. Ricklefs, M. Wikelski, K. A. Lee, and J. D. Brawn. 2010. Corticosterone, testosterone and life-history strategies of birds. *Proceedings of the Royal Society B: Biological Sciences* 277: 3203–3212.
- Hill, J. A., D. A. Enstrom, E. D. Ketterson, V. Nolan, and C. Ziegenfus. 1999. Mate choice based on static versus dynamic secondary sexual traits in the dark-eyed junco. *Behavioral Ecology* 10:91–96.
- Hoffmann, A. A., and C. M. Sgro. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.
- Holberton, R. L., K. P. Able, and J. C. Wingfield. 1989. Status signaling in dark-eyed juncos, *Junco hyemalis*: plumage manipulations and hormonal correlates of dominance. *Animal Behaviour* 37:681–689.
- Horton, B. M., J. Yoon, C. K. Ghahambor, I. T. Moore, and T. Scott Sillett. 2010. Seasonal and population variation in male testosterone levels in breeding orange-crowned warblers (*Vermivora celata*). *General and Comparative Endocrinology* 168:333–339.
- Jawor, J. M., J. W. McGlothlin, J. M. Casto, T. J. Greives, E. A. Snajdr, G. E. Bentley, and E. D. Ketterson. 2006. Seasonal and individual variation in response to GnRH challenge in male dark-eyed juncos (*Junco hyemalis*). *General and Comparative Endocrinology* 149: 182–189.
- Ketterson, E. D., J. W. Atwell, and J. W. McGlothlin. 2009. Phenotypic integration and independence: hormones, performance, and response to environmental change. *Integrative and Comparative Biology* 49:365–379.
- Ketterson, E. D., and V. Nolan. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* 154(suppl.): S4–S25.
- Ketterson, E. D., V. Nolan, J. M. Casto, C. A. Buerkle, E. D. Clotfelter, J. L. Grindstaff, K. J. Jones, et al. 2001. Testosterone, phenotype and fitness: a research program in evolutionary behavioral endocrinology. Pages 19–40 in A. Dawson and C. M. Chaturvedi, eds. *Avian endocrinology*. Alpha Science, Pangbourne, UK.
- Kitano, J., S. C. Lema, J. A. Luckenbach, S. Mori, Y. Kawagishi, M. Kusakabe, P. Swanson, and C. L. Peichel. 2010. Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. *Current Biology* 20:2124–2130.
- Knudsen, E., A. Linden, C. Both, N. Jonzen, F. Pulido, N. Saino, W. J. Sutherland, et al. 2011. Challenging claims in the study of migratory birds and climate change. *Biological Reviews* 86:928–946.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain–body size allometry. *Evolution* 33:402–416.
- Liebl, A. L., and L. B. Martin. 2012. Exploratory behaviour and stressor hyper-responsiveness facilitate range expansion of an introduced songbird. *Proceedings of the Royal Society B: Biological Sciences* 279:4375–4381.
- Lindsay, W. R., M. S. Webster, and H. Schwabl. 2011. Sexually selected male plumage color is testosterone dependent in a tropical passerine bird, the red-backed fairy-wren (*Malurus melanocephalus*). *PLoS ONE* 6:e26067.
- Magrath, M. J. L., and J. Komdeur. 2003. Is male care compromised by additional mating opportunity? *Trends in Ecology and Evolution* 18:424–430.
- Martin, L. B., and L. Fitzgerald. 2005. A taste for novelty in invading house sparrows, *Passer domesticus*. *Behavioral Ecology* 16:702–707.
- Martins, T. L. F., M. L. Roberts, I. Giblin, R. Huxham, and M. R. Evans. 2007. Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches. *Hormones and Behavior* 52:445–453.
- McGlothlin, J. W., J. M. Jawor, T. J. Greives, J. M. Casto, J. L. Phillips, and E. D. Ketterson. 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. *Journal of Evolutionary Biology* 21:39–48.
- McGlothlin, J. W., J. M. Jawor, and E. D. Ketterson. 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *American Naturalist* 170:864–875.
- McGlothlin, J. W., P. G. Parker, V. Nolan, and E. D. Ketterson. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. *Evolution* 59: 658–671.
- Miller, A. H. 1941. Speciation in the avian genus *Junco*. University of California Publications in Zoology 44:173–434.
- Miranda, A. C., H. Schielzeth, T. Sonntag, and J. Partecke. 2013. Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? *Global Change Biology* 19: 2634–2644.
- Møller, A. P., W. Fiedler, and P. Berthold. 2010. Effects of climate change on birds. Oxford University Press, New York.
- Newman, M. M., P. J. Yeh, and T. D. Price. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. *Animal Behaviour* 71:893–899.
- Nolan, V., E. D. Ketterson, D. A. Cristol, C. M. Rogers, E. D. Clotfelter, R. C. Titus, S. J. Schoech, and E. Snajdr. 2002. Dark-eyed junco (*Junco hyemalis*). Pages 1–44 in A. Poole and F. Gill, eds. *The birds of North America*. Vol. 716. Academy of Natural Sciences and American Ornithologists Union, Philadelphia.
- Partecke, J., and E. Gwinner. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* 88:882–890.
- Partecke, J., I. Schwabl, and E. Gwinner. 2006. Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. *Ecology* 87:1945–1952.
- Partecke, J., T. Van't Hof, and E. Gwinner. 2004. Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences? *Proceedings of the Royal Society B: Biological Sciences* 271:1995–2001.
- . 2005. Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds *Turdus merula*. *Journal of Avian Biology* 36:295–305.
- Phillips, B. L., G. P. Brown, and R. Shine. 2010. Life-history evolution in range-shifting populations. *Ecology* 91:1617–1627.
- Price, T. D. 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. *Journal of Experimental Biology* 209: 2368–2376.
- Price, T. D., A. Qvarnstrom, and D. E. Irwin. 2003. The role of

- phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* 270:1433–1440.
- Price, T. D., P. J. Yeh, and B. Harr. 2008. Phenotypic plasticity and the evolution of a socially selected trait following colonization of a novel environment. *American Naturalist* 172(suppl.):S49–S62.
- Rasner, C. A., P. Yeh, L. S. Eggert, K. E. Hunt, D. S. Woodruff, and T. D. Price. 2004. Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. *Molecular Ecology* 13:671–681.
- Reale, D., D. Garant, M. M. Humphries, P. Bergeron, V. Careau, and P.-O. Montiglio. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:4051–4063.
- Reed, T. E., D. E. Schindler, and R. S. Waples. 2011. Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conservation Biology* 25:56–63.
- Rosvall, K. A., D. G. Reichard, S. M. Ferguson, D. J. Whittaker, and E. D. Ketterson. 2012. Robust behavioral effects of song playback in the absence of testosterone or corticosterone release. *Hormones and Behavior* 62:418–425.
- Safran, R. J., J. S. Adelman, K. J. McGraw, and M. Hau. 2008. Sexual signal exaggeration affects physiological state in male barn swallows. *Current Biology* 18:R461–R462.
- Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766–1774.
- Shochat, E., P. S. Warren, S. H. Faeth, N. E. McIntyre, and D. Hope. 2006. From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution* 21:186–191.
- Sih, A., M. C. O. Ferrari, and D. J. Harris. 2011. Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications* 4:367–387.
- Sinervo, B., D. B. Miles, W. A. Frankino, M. Klukowski, and D. F. DeNardo. 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior* 38:222–233.
- Sinervo, B., and E. Svensson. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83:432–442.
- Strasser, R., and H. Schwabl. 2004. Yolk testosterone organizes behavior and male plumage coloration in house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* 56:491–497.
- Tuomainen, U., and U. Candolin. 2011. Behavioural responses to human-induced environmental change. *Biological Reviews* 86: 640–657.
- Ukena, K., T. Ubuka, and K. Tsutsui. 2003. Distribution of a novel avian gonadotropin-inhibitory hormone in the quail brain. *Cell and Tissue Research* 312:73–79.
- Unitt, P. 2005. San Diego County bird atlas. San Diego Natural History Museum, San Diego, CA.
- van Oers, K., K. L. Buchanan, T. E. Thomas, and P. J. Drent. 2011. Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality. *Animal Behaviour* 81:1055–1061.
- West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York.
- Whittaker, D. J., A. L. Dapper, M. P. Peterson, J. W. Atwell, and E. D. Ketterson. 2012. Maintenance of MHC Class IIB diversity in a recently established songbird population. *Journal of Avian Biology* 43:109–118.
- Wingfield, J. C., R. E. Hegner, A. M. Dufty, and G. F. Ball. 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist* 136:829–846.
- Wingfield, J. C., S. E. Lynn, and K. K. Soma. 2001. Avoiding the “costs” of testosterone: ecological bases of hormone-behavior interactions. *Brain Behavior and Evolution* 57:239–251.
- Yeh, P. J. 2004. Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution* 58:166–174.
- Yeh, P. J., and T. D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *American Naturalist* 164:531–542.

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A male dark-eyed junco (*Junco hyemalis thurberi*) sings from his perch atop a utility box on the University of California–San Diego campus in La Jolla, California. Photo credit: Jonathan Atwell/www.juncoproject.org.