Does Differential Access to Protein Influence Differences in Timing of Breeding of Florida Scrub-Jays *(Aphelocoma coerulescens)* in Suburban and Wildland Habitats?

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Abstract —Timing of breeding in Florida Scrub-Jays (*Aphelocoma coerulescens*) varies both within and between years. Social status and breeding experience may explain much of the within-year variation, but the availability of certain foods may partially explain between-year patterns. Scrub-jays in suburban habitats with access to unlimited human-provided foods breed earlier and with less between-year variation in timing of breeding than jays in wildland habitats. We hypothesized that those differences in timing of breeding result from access to human-provided foods in the suburban site. Human-provided food may influence timing of breeding by improving the overall body condition of females, or it may influence breeding by providing nutrients essential for breeding. If condition mediated, breeding females in the two habitats should differ in certain physiological parameters relative to time before egg laying and calendar date. If the effect is not related to body condition, we expect differences in pre-breeding females relative to calendar date, but not in relation to time before egg laying. To test those predictions, we measured plasma levels of total protein, calcium, luteinizing hormone, and estradiol. We also measured variables associated with body condition—body mass, a size-corrected condition index, and total body lipids. Most variables tended to increase with both days before laying and calendar date, except total body lipids, which decreased. Suburban females had higher levels of plasma protein relative to both days before egg laying and calendar date than female breeders in the wildland habitat. Luteinizing hormone differed between sites relative to calendar date but not days before laying. Our data suggest that suburban scrub-jays with access to predictable sources of high-quality human-provided foods accumulate endogenous protein that can be used to breed earlier. *Received 25 January 2002, accepted 14 June 2003.*

ONE OF THE most important decisions made by seasonally breeding animals is when to initiate their reproductive effort in a given year. Reproduction should occur when environmental factors are suitable (i.e. when resources are available to meet the energetic and nutritional demands of the mother and her growing young). To time reproduction to coincide with resources, animals rely on environmental cues that reliably predict favorable conditions. Many temperate-zone birds use an initial predictive cue, such as photoperiod, as the primary predictor of the onset of favorable conditions, but numerous other supplemental cues are known to play a role in fine-tuning the timing of breeding within the temporal window when environmental conditions are favorable for reproduction (for reviews see Farner 1985, Wingfield and Kenagy 1991, Wingfield et al. 1992, Wingfield and

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Farner 1993). Nonphotic cues may include temperature, rainfall, humidity, food abundance or food type (i. e. changes in availability of different types of food), and social environment (Wingfield 1983, Wingfield et al. 1994).

Many of those cues, especially climatic variables, are likely to affect relatively large spatial areas in the same manner. Therefore, birds within the same population, or even adjacent populations, will experience a similar set of cues and, as a result, initiate breeding at approximately the same time. Many temperate-zone birds display a relatively high degree of within-population breeding synchrony. Although photoperiod remains constant between years, climatic conditions often vary markedly, and that may lead to between-year variation in the onset of reproduction. Other cues, such as availability of food and social and demographic factors, can be expected to vary locally leading to both between- and within-population variation in timing of breeding.

In natural habitats, Florida Scrub-Jays (*Aphelocoma coerulescens*) exhibit considerable within- and between-year variation in clutch initiation date (see Woolfenden and Fitzpatrick 1984, 1990, 1996; Schoech 1996). However, scrub-jays that occur in a suburban matrix breed earlier than those in natural habitats and show relatively little between-year variation in clutch initiation dates (Bowman et al. 1998). Scrub-jays in suburban habitats have access to numerous sources of human-provided foods, including bird seed, pet foods, food waste, and peanuts provided specifically for scrub-jays by human residents that are unavailable to scrub-jays in natural habitats. Numerous studies, including two on Florida Scrub-Jays (Schoech 1996, Reynolds et al. 2003), have shown that access to supplemental food can advance laying date in birds (for review see Meijer and Drent 1999).

Over 30% of the diet of breeding female scrub-jays in suburban habitats during the one to two months preceding reproduction consists of human-provided foods (Fleischer et al. 2003). Those observations led us to conclude that access to human-provided food by suburban scrub-jays may be an important factor in the between-population differences in the timing of breeding. We hypothe-sized that year-round access to human-provided foods could improve the overall body condition of scrub-jays, thereby providing them with sufficient endogenous resources to initiate breeding shortly after stimulus by an initial predictive cue (i.e. to recrudesce their gonads and form ova follow-ing stimulation of the hypothalamo–pituitary–gonadal [HPG] axis). Jays in wildlands would have to wait for local resources to increase before initiating breeding. Alternatively, access to human-provided foods could provide jays with enough exogenous resources, regardless of their endogenous resources, so that once an initial predictive cue such as increasing daylength informed a female that the time to breed was approaching, breeding was unimpeded by limited local resources. If the endogenous resources hypothesis is correct, suburban females should have more endogenous resources than wildland females long before initial predictive cues occur and thus certain phys-iological measures of prebreeding females should differ between the two populations relative to both calendar date and days before egg laying (Fig. 1; H1). If the exogenous resource hypothesis is correct, then birds in both habitats are in similar physiological condition when initial predictive cues occur, but suburban females begin improving their body condition immediately because local resources are not limiting. In that case, physiological differences between females in the two habitats should exist relative only to calendar date (Fig. 1; H_0); in both populations, breeding should occur shortly after exogenous resources are sufficient to fuel the costs of reproduction.

To differentiate between those hypotheses, we examined several variables predicted to reflect the differences in food availability, and presumably diet, between the two populations. During the two months immediately preceding the breeding season we compared the following in female breeder Florida Scrub-Jays in the two populations: (1) total plasma protein, a measure of protein intake and an indicator of body condition; (2) total body lipids, another measure of condition; (3) body mass; (4) a morphometric index of body condition based on mass corrected for body size; (5) plasma levels of calcium, an essential component for eggshell production and a number of physiological processes; and plasma levels of two reproductive hormones, namely (6) luteinizing hormone (LH), and (7) 17β-estradiol (E_2).
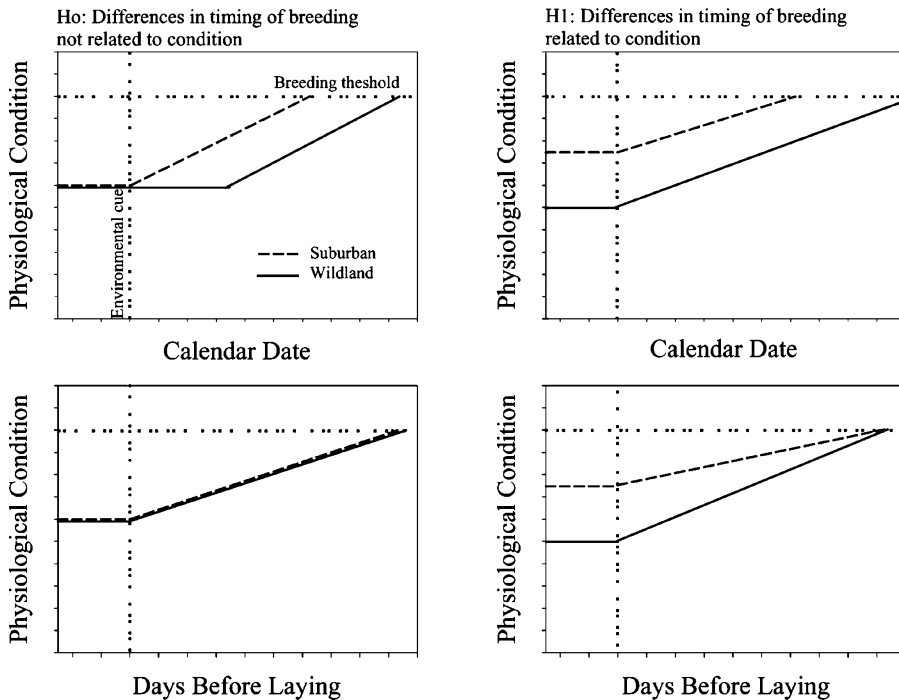
Fig. 1 Hypothetical trends in various physiological parameters relative to days before egg laying and calendar date in two Florida Scrub-Jay populations, one in suburban habitats that consistently breeds earlier than a population in wildland habitats. The two hypotheses are Ho (physiological parameters do not differ between sites and are unrelated to differences in timing of breeding; therefore, they should differ only in respect to calendar date and not days before laying) and H1 (physiological parameters differ between sites and are related to differences in timing of breeding; therefore, they should differ in respect to both calendar date and days before laying.)

Methods

Study population.—Florida Scrub-Jays were studied in a natural scrub preserve at Archbold Biological Station (hereafter wildlands") (27°10′N, 81°21′W, elevation 38–68 m) and at a nearby (<8 km) suburban development in Highlands County, Florida, during the 1998 breeding season. All scrub-jays in both populations were banded with a unique combination of color and federal aluminum bands. The sex, breeding status (breeder or nonbreeding helper), and group association of all scrub-jays in both populations were known from ongoing long-term studies. All nests were located during the nest-building stage or incubation (for more information on the wildland population, see Schoech et al. 1991, 1996; Mumme 1992; Schoech 1996; for further details on the suburban population see Bowman et al. 1998, Bowman and Woolfenden 2001).

Capture and blood sampling.—Beginning in mid-January, scrub-jays from the two populations were captured in Potter traps baited with peanuts. Traps were monitored continuously and each scrub-jay was removed within seconds of capture. Only female breeders were used and individuals were sampled only once between 21 January and 21 April 1998. To control for diel fluctuations in the variables of interest, all scrub-jays were captured between 0700 and 1100 hours EST. Immediately following removal from the trap, a blood sample was collected from the brachial vein after venipuncture with a 26 gauge needle. Samples were stored on ice until returned to the laboratory (within 1–4 h). Samples were centrifuged, and the plasma harvested for later assay. A small volume (20 µL) of plasma was retained to evaluate plasma protein levels (see below). The remaining volume

was frozen and stored at $-20°C$ until shipped to Indiana University for assay of plasma levels of calcium and estradiol or to Princeton University for assay of luteinizing hormone.

Immediately after blood samples were taken, scrub-jays were anaesthetized and total body lipids were determined (see below for details). While recovering from the anaesthetic, individuals were weighed to the nearest 0.1 g with an Avinet (Dryden, New York) spring balance, and several morphological variables, including wing-chord and tail length, a series of bill measurements, head-breadth, and overall head plus bill length, were measured to the nearest 0.1 mm. Birds were released at their capture site after the effects of anaesthesia had completely abated, usually within 0.5 h of initial capture. All procedures were sanctioned by the Bloomington Institutional Animal Care and Use Committee of Indiana University.

Physiological parameters.—Plasma protein concentration was measured with a handheld clinical refractometer (Model A 300 CL, Atago Company, Kirkland, Washington) that allows determination of the amount of dissolved solute in a small volume of plasma ($20 \mu L$), based upon the degree to which light passing through the sample is refracted. To validate protein estimates using refractometry, protein levels were also determined for 10 samples using the Biuret method of protein determination (Sigma Total Protein diagnostic kit, procedure 541; Sigma-Aldrich Corporation, St. Louis, Missouri). Protein levels determined by the two methods were similar (linear regression, $r^2 = 0.69$, $F = 17.3$, df $= 8$, $P < 0.01$).

Immediately after blood samples were taken, body lipids were assessed with the total body electrical conductivity (TOBEC) method. This allows determination of an animal's lean mass and from this, total body lipids can be estimated (Kenagy and Barnes 1988, Walsberg 1988, Roby 1991, Schoech 1996). Accurate use of the instrument requires that subjects are positioned uniformly and kept in the same position for the duration of the scanning procedure. Therefore, all scrub-jays were anaesthetized with Metophane (inhaled) and held in a nylon stocking during the procedure.

When calibrated, that method is useful for within-species comparisons of relative body lipid content, although the technique has been criticized (see Morton et al. 1991, Asch and Roby 1995). Calibration of the instrument for a given species necessitates that on one occasion a number of individuals be scanned, killed, and total body fat content measured directly with a lipid extraction technique (e.g. chloroform or ether in a Soxhlet apparatus). For this study, an equation derived from the congeneric Western Scrub-Jay (*A. californica*) was used to estimate total body lipid in Florida Scrub-Jays (see Schoech 1996 for calibration, validation, and methodological details).

Body mass divided by a linear measurement is often used as an index of condition. Previously, discriminant function analysis found that overall head plus bill length was the best predictor of sex and, therefore, body size in this visually monomorphic species (S.J. Schoech unpubl. data). Accordingly, body mass was divided by that linear measurement as an index of body condition.

Determination of plasma calcium concentration was made with a kit obtained from Sigma Diagnostics (procedure 587). Calcium reacts in a dose dependent fashion with o-cresolphtalein complexone to produce a red complex. The intensity of the resulting color of the solution is directly proportional to the calcium concentration in the plasma sample. The color intensity was determined with a spectrophotometer with absorbance set at 575 nm.

Luteinizing hormone was assayed in the laboratory of Dr. T. Hahn at Princeton University. The assay is a post-precipitation double antibody RIA that uses purified chicken LH as a standard and rabbit-reared antisera against LH (Follett et al. 1972, 1975; Sharp et al. 1987). Components were provided by Dr. P. Sharp of the Agricultural Research Council, Roslyn, Scotland. Radiolabeling of LH with ^{125}I was done using the chloramine-T method. All samples were run in a single assay in duplicate with volumes that ranged from 10 to $20 \mu L$ and averaged $18.9 \mu L$. Intra-assay coefficient of variation was 5.5%.

Plasma levels of the sex steroid hormone estradiol were measured in the laboratory of Dr. E. Ketterson at Indiana University following transport on dry ice from the field site in Florida. This competitive binding radioimmunoassay has been conducted in the Ketterson laboratory for several

years (Ketterson et al. 1992; Schoech et al. 1998, 1999). Further details on the methods and reliability criteria can be found elsewhere (see Wingfield and Farner 1975, Ball and Wingfield 1987, Schoech et al. 1991).

In brief, assay of E_2 was conducted in the following manner. Approximately 2,000 counts min^{-1} of radiolabeled E_2 were added to the unknown sample to allow calculation of the percentage of hormone recovered following extraction and column chromatography (see below). Following overnight equilibration of the labeled hormone with the plasma sample, steroids were extracted from the aqueous phase using anhydrous diethyl ether. Snap freezing of the ether and aqueous mixture in acetone super-chilled with dry ice allows the steroid-containing organic phase to be easily decanted. Samples were then dried down under nitrogen gas and reconstituted in an ethyl acetate and iso-octane mixture (10:90 by volume). That solution was then forced into celite-glycol packed minicolumns with nitrogen gas. Steroids were separated from one another by adding ethyl acetate and iso-octane mixtures of increasing polarity that were moved through the columns with nitrogen gas. Upon collection of the desired fraction, samples contained within the elute were dried, reconstituted in buffer, and subjected to a standard competitive-binding assay.

All estradiol samples were run in a single assay. Mean plasma volume used was $178\,\mu\text{L}$; recovery averaged 79.2% and intra-assay variation was 6.6%. Tritiated E_2 was obtained from New England Nuclear Research Products (Boston, Massachusetts), standard from Sigma, and antibody from Arnel Products (New York, New York).

Data analyses.—Between-population differences in timing of first-clutch initiation were determined using a *t*-test. To distinguish between our alternative hypotheses, all physiological and morphometric measures between the two populations were compared relative to first-clutch initiation date (days before laying) and calendar date. Regression was used to test data for linearity; most data were nonlinear but were successfully linearized by plotting the natural log of each variable. Analyses of covariance (ANCOVA) were then used on the transformed data with site (suburbs or wildlands) as the factor, days before laying or calendar date as the covariate, and physiological or morphological measures as the dependent variable to test whether the measures examined differed between sites or across time. In a few instances, natural-log transformation failed to linearize data, but ANCOVA were performed on the nonlinear data. ANCOVA can be fairly robust to violations of assumptions, especially experimental designs with heterogenous slopes (Klockars and Beretvas 2001). Our data met all other assumptions, our sample sizes were relatively large, and the power of our statistical tests exceeded 0.8; thus we felt that analytical approach was justified. Interactions between independent factors were tested for, and where no interaction existed, data were reanalyzed without the interaction term. When no interaction existed, data reported are for models without the interaction term. The overall fit ($adj\,R^2$) for each model is included. All analyses were conducted with SPSS for WINDOWS (SPSS 1999).

Results

In total, we obtained blood samples and measurements from 35 and 46 females from the suburban and wildland populations, respectively. Of those, 32 females in the suburbs and 36 females in the wildlands eventually laid eggs. Samples from the suburban population were collected between 21 January and 25 March and from 88 days to 1 day before egg laying. In the wildlands, samples were obtained between 29 January and 21 April and from 90 days to 1 day before egg laying.

Timing of reproduction.—Florida Scrub-Jays in suburban habitats initiated first clutches significantly earlier than jays in the natural habitat (Student's *t*-test, $t = -6.52$, $P < 0.001$, $n = 68$; Fig. 2). Of the 32 females that laid eggs in the suburbs, the mean laying date of the first egg in the first clutch was 24 March ± 2.3 (SE) days, and laying dates of first eggs ranged from 5 March through 23 April. In contrast, of the 36 females that laid eggs in the natural habitat, the mean laying

Fig. 2 Horizontal box plot of the timing of firstlaid clutches of the Florida Scrub-Jay populations in suburban and wildland habitats. Vertical lines within the box represent the median laying date. The box represents the middle 50% of the population and error bars represent the first 10 and 90% of the populations. Filled circles represent outliers that began laying very early or very late
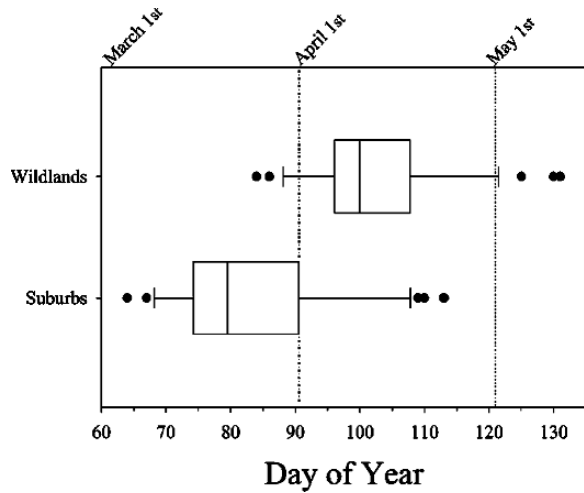
date was 13 April ± 1.95 (SE) days, and laying dates of first eggs ranged from 25 March to 11 May. Although dates of breeding differed, the distribution of laying dates within each population was similar. In each population, breeding was relatively synchronous with 50% of all female breeders laying within 15 days of the first egg laid in each population (Fig. 2).

Plasma protein.—Plasma protein levels increased as laying date neared ($F = 57.4$, df $= 1$ and 66, $P < 0.001$) and differed by site ($F = 5.0$, df $= 1$ and 66, $P = 0.03$). In addition, a significant interaction existed ($F = 5.9$, df $= 1$ and 63, $P = 0.02$; Fig 3A). The corrected model was highly significant ($adj\ R^2 = 0.49$, $P < 0.001$). Plasma protein levels were higher in the suburbs long before egg laying but increased more slowly than in the wildlands. Plasma protein levels increased with calendar date ($F = 25.7$, df $= 1$ and 80, $P < 0.001$) and were significantly higher in the suburbs than in the wildlands ($F = 4.4$, df $= 1$ and 80, $P = 0.04$). No interaction between site and calendar date existed ($F = 1.2$, df $= 1$ and 78, $P = 0.27$; Fig. 4A). The corrected model was significant ($adj\ R^2 = 0.23$, $P < 0.001$).

Several previous publications have equated plasma protein levels with overall body condition (Dawson and Bortolotti 1997, Ots et al. 1998). We, therefore, examined the relationship between plasma protein levels and body condition index. Plasma protein levels were significantly correlated with the body condition index in both populations (Pearson correlation: natural habitat, $r = 0.52$, $P = 0.01$; suburban habitat, $r = 0.37$, $P = 0.04$).

Total body lipids.—Total body lipids decreased as laying date neared ($F = 4.0$, df $= 1$ and 66, $P = 0.05$), but did not differ by site ($F = 0.0$, df $= 1$ and 66, $P = 0.99$), nor was there a significant interaction ($F = 0.5$, df $= 1$ and 63, $P = 0.83$; Fig. 3B). However, the corrected model was not significant ($adj\ R^2 = 0.03$, $P = 0.14$). We found no significant variation in total body lipids relative to calendar date ($F = 0.6$, df $= 1$ and 80, $P = 0.43$), site ($F = 0.1$, df $= 1$ and 80, $P = 0.83$), nor was there an interaction ($F = 1.3$, df $= 1$ and 77, $P = 0.27$; Fig 4B). The corrected model was not significant ($adj\ R^2 = -0.02$, $P = 0.73$)

Body condition and mass.—Body condition increased as laying date neared ($F = 46.8$, df $= 1$ and 64, $P < 0.001$) but did not differ between site ($F = 0.6$, df $= 1$ and 66, $P = 0.43$) nor was there a significant interaction ($F = 1.8$, df $= 1$ and 61, $P = 0.19$; Fig. 3C). The corrected model was significant ($adj\ R^2 = 0.41$, $P < 0.001$). Body condition increased with calendar date ($F = 8.2$, df $= 1$ and 78, $P = 0.01$) but did not differ by site ($F = 0.3$, df $= 1$ and 78, $P = 0.60$) nor was there a significant interaction (F $= 0.1$, df $= 1$ and 75, $P = 0.73$; Fig. 4C). The corrected model was significant ($adj\ R^2 = 0.17$, $P = 0.02$).

Body mass increased as laying date neared ($F = 50.8$, df $= 1$ and 66, $P < 0.001$) but did not differ by site ($F = 2.1$, df $= 1$ and 66, $P = 0.15$) nor was there a significant interaction
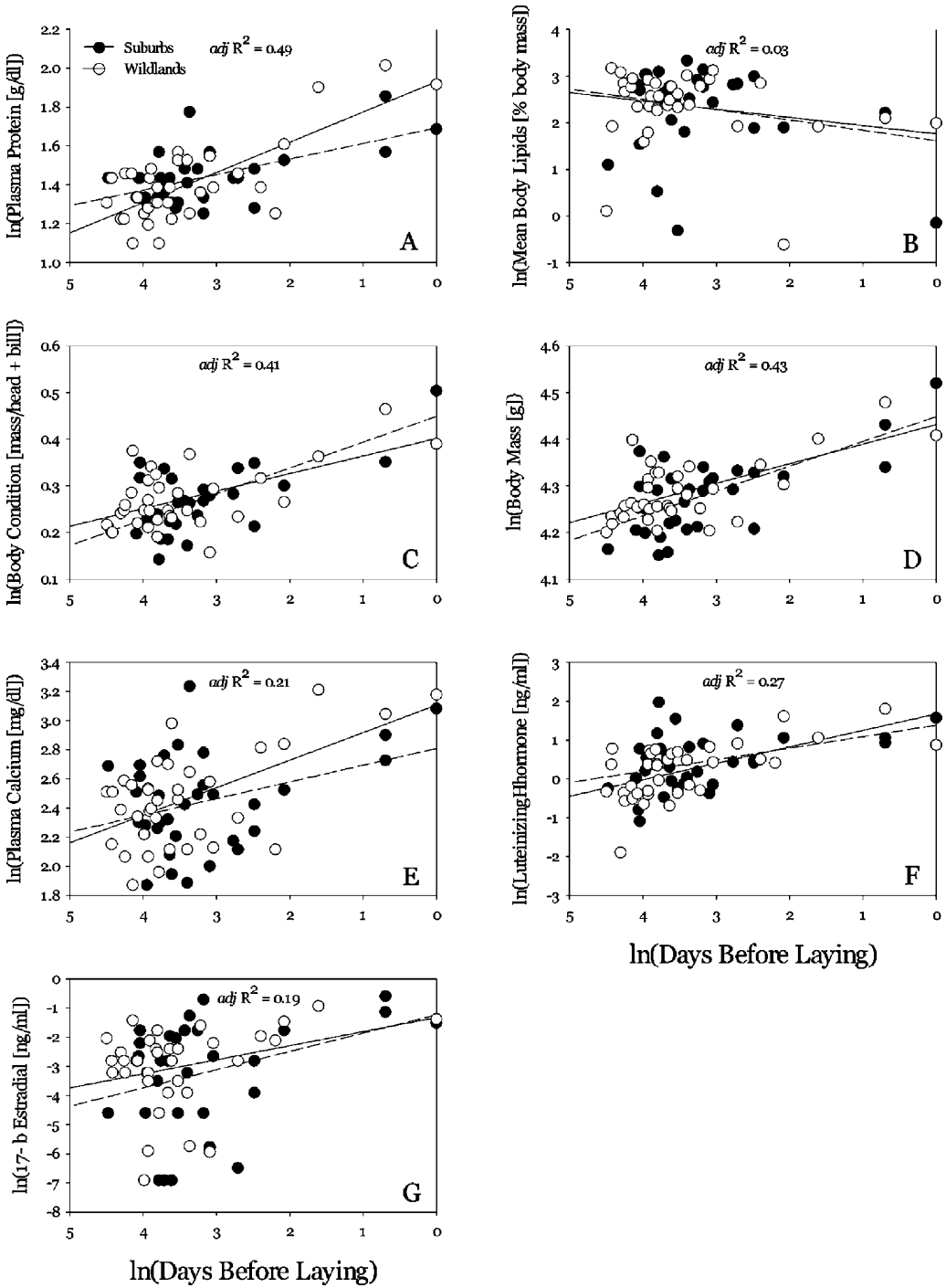
Fig. 3 A between-population comparison of female Florida Scrub-Jays relative to days before egg laying and (A) plasma protein levels, (B) total body lipids, (C) a size-corrected condition index, (D) body mass, (E) plasma calcium levels, (F) plasma luteinizing hormone levels, and (G) plasma estradiol levels. Suburban females are represented by closed circles and wildland females are represented by open circles. The dashed line represents the best-fit linear function for the suburban data and the solid line represents the same for wildland females
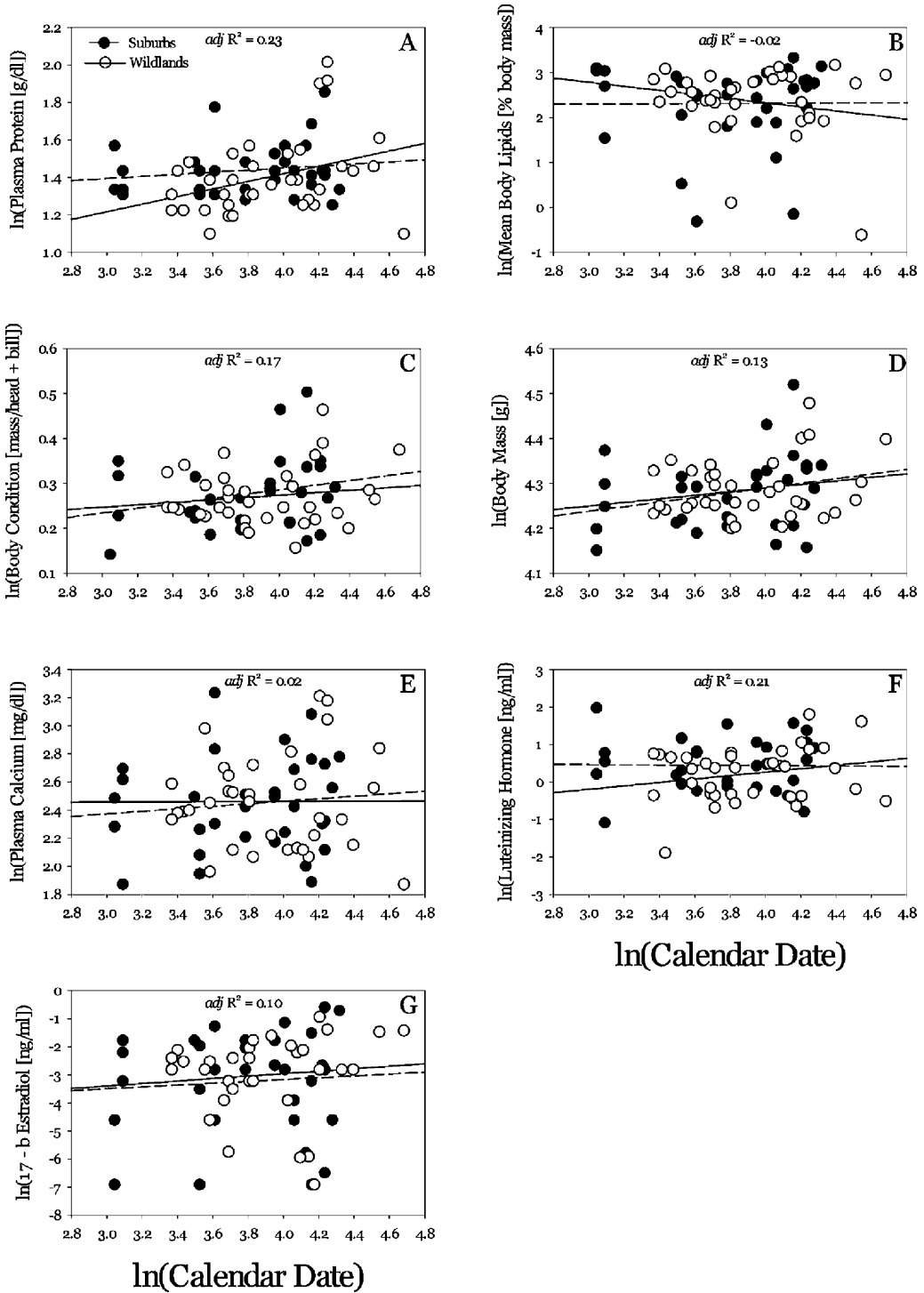
Fig. 4 A between-population comparison of female Florida Scrub-Jays relative to calendar date and (A) plasma protein levels, (B) total body lipids, (C) a size-corrected condition index, (D) body mass, (E) plasma calcium levels, (F) plasma luteinizing hormone levels, and (G) plasma estradiol levels. Suburban females are represented by closed circles and wildland females are represented by open circles. The dashed line represents the best-fit linear function for data from suburban females and the solid line represents the same for wildland females

($F = 0.7$, df $= 1$ and 63, $P = 0.41$; Fig. 3D). The corrected model was significant ($adj\ R^2 = 0.43$, $P < 0.001$). Body mass increased with calendar date ($F = 13.4$, df $= 1$ and 80, $P < 0.001$) but did not differ by site ($F = 0.4$, df $= 1$ and 80, $P = 0.52$) nor was there a significant interaction ($F = 0.4$, df $= 1$ and 77, $P = 0.52$; Fig. 4D). The corrected model was significant ($adj\ R^2 = 0.13$, $P = 0.02$)

   Plasma calcium.—Plasma calcium increased as laying date neared ($F = 19.0$, df $= 1$ and 67, $P < 0.001$) but did not differ by site ($F = 0.6$, df $= 1$ and 67, $P = 0.46$) nor was there a significant interaction ($F = 1.1$, df $= 1$ and 64, $P = 0.29$; Fig. 3E). The corrected model was significant ($adj\ R^2 = 0.21$, $P < 0.001$). Plasma calcium increased with calendar date ($F = 4.1$, df $= 1$ and 80, $P = 0.04$) but did not differ by site ($F = 0.3$, df $= 1$ and 80, $P = 0.57$) nor was there a significant interaction ($F = 1.1$, df $= 1$ and 78, $P = 0.31$; Fig. 4E). The corrected model was not significant ($adj\ R^2 = 0.02$, $P = 0.14$).

   Luteinizing hormone.—Luteinizing hormone increased as laying date neared ($F = 24.3$, df $= 1$ and 66, $P < 0.001$) but did not differ by site ($F = 0.7$, df $= 1$ and 66, $P = 0.40$) nor was there a significant interaction ($F = 0.3$, df $= 1$ and 63, $P = 0.76$; Fig. 3F). The corrected model was significant ($adj\ R^2 = 0.27$, $P < 0.001$). Luteinizing hormone increased with calendar date ($F = 21.0$, df $= 1$ and 80, $P < 0.001$) and was significantly higher in the suburbs ($F = 6.7$, df 1 and 80, $P = 0.01$), but there was no significant interaction ($F = 0.4$, df $= 1$ and 77, $P = 0.53$; Fig. 4F). The corrected model was significant ($adj\ R^2 = 0.21$, $P < 0.001$).

   17β-Estradiol.—Estradiol increased as laying date neared ($F = 7.9$, df $= 1$ and 63, $P = 0.01$) but did not differ by site ($F = 1.0$, df $= 1$ and 63, $P = 0.33$) nor was there a significant interaction ($F = 0.1$, df $= 1$ and 60, $P = 0.72$; Fig. 3G). The corrected model was significant ($adj\ R^2 = 0.19$, $P = 0.02$). We found no significant variation in estradiol relative to calendar date ($F = 2.7$, df $= 1$ and 77, $P = 0.10$), site ($F = 0.5$, df $= 1$ and 77, $P = 0.49$), or their interaction ($F = 0.2$, df $= 1$ and 74, $P = 0.67$; Fig 4G). The corrected model was not significant ($adj\ R^2 = 0.10$, $P = 0.25$).

Discussion

We found that scrub-jays in the suburbs nested earlier than jays in wildlands and that plasma levels of protein differed between sites relative to both days before egg laying and calendar date. At least for plasma protein, those patterns are consistent with our hypothesis that in suburban habitats human-provided foods improve the condition of adult scrub-jays, providing them with additional endogenous resources that enable them to breed earlier. However, we found no other condition-related differences between sites.

   Few supplementation studies have attempted to examine the relative importance of different nutrients on the timing of breeding. Of those, all have attempted to compare a high protein diet with a high energy diet, and although supplemented birds began breeding earlier than controls in all cases, the specific effects of different nutrients could not be determined (Bolton et al. 1992, Nager et al. 1997, Ramsay and Houston 1997). Human-provided foods make up 30% of the diet of prebreeding females in suburbs and of that, peanuts constitute 30% (Fleischer et al. 2003). With the exception of peanuts, which are relatively rich in proteins (Karasov 1990), we have few data on the nutritional composition of the human-provided foods consumed by suburban scrub-jays. In contrast, adult females in wildlands depend heavily on acorns cached during the previous fall (Woolfenden and Fitzpatrick 1984, 1990). They may augment that diet with arthropods and small vertebrates, but both of those food sources can be scarce in winter. Although acorns also are relatively rich in proteins, tannins in acorns may reduce the ability of scrub-jays to assimilate proteins (Koenig 1991, Fleck and Woolfenden 1997). Thus, it seems reasonable to postulate that jays in wildlands might be

protein limited during years in which environmental conditions delay or decrease the availability of protein-rich food sources, such as arthropods; whereas jays in suburbs have predictable, essentially *ad libitum*, access to high-protein foods.

In both populations, plasma protein increased with calendar date and as laying neared; however, the rate of increase was slower in suburbs. In many birds, elevated levels of plasma protein as laying approaches reflects an increase in vitel-logenin (White 1991). This protein is produced in the liver in response to endocrine signals (primarily estradiol), transported to the ovary via the bloodstream, and incorporated into the yolk to be mobilized later as an essential nutrient by the developing embryo (Jackson et al. 1977, Ho 1991, Deeley et al. 1993, Carey 1996). It may be that plasma protein levels that exist at the onset of vitellogenesis influence the rate of additional protein production; thus suburban birds would likely add protein at a slower rate (Figs. 3A and 4A).

We also found that LH was higher in the suburbs relative to calendar date, but not days before laying. That pattern suggests that site-specific differences in LH are a result of differences in timing of breeding rather than a cause of the difference. In many seasonally breeding animals, environmental changes cause an increase in hypothalamic secretion of gonadotropin-releasing hormone (GnRH) that results in increased pituitary release of LH that, in turn, induces increases in other reproductive hormones (for reviews see Wingfield and Kenagy 1991, Wingfield and Farner 1993). Both field and laboratory studies confirm that changes in environmental conditions that precede conditions favorable for reproduction precipitate those endocrine changes (for reviews see Immelmann 1971, 1973; Farner and Follett 1979; Wingfield and Kenagy 1991; Wingfield et al. 1993, 2000; Hahn et al. 1997). Dietary restrictions are well known to negatively affect the reproductive axis (see Tanabe et al. 1981, Hocking et al. 1987, Katanbaf et al. 1989, Wade and Schneider 1992, Hocking 1996, Hocking and Bernard 1998, Renema et al. 1999). In wildlands, during years in which peak food availability is delayed, those endocrine changes may be dampened, delaying gonadal recrudescence and ooge-nesis and, in turn, delaying breeding (Balthazart 1983, Wingfield and Farner 1993, Schoech and Lipar 1997). We suggest that the temporal differences in LH between the two sites simply reflect the differences in timing of breeding between the two sites that result from differential access to protein-rich foods.

Surprisingly, we found few differences in condition-related or other micronutrient measures between sites relative to calendar date, even though many of those measures tended to increase during the season. This suggests that those variables, although associated with breeding, have a relatively small influence on timing of breeding. Some researchers have postulated that plasma protein levels reflect overall body condition. For example, Ots et al. (1998) concluded that urban-dwelling male Great Tits (*Parus major*), which had higher plasma protein levels than nearby rural birds, were in better condition. Similarly, Dawson and Bortolotti (1997) suggested that plasma protein values can be used as an index of condition in American Kestrels (*Falco sparverius*). Our data show that plasma protein levels were correlated with body condition, but body condition *per se* did not appear to be affected by food supplementation nor appear to be related to the differences in timing of breeding between sites. It is possible that other environmental conditions in suburban habitats also affect body condition, independently of plasma protein levels.

It is somewhat surprising that we found no between-population differences in plasma calcium levels or total body lipids. In the suburbs, calcium is easily available; all roads are built upon a crushed shell base. Calcium is likely highly limited in the low pH environments typical of wildland scrub. Although natural calcium sources may be scarce in scrub habitats, birds may know where to find it when necessary during the breeding season. It may only be when natural sources of calcium are reduced through anthropogenic effects that reproduction is limited by calcium (Graveland et al. 1994). Schoech (1996) found that scrub-jays provided supplemental food in wildlands bred earlier and had higher levels of total body lipids than did control birds. Peanuts are relatively rich in both protein and lipids (Karasov 1990); thus it is somewhat surprising that we did not see a similar pattern in suburban jays.

Equally intriguing is our finding that total body lipids appeared to decline prior to egg laying in both populations (see Fig. 3B), although our model explained relatively little of the variation in the data. Whereas some avian species rely on endogenous reserves, most passerines appear to depend on exogenous resources through increased food intake to fuel reproduction (Perrins 1970, 1996). That apparent dichotomy in strategies to fuel reproduction, termed capital versus income strategies (Drent and Daan 1980, Meijer and Drent 1999), may be better viewed as a continuum between the two extremes of total reliance on either endogenous or exogenous resources. For example, Williams and Ternan (1999) found that female Zebra Finches (*Taeniopygia guttata*) reduce locomotor activity rather than increase food intake during oogenesis, and it is likely that other species meet part of the increased energetic or nutritional demands of oogenesis by reducing their daily energetic expenditures by reallocating time spent in various activities. Prebreeding female scrub-jays in suburban habitats reduce the proportion of time they spend foraging while increasing time perched (Fleischer et al. 2003), which suggests that when provided with supplemental food, they too reduce and reallocate their daily energy expenditure. Similarly, food-supplemented wildland jays spend less time foraging than unsupplemented jays (S. J. Schoech and R. Bowman unpubl. data).

Given the proximity of these two populations, it seems unlikely that the difference in timing of breeding could be explained by differences in any of the seasonally variable abiotic environmental factors used by birds to time reproduction (e.g. photoperiod, temperature, rainfall, or relative humidity), though suburban light pollution and heat island effects could be important and should be considered. However, observations that the phenology of oak leaf-out does not differ between sites (R. Bowman unpubl. data) are inconsistent with the hypothesis that temperature differences exist between the suburban and wildland study sites. Suburban habitats are lighter at night than the wildlands (R. Bowman and S. J. Schoech unpubl. data). Although constant dim light can affect biological rhythms in birds (Kumar et al. 2000), there is little information on whether low levels of nighttime light accelerate the seasonal physiological changes that occur in breeding birds.

The data presented here support the hypothesis that differential access to exogenous protein sources between suburban and natural habitats underlies the observed between-population differences in timing of breeding. Variation of plasma protein explained a much higher proportion of variation in breeding date than did models for other variables; however, it seems likely that other environmental effects, in addition to food, may influence timing of reproduction. For example, human-provided foods are spatially and temporally predictable, and as a result, suburban scrub-jays are much more efficient foragers than are jays in wildland habitats. Foraging efficiency may be a perceptual cue that jays use to predict future levels of resource abundance. In tits, capture rates of lepidopteran larva likely provide a cue for initiating breeding because rising capture rates accurately predict the increase in resource availability (Perrins 1991). However, human-provided foods are unlikely to be a reliable cue of future foods that are suitable for nestlings, and as a result, the advancement of reproduction might have negative effects on the fitness of both parents and offspring, as has been observed in Blue Tits (*Parus caeruleus*; Nilsson 1994) and European Coots (*Fulica atra*; Brinkhof 1995). Clearly, further research is needed to distinguish between those competing hypotheses, but our data suggest a role for protein in the decision of when a female initiates her clutch.

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