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HEMATOCRIT VARIATION IN RESPONSE TO ALTITUDE CHANGES IN WILD BIRDS: A REPEATED-MEASURES DESIGN

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Abstract. Seasonal migration of a bird from lowlands to montane habitats should induce an increase in its hematocrit value. This relationship has been studied mainly in domestic species, however, and data on wild birds are mostly correlational. We report our results on variation in hematocrit values of the Citril Finch (*Serinus citrinella*), which migrates altitudinally. Data were taken from wild birds trapped within the same season at different altitudes and from an experiment in which Citril Finches were transported to different altitudes (600 to 2000 m above sea level). On average, hematocrit values from Citril Finches trapped at higher altitudes in the wild were 3% higher than those of birds collected at lower levels. In the experiment in which Citril Finches were transported to different altitudes, individual birds were measured repeatedly, so that the same individuals acted alternately as controls or experimental subjects. Hematocrit values of experimental and control individuals did not vary initially but varied over the course of the experiment, decreasing when birds were moved to lower altitudes and increasing when they were moved to higher altitudes by an average of 6%. These results indicate that wild birds that migrate altitudinally must contend with an increase in hematocrit values. Transporting the birds to altitudes within the species' range allowed us to exclude the possibility that the increase in hematocrit from lower to higher altitudes was the result of the flight itself and demonstrates an adjustment in hematocrit not induced by the exercise of flight.

Key words: *hematocrit value, hct, Citril Finch, altitudinal migration, repeated-measures experimental design,* Serinus citrinella.

Variación en el Hematocrito como Respuesta a Cambios de Altitud en Aves Silvestres: Un Diseño de Medidas Repetidas

Resumen. La migración estacional de un ave entre ambientes de tierras bajas y de montaña debería inducir un incremento en su valor de hematocrito. Sin embargo, esta relación se ha estudiado principalmente en especies domésticas y los datos sobre aves silvestres estan principalmente basados en correlaciones. En este trabajo presentamos resultados sobre la variación en valores de hematocrito de *Serinus citrinella*, una especie que migra altitudinalmente. Los datos fueron tomados de aves silvestres capturadas en la misma estación a diferentes altitudes y de algunos individuos que fueron transportados experimentalmente a altitudes diferentes (600 a 2000 m sobre el nivel del mar). En promedio, los valores de hematocrito de los individuos capturados a mayores altitudes en el campo fueron 3% mayores que los de aves capturadas a altitudes menores. En el experimento en el que las aves fueron transportadas a diferentes altitudes, los mismos individuos fueron estudiados con un diseño de medidas repetidas, de manera que cada individuo actuaba alternadamente como control o sujeto experimental. Los valores de hematocrito de los individuos experimentales y de control no variaron inicialmente pero sí lo hicieron en el transcurso del experimento, disminuyendo cuando las aves fueron transportadas a altitudes menores y aumentando cuando fueron transportadas a altitudes mayores en un promedio de 6%. Estos resultados indican que las aves silvestres que migran altitudinalmente deben lidiar con un aumento en los valores de hematocrito. Transportar a las aves a los extremos de altitud dentro de los rangos de variación de la especie nos permitió excluir la posibilidad de que el aumento en el hematocrito de altitudes menores a altitudes mayores fuera un resultado del vuelo en si mismo y demostró un ajuste en el hematocrito que no fue inducido por el ejercicio del vuelo.

INTRODUCTION

Seasonal migration in which a bird moves from lowlands to montane habitats greatly increases its metabolic costs (Gwinner 1998). In montane environments, barometric pressures, partial pressures of oxygen, and temperatures are lower than at lower elevations, increasing a bird's metabolism (Swanson 1990). As a consequence, birds migrating to higher altitudes have to increase the supply of oxygen to their tissues, hence increasing hematocrit values by 7–8% in a move to altitudes of 3000 m above sea level (Jaeger and McGrath 1974, Carey and Morton 1976, Keys et al. 1986, Clemens 1988, 1990, Morton 1994, Balog et al. 2000, Fair et al. 2007). In not all species studied, however, has an increase in hematocrit has been recorded (Carpenter 1975, Morton 1976, Carey et al. 1993, Ruiz et al. 1995).

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Although there is extensive literature on variation in hematocrit values in response to variations in altitude, most works to date refer to domestic birds, either transported to mountain tops or acclimated to artificially reduced barometric pressures or low oxygen concentrations in the laboratory (McGrath 1971, Jaeger and McGrath 1974, Weinstein et al. 1985, Yersin et al. 1992, Maginniss et al. 1997). Currently available data on wild birds either refer to embryos (e.g., Carey et al. 1993) or compare different species or individuals within the same species inhabiting different habitats or altitudes (Carey and Morton 1976, Clemens 1988).

As variation in hematocrit values in response to altitudinal movements plays a significant role in the biology of birds that migrate seasonally, this topic should ideally be studied with wild birds, under natural experimental conditions, and with repeated measures. A study so designed is the only way any confounding effect, such as sex (Morton 1976, Keys et al. 1986), body condition (Potti et al. 1999, Hunter and Powers 1980, Donnelly and Sullivan 1998) individual variation, or season (Morton 1976, Hunter and Powers 1980, Swanson 1990) can be overridden. Such a design could also control for collateral effects of captivity, such as a decrease (because of stress) or an increase (because of food and water available *ad libitum*) in erythrocyte production (Keys et al. 1986), which can affect hematocrit values.

The Citril Finch (*Serinus citrinella*) is a small (12 g) carduline finch inhabiting the boreal zones of the mountains of temperate western Europe. The Pyrenees support the highest densities of the species in Europe, at altitudes ranging from 1800 to 2500 m above sea level (Baccetti and Märki 1997, Borras and Senar 2003). The Citril Finch typically abandons the mountains by the end of autumn, moving to lower altitudes (0–1000 m) for the winter before returning to the mountains with spring (Borras and Junyent 1993). Because of this altitudinal migration it is therefore an ideal species for study of variations in hematocrit values.

We studied the variations in hematocrit values of Citril Finches inhabiting different altitudes. Furthermore, to establish causal relationships, we transported birds to different altitudes. We used a within-individual experimental design (repeated measures) so that one individual acted alternately as control or experimental subject. This design allowed us to test for a causal link between variations in hematocrit values and altitude in a wild altitudinal migrant. Although any variation in hematocrit is likely the result of a reduction in both temperature and partial pressure of oxygen at higher altitudes, altitude is the variable of biological significance and interest we analyze.

Hematocrit is the ratio of volume of total red blood cells to total volume of blood. Therefore hematocrit can change not only as the result of variation in altitude but also from swelling or shrinkage of the erythrocytes or a change in the volume of vascular fluid. Hemoglobin concentration is the true variable that reflects the blood's capacity to carry oxygen. Because of the characteristics of our experiment (see below), however, we assumed that hematocrit value reflects hemoglobin concentration accurately. Additionally, the hematocrit and oxygen-carrying capacity (and/or hemoglobin concentration) are generally correlated (Birchard 1997).

MATERIAL AND METHODS

HEMATOCRIT VALUE FROM WILD BIRDS

We trapped Citril Finches in April and May, 2003, 2005, and 2006, simultaneously at both low (500–800 m) and high (2000 m) elevations. We aged (as yearlings or adults) and sexed the birds according to Svensson (1992) and Jenni and Winkler (1994). We sampled blood from the wing vein by venipuncture, taking a single capillary tube of blood from each individual. Samples were refrigerated vertically, and hematocrit values were later measured in the laboratory. On average, the time from collection to centrifugation was ~5 hr for all samples. Samples were centrifuged at 11 000 rpm for 10 min. The column of cells to plasma was measured with calipers at a precision of 0.01 mm. We analyzed results for males only ($n = 98$) to avoid the confounding effect of sex and because the sample of females was smaller. We detected no variation in hematocrit values by year ($F_{2,54} = 1.19$, $P = 0.31$; effect of age controlled for age by inclusion in ANOVA; n by year: 2003, 4; 2005, 37; 2006, 19), so we pooled data from the three years.

HEMATOCRIT VALUE FROM EXPERIMENTALLY TRANSPORTED BIRDS

For the experiment with captive birds, we trapped Citril Finches on 3 July 2006 around Vansa (elevation 2000 m; Port del Comte, Lleida Pyrenees, northeastern Spain) (Senar et al. 2002). Only birds with juvenile plumage were used for the experiment (i.e., hatched within the year; EURING age 3J). The experiment consisted of caging the birds into two groups of nine. They were allowed food and water *ad libitum*. The food was commercial canary food, with the addition of seeds of niger (*Guizotia abyssinica*) and rape (*Brassica napus*) as substitutes for the seeds of the Asteraceae and Brassicaceae that the species normally eats in the wild. During the experiment, one of the birds escaped, causing an imbalance in numbers between the two groups. The cages measured $120 \times 50 \times 40$ cm. Hematocrit values were obtained as explained above. One of the groups was moved to lower altitudes (Clariana de Cardener, Solsonés, elevation 600 m), the other, the control group, to the Bofia area, a distance of 5 km but also at 2000 m. The cages were set outdoors inside a larger roofed cage to protect the birds from rain, wind, and predators. One month later, we again measured hematocrit value and reversed the experiment, moving birds from the low site to the high one and those from the high site to the low one. One month later we again measured hematocrit values. This procedure allowed for the same individual to act as a control and as an experimental subject alternately. Since birds' hematocrit values adjust in short periods, even days (Jaeger and McGrath 1974, Weathers and

Snyder 1974, Clemens 1990), our delay of one month is conservative and sufficient to detect differences in this variable.

TEMPERATURE VARIATION ASSOCIATED WITH ALTITUDE

Temperature data for 2003–2006, from the web page of the Servei Meteorològic de Catalunya (http://www.meteo.cat/mediambxemec/servmet/index.html), are from two weather stations, Clariana de Cardener (Solsonés, elevation 600 m) and Port del Comte (elevation 2000 m), both near the sites of our experiment with captive birds. Pairing the average temperature in April and May at each locality, we analyzed the data with a repeated-measures ANOVA, where locality was one factor and month was another. The average temperature at the higher elevation was significantly less than that at the lower elevation: average temperature, high elevation, 2.3 °C (SE.0.59); low elevation, 13.7 °C (SE 0.54) (altitude $F_{1,6}$ = 4785.49, $P < 0.001$; month $F_{1,6} = 18.32$, $P < 0.01$; interaction $F_{1,6} = 0.02$, $P = 0.88$). Similarly, during the experiment (July–September 2006), the temperature at the higher locality was significantly less than at the lower locality: average temperature, high elevation, 10.4 °C (SE 1.52); low elevation, 21.2 °C (SE = 1.65); $F_{1,2} = 1249.71$, $P < 0.001$).

STATISTICAL METHODS

In the analysis of the hematocrit value of wild birds, the dependent variable was hematocrit, and independent variables included the elevation at which birds were trapped (low or high) and the bird's age (yearling or adult). We tested the hematocrit values' departure from normality with a Kolmogorov–Smirnov test. Since no departure was detected (see results), we used a parametric two-way ANOVA.

We analyzed the hematocrit variation in experimental captive birds with a repeated-measures ANOVA. The hematocrit value was the dependent variable, and the independent variables included treatment (whether birds stayed at the low or the high elevation) and period (at capture, after 1 month, or after 2 months). Treatment was reversed after 1 month, so that all the birds experienced both treatments but at a different period. This procedure allowed us to study the effect of altitude within an individual, with a repeated-measures design, controlling for the effect of the order in which each bird received each treatment (i.e., period).

RESULTS

HEMATOCRIT VALUES OF WILD BIRDS

The average hematocrit value of wild Citril Finches in spring (April–May) was 57.0% (SE 0.37, $n = 98$, average value for both low- and high-elevation populations; Kolmogorov–Smirnov test for departure from normality: $d = 0.06$, $P = 0.20$). Birds trapped at the higher elevation had on average a hematocrit value 3% higher than those at the lower elevation (Fig. 1) (ANOVA factor elevation: $F_{1,94} = 15.12$, $P < 0.001$). We detected no differences by age (ANOVA factor age: $F_{1,94} = 0.01$, $P = 0.94$, interaction elevation × age: $F_{1,94} = 1.22$, $P = 0.27$).

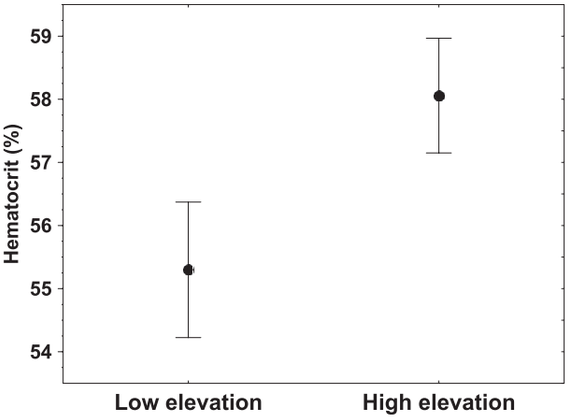


FIGURE 1. Variation in hematocrit values of the Citril Finch, comparing birds from low elevations (500–800 m) and subalpine high elevations (2000 m). Values have been standardized according to age by ANOVA.

HEMATOCRIT VALUE OF EXPERIMENTALLY TRANSPORTED BIRDS

The initial hematocrit value of experimental and control birds did not differ (mean 55.1%, SE 0.64; planned comparison: $F_{1,15} = 0.21$, $P = 0.66$). But the values changed with altitude manipulation over the course of the experiment (repeated-measures ANOVA, treatment × period interaction: $F_{2,30} = 16.11$, $P < 0.001$, $n = 17$ birds; Fig. 2). Overall, the hematocrit

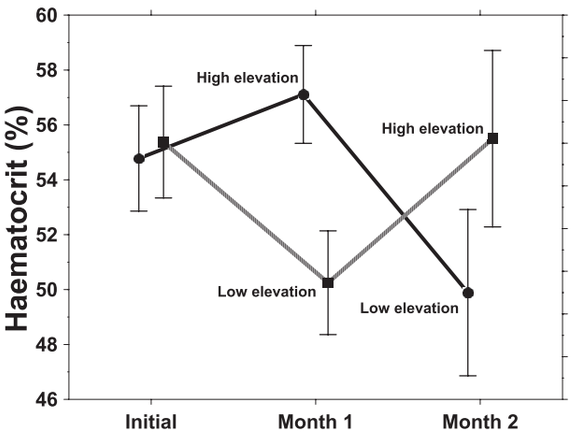


FIGURE 2. Variation in hematocrit values of the Citril Finch through the experiment, comparing birds moved from lowlands (600 m) to highlands (2000 m) in a simulated altitudinal migration and control birds (moved within the highlands). One month later the treatment was reversed, so that the control birds were moved within the lowlands and the birds used in the previous experimental birds were moved from lowlands to highlands.

value of a bird experimentally moved to lower elevation (600 m) was lower than when it acted as a control, remaining at high elevation (2000 m) (repeated-measures ANOVA comparing experimental vs. control hematocrit value: high elevation, 56.4%, SE 1.01; low elevation, 50.1%, SE 0.61; $F_{1,16} = 45.4$, $P < 0.001$).

DISCUSSION

Hematocrit values of wild Citril Finches trapped in high-elevation subalpine habitats (2000 m) were on average 3% higher than those of birds captured within the same period but at lower elevations (500–800 m). The change in hematocrit value could reflect altitude acclimation, as found in other species of birds, but could also reflect the effort involved in migration (Jenni et al. 2006). However, by transporting the birds altitudinally within the species' range, our experiment allowed us to exclude the possibility that the increase in hematocrit from lower to higher elevations was the result of the flight itself. The result is robust because of the repeated-measures design that demonstrates for the first time in a songbird adjustment in hematocrit not induced by the exercise of flight. Our results confirm the effect of altitude on hematocrit data observed in domestic birds (Yersin et al. 1992) and correlative studies (Carey and Morton 1976). The change we found in hematocrit values in response to different altitudes (6%) is similar to that reported in other species in other situations that consume energy: Potti et al. (1999) found the hematocrit value of Pied Flycatchers (*Ficedula hypoleuca*) in mite-infested nests to be 6% less than that of healthy birds, and Keys et al. (1986) found that the hematocrit values of female Brown-headed Cowbirds (*Molothrus ater*) decreased by 5.5% before and during egg production. Bordel and Haase (1993) found that hematocrit values of homing pigeons (*Columba livia*) in sustained flight decreased by 3.4% (Bordel and Haase 1993), and Jenni et al. (2006) found that in several passerines hematocrit decreased by 3% during long-distance migration (Jenni et al. 2006). Dark-eyed Juncos (*Junco hyemalis*) experience seasonal fluctuations in hematocrit values of 5.4% as a consequence of temperature variations (Swanson 1990). Furthermore, Fair et al. (2007) stressed the increase in hematocrit in energetically demanding situations like migration, reproduction, and parasitism. The change in hematocrit in the Citril Finch in relation to different elevations therefore suggests that altitudinal migration has a cost to fitness that may be similar to that of other highly energy-demanding events in the life history of birds.

The increase in hematocrit as the result of an increase in elevation could be due either to hypoxia or to thermoregulation in response to the decrease in temperature (Carey and Morton 1976). Whatever the reason, however, our data support the hypothesis that the typical altitudinal migration of the Citril Finch in spring may entail a physiological cost related to an increase in hematocrit.

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