

# Life-history variation of a neotropical thrush challenges food limitation theory

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Since David Lack first proposed that birds rear as many young as they can nourish, food limitation has been accepted as the primary explanation for variation in clutch size and other life-history traits in birds. The importance of food limitation in life-history variation, however, was recently questioned on theoretical grounds. Here, we show that clutch size differences between two populations of a neotropical thrush were contrary to expectations under Lack's food limitation hypothesis. Larger clutch sizes were found in a population with higher nestling starvation rate (i.e. greater food limitation). We experimentally equalized clutches between populations to verify this difference in food limitation. Our experiment confirmed greater food limitation in the population with larger mean clutch size. In addition, incubation bout length and nestling growth rate were also contrary to predictions of food limitation theory. Our results demonstrate the inability of food limitation to explain differences in several life-history traits: clutch size, incubation behaviour, parental feeding rate and nestling growth rate. These life-history traits were better explained by inter-population differences in nest predation rates. Food limitation may be less important to life history evolution in birds than suggested by traditional theory.

**Keywords:** life-history traits; food limitation; nest predation; Rufous-bellied thrush; clutch size; parental behaviour

## 1. INTRODUCTION

A major focus of life-history theory is to try and understand the underlying causes of variation in clutch size and other life-history traits (Roff 1992). Food limitation is widely considered to be the major determinant of clutch size and other life-history traits in birds (Lack 1947; reviewed in Martin 1987). Greater food limitation can reduce the number of young that parents attempt to raise because of mortality from greater starvation as well as impaired growth that can affect survival after fledging (Lack 1947; Martin 1987; Saether 1994). Much of the evidence for food limitation, however, is based on studies within single populations, which may overemphasize the importance of food because the variation and possible effects of other sources of selection may be minimized (Martin 2004). Nest predation, for example, which accounts for most reproductive mortality in birds (Martin 1993), may vary more among than within species and among than within populations (Martin 1995). Comparative studies among species have indeed suggested that nest sites and associated nest predation play a stronger role than food limitation in life-history variation (Martin 1995; Owens & Bennett 1995; Martin *et al.* 2000a; reviewed in Martin 2004; but see Saether 1994). Studies of life-history differences among rather than within populations similarly may provide alternative perspectives for the relative importance of food versus other selection pressures.

Here, we report on the importance of food limitation versus nest predation on life-history differences between two populations of a neotropical thrush, the Rufous-bellied thrush (*Turdus rufiventris*), separated by 1200 km in Argentina, South America.

We initially observed strong differences in clutch size between the two populations (mean clutch size  $\pm$  s.e. for Salta =  $2.7 \pm 0.05$ , range: 2–3,  $N=99$ ; for Buenos Aires =  $3.2 \pm 0.06$ , range: 2–5,  $N=84$ ;  $F=34.514$ ,  $p<0.001$ ). We subsequently examined additional life-history traits because they provide contrasting predictions about responses to food limitation versus nest predation (Martin 1996, 2004), and thereby increase the power of our tests. Greater food limitation, for example, may not only favour smaller clutch sizes, but may also favour shorter on-bouts and longer off-bouts during incubation, lower parental visitation rates to feed young and slower growth of chicks (Lack 1947; Martin 1987, 1996; Saether 1994; Conway & Martin 2000).

Greater nest predation also favours smaller clutch sizes (Skutch 1949; Slagsvold 1982), but nest predation is expected to favour a different pattern of incubation bout lengths, parental feeding rates, and nestling growth rates. In particular, predation by visually oriented predators selects against high activity at the nest (Skutch 1949; Martin *et al.* 2000a,b), which can favour longer on- and off-bouts during incubation (Weathers & Sullivan 1989; Conway & Martin 2000) and lower parental visitation rates to feed nestlings (Martin *et al.* 2000a,b). In addition, young should grow faster to reduce accumulating daily risk of predation (Bosque & Bosque 1995; Remeš &

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Martin 2002). The predicted increases in on-bouts and growth rates are contrary to expectations from the food limitation hypothesis (Martin 1995, 1996; Conway & Martin 2000). Consequently, the life-history syndrome (i.e. covarying expression of a suite of life-history traits; Martin 2004) is expected to differ under food limitation versus nest predation, allowing us to test these alternatives.

Mortality from food limitation versus nest predation represents the selection pressures that favour these alternative life-history syndromes. Inference concerning the importance of a selection pressure (i.e. food limitation or nest predation) is strengthened if predicted differences in a life-history syndrome are shown to directly covary with the source of selection (i.e. mortality) predicted to favour the syndrome. Consequently, we examined differences in mortality between our populations by directly measuring nest predation and starvation rates. We then compared differences in these specific forms of mortality relative to predicted life-history syndromes between our two populations of neotropical thrushes.

## 2. MATERIAL AND METHODS

### (a) *Study areas and species*

We conducted our study on two sites in Argentina: a subtropical forest located in El Rey National Park, Salta Province (Martin *et al.* 2000a) on the western side of Argentina and a subtropical, forested savannah in Escobar, Buenos Aires province on the eastern side, from October to December 1997–2001. In both locations, we studied *T. rufiventris*, a sedentary resident species that is sexually monomorphic and weighs 70–80 g. We chose this species because it is common in a wide array of subtropical forest types in eastern Bolivia, Paraguay, northern Argentina and eastern Brazil (Ridgely & Tudor 1989).

Rufous-bellied thrushes breed in Argentina from early October to early January. They are typically single brooded, although a few early breeders re-nest after fledging a successful brood. The peak in nest initiation activity generally occurs in the first 15 days of October. Thrushes normally start incubating after laying their second egg. Thus, when the clutch is two eggs, the nestlings will hatch synchronously, but in clutches of three or four eggs, they hatch asynchronously (in 24-h intervals after the hatching of the first two nestlings). This particular incubation behaviour can yield differences in hatching asynchrony between populations that differ in clutch size, which is the case between the populations of Salta and Buenos Aires.

We intensively studied the two populations (312 total nests) to examine differences in life-history syndromes. We quantified the expression of a suite of life-history traits (i.e. clutch size, incubation bout lengths, parental activity at the nest, nestling growth rates) because traits tend to covary as components of a syndrome that may be selected by differing selection pressures (Martin 2004). In addition, we measured mortality from food limitation (i.e. starvation) versus nest predation, as the putative selection pressures predicted to favour these life-history syndromes.

### (b) *Nest location and monitoring*

We searched for thrush nests from the beginning of October to the end of December from 1997 to 1999 and 2001 at the Salta site (Martin *et al.* 2000a) and from 1998 to 1999 and

2001 at the Buenos Aires site. Nests were located using behavioural cues of the adults and by systematic search and were monitored every 2–4 days to assess the fate of the nests (Martin *et al.* 2000a). Nests were considered depredated if eggs or young disappeared when too young to fledge (nestlings usually fledge at around 15 days of age). Nests were considered successful if at least one nestling fledged. Clutch size was recorded when it did not change over two visits at least 2 days apart. Clutch sizes were not used from nests found during the nestling stage, nests that were depredated during laying or nests of heights that made it impossible to check their contents. We tested for clutch size differences between populations with a one-way ANOVA. Because some nests were found after the onset of incubation, we estimated daily predation rates (Mayfield 1975; Hensler & Nichols 1981) for all nests found. Nests that were abandoned before egg laying or that we were unable to determine the status of were not used for daily predation estimates. We used the CONTRAST program to compare daily predation rates (Hines & Sauer 1989).

Survival rate of nestlings for both populations was generated by the Kaplan–Meier survival analysis (SPSS v. 12). This analysis estimates conditional probabilities for each point in time where an event has occurred (mortality due to starvation or predation) and then estimates the survival rates at each time. Nestling survival was compared between populations using a log rank test.

### (c) *Video monitoring*

Parental activity during incubation and nestling periods was recorded using Hi-8-mm video cameras at the nest in the first 6 h after first light (Martin & Ghalambor 1999; Martin *et al.* 2000a). This approach standardized for time of day and duration of measurements. Videotapes were scored in the laboratory for length of incubation on- and off-bouts, and parental visitation rates during incubation and nestling periods. Nests were filmed during the first 5 days of incubation after clutch completion to control for any potential age effects, although subsequent detailed studies of incubation behaviour during early versus late incubation in a variety of species showed little change (Martin, unpublished data). Parental activity during the nestling stage was measured on the day that nestling primary feathers broke their sheaths or a day later to control for stage of development. We ultimately found that length of incubation bouts and parental activity in incubation and nestling stages did not differ relative to clutch size (Martin, unpublished data), but we only examined nests with three-egg clutches in order to ensure that any possible effect of clutch size was controlled. Nests were the sampling unit such that mean visits per hour for the incubation and nestling periods, as well as mean duration of incubation on- and off-bouts were calculated over the entire 6 h sampling period of each nest for the statistical comparisons using ANOVA.

### (d) *Experimental protocol for clutch manipulations*

We sought to examine starvation rates as the source of mortality selection from food limitation. To eliminate any population differences in clutch size and hatching asynchrony as possible influences on starvation rates, we experimentally manipulated clutches in 2001. We equalized clutch sizes between populations and synchronized hatching by replacing natural eggs with naturally coloured plaster eggs as they were laid. We held the removed eggs in cotton-filled film canisters

placed in a hole in the ground to maintain stable temperature conditions. Within 1 h of the last egg being laid (through intensive monitoring), we replaced the plaster eggs with a clutch size of three original eggs. This approach yielded clutch sizes of the same size and ensured synchronous hatching in both populations to control both variables for comparisons of starvation rates. This procedure did not affect hatching success. These experiments required that we find nests before laying began to enable swapping of eggs within an hour of being laid. The stringent nature of timing required for these experiments limited the sample sizes, but we conducted experiments on all nests found before laying in 2001. A total of 27 nests were found before laying in Salta and 56 in Buenos Aires in 2001. We were able to equalize and synchronize eggs in 17 nests in Salta ( $N_{\text{eggs}}=51$ ) and 43 in Buenos Aires ( $N_{\text{eggs}}=129$ ). These clutches were distributed across the entire season such that seasonal distribution of experimental clutches did not differ from clutches initiated in the respective populations at large ( $F_{\text{Salta}}=0.136$ ,  $p=0.713$ ;  $F_{\text{Buenos Aires}}=1.246$ ,  $p=0.267$ ). Of the 17 nests at Salta, eight were depredated, two were found destroyed after a storm and seven were successful. In Buenos Aires, 15 of the 43 nests were predated, eight nests were destroyed by bad weather conditions, all nestlings died from starvation in five nests and 15 nests successfully fledged at least one young. All experimental eggs in nests that did not undergo predation during incubation eventually hatched ( $N_{\text{eggs}}=66$ ).

#### (e) Growth measurements

We examined growth rates of nestlings in the experimental nests to control for any possible effects of clutch size or hatching asynchrony on growth rates. We weighed nestlings and measured length of their tarsi every other day, starting the day after hatch, up to the day at which they might force fledge (between 10 and 12 days of age, which also is the age at which nestlings reach their asymptotic mass). We calculated growth rates for measures of mass and tarsus on nestlings at all successful experimental nests ( $N_{\text{nestlings Salta}}=7, 21$ ;  $N_{\text{nestlings Buenos Aires}}=15, 45$ ) by fitting our data to the logistic growth curve following Remeš & Martin (2002). Use of successful nests caused exclusion of the five nests where all young died of starvation in the Buenos Aires population (see above); this made our analysis conservative as these young grew slowly (personal observation), but calculation of an appropriate growth rate was compromised because they died prior to reaching an asymptote (see Remeš & Martin 2002). We analysed differences in growth rates of successful nests between populations using an independent samples *t*-test, with nests as the sampling unit.

### 3. RESULTS

Food limitation did not explain life-history differences between populations, even for clutch size alone. The population with larger clutches had higher starvation rates (figure 1*a*). This greater starvation was not a simple result of the larger clutch sizes and greater hatching asynchrony in this population. Starvation remained higher in the large-clutch population even in experimentally equalized and synchronized clutches (figure 1*b*). Thus, both broad samples across 3 years and stringent experiments indicated that food limitation was greater in the population with larger clutch size, contrary to expectations from food limitation theory.

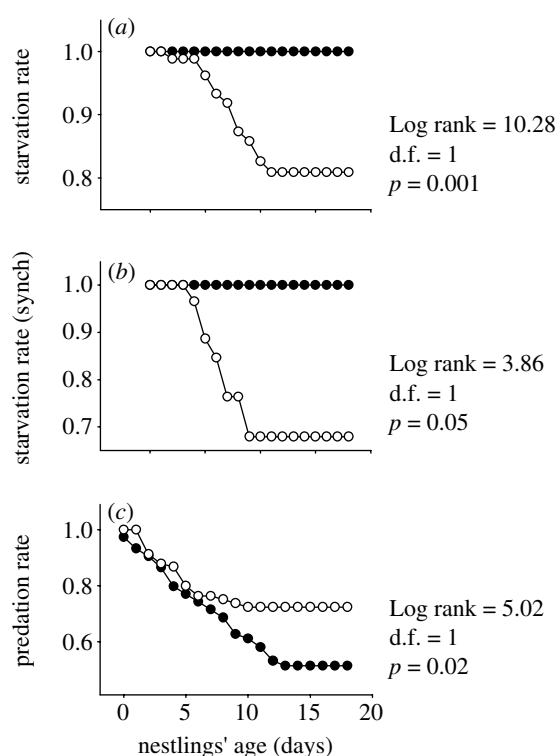


Figure 1. Nestling survival rates of Rufous-bellied thrush as a function of food limitation and nest predation in a population breeding in Salta (filled circles) and a population breeding in Buenos Aires (open circles). (a) Nestling starvation rates were greater at the Buenos Aires site at unmanipulated nests (all years and clutch sizes pooled,  $N_{\text{nestlings Salta}}=74$ ,  $N_{\text{nestlings Buenos Aires}}=91$ ). (b) Nestling starvation rates were greater at the Buenos Aires site even when clutch size was equalized to three eggs at both sites and synchronized ( $N_{\text{nestlings Salta}}=7$ ,  $N_{\text{nestlings Buenos Aires}}=15$ ). (c) Nestling predation rates were greater at the Salta site ( $N_{\text{nestlings Salta}}=74$ ,  $N_{\text{nestlings Buenos Aires}}=91$ ). Overall daily predation rates were higher in Salta than in Buenos Aires (Salta =  $0.053 \pm 0.005$ ,  $N=182$ , Buenos Aires =  $0.018 \pm 0.003$ ,  $N=130$ ,  $\chi^2=25.43$ , d.f. = 1,  $p<0.0001$ ). These differences were maintained both during incubation and nestling periods (incubation: Salta =  $0.056 \pm 0.008$ ,  $N=104$ , Buenos Aires =  $0.019 \pm 0.005$ ,  $N=62$ ,  $\chi^2=13.6$ , d.f. = 1,  $p=0.0002$ ; nestling: Salta =  $0.069 \pm 0.011$ ,  $N=66$ , Buenos Aires =  $0.017 \pm 0.005$ ,  $N=53$ ,  $\chi^2=95.0$ , d.f. = 1,  $p<0.0001$ ).

The population with greater starvation rates had shorter on- and off-bouts during incubation (figure 2*a,b*) and slower nestling growth (figure 3). This population also had higher total feeding visitation rates (figure 2*d*), although we found that feeding rate per nestling did not differ between populations (figure 2*e*). Under the reasonable assumption that higher starvation rates reflect greater food limitation, then the larger clutch sizes, shorter incubation off-bouts (figure 2*b*), and higher or equal parental feeding rates (figure 2*d,e*) in this population are opposite to expectations from food limitation theory (Lack 1947; Martin 1987, 1996; Saether 1994; Conway & Martin 2000). Even if we take the unlikely view that larger clutch sizes reflect reduced food limitation in some way unmeasured by starvation rates, then the shorter on-bouts and slower nestling growth in this population still do not follow from food limitation theory (Lack 1947; Martin 1987, 1996; Saether 1994; Conway & Martin 2000). Thus, differences

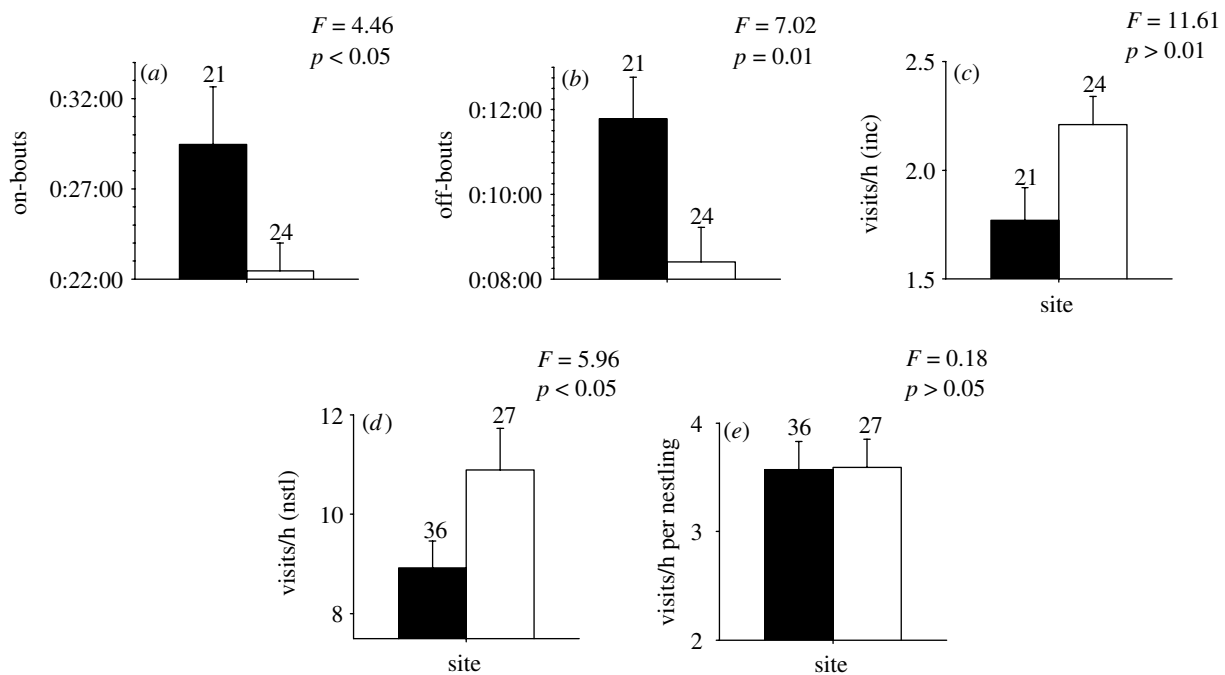


Figure 2. Parental behaviour of Rufous-bellied thrush during incubation and nestling periods of unmanipulated nests in a population with larger clutch size (Buenos Aires, open bars) compared with one with smaller clutch size (Salta, filled bars). (a) Duration of incubation on-bouts (i.e. length of time, in minutes, that a female sits on the nest in a given bout) is longer in the smaller clutch population. (b) Duration of incubation off-bouts (i.e. length of time, in minutes, a female is off the nest to forage during a break) is longer in the smaller clutch population. (c) Parental activity during incubation (number of visits per hour) was lower in Salta, the site with smaller clutch size. (d) Total number of visits per hour by parents to feed their young is greater in the smaller clutch population. (e) Number of visits per hour by parents to feed their young on a per nestling basis does not differ between populations. Data are presented as population means  $\pm$  s.e. Numbers above bars represent the number of nests sampled.

in life-history syndromes between populations could not be explained by food limitation.

In contrast, differences in these life-history syndromes between populations were predicted categorically by nest predation. The population with greater nest predation (figure 1c) had smaller clutch sizes, longer on- and off-bouts during incubation, faster nestling growth and lower parental visitation rates during incubation and nestling periods (figures 2 and 3). All traits varied in the direction predicted by observed differences in the putative selection pressure (i.e. nest predation rates) and nest predation theory (Skutch 1949; Slagsvold 1982; Bosque & Bosque 1995; Martin 1995, 1996; Conway & Martin 2000; Martin *et al.* 2000a).

#### 4. DISCUSSION

We believe that the contrary nature of our results with respect to food limitation relative to results of other studies reflects the interpopulation approach of our study. Studies of food limitation have historically focused on variation within single populations (Martin 2004; but see Saether 1994). In the two cases where clutch size was found to vary nonadaptively with food abundance, comparisons were made between habitats (Blondel *et al.* 1993; Nour *et al.* 1998). In these cases, the studies were still of single populations, where the habitats were in close proximity and gene flow was argued to simply constrain adaptive variation in clutch size; clutch size remained similar between habitats with differing food availability. Nonetheless, they provide the first hints that food limitation cannot simply explain life-history

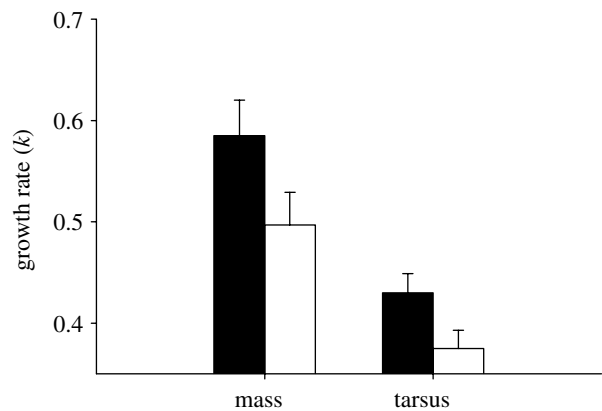


Figure 3. Growth rates in mass and tarsus length of nestlings of Rufous-bellied thrush in experimentally equalized and synchronized nests. The site with smaller clutch size is denoted with filled bars (Salta,  $N_{\text{nests}} = 7, N_{\text{nstd}} = 21$ ). The site with larger clutch size is denoted with open bars (Buenos Aires,  $N_{\text{nests}} = 15, N_{\text{nstd}} = 45$ ). Nestlings in Salta grew faster than nestlings in Buenos Aires based on tarsus length ( $t$ -value = 2.101,  $p < 0.05$ ) and marginally faster based on mass ( $t$ -value = 1.858,  $p < 0.10$ ). Data are presented as means  $\pm$  s.e.

variation when we look beyond a single population within a single habitat type.

Constraints on variation in life-history expression due to gene flow cannot explain our results. First, clutch size was not the same between populations. Second, we studied populations at great distances (1200 km) from each other to minimize gene flow issues. Our results clearly show that, contrary to traditional food limitation

theory (Lack 1947; Martin 1987; Saether 1994), clutch size is not optimized relative to food limitation between our populations. Moreover, the study of a suite of traits (i.e. a life-history syndrome) further demonstrated the inability of food limitation to explain life-history differences between populations. Instead, the syndromes documented in our two populations follow observed differences in nest predation rates and predicted nest predation theory. These results, thus, may suggest that nest predation can over-ride the effects of food in shaping life-history differences among populations. The covariation of the putative selection pressure (i.e. nest predation) with the predicted life-history syndrome increases inference, but the correlative nature of our results cannot allow definitive conclusions about the importance of nest predation. Nonetheless, our experimental confirmation of starvation rate differences clearly demonstrates the inability of food limitation theory to explain variation in clutch size and life-history syndromes between populations. Food limitation may be much less important to life-history evolution in birds than suggested by traditional theory. Greater attention to other selection pressures is needed, and we believe that examination of multiple life-history traits (or life-history syndromes) among populations adds significant strength to understanding life-history variation that is not provided by study of single populations of clutch size alone (Martin 2004).

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