Circulating corticosterone levels in breeding blue tits *Parus caeruleus* differ between island and mainland populations and between habitats

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Abstract

Little is known about whether adaptations to an insular life also involve adaptations in basal corticosterone levels or in the adrenocortical stress response, thus being part of a genetically based island syndrome. However, differences in corticosterone between island and mainland may also be a direct phenotypic response to differences in environmental conditions or may depend on individual characteristics of the animal such as body condition or parental investment. In this paper, we investigated whether insular (Island of Corsica) and mainland (nearby Southern France) blue tits *Parus caeruleus* populations differed in baseline and handling-stress induced corticosterone levels during the breeding season as a response to biological changes of insular biota. We also examined whether corticosterone levels of both mainland and insular blue tits differed between birds living in two different habitats (summergreen and evergreen oak woods) that differ in food availability and whether individual characteristics affected corticosterone levels. We found (a) differences in baseline corticosterone plasma levels between Corsica and the mainland, independent of regional differences in fat scores, (b) a regional difference in the relationship between corticosterone levels and brood size, (c) a difference in the rapidity of onset of the stress response to handling between habitats, independent of region, and (d) a negative relationship between body fat stores and baseline corticosterone levels independent of region. Reduced baseline corticosterone levels on Corsica may be a component of the insular syndrome, allowing birds to be less aggressive and to enhance parental investment despite higher breeding densities. We suggest that baseline corticosterone levels are only elevated if food availability affects directly the parents. However, when conditions deteriorate unexpectedly (as mimicked by handling stress), food allocation between parents and offspring needs to be re-adjusted in favor of the parents, possibly by increased circulating corticosterone levels. The switch to self-maintenance seems to be modified by the amount of body energy stores.

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1. Introduction

Little is known about the variation in corticosterone levels between geographically separated populations, subspecies or species. Most studies concentrated on birds breeding in extreme environments (arctic, taiga, desert, high mountains) and examined whether a reduced acute stress response during breeding facilities breeding under harsh conditions compared with populations breeding in more benign environments (Wingfield et al., 1992, 1995b; Wingfield and Romero, 1999; Wingfield and Romero, 2002; but see Wingfield and Romero, 1999; Silverin and Wingfield, 2001; Breuner et al., 2003 for studies not supporting this hypothesis).

Islands are another environment to which specific behavioral, ecological and reproductive adaptations have
been found (the so-called island syndrome). Birds living on islands face other ecological conditions than their mainland conspecifics, e.g. reduced species diversity leading to fewer predators and less interspecific competition (Whittaker, 1998; Blondel, 2000). Often, population densities are higher and intraspecific aggression is reduced compared with the mainland (see Stamps and Buechner, 1985).

Surprisingly, only a few studies compared the physiology of island and mainland populations, and in particular the question whether adaptations to an insular life also involve adaptations in basal corticosterone levels or in the adrenocortical stress response. Clinchy et al., 2004 found differences in baseline and stress-induced corticosterone levels between island and mainland populations of the song sparrow Melospiza melodia. To and Tamarin (1977) looked at the weight of the adrenal gland in an island vole species and its close relative on the mainland.

However, because environmental conditions, and in particular habitat quality, may differ between islands and mainland, differences in basal corticosterone levels or in the adrenocortical stress response may be phenotypic adaptations to habitat quality and not part of a genetically based island syndrome. Indeed, habitat quality was found to affect corticosterone levels in birds. In suboptimal habitats, baseline corticosterone levels have been found to be elevated to moderate levels compared to nearby optimal habitats (Wingfield et al., 1995a; Wasser et al., 1997; Marra and Holberton, 1998; Kitaysky et al., 1999).

Furthermore, baseline and acute-stress-induced corticosterone levels may also depend on individual characteristics (e.g. sex, social status, body energy stores, life cycle stage, time of day, or degree of parental investment; Romero, 2002; Goymann and Wingfield, 2004). Hence, differences between island and mainland may be proximally caused by an adaptation to the locally changing environment and the particular state of the animal (e.g. Wingfield and Romero, 1999).

In the western Mediterranean region, we had the opportunity to investigate differences in baseline corticosterone levels and in the adrenocortical stress response between well-studied island and mainland populations of blue tits Parus caeruleus, while at the same time examining in each of these two regions whether there were differences between two habitats of different quality and taking into account individual characteristics of the birds such as energy stores and the degree of parental investment.

In the western Mediterranean region, mainland and insular blue tits show many differences, many of these can be attributed to insular effects. Blue tits breeding on Corsica, one of the western Mediterranean islands, are about 15% smaller in size, darker in plumage and have a different song (Doutrelant et al., 2001) than the nominate mainland subspecies and are assigned to the subspecies P. c. ogliastrae. Densities on Corsica are higher than on the mainland (Blondel et al., 1988; Lambrechts et al., 1997) and Protocalliphora larvae, an ectoparasite affecting nestlings, more frequent (Hurtrez-Boussès, 1996; A. Men-nerat, pers. comm.). Blue tits on Corsica have smaller clutches (Blondel et al., 1993) and are less aggressive (Perret and Blondel, 1993) compared with their mainland conspecifics.

On both Corsica and the mainland of Southern France, blue tits breed in two habitats, dominated by either summergreen or evergreen oak Quercus species which differ mainly in food availability. Blue tits feed their nestlings predominantly with leaf-eating caterpillars and generally synchronize the nestling stage with the seasonal caterpillar peak which occurs during the spring flush of new leaves. The summergreen downy oaks Quercus humilis renew their entire foliage each year enabling a high abundance of caterpillars during leafing at the beginning of May. By contrast, the evergreen holm oak Quercus ilex renews only one third of its foliage each year not until the beginning of June, leading to a late and reduced caterpillar supply (Blondel et al., 1993). As a result, in the evergreen habitat food availability is lower and distances to collect food for nestlings are higher than in the summergreen downy oak woods (Blondel et al., 1991, 1993; Tremblay et al., 2005). Moreover, heat stress is higher in the evergreen habitat, because the breeding season is later. Besides the amount of caterpillars potentially available at the seasonal peak, the extent of synchronization of blue tit broods with the caterpillar peak is a determinant of habitat quality. On the Mediterranean island of Corsica, blue tits can match the maximum food demand of their broods with the caterpillar peak in both habitat types, because reduced dispersal on islands is conducive to local specialisation of tits to their habitats (Blondel et al., 1999). On the mainland near Montpellier, blue tits breeding in the summergreen habitat are also well synchronized with the caterpillar peak, but tits inhabiting the interspersed evergreen habitat patches mismatch the maximal caterpillar abundance and breed two weeks too early because of gene flow across habitats (e.g. Blondel et al., 1993).

In this paper, we examined whether insular (Corsica) and mainland (Southern France) blue tits differed in baseline and stress-induced corticosterone levels during the breeding season and we examined in each of these two regions whether blue tits living in summergreen habitats differed from those living in evergreen habitats. Other factors known to affect baseline or stress-induced corticosterone levels at a given place, namely sex, body condition, and the degree of parental investment were also taken into account.

2. Materials and methods

2.1. Study species and study site

The Blue tit Parus caeruleus (Cyanistes caeruleus) is a small hole-nesting passerine breeding throughout the western Palearctic between 35° and 65°N and preferring broad-leaved vegetation. In the Mediterranean region, the Blue tit is sedentary (Cramp, 1993) with natal dispersal distances of up to about 1–2 km (Matthysen et al., 2005; Blondel et al., 2006). Blue tits on mainland Southern France belong to the nominate
subsidiary, which occurs in central and northern Europe. Corsican blue tits are assigned to the subspecies *P. c. ogliastrae*, which can be found in the southern parts of the Iberian peninsula and on the western Mediterranean islands (Cramp, 1993). The two subspecies have been found to belong to two different mitochondrial lineages. Their divergence was estimated to have occurred in the mid-Pleistocene, approximately 650,000 years ago, probably from different refugia during the glacial period (Kvist et al., 1999).

The female incubates the eggs (about 5–8 eggs on Corsica and 8–10 on the mainland, depending on the habitat) and broods the nestlings. Both parents feed nestlings and fledglings. Nestlings remain in the nest for about three weeks.

Fieldwork was performed in Southern France and on the island of Corsica (Fig. 1) during the entire breeding seasons 2001 and 2003, from the beginning of May until the end of June. On the mainland, blue tits breeding in a deciduous downy oak *Quercus humilis* were examined. On the Mediterranean island of Corsica, the two main study sites were a downy oak wood 15 km east of Calvi (Muro, 42°36′N, 8°58′E) and a holm oak forest 20 km south of Calvi (Pirio, 42°24′N, 8°44′E). In addition, individuals breeding in several small study plots between Muro and Pirio were included (downy oak stands: Avapessa, Feliceto and Pietra; holm oak woods: Arinelle, Filaniu, Grassa and Prezzauna). All the blue tits we examined were breeding in nestboxes provided for several years as part of a long-term population study (Blondel et al., 1993). Most of the small study plots have been investigated from 2000 onwards, and are situated near Muro (except Prezzauna that is situated between Muro and Pirio). Coordinates, habitat characteristics and the life-history traits in these study plots are provided in Lambrechts et al. (2004).

### 2.2. Capture and blood sampling

All the nestboxes were controlled at least once a week for measuring breeding traits such as clutch size, hatching date and fledging success. When the nestlings were 9–15 days old, a period during which feeding effort by adults does not change much because nestlings have approximately attained their fledging weight, the parents were captured in the nest box (for details see Müller et al., 2006). Blood was taken by puncturing the alar vein and collected with heparinised microcapillaries or (more rarely) with a heparinised syringe from the jugular vein. The time difference between capture in the nest box and blood sampling, hereafter called Time after capture, was measured to the nearest 10 s and was between 1 and 8 min. Within 2 h, the blood was centrifuged and the plasma stored in liquid nitrogen or on dry ice. After their transfer to the laboratory, the samples were stored at −20 °C until analysis.

In total, blood samples from 290 breeding blue tits (54% females) were obtained, 133 from Southern France (summergreen habitat *n* = 94, evergreen habitat *n* = 39) and 157 from Corsica (summergreen habitat *n* = 60, evergreen habitat *n* = 97). As shown earlier (Müller et al., 2006), plasma corticosterone levels do not increase within 3 min after capture. Such baseline levels (within 3 min after capture) were obtained from 74 birds, 25 from Southern France (summergreen habitat *n* = 16, evergreen habitat *n* = 9), and 49 from Corsica (summergreen habitat *n* = 26, evergreen habitat *n* = 23).

### 2.3. Size, body condition and parental investment

Sex was determined by the presence of the brood patch in females. Second calendar year birds (yearlings) were distinguished from older birds after Jenni and Winkler (1994). Wing length was measured to the nearest 0.5 mm. Because the wing length differs between the two subspecies, sexes and age classes, we used the difference from the mean of each subspecies—sex–age class as a measure of relative wing length.

Fat stores were estimated by assigning the visible amount of subcutaneous fat between the furcula and on the abdomen to one of 31 fat scores, ranging from 0 to 8 (Kaiser, 1993). These scores correlate well with the amount of fat extracted from whole birds (Kaiser, 1993). As is usual in small passerines feeding their nestlings, birds were rather lean so that fat scores never exceeded a score of 3.

As a crude measure of parental investment, we determined the number of nestlings at capture, because feeding rates increase as the number of nestlings increases (e.g. Perrins, 1979; Nur, 1984). Clutch size, and thus the number of nestlings, depends on region and habitat type (Blondel et al., 2001). Therefore, the relative number of nestlings at capture was used in the statistical analyses, calculated as the difference from the mean of the respective region and habitat of the study years.

### 2.4. Hormone assay

Plasma corticosterone concentration was determined using an enzyme immuno assay (Munro and Stabenfeldt, 1984; Munro and Lasley, 1988). Corticosterone in 5 μl plasma and 195 μl water was extracted with 4 ml dichloromethane, re-dissolved in phosphate buffer and given in triplicates in the enzyme immuno assay. The dilution of the corticosterone antibody (Chemicon; cross-reactivity: 11-dehydrocorticosterone 0.35%, Progesterone 0.004%, 18-OH-DOC 0.01%, Cortisol 0.12%, 18-OH-B 0.02% and Aldosterone 0.06%) was 1:8000. HRP (1:400,000) linked to corticosterone served as enzyme label and ABTS as substrate. The concentration of corticosterone in plasma samples was calculated by using the standard curve run in duplicate on each plate. Plasma pools from chickens with two different corticosterone concentrations were included as internal controls on each plate. If the amount of plasma allowed, all samples were analysed twice, and the mean applied for data analysis. If the concentration was below the detection threshold, the value of the lowest detectable concentration (2.15 ng ml−1) was assigned. Intra-assay variation ranged from 5.2% to 12.5% and inter-assay variation from 7.7% to 19.2%, depending on the concentration of the internal control and the year of determination.

### 2.5. Statistical analysis

The effects of various covariates and factors (see above and Table 1) on plasma corticosterone levels were evaluated using a Mixed Model Analysis (Residual Maximum Likelihood Analysis REML; Patterson and
Table 1
Dependence of handling-induced corticosterone levels (square root-transformed, \(n = 227\)) on the region, the habitat and various other parameters and their interactions analysed in a Mixed Model (see Methods, deviance 169.86, df = 204).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Effect ± SE</th>
<th>Wald statistic</th>
<th>df</th>
<th>(\chi^2)-probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after capture</td>
<td>0.285 ± 0.15</td>
<td>162.03</td>
<td>1</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Region</td>
<td>0.586 ± 0.20</td>
<td>37.79</td>
<td>1</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Habitat</td>
<td>0.045 ± 0.20</td>
<td>1.12</td>
<td>1</td>
<td>0.291</td>
</tr>
<tr>
<td>Region (\times) habitat</td>
<td>0.071 ± 0.28</td>
<td>0.34</td>
<td>1</td>
<td>0.558</td>
</tr>
<tr>
<td>Time after capture (\times) region</td>
<td>0.073 ± 0.16</td>
<td>0.87</td>
<td>1</td>
<td>0.350</td>
</tr>
<tr>
<td>Time after capture (\times) habitat</td>
<td>0.232 ± 0.16</td>
<td>6.75</td>
<td>1</td>
<td>0.009</td>
</tr>
<tr>
<td>Time after capture (\times) region (\times) habitat</td>
<td>−0.061 ± 0.20</td>
<td>0.39</td>
<td>1</td>
<td>0.533</td>
</tr>
<tr>
<td>Sex</td>
<td>0.038 ± 0.09</td>
<td>0.01</td>
<td>1</td>
<td>0.927</td>
</tr>
<tr>
<td>Fat score</td>
<td>−0.298 ± 0.09</td>
<td>15.27</td>
<td>1</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Relative wing length</td>
<td>0.056 ± 0.04</td>
<td>2.52</td>
<td>1</td>
<td>0.113</td>
</tr>
<tr>
<td>Relative number of nestlings</td>
<td>0.141 ± 0.06</td>
<td>2.24</td>
<td>1</td>
<td>0.135</td>
</tr>
<tr>
<td>Time after capture (\times) sex</td>
<td>−0.033 ± 0.07</td>
<td>0.10</td>
<td>1</td>
<td>0.756</td>
</tr>
<tr>
<td>Time after capture (\times) fat score</td>
<td>−0.026 ± 0.06</td>
<td>0.01</td>
<td>1</td>
<td>0.922</td>
</tr>
<tr>
<td>Time after capture (\times) relative wing length</td>
<td>0.045 ± 0.03</td>
<td>2.87</td>
<td>1</td>
<td>0.090</td>
</tr>
<tr>
<td>Time after capture (\times) relative number of nestlings</td>
<td>0.019 ± 0.02</td>
<td>1.46</td>
<td>1</td>
<td>0.227</td>
</tr>
<tr>
<td>Region (\times) relative number of nestlings</td>
<td>−0.137 ± 0.07</td>
<td>6.74</td>
<td>1</td>
<td>0.009</td>
</tr>
<tr>
<td>Habitat (\times) relative number of nestlings</td>
<td>0.005 ± 0.08</td>
<td>0.11</td>
<td>1</td>
<td>0.743</td>
</tr>
<tr>
<td>Region (\times) habitat (\times) relative number of nestlings</td>
<td>0.079 ± 0.14</td>
<td>0.41</td>
<td>1</td>
<td>0.524</td>
</tr>
<tr>
<td>Sex (\times) relative number of nestlings</td>
<td>−0.070 ± 0.05</td>
<td>2.08</td>
<td>1</td>
<td>0.150</td>
</tr>
</tbody>
</table>

The effect for the variable region is given for the mainland (versus Corsica), for the variable habitat for evergreen woods (versus summergreen woods) and for the variable sex for females (versus males).

3. Results

In the full model (Table 1), not surprisingly Time after capture was strongly positively related to plasma corticosterone levels. After accounting for Time after capture, plasma corticosterone levels differed significantly between region with blue tits from the mainland having higher levels than Corsican blue tits. The rate of increase in circulating corticosterone concentration up to 8 min after capture (during handling), tested as the interaction between Time after capture and Habitat, differed significantly between habitat types. In both regions, blue tits breeding in the evergreen habitats showed a steeper increase in corticosterone levels in the first 8 min after capture than blue tits of the summergreen habitat. This stronger corticosterone response to capture and handling resulted in higher corticosterone levels in the evergreen habitats compared with the summergreen habitats 6–8 min after capture. In contrast, the handling-induced increase in corticosterone was similar between regions (interaction terms Time after capture × region and Time after capture × region × habitat not significant).

These results are confirmed when analysing the larger, and thus statistically more powerful, data set including birds with missing data on fat score and brood size (\(n = 290\); Fig. 2). Again, Time after capture (\(p < 0.001\) and Region (\(p < 0.001\) and the interaction term Time after capture × Habitat (\(p = 0.006\)) were highly significant, while the interaction terms Time after capture × Region and Time after capture × Region × Habitat were not (\(p = 0.9\) for both).

When restricting the analysis to the birds blood-sampled within 3 min, thus representing baseline levels (Müller et al., 2006), baseline corticosterone levels differed significantly between regions (Wald = 16.96, df = 1, \(p < 0.001\)). Corsican blue tits of the subspecies Parus caeruleus ogliasta-ræe had lower baseline corticosterone levels (5.84 ± 0.39 ng ml\(^{-1}\), \(n = 49\)) than individuals of the nominate subspecies near Montpellier (8.93 ± 0.69 ng ml\(^{-1}\), \(n = 25\)). There was no significant difference between
baseline corticosterone levels of blue tits living in summergreen downy oak habitats and those living in evergreen holm oak habitats (Fig. 3).

We tried to explain the remaining variation in corticosterone levels after correction for Time after capture, Region and Habitat by introducing the additional variables sex, size, fat score and brood size and interaction terms into the Mixed Model (Table 1; when we restricted the analyses to the blood samples collected within 3 min (n = 64), we obtained very similar results).

Sex and relative wing length had no significant effect on corticosterone. The increase in corticosterone levels as a response to handling did not vary with sex nor with relative wing length (interactions of Time after capture with Sex and Relative wing length, respectively, not significant). The number of nestlings at capture was related to corticosterone levels in one region (as interaction term between Region and Number of nestlings at capture; Wald = 6.74, df = 1, p = 0.009; Table 1). Corticosterone levels, after being corrected for Time after capture, increased with the relative number of nestlings at capture on Corsica, while on the mainland there was no relationship between corticosterone residuals and number of nestlings at capture. This same result was also obtained when analysing blood-samples obtained within 3 min (Fig. 4).

Fig. 3. Baseline corticosterone levels (mean ± SE) of blue tits breeding in habitats of different quality on Corsica and mainland Southern France. Individuals of the subspecies *P. c. ogliastrae* on the Mediterranean island Corsica (n = 49) had significantly lower levels than their conspecifics of the nominate subspecies in Southern France near Montpellier (n = 25). There was no significant difference between individuals breeding in the summergreen downy oak habitat (Corsica n = 26, Mainland n = 16) and those breeding in the evergreen holm oak habitat (Corsica n = 23, Mainland n = 9).

Fig. 4. Relation between baseline corticosterone levels of blue tit parents and relative number of nestlings at capture on Corsica and mainland Southern France. The relative number of nestlings at capture is the deviance from the mean of the region and habitat concerned.
As shown earlier (Müller et al., 2006), fat scores were significantly related to corticosterone levels (Table 1), but the increase in corticosterone levels as a response to handling was not related to fat score (interaction term Fat score $\times$ Time after capture not significant, $p = 0.92$). Because fat scores were lower on the mainland ($0.96 \pm 0.05, n = 113$) than on Corsica ($1.29 \pm 0.06, n = 114$), the higher corticosterone levels of blue tits on the mainland may be explained by their lower fat scores. Therefore, we ran an additional Mixed Model including Fat score as an explanatory variable after Time after capture, but before the Region and Habitat. As expected, the relation between fat score and corticosterone level was significant (effect of fat score: Wald = 33.35, df = 1, $p < 0.001$); but explained only one third of the variation in corticosterone levels between regions. In that model, the effect of the region was still highly significant (Wald = 24.25, df = 1, $p < 0.001$), indicating an additive effect of the region and fat score on corticosterone levels.

Caterpillar availability decreased as the season progressed in all habitats with the exception of the mainland evergreen habitat, where it increased (own unpublished data). Therefore, we tested for any seasonal change in corticosterone levels in all regions and habitats separately (because breeding season differs between habitats) by introducing capture date into the Mixed Model. However, we did not find any indication that corticosterone levels changed as the season progressed, nor was there a difference in the increase in corticosterone levels between the first and the second half of the breeding season.

4. Discussion

In this study we found (a) differences in baseline corticosterone plasma levels between the island of Corsica and the mainland, independent of regional differences in fat scores (Fig. 3), but a similar rate of increase at the onset of the adrenocortical response to capture and handling (Fig. 2), (b) a regional difference in the relationship between corticosterone levels and brood size (Fig. 4), (c) a difference in the rapidity of onset of the stress response to handling between habitats, independent of region, and (d) a negative relationship between body fat stores and baseline corticosterone levels independent of region.

4.1. Differences in corticosterone levels between island and mainland

The question is whether the lower baseline levels of corticosterone found on Corsica are a direct response to the particular conditions on the island (and thus part of phenotypic plasticity) or whether they are part of the genetic differences of the island population, thus part of the island syndrome, or whether they are part of the genetic differences of the subspecies P. c. ogliastrae that also occurs in Southern Spain on the mainland (e.g. Kvist et al., 1999). Ideally, this question should be resolved with a common garden experiment, but it is quite difficult to obtain baseline corticosterone levels of which one is certain that they are not affected by captivity (e.g. Marra et al., 1995).

The fact that we did not find any difference between habitats both on Corsica and on the mainland may indicate that baseline circulating corticosterone levels are not readily adapted phenotypically to the prevailing conditions, but that they differ genetically. Corsican and mainland blue tits are assigned to different subspecies which evolved during the mid-Pleistocene. One reason for genetic differences in hormone levels between the two populations could be an accidental genetic drift. Another explanation is that the low baseline levels on Corsica are part of the genetic adaptation of the subspecies ogliastrae or the Corsican island population. A comparison between circulating corticosterone between mainland and island P. c. ogliastrae could reveal whether the lower corticosterone levels found on Corsica are characteristic of the island situation on Corsica or of the subspecies.

Low corticosterone levels on Corsica may be part of the genetic adaptation to evolutionary pressures that differ from those on the mainland. Evolutionary pressures may arise from islands having fewer predators and interspecific competitors and, thus, higher population densities (Williamson, 1981; Adler and Levins, 1994), as is the case in blue tits on Corsica compared to the mainland (Blondel et al., 1988; Lambrechts et al., 1997). Blue tits on Corsica apparently adapt to this situation by reducing their aggressiveness and the number of offspring, but increasing the quality of offspring through a longer nestling period (Perret and Blondel, 1993). A generally higher ectoparasite load on nestlings by Protocalliphora larvae in Corsica may be a further reason to reduce clutch size and increase parental care.

Baseline corticosterone levels are known to raise under moderately stressful conditions, such as high density or low food availability, (Marra and Holberton, 1998; Silverin, 1998; Kitaysky et al., 1999) and are positively correlated with aggression (Guminski Sorenson et al., 1997; Kitaysky et al., 2003; Van Duyse et al., 2004). Under the conditions of Corsica, such elevated levels would normally reduce parental investment (Silverin, 1986), rather than enhance. Low baseline corticosterone levels may thus be a component of the adaptation to live under these particular island conditions and to produce less, but higher quality offspring.

It is interesting that the low baseline corticosterone levels on Corsica occurred in the evergreen habitat (Pirio) with less food available and a hotter climate as well as in the summergreen habitat (Muro) with a superabundant caterpillar supply and a milder climate during the earlier breeding season. This suggests that the conditions prevailing during chick rearing are not the proximate cause of the low baseline corticosterone levels on Corsica. It would, therefore, be interesting to measure baseline corticosterone during the entire annual cycle.

Baseline and stress-induced corticosterone levels were dependent on the relative number of nestlings in a
region-specific manner. While there was no dependence of corticosterone levels on the number of nestlings on the mainland, corticosterone levels increased with increasing number of nestlings on Corsica. Surprisingly, the relationships were similar between habitats in both regions, suggesting that habitat quality was not a major reason. Perhaps, baseline levels on the mainland are already so high, that a possibly small effect of the number of nestlings is masked. Another reason may be that feeding rates per chick are higher on Corsica because there are more *Protopalliphora* larvae in the nest (Hurtrez-Boussès et al., 1998). Thus, to feed a higher than average number of nestlings may need a higher baseline corticosterone level on Corsica, but not on the mainland. Moderately elevated baseline corticosterone levels are known to increase foraging (Astheimer et al., 1992).

4.2. Differences in corticosterone levels between habitats and fat stores

Blue tits inhabiting the evergreen habitats did not differ in baseline corticosterone levels from conspecifics living in the summergreen habitats on both Corsica and the mainland. This is in contrast to findings from other studies (Marra and Holberton, 1998; Kitaysky et al., 1999; Wasser et al., 1997), showing increased baseline levels in suboptimal habitats. However, blue tits in both regions inhabiting the evergreen habitats showed a stronger increase at the onset of the adrenocortical response to capture and handling than their conspecifics living in the summergreen habitats.

Evergreen habitats differ from summergreen habitats primarily by a lower food availability (Blondel et al., 1993). Blue tits adjust to the lower food supply by having a lower clutch size (in our study 10.0 in summergreen and 8.4 in evergreen woods on the mainland; 7.9 in summergreen and 5.4 in evergreen woods on Corsica). As a result, foraging effort in summergreen and evergreen habitats on Corsica is similar (Tremblay et al., 2005). During the sprouting of new leaves, caterpillars are superabundant, especially in the summergreen Mediterranean downy oak forests where breeding success is not food limited (Tremblay et al., 2003). In the evergreen holm oak habitat, caterpillars are less abundant, but still food availability is high. Thus, the parents themselves are not food-stressed and this may explain why baseline corticosterone levels are not elevated. In contrast, in studies that found an effect of habitat quality on baseline corticosterone levels, the birds suffered directly from reduced food availability: American redstarts *Setophaga ruticilla* during the non-breeding season in a dry habitat in Jamaica (Marra and Holberton, 1998); parent barn swallows *Hirundo rustica* during cold and rainy periods (Jenni-Eiermann et al., unpublished) and adult kitiwakes *Rissa tridactyla* in a food-poor colony (Kitaysky et al., 1999). Also, Northern spotted owls *Strix occidentalis caurina* probably suffered directly from disturbance (Wasser et al., 1997). Therefore, we suggest that baseline corticosterone levels are only elevated if habitat quality affects directly the individual and not when the costs of suboptimal habitat quality are paid only by the nestlings.

However, when conditions deteriorate unexpectedly (rainy weather, disturbance), food allocation between parent and offspring needs to be adjusted. Increased corticosterone levels possibly adjust the balance of food allocation in favor of the parent and at the expense of the nestlings (Jenni-Eiermann et al., unpublished).

This may be the reason why blue tits in evergreen habitats exhibited a steeper increase in corticosterone levels during the first minutes of capture and handling than the blue tits in the summergreen habitat. As a response to an unexpected acute stressor, they have to switch to self-maintenance more strongly in the evergreen habitat than in the summergreen habitat. Moderately elevated corticosterone levels reduce parental care and high corticosterone levels can even cause brood abandonment (Silverin, 1986).

Higher stress-induced glucocorticoid levels have also been found in more food-stressed song-sparrows feeding nestlings (Clinchy et al., 2004) and in snowshoe hares (Boonstra et al., 1998).

The switch to self-maintenance seems to be modified by the amount of body energy stores. As shown earlier (Müller et al., 2006), corticosterone levels in blue tits were dependent on fat stores. By reducing their own body energy stores, parents are able to buffer unexpected reductions in food availability for a limited time. However, when body energy stores are very low, they have to switch to self-maintenance by increasing corticosterone levels (see Jenni-Eiermann et al., unpublished).

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