Adrenocortical responses to stress in breeding Pied Flycatchers
_Ficedula hypoleuca_: relation to latitude, sex and mating status

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The adrenocortical response to acute stress, as measured by increases in plasma levels of corticosterone following capture and handling, has been shown to vary between individuals within a population as well as with season in a variety of species. However, the ecological bases for such endocrine phenomena remain unclear. One hypothesis predicts that populations of birds breeding under extreme conditions such as in sub-arctic habitats where the nesting season is short, decrease the amplitude of the adrenocortical response to acute stress so as to allow non-interrupted breeding under potentially severe conditions such as bad weather conditions and/or food shortage. To test this hypothesis we measured increases in circulating levels of corticosterone following capture and handling in populations of Pied Flycatchers _Ficedula hypoleuca_ breeding in southern (Göteborg) and northern (Ammarnäs) Sweden. We also sampled breeding females that did not have a male helping them to feed young (only in south Sweden). As predicted by the hypothesis we found male and female Flycatchers breeding in northern Sweden to be adapted to the short breeding season and harsh unpredictable sub-arctic climate by having a reduced adrenocortical response to stress, as indicated by the pattern of corticosterone levels in blood. Maximum corticosterone levels generated by the capture stress protocol were significantly higher in single females than all other groups. Unlike other passerines studied at high latitudes Pied Flycatchers showed no significant relationships between corticosterone levels and body mass or fat score.

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A broad spectrum of stressful stimuli markedly increases corticosterone secretion in birds (Holmes and Phillips 1976, Greenberg and Wingfield 1987). If stress conditions become chronic, continued high levels of corticosterone can lead to inhibition of reproduction, severe debilitation and even death (Harvey et al. 1984, Moore and Miller 1984, Greenberg and Wingfield 1987, Sapolsky 1992). On the other hand, the initial rise in glucocorticoid secretion may be beneficial since it appears to trigger physiological and behavioural changes related to emergency activities that directly potentiate survival in the face of unpredictable environmental events and, most importantly, avoid the deleterious effects of chronic stress (Sapolsky 1992, Wingfield 1988, 1994). Corticosterone can suppress reproductive and territorial behaviours; increase foraging and irruptive “escape”-like behaviour, mobilize glucose and save energy by increasing night restfulness (see Asheimer et al. 1992, Wingfield 1994, Wingfield et al. 1995 for details). These behaviours comprise “facultative physiological and behavioural patterns” in response to environmental stress. Moreover, avian populations in the field had elevated circulating levels of corticosterone when in this “facultative physiological and behavioural pattern” in response to a potentially stressful stimulus such as severe weather (Wingfield 1988, 1994).

It is also clear that many avian populations modulate their adrenocortical responsiveness to stress either sea-
sonally (i.e. as a function of reproductive status), or within a population (i.e. individual variation). One hypothesis states that avian populations breeding in severe environments, especially when the breeding season is very short, suppress the adrenocortical response to stress to allow reproduction to proceed in the face of potentially stressful conditions (Wingfield 1994). It also appears that onset of parental behaviour is a strong ecological correlate of stress response inhibition (Wingfield et al. 1995, Silverin 1998). Field endocrinology techniques allow us to sample free-living individuals of the same species in different localities and seasons. In this way we can confirm the ecological bases of such endocrine phenomena, and control for phylogenetic differences.

Birds breeding in the north where the breeding season is very short, and the weather unpredictable, must be able to withstand low temperatures and temporary stress. Here we test the hypothesis that avian species nesting in a sub-arctic region suppress the adrenocortical response to stress (Wingfield 1988, 1994). The hypothesis was tested by comparing the elevation of corticosterone levels in blood of breeding Pied Flycatchers Ficedula hypoleuca at a temperate zone breeding site in southern Sweden with those of a population nesting at a sub-arctic site in Swedish Lapland. Both males and females feed young so we can compare sex and latitude while holding parental behaviour constant. We also were able to sample a group of female Pied Flycatchers that were feeding young alone. Previous work has shown that these females tend to have higher baseline levels of corticosterone (Silverin 1982), and thus may already be stressed.

Methods

Study sites

Pied Flycatchers were caught in Japanese mist nets or in nest box traps in forests near Göteborg (57°42′N), south west Sweden. These sites were Gunnebo, an area with mixed forest and a lake; the Botanical Garden, with rock outcrops and extensive mixed forest including European species as well as trees and plants from the Holarctic region; and Hysnå, consisting of coniferous boreal forest with scattered broadleaf trees and extensive bogs. High latitude study areas were in an elfin birch forest Betula pubescens in the vicinity of Ammarnäs in northern Sweden (63°58′N).

Sampling procedures

All birds were removed from the mist net as soon as possible after capture and an initial blood sample collected from a wing vein into a heparinized capillary tube. Each sample (see also below) was about 30-40 μl in volume. Capillary tubes were sealed at one end with molder clay and stored on ice until return to a field station (within 1-5 h). Here blood was centrifuged, plasma harvested and stored at -20°C. After blood samples were collected, all birds were banded with a numbered aluminium ring, body mass measured to the nearest 0.1 g, and wing length measured to the nearest 0.5 mm. Fat depots in the furcular pit and on the abdomen were assessed using an arbitrary scale (Wingfield and Farner 1978) of 0-5 where 0 = no fat, and 5 = gross bulging fat bodies. For each bird the average of furcular and abdominal fat scores was used. All birds captured in this study were in the parental phase of breeding and were either captured during the last days of incubation (females only) or during the first days after hatching (both sexes). All Pied Flycatchers captured were breeding in artificial nest boxes that could be checked easily to confirm breeding status. At some nest boxes, only a single female was seen feeding young. Single females included in the present study were captured while feeding 2-4 day-old nestlings. All single females originate from the population in southern Sweden. Data from these birds were treated separately since their endocrine status has been shown to be different from that of paired females (Silverin 1982).

Capture stress protocol

When comparing the intensity of adrenocortical responses to stress between groups of breeding Pied Flycatchers, it was critical that all individuals be stressed in as close to identical manner as possible. Capture, handling and restraint are known to elicit a marked increase in circulating corticosterone in wild birds as well as other vertebrates (Holmes and Phillips 1976, Harvey et al. 1984, Wingfield et al. 1982). Increased circulating corticosterone following capture was thus assumed to be comparable across sex, latitude and mating status. This protocol has been applied to a wide spectrum of species (see Schwabl et al. 1991, Wingfield et al. 1992, Wingfield 1994 for details). Approximately 30-40 μl of whole blood was collected from a wing vein as soon as possible after capture (time was noted as soon as the bird hit the net, or when the nest-box trap was released, and again when the first sample was collected). Most samples were collected within 1-2 min and this time group was designated Min 1 for convenience in the figures. Additional samples were then collected at 5, 20, and 60 min following capture (i.e., Min 5, Min 20 and Min 60 in the figures). Plasma levels of corticosterone in these samples indicated the degree and time course of the response to capture, handling and restraint.
Corticosterone assay

After extraction in dichloromethane, plasma levels of corticosterone were measured by a direct radioimmunoassay method identical to that described by Wingfield et al. (1992). All samples were measured in duplicate, and all were run in one assay to avoid intersay variations. Included in the assay were three solvent blanks, and three samples from a plasma pool as a check on reliability criteria. Based on these plasma pool samples the accuracy of measurements was 96.3%.

Statistical analysis

Changes in corticosterone levels with time after capture were compared by two-way analysis of variance (ANOVA) for populations (latitude, sex and mating status), and for repeated measures (i.e. time after capture). Within a group, changes in corticosterone were compared with Fisher’s Protected Least Significant Difference test (PLSD). Comparisons among populations, where appropriate, were made with Student’s t-tests, unpaired and two-tailed.

Comparisons of body mass, fat scores, initial and maximum corticosterone levels generated during the capture stress protocol, percent increase and rate of increase (i.e. ng/ml/min) of corticosterone among populations following capture were all compared with Student’s t-test or, where appropriate, with the non-parametric Mann-Whitney U-test.

To further analyse the dynamics of changes in plasma corticosterone levels during the capture stress protocol, we correlated body mass and fat score with the dynamics of corticosterone changes. Past investigations have revealed major differences in adrenocortical responsiveness to stress with body condition (see Wingfield 1994, Wingfield et al. 1995 for full discussion). Spearman Rank Correlation Coefficients were calculated to indicate significant relationships.

Results

Adrenocortical responses to capture stress

Two-way analysis of variance (ANOVA) for repeated measures revealed that all groups of Pied Flycatchers showed significant increases in circulating corticosterone levels following capture, handling and restraint (Fig. 1, F = 39.79, df = 3, p < 0.0001). There were highly significant differences between groups (F = 4.57, df = 4, p < 0.006, Fig. 1), and also a significant interaction between groups and time after capture (F = 3.059, df = 12, p < 0.002). Paired males and females at both sites showed a significant increase in corticosterone levels by 5 min after capture (p < 0.05, Fisher’s PLSD test). At Göteborg, corticosterone levels in both males and females continued to rise through 60 min of capture stress (Fisher’s PLSD test, Fig. 1) but at Ammarnäs, they had decreased by Min 60 and significantly so in females (Fisher’s PLSD test, Fig. 1). In single females, corticosterone levels did not rise significantly until Min 20 post capture (Fig. 1, p < 0.05, Fisher’s PLSD test) and continued to rise by Min 60 (Fisher’s PLSD test). Note that in the lower panel of Fig. 1 the initial levels of corticosterone (i.e. Min 1) remained low throughout the hours of the day when samples were collected indicating that any diurnal changes in baseline levels of corticosterone did not confound the increase during the capture stress protocol.

Fig. 1. Changes in corticosterone following capture, handling and restraint (upper panel) in male and female Pied Flycatchers captured in southern Sweden (Göteborg – 57°42’ N) and in northern Sweden (Ammarnäs – 63°58’ N), and single females. Points are means and vertical lines the standard errors. N = 8 and 9 for males and females at Göteborg; 5 and 8 for males and females at Ammarnäs; and 5 for single females which were all captured at Göteborg. The lower panel shows individual corticosterone levels at time Min 1 for all groups as a function of time of day.
Comparisons among populations

One-way ANOVA of body mass (Fig. 2, upper panel, $F = 3.14, df = 4, p < 0.03$) revealed that females at Ammarnäs were heavier than males at both sites and mated females at Göteborg (Fisher’s PLSD tests, $p < 0.05$ in both cases). Fat scores were also significantly different (Fig. 2, lower panel, $F = 4.381, df = 4, p < 0.006$). Mated birds at Ammarnäs were fatter than mated birds at Göteborg (Fisher’s PLSD tests, $p < 0.05$ in all cases). Initial plasma levels of corticosterone (i.e. Min 1) did not differ among the groups (Fig. 3, upper panel, $F = 0.975, df = 4, p = 0.435$), but maximum corticosterone levels generated during the capture stress protocol were different (Fig. 3 upper panel, $F = 5.754, df = 4, p < 0.002$). Single females had higher maximum levels of corticosterone than all other groups ($p < 0.05$, Scheffe F tests and Fisher’s PLSD tests). Mated males and females at the two sites had similar maximum levels. However, if corticosterone levels at 60 min were compared, there were significant differences between the two sites (Fig. 3 lower panel, $F = 7.548, df = 4, p < 0.0003$). Again, single females had higher corticosterone levels at 60 Min post capture than all other groups (Scheffe F test, $p < 0.05$), and both males and females at Ammarnäs had lower corticosterone levels at Min 60 than both sexes at Göteborg (Fisher’s PLSD tests, $p < 0.05$ in all cases).

Correlations of corticosterone dynamics with body mass and fat score

There were no significant relationships between initial corticosterone level, percent increase, rate of increase or maximum level generated during the stress protocol with body mass, or fat score in any of the groups (data not shown).

Discussion

The breeding biology and reproductive endocrinology of the Pied Flycatcher are very well known (e.g. Silverin 1983a, b, 1990, Lundberg and Alatalo 1992), and the
results from the present study may be explained and understood in view of this knowledge. The main breeding areas for Pied Flycatchers are central Europe and southern Scandinavia. The sub-arctic birch forest therefore is a marginal breeding habitat for the Pied Flycatcher (Järvinen 1983, Thingstad 1992). Breeding success of northern Pied Flycatchers is low, and much more variable between years, than for Flycatchers breeding in southern Scandinavia (Källander 1975, Järvinen 1980). Järvinen (1983) therefore argued that the Pied Flycatcher probably is not well adapted to breeding in northern Scandinavia. However, the present study shows that Pied Flycatchers breeding in the harsh sub-arctic birch forest are adapted to this unpredictable environment at least by having a lower stress response than Pied Flycatchers breeding in more benign habitats in southern Sweden. The results from the present study also contradict Järvinen’s (1993) conclusions that Pied Flycatchers breeding in southern Scandinavia should be less sensitive to unpredictable weather than those breeding in the north as they on average experience warmer temperatures during the breeding season. As indicated by the present results the situation in fact is the other way around. The difference in the stress response between Pied Flycatchers breeding in southern and northern Sweden, i.e. a reduced response in the north, agrees with observations made on Willow Warblers Phylloscopus trochilus breeding in the same two areas, but not with results from Chaffinches Fringilla coelebs breeding at Göteborg and Bramblings F. montifringilla breeding at Ammarnäs (Silverin et al. 1997, Silverin and Wingfield unpubl. data). Both Willow Warblers and Pied Flycatchers are strictly insectivorous and short-lived (approximately 1.5 years). They are both long-distance migrants spending only the period late April – late August in Sweden, and they never lay second clutches. Chaffinches and Bramblings, on the other hand, are short-distance migrants with significantly longer life expectancies (2–3 years) than both Pied Flycatchers and Willow Warblers. Furthermore, Chaffinches and Bramblings can produce eggs during a longer period, and they spend most of the year in Sweden.

How would Pied Flycatchers breeding in an unpredictable sub-arctic environment benefit from a reduced stress response? A short answer to this question is that it reduces the chances of parents abandoning their nests due to bad weather conditions and/or food shortage. Pied Flycatchers change their parental behaviour as a result of increased plasma levels of corticosterone (Silverin 1986, 1998). Parents given silastic implants containing corticosterone (increasing plasma levels to 60–70 ng/ml) during the nesting period have drastically reduced reproductive success. This plasma level is slightly below the highest level found in an intact free-living Pied Flycatcher. Reproductive success is reduced because parents having high corticosterone levels feed their nestlings less frequently than parents with low (up to 15–25 ng/ml) plasma levels of corticosterone. Instead of giving the food to the nestlings they eat a large part of the food they collect themselves, indicated by the fact that these parents do not lose weight during the nestling period. An experimental increase of corticosterone levels to even higher levels than 60–70 ng/ml results in parents abandoning the nest (Silverin 1986, 1998). The conclusion drawn from these field experiments, namely that high corticosterone levels may redirect behaviour away from reproduction towards survival, has gained support from ecological studies. Field observations on intact Pied Flycatcher populations show that a conflict between parents and nestlings may emerge when food resources are decreasing, i.e. under a circumstance when plasma levels of corticosterone are expected to be high, and that parents in such a situation may sacrifice their broods for their own survival (Järvinen and Ylímaunu 1986).

Several environmental factors are potential stressors to a breeding bird. One major factor affecting reproduction, as well as corticosterone secretion, is weather. As an increase in the corticosterone secretion may result in parents abandoning their nests Pied Flycatchers breeding in unpredictable sub-arctic areas should have a reduced stress response so that they do not risk their reproductive success, especially since the variability in the daily mean temperature is much greater at Ammarnäs in northern Sweden than at Göteborg in southern Sweden. It would be especially important to have a reduced stress response during the second half of June, i.e. at the time the present study was performed, since this is the time when temperature variations reach their peak in the north. From this point of view it is not that important for Pied Flycatchers in southern Sweden to have a reduced stress response since temperature variation has no pronounced peak during the breeding season in southern Scandinavia. For example, in northern Finland the risk for temperatures <5°C is 28% during the breeding period, whereas the corresponding figure for southern Finland is only 3% (Järvinen 1993). However, despite this physiological adaptation cold and rainy summers sometimes result in complete nestling failure for Pied Flycatchers breeding at their most northern and altitudinal limits (Järvinen 1980, 1983, 1993, Hildén et al. 1982, Järvinen and Ylímaunu 1986).

Pied Flycatchers breeding in southern Sweden showed a rapid response to handling stress. This is what the hypothesis predicted. However, there are reasons why one could expect also Pied Flycatchers breeding in southern Sweden to have a low stress response, and thereby a lowered risk for nest desertion. One very obvious reason is the fact that, in contrast to most other species studied, gonads regress extremely early in Pied Flycatchers (Silverin 1975, 1980). Testes and ovary start to regress already during the last days of the average incubation period for the population, and al-
ready 1–2 days after the eggs have hatched gonads are completely regressed. This is true for Pied Flycatchers breeding both in northern and southern Sweden. Consequently, Scandinavian Pied Flycatchers never lay a second clutch, and field experiments indicate that they normally cannot lay a repeat clutch if the first brood is destroyed (Silverin 1980). In view of this even birds in southern Sweden should avoid getting distressed during the nestling period as their ability to lay a new clutch is limited in time and only granted those that started breeding very early.

Why then do we see this difference in the stress response between Pied Flycatchers breeding in northern and southern Sweden? Despite similarities in the timing of gonadal regression, Flycatchers breeding in the north have a much shorter time available for egg-laying. At Åmmarnäs, northern Sweden the period between the onset of laying of the first and the last clutch in a single year has never been observed to exceed 23 days (Svensson 1985). At Göteborg, south Sweden, the laying period is about twice as long, giving the very early breeders an opportunity to replace a destroyed or robbed clutch. This circumstance could be the reason why selection pressure is lower for a reduced stress response in Pied Flycatchers in southern Sweden. In accordance with this, repeat laying is much more rare in northern Scandinavia (Berndt and Sternberg 1972, Järvinen 1983, Lifjeld and Slagsvold 1988, Silverin, unpubl. data). Furthermore, as Pied Flycatchers breeding in northern Sweden start egg-laying 2–3 weeks later than in southern Sweden, and as parents may start moulting already during the nestling period, there is a shortage of time for Pied Flycatchers breeding in the north (Hyytiä and Vikberg 1973, Ojanen and Orell 1982, Silverin unpubl. data). Consequently, northern flycatchers should have a mechanism preventing them from abandoning their nest in case of for example bad weather. A reduced stress response is such a mechanism, and the present results show that such a mechanism has evolved. Once evolved it is likely that it is maintained within the population since there is very little gene flow even between close breeding populations (Järvinen 1993). Pied Flycatchers are well known for having a high breeding-site fidelity (Lundberg and Alatalo 1992). Once again, field observations support our results showing that northern flycatchers have reduced their stress response. In years with extremely cold weather in northern Scandinavia, when only about half of the eggs hatch and nesting success is only about 25%, field data indicate that Pied Flycatcher parents invest in the current reproduction, even at the cost of their own survival and fecundity, as they do not easily abandon their nests, except during the nest-building period. There are even several observations that a parent may actually die on the nest (Järvinen 1993, Silverin unpubl. data).

The Pied Flycatcher is a polygamous species where the male very often abandons his mate at the time she lays her first egg and establishes a secondary territory some 100 m away where he tries to attract a second female (Silverin 1983b). The proportion of polyterritorial and successfully polygynous males does however differ dramatically between areas. At Göteborg, south west Sweden, about 95% of the males are polyterritorial and about 30–35% of these polyterritorial males succeed in getting a second female. At Åmmarnäs, northern Sweden, the corresponding figures are about 30% and 0–25% (Askennmo 1977, Lyholm 1984, Silverin 1983a, b). Secondary females have a significantly lower breeding success than simultaneously laying monogamous females (Alatalo and Lundberg 1984) because the male almost exclusively assists only his first female in feeding the young. If his two females start egg-laying very close in time, the secondary female may also receive assistance (Askennmo 1977, Alatalo and Lundberg 1984, Lifjeld and Slagsvold 1989). In view of this it is not surprising that secondary females showed a stronger stress response than any other category of breeding flycatchers, and it can be predicted that single females should more easily abandon their nests than females having male assistance.

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