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## Hormone levels predict individual differences in reproductive success in a passerine bird



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2 **Hormone levels predict individual differences in reproductive success in a passerine bird**

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18 **Summary**

19 **Hormones mediate major physiological and behavioural components of the**  
20 **reproductive phenotype of individuals. To understand basic evolutionary processes in the**  
21 **hormonal regulation of reproductive traits we need to know whether, and during which**  
22 **reproductive phases, individual variation in hormone concentrations relates to fitness in**  
23 **natural populations. We related circulating concentrations of prolactin and corticosterone**  
24 **to parental behaviour and reproductive success during both the pre-breeding and chick-**  
25 **rearing stages in both individuals of pairs of free-living house sparrows, *Passer domesticus*.**  
26 **Prolactin and baseline corticosterone concentrations in pre-breeding females, and prolactin**  
27 **concentrations in pre-breeding males predicted total number of fledglings. When the**  
28 **strong effect of lay date on total fledgling number was corrected for, only pre-breeding**  
29 **baseline corticosterone, but not prolactin, was negatively correlated with the reproductive**  
30 **success of females. During the breeding season, nestling provisioning rates of both sexes**  
31 **were negatively correlated with stress-induced corticosterone levels. Lastly, individuals of**  
32 **both sexes with low baseline corticosterone before and high baseline corticosterone during**  
33 **breeding raised the most offspring, suggesting that plasticity of this trait contributes to**  
34 **reproductive success. Thus hormone concentrations both before and during breeding as**  
35 **well as their seasonal dynamics predict reproductive success, suggesting that individual**  
36 **variation in absolute concentrations and in plasticity is functionally significant and, if**  
37 **heritable, may be a target of selection.**

38 **Key words:** stress, corticosterone, prolactin, *Passer domesticus*, parental investment

## 39 **Introduction**

40           Hormones regulate many aspects of an individual's phenotype, including various  
41 physiological and behavioural traits (Adkins-Regan 2005). A full understanding of the evolution  
42 of fitness-relevant traits such as reproductive investment therefore requires a corresponding  
43 knowledge of the evolution of the endocrine mechanisms that control the expression of the  
44 phenotype (Ketterson & Nolan 1992; Wingfield *et al.* 1998; Zera *et al.* 2007). One important  
45 component of studies in evolutionary physiology is heritable individual variation, especially in  
46 relation to individual fitness (Sinervo & Licht 1991; Williams 2008; Bonier *et al.* 2009a), as it is  
47 the raw material of selection (Bennett 1987; Kempenaers *et al.* 2008; Williams 2008).  
48 Furthermore, knowledge of the dynamics of endocrine signaling in relation to the reproductive  
49 investment of individuals will increase our understanding of reproductive decision-making and  
50 life-history trade-offs (e.g. Sinervo & Licht 1991; Zera & Harshman 2001; Dingemanse *et al.*  
51 2010; McGlothlin *et al.* 2010).

52           Recent studies have demonstrated relationships between individual variation in  
53 circulating concentrations of hormones, behaviour and fitness during the breeding phase. For  
54 example, individual variation in plasma testosterone concentrations relates to male alternative  
55 reproductive strategies, territorial behaviour, paternal behaviour, reproductive success and  
56 survival in several vertebrates (e.g., Sinervo *et al.* 2000; Trainor & Marler 2001; Reed *et al.* 2006;  
57 Kempenaers *et al.* 2008). Individual variation in plasma prolactin (Prl) concentrations correlates  
58 with nestling provisioning rates in birds (Badyaev & Duckworth 2005; Chastel *et al.* 2005) and  
59 with alternative male reproductive tactics in mammals (Schradin 2008). In birds, individual  
60 variation in baseline concentrations of corticosterone (Cort0) correlates with parental care,  
61 timing of breeding and reproductive success, although the direction of the relationship appears to

62 be species-specific and dependent on sex and reproductive stage (Angelier & Chastel 2009;  
63 Bonier *et al.* 2007, 2009a; Schoech *et al.* 2009; see also Foerster & Montfort 2010). Furthermore,  
64 stress-induced concentrations of corticosterone (maxCort) tend to show a negative relationship  
65 with reproductive behaviour (Love *et al.* 2004; Lendvai *et al.* 2007).

66         These studies suggest that correlations between individual variation in concentrations of  
67 single hormones and reproductive performance during the breeding season are functionally  
68 significant (Silverin *et al.* 1997; Angelier *et al.* 2009). However, they do not take into account  
69 that seasonal changes in the concentrations of two or more hormones may have interactive  
70 effects on reproductive performance. Furthermore, major decisions about reproductive  
71 investment often are made during the pre-breeding season where we know much less about the  
72 relationship between hormones and reproductive phenotype. For example, in birds, lay date, a  
73 trait often closely linked with reproductive fitness (Horak *et al.* 1997) is set before the start of the  
74 breeding season (Meijer *et al.* 1990; Cresswell & McCleery 2003). To our knowledge only two  
75 studies have examined the relationship between the natural variation in concentration of  
76 circulating hormones during the pre-breeding season and subsequent reproductive investment. In  
77 one of these studies, female marine iguanas (*Amblyrhynchus cristatus*) with low Cort0 and  
78 maxCort during the pre-breeding season were more likely to breed that year than those with high  
79 Cort0 and maxCort (Vitousek *et al.* 2010), while in the second study, female snow petrels  
80 (*Pagodroma nivea*) with elevated pre-breeding Cort0 had a high probability of skipping  
81 breeding that year (Goutte *et al.* 2010). Additionally, experimental treatment of female side-  
82 blotched lizards (*Uta stansburiana*) with Cort prior to the breeding season altered their tendency  
83 to reproduce, although in opposite directions depending on the reproductive strategy/morph of  
84 individuals (Lancaster *et al.* 2008).

85           Here we determined whether Prl, Cort0, and maxCort of individuals measured during  
86 both the pre-breeding and the breeding season are related to individual differences in  
87 reproductive investment and success in breeding pairs of free-living house sparrow (*Passer*  
88 *domesticus*). House sparrows show marked individual variation in number of clutches laid per  
89 season, parental feeding rates, and juvenile recruitment rates (Ringsby *et al.* 2009). We focused  
90 on Cort and Prl as interactive mediators of reproductive decisions and trade-offs in light of their  
91 opposing actions on reproductive investment (Buntin 1996; Love *et al.* 2004; Lendvai *et al.* 2007;  
92 Angelier *et al.* 2009; Angelier and Chastel, 2009). In birds, Prl secretion is stimulated by  
93 increasing photoperiods (Sharp *et al.* 1998), with further increases at the onset of incubation  
94 (Dawson & Goldsmith 1985). Prl promotes parental care, thereby modulating the seasonal  
95 adjustments of reproductive effort (Buntin 1996; Sockman *et al.* 2006). Cort0 typically increases  
96 as an animal works harder, acting as a metabolic hormone by supporting energetically  
97 demanding processes (e.g., Sapolsky *et al.* 2000; Bonier *et al.* 2009b). Cort concentrations can  
98 increase within 3 minutes when an individual experiences adverse conditions, and then typically  
99 shut down non-essential processes such as reproduction to promote survival functions (Sapolsky  
100 *et al.* 2000; Wingfield & Romero 2001; Wingfield & Sapolsky 2003).

101           In the current study we first determined whether individuals have consistent hormone  
102 concentrations, by calculating repeatabilities for pre-breeding and breeding season hormone  
103 concentrations. We also examined the level of correlation in hormone concentrations between  
104 members of a pair. Second, to establish whether variations in hormone concentrations relate to  
105 fitness we determined whether hormonal traits obtained during the pre-breeding and the breeding  
106 season were related to the reproductive success of an individual. Third, during the parental phase

107 we determined the relationship between hormone concentrations and behavioral measures of  
108 parental investment such as nestling feeding rates.

109

## 110 **Methods**

### 111 Study species and behavioural observations

112 We carried out the study between March and August 2008 on a free-living population of  
113 house sparrows that bred in nest-boxes of two large barns at a farm co-op in Belle Mead, New  
114 Jersey, USA (40°28'N, 74°39'W). We captured adults in mist nets, and upon first capture, we  
115 individually marked them with a numbered aluminum ring and a unique combination of colored  
116 leg bands. We monitored nests daily to determine laying dates, clutch sizes, and number of  
117 hatchlings. Parental food provisioning rate (hereafter termed 'feeding rate') was determined for  
118 each individual by continuous scan observations (Altmann 1974) from a central location (about  
119 100m from nests) from 0700-0800h during days 11 or 12 of the nestling phase of the first clutch  
120 of each pair. Scans were made on sunny days when there were no detectable disturbances nearby.  
121 House sparrows are sexually dimorphic and easily distinguished (Summers-Smith 1963). We  
122 captured and blood sampled 49 adult birds on March 9<sup>th</sup> before the breeding season with mist  
123 nets, 24 days before the first eggs were laid in the study population. Of these, 20 females and 20  
124 males were pair-bonded and nested in nest-boxes inside the barn. We recaptured both members  
125 of each pair during the breeding season using manually triggered spring-loaded traps shutting the  
126 entrance hole as they entered the nest to feed 8-10 day old nestlings of their first clutch (between  
127 April 27<sup>th</sup> and June 2<sup>nd</sup>). Pairs remained bonded for the duration of the breeding season. We  
128 searched the field site (51ha) every other day between March and August and every week  
129 between August and late-October for additional nests. This sedentary population of house



130 sparrows relies upon the study site for food and available nesting habitat, making it unlikely that  
131 additional nests outside the core study area were not found. Nest-boxes were located at about a  
132 10 m height inside enclosed storage barns, and there was no nest predation. All procedures used  
133 in this study were approved by the Princeton University Institutional Animal Care and Use  
134 Committee.

135

### 136 Measurement of hormone concentrations

137 Immediately after each capture, a blood sample (total <200  $\mu$ l) was collected from each  
138 individual from the brachial vein by venipuncture for the determination of Cort0 and Prl, and the  
139 time required to do so from hitting the mist net, or springing the nest trap to completing  
140 collection, was recorded. The first 70-100  $\mu$ l of blood collected were used for measurement of  
141 Cort0 (mean handling time:  $2.0 \pm 0.2$  minutes, maximum: 3.3 minutes), while the second 70-100  
142  $\mu$ l were collected for Prl determination (mean handling time  $3.5 \pm 0.3$  minutes maximum: 6.03  
143 minutes). Cort0 and Prl concentrations in these blood samples were not related to handling time  
144 (Cort0:  $r = -0.22$ ,  $p = 0.20$ ,  $N = 80$ ; Prl:  $r = -0.14$ ,  $p = 0.31$ ,  $N = 80$ ). We then used a standard capture-  
145 handling-restraint protocol (see Wingfield & Romero 2001) to determine maxCort  
146 concentrations. For this, following the initial collection of blood samples, we placed each bird in  
147 a cloth bag and collected a final blood sample (<70  $\mu$ l) 30 min later. We chose 30 min as the time  
148 for the final sample because previous studies on this species have shown that Cort concentrations  
149 reach the maximum values at this time (Breuner & Orchinik 2001). We then measured tarsus  
150 length ( $\pm 0.1$  mm) and body mass ( $\pm 0.1$  g) before releasing the birds at the site of capture. The  
151 blood samples were kept on ice and centrifuged (3000rpm/1276g, 10 min) within 3 hours, and  
152 the plasma was separated and stored at  $-20^{\circ}\text{C}$  for hormone analyses.

153

154 Hormone assays

155           Circulating Cort concentrations were determined in a single radioimmunoassay (Gill *et al.*  
156 2008). Cort antibody was purchased from Esoterix Endocrinology, CA. All samples were  
157 assayed in duplicate. Average recovery after extraction with dichloromethane of samples spiked  
158 with a small amount of radio-labelled hormone was  $82.9 \pm 1.8\%$ ; final concentrations were  
159 corrected for individual extraction efficiencies. The lower limit of detection of the assay was  
160 at 1.99 ng/ml; the intra-assay coefficient of variation as estimated by taking replicates Cort  
161 standards of known concentrations through the entire assay procedure (one at low and one at  
162 medium concentration were included in the beginning and the end of the assay, respectively) was  
163 13.6%. Plasma Prl concentrations were determined using a direct recombinant-derived starling  
164 (*Sturnus vulgaris*) Prl radioimmunoassay (Bentley *et al.* 1997). Samples were assayed in  
165 duplicate when there was sufficient sample volume, but in most cases there was not. The reaction  
166 volume was 60 $\mu$ l, comprising 20 $\mu$ l of plasma sample or standard, 20 $\mu$ l of primary rabbit  
167 antibody to starling Prl (1:24000), and 20 $\mu$ l of I<sup>125</sup>-labelled Prl (15000 cpm). The primary  
168 antibody was precipitated to separate free and bound I<sup>125</sup> label using 20 $\mu$ l of donkey anti-rabbit  
169 precipitating serum and 20 $\mu$ l of non-immune rabbit serum. All samples were measured in a  
170 single assay. The intra-assay coefficient of variation was 8.5 %; the minimum detectable dose  
171 was 1.0 ng/ml, with a 50% displacement at 12.14ng/ml.

172

173 Data analysis

174           Data for both sexes were analysed separately to avoid pseudo-replication of data on  
175 fledgling numbers from the same nest/pair. Pre-breeding and breeding season data were also

176 analysed separately. Data for total fledgling number followed a normal distribution (Shapiro-  
177 Wilk test;  $n=40$ ,  $z=1.54$ ,  $p>0.07$ ). We used multiple regression models to predict total fledgling  
178 numbers from the variables: Cort0, maxCort, Prl concentrations. Because lay date was highly  
179 correlated with total number of fledglings, we controlled for this by adding lay date into the  
180 model. We initially included all three hormonal traits in the model and then used backward  
181 elimination to remove any non-significant correlations. Body condition was calculated by using  
182 residuals from a linear regression of body mass against tarsus length and was included in all  
183 models to control for effects of body condition on reproductive success. We also ran all analyses  
184 with body mass and tarsus length included as separate co-variates in the models. Both methods  
185 gave similar results (example of one model:  $r^2=0.9258$  including body condition and  $r^2=0.9292$   
186 with body mass and tarsus length), and we opted to include body condition as calculated from  
187 residuals as above in our models because in our data set body mass and tarsus length were  
188 linearly correlated ( $r=0.61$ ,  $p=0.0008$ ). Omitting body condition from our models entirely gave  
189 very similar results to the ones reported below. Changes in hormone concentrations were  
190 calculated as breeding-season minus pre-breeding season concentrations, and we used backward  
191 elimination to generate the best model that predicted total fledgling numbers from the changes in  
192 hormone concentrations. The ideal statistical approach to analyse our data set would have been  
193 to include all variables, both sexes and both seasons into one single model to determine the  
194 relative importance of each parameter. However, our sample sizes precluded such models and  
195 therefore necessitated the use of separate models for each sex and breeding stage. Pearson's  
196 correlations were used to test if the behaviours and hormone concentrations in adult pairs were  
197 correlated. Repeatability of hormone concentrations between pre-breeding to breeding seasons  
198 were calculated from between and within group variances derived from one-way ANOVAs

199 according to Lessells and Boag (1987). Analyses were performed using STATA 9.0 (College  
200 Station, TX, USA). Sample sizes for females and males in both seasons were  $n=20$ , respectively.  
201 Data are given as means $\pm$ 1SEM.

202

## 203 **Results**

### 204 Reproductive characteristics and individual hormone consistencies

205 Pairs began displaying courtship behaviour in February and the first egg was laid on  
206 April 2<sup>nd</sup>. The mean first clutch initiation date for pairs that laid three clutches was April 6<sup>th</sup>  $\pm$ 1  
207 (n=8), for pairs that laid two clutches, April 20<sup>th</sup>  $\pm$ 3 (n=8), and for pairs that laid one clutch, May  
208 14<sup>th</sup>  $\pm$  3 days (n=4). Early laying females produced a greater total number of clutches (and thus  
209 total number of eggs) during the season ( $r=-0.91$ ,  $p<0.0001$ ,  $n=20$ ). Average clutch size was 4.56  
210  $\pm$  0.72 (range 4-6) with 96.5% of the eggs hatching. Mean clutch sizes of females that laid  
211 different number of clutches did not differ ( $\chi^2=4.42$ ,  $df=2$ ,  $p=0.11$ ) so the main difference in  
212 reproductive output was in the number of clutches laid.

213 Prl concentrations in the same individual were repeatable (variation between pre- and  
214 during-breeding concentrations within an individual was lower than variation among individuals)  
215 in males ( $r=0.65$ ,  $df=19$ ,  $F=2.55$ ,  $p=0.02$ ) and in females ( $r=0.68$ ,  $df=19$ ,  $F=6.24$ ,  $p<0.0001$ ) from  
216 the pre-breeding to the nestling stages of the reproductive cycle. Cort0 was not repeatable in  
217 males ( $r=-0.04$ ,  $df=19$ ,  $F=0.28$ ,  $p=0.89$ ) nor in females ( $r=-0.04$ ,  $df=19$ ,  $F=0.28$ ,  $p=0.88$ ) from the  
218 pre-breeding to the nestling stages. MaxCort was not repeatable in females ( $r^2=0.29$ ,  $df=19$ ,  
219  $F=0.56$ ,  $p=0.11$ ) nor in males ( $r^2=0.61$ ,  $df=19$ ,  $F=0.58$ ,  $p=0.79$ ).

220 Hormone levels of the members of a pair were positively correlated with each other, both  
221 before the breeding season (Prl:  $r=0.78$ ,  $p<0.0001$ ; Cort0:  $r=0.77$ ,  $p<0.0001$ ; maxCort:  $r=0.47$ ,

222 p=0.01) and during the breeding season (Prl:  $r=0.53$ ,  $p=0.003$ ; Cort0:  $r=0.50$ ,  $p=0.004$ ; maxCort:  
223  $r=0.49$ ,  $p=0.008$ ). Feeding rates were also positively correlated between members of a pair  
224 ( $r=0.77$ ,  $n=20$ ,  $p<0.0001$ ).

225

### 226 Hormones and reproductive success

227 Pre-breeding body condition was negatively correlated with pre-breeding Cort0 (females:  
228  $r=-0.47$ ,  $p=0.035$ ; males:  $r=-0.53$ ,  $p=0.015$ ) and positively correlated with breeding Cort0 levels  
229 (females:  $r=0.66$ ,  $p=0.0017$ ; males:  $r=0.48$ ,  $p=0.034$ ).

230 In females, both Cort0 and Prl concentrations, and in males Prl, but not Cort0 during the  
231 pre-breeding season predicted total number of fledglings for the entire season (overall model:  
232 females:  $F=61.20$ ,  $df=5$ ,  $p<0.00001$ ,  $r^2=0.93$ ; males:  $df=3$ ,  $F=27.62$ ,  $p<0.00001$ ,  $r^2=0.81$ ; see  
233 Table 1). Females with low Cort0 and high Prl concentrations during the pre-breeding season  
234 fledged the most offspring, while in males only low pre-breeding Cort0 was associated with  
235 increased reproductive success (Fig. 1).

236 As indicated by bivariate correlations, lay date was the strongest predictor of the number  
237 of fledglings (pairs:  $r=-0.86$ ,  $p<0.0001$ ). Bivariate correlations showed that Prl was more closely  
238 associated with lay date than Cort0 (Prl with lay date: females:  $r=-0.69$ ,  $p<0.0007$ , males:  $r=-0.70$ ,  
239  $p=0.0006$ ; Cort0 with lay date: females:  $r=0.39$ ,  $p=0.093$ , males:  $r=0.45$ ,  $p=0.044$ ). To  
240 understand which hormonal traits are associated with lay date and thereby with fitness as  
241 opposed to traits that contribute to fitness independently of lay date, we computed the residuals  
242 from a regression of the number of fledglings and lay date. This fitness variable was thereby  
243 'corrected' for lay date and included in a modified version of the multiple regression model.  
244 Using this model, it was found in females that hormones contributed to explaining the variance

245 of the 'corrected' number of fledglings ( $F=4.00$ ,  $p=0.027$ ,  $df=4$ ,  $r^2=0.32$ ), whereas in males,  
246 hormones had no significant effect on this variance ( $F=2.22$ ,  $p=0.13$ ,  $r^2=0.16$ ; Table 2). In  
247 females, pre-breeding Cort0 was negatively correlated with the 'corrected' number of fledglings,  
248 i.e. females that had the largest fledgling numbers regardless of lay date had the lowest pre-  
249 breeding Cort0 ( $F=6.82$ ,  $r^2=0.45$ ,  $p=0.006$ ; Figure 2).

250 Hormone concentrations during feeding of the first clutch significantly predicted total  
251 number of fledglings (females:  $F=32.52$ ,  $df=3$ ,  $r^2=0.83$ ,  $p<0.0001$ ; males:  $F=24.13$ ,  $df=3$ ,  
252  $r^2=0.79$ ,  $p<0.0001$ ). However, only Prl concentrations were significant and thus included in this  
253 model: individuals with the highest Prl while rearing their first clutch fledged the most young  
254 during the entire breeding season (females:  $r^2=0.74$ ,  $t=2.75$ ,  $p=0.014$ ; males:  $r^2=0.81$ ,  $t=2.45$ ,  
255  $p=0.026$ ).

256 The relationship between Cort0 and total number of fledglings changed between the pre-  
257 breeding and the breeding seasons in both sexes. Moreover, the direction of the change in Cort0  
258 was important for fitness: individuals that had low Cort0 during pre-breeding but high Cort0  
259 during the breeding season fledged more young during the entire season than individuals that had  
260 high pre-breeding Cort0 and low breeding Cort0 (females:  $F=5.65$ ,  $p=0.01$ ,  $r^2=0.40$ ; males:  
261  $F=8.47$ ,  $p=0.003$ ,  $r^2=0.50$ ; Figure 3).

262

### 263 Hormones and parental behaviour

264 Feeding rates per hour and feeding rates per hour per young were positively correlated  
265 ( $r=0.87$ ,  $n=40$ ,  $p<0.0001$ ) because for the first brood, 80% of the observed pairs fledged five  
266 young. Thus, we opted to use feeding rate per hour to quantify parental investment for each adult.  
267 Feeding rates of nestlings from the first clutch were predicted by breeding maxCort levels in

268 both females ( $df=1$ ,  $F=26.73$ ,  $r^2=0.58$ ,  $p<0.0001$ ) and males ( $F=17.74$ ,  $df=2$ ,  $r^2=0.64$ ,  $p<0.0001$ ),  
269 with individuals that reached the highest maxCort concentrations showing lower feeding rates  
270 (Fig. 4). In initial bivariate analyses, Prl correlated positively with feeding rates (females:  $r=0.63$ ,  
271  $p=0.003$ ; males:  $r=0.68$ ,  $p=0.001$ ), but Prl was not a significant variable when included together  
272 with maxCort in the above model.

273

## 274 Discussion

275 This study shows that individual variation in baseline corticosterone (Cort0)  
276 concentrations several weeks before first eggs were being laid and in prolactin (Prl) during the  
277 parental phase of the first clutch predicted individual reproductive success during the entire  
278 season. Furthermore, not only were absolute hormone concentrations important in determining  
279 fitness, seasonal dynamics in Cort0 concentrations also predicted reproductive success.

280

### 281 Hormones and reproductive success

282 Individuals of both sexes with the highest pre-breeding Prl concentrations had the  
283 greatest total reproductive output (Fig. 1a). However, Prl appeared to be most strongly related to  
284 lay date, which in turn is a strong determinant of overall reproductive success in a season  
285 (Hegner & Wingfield 1987; Gienapp & Visser 2006). This relationship could be caused by  
286 several processes. Prl increases in response to increasing day-length prior to the breeding season  
287 (Sharp & Sreekumar 2001), and birds laying early clutches might have a seasonally accelerated  
288 photoperiodic induction of Prl secretion. Alternatively, at the time of sampling, individuals with  
289 early lay dates might have been at a more advanced stage of preparedness for breeding, and Prl  
290 secretion may have been stimulated to a greater extent, for example, by the presence of a nest

291 (Dawson & Goldsmith 1985). In American kestrels (*Falco sparverius*) and pheasants (*Phasianus*  
292 *colchicus*), Prl concentrations rise with proximity to the onset of incubation (Breitenbach *et al.*  
293 1965; Sockman *et al.* 2000). Our data do not allow us to determine whether individual variation  
294 in pre-breeding concentrations of Prl reflect genetic differences that also determine lay date,  
295 whether Prl is causally involved in determining the decision of when to lay, or whether Prl  
296 concentrations were the consequence of reproductive decisions having already been made.  
297 Experimental approaches such as manipulation of lay date, clutch size or Prl concentrations will  
298 be required to distinguish between these possibilities.

299         When we controlled total numbers of young fledged for lay date in the present study, the  
300 residual variance for females was only explained by pre-breeding Cort0, in that females with low  
301 pre-breeding Cort0 had higher total fledgling numbers during the breeding season (Fig. 2). Pre-  
302 breeding Cort0 appeared inversely related to female quality, as females with lower Cort0 had  
303 higher body condition and higher subsequent reproductive output, irrespective of lay date. This  
304 finding is consistent with that of Vitousek *et al.* (2010), in which female reptiles with lower pre-  
305 breeding Cort0 also had higher reproductive output during the breeding season. In our study we  
306 could not determine age, experience or genetic make-up of individuals, and hence were not able  
307 to quantify the potential importance of those factors on reproductive performance (O'Dwyer *et al.*  
308 2006; Wilson & Nussey 2009). However, among birds that laid the same number of clutches  
309 there was ample individual variation in reproductive success (Fig. 1), of which pre-breeding  
310 Cort0 explained a considerable part.

311         Even more intriguing is the finding that individuals with low pre-breeding but high  
312 breeding Cort0 concentrations raised more fledglings during the entire breeding season than  
313 individuals with a similar degree of plasticity but in the opposite direction (high pre-breeding and



314 low breeding Cort0; Fig. 3). This suggests that a certain type of plasticity, specifically an up-  
315 regulation, of Cort0 in the course of the reproductive season is an important component of  
316 reproductive success. An alternative hypothesis is that birds with low Cort0 were more likely to  
317 initiate more clutches, and the act of raising more nestlings is what is driving the Cort0 increase.  
318 Increased Cort0 during the breeding season might support the challenges of provisioning a brood  
319 by promoting the utilisation of resources to address high energetic demands (Romero 2002;  
320 Landys *et al.* 2006). Indeed, an up-regulation of Cort0 was also observed in female tree-  
321 swallows (*Tachycineta bicolor*) with higher reproductive success from incubation to chick  
322 rearing (Bonier *et al.* 2009b). However, in white-crowned sparrows (*Zonotrichia leucophrys*),  
323 females with lowest breeding Cort0 had the highest reproductive success (although this was not  
324 observed in males; Bonier *et al.* 2007).

325 MaxCort was not related to reproductive success during the pre-breeding or the breeding  
326 season when included together with Cort0 and Prl in statistical tests (although it was related to  
327 parental behaviour; Fig. 4). This suggests that the functional role of maxCort differs from that of  
328 Cort0 (Sapolsky *et al.* 2000; Romero 2004; Hau *et al.* 2010). In the current study, maxCort  
329 appears unrelated to reproductive decision-making in the pre-breeding period and instead may be  
330 a modulator of reproductive effort once breeding is under way (see below). Instead, Prl  
331 concentrations in both sexes during the breeding season (while raising the young of their first  
332 brood) were positively associated with the total number of fledglings produced during that year.  
333 This could be because birds with high prolactin are more likely to raise subsequent broods. In  
334 single-brooded starlings, prolactin concentrations decreased in both sexes after the parental stage  
335 (Dawson & Goldsmith 1982) whereas in double-brooded song sparrows (*Melospiza melodia*),

336 prolactin remained high between the two broods and did not decrease until after the second  
337 parental stage (Wingfield & Goldsmith 1990).

338         Although clutch numbers and sizes are under female control, hormone concentrations of  
339 males caught during the pre-breeding season correlated with those of their female partner, raising  
340 the possibility that individuals of similar quality and/or reproductive state may pair bond  
341 associatively (e.g., Moore *et al.* 2005). Alternatively, hormone profiles of males and female  
342 might become more correlated after pairing.

343

#### 344 Hormones and parental behaviour

345         In the current study, maxCort concentrations during the breeding season showed an  
346 inverse relationship with feeding visits to the nest: individuals of both sexes that reached lower  
347 maxCort concentrations during a standardised capture-restraint protocol showed higher nestling  
348 provisioning rates than individuals that reached higher maxCort concentrations. This is in  
349 agreement with other studies showing that maxCort during the breeding season in individuals of  
350 different species correlates inversely with parental effort (Silverin 1986; Wingfield *et al.* 1995;  
351 Angelier *et al.* 2009). Further experiments are needed to establish whether individuals with lower  
352 maxCort concentrations actively suppress their stress response or whether their stress response is  
353 lower because of their state and/or reproductive strategy (see Lendvai *et al.* 2007; Romero *et al.*  
354 2009). In other studies, Prl correlated with nestling feeding rate when measured on its own (see  
355 Buntin 1996), whereas in our study, when measured together with maxCort, the latter hormone  
356 was more important in explaining parental effort. This highlights the importance of studying  
357 multiple endocrine signals in conjunction to fully understand how hormones mediate behaviour.

358

**359 Conclusion**

360 This study suggests that in free-living house sparrows, circulating hormone  
361 concentrations during the pre-breeding and the breeding season can translate into individual  
362 variation in reproductive performance upon which selection could act (e.g., McGlothlin &  
363 Ketterson 2008). For Cort0, both absolute levels within a reproductive stage as well as seasonal  
364 plasticity were positively correlated with reproductive success. Whether and to which degree  
365 absolute hormone concentrations or plasticity in endocrine responses is heritable, and thus  
366 amenable to selection, remains to be established. Heritabilities of Cort0 and Prl concentrations  
367 are still unclear; although it is tantalising that Prl concentrations in this study were consistent  
368 within individuals and that maxCort concentrations in birds appear to have a heritable  
369 component (Satterlee & Johnson 1988; Evans *et al.* 2006). Furthermore, plastic physiological  
370 responses, which can be equated with reaction norms, can be heritable (e.g., Visser *et al.* 1998;  
371 Nussey *et al.* 2007). It will be important in future studies to determine the degree of among-year  
372 repeatability and heritability in hormone concentrations, or their plasticity, to determine the  
373 evolutionary potential of hormonal traits. Further studies are also required to determine whether  
374 the reproductive success of both males and females is directly related to their own hormone  
375 concentrations, or whether there are indirect effects through its mate's phenotype. Finally,  
376 although we found relationships between hormonal traits and reproductive success in males, we  
377 could not determine the rate of extra pair fertilisation (EPF) in our population and thus the real  
378 reproductive success for each individual male. The EPF rate in house sparrows in a population  
379 can be around 20% (Whitekiller *et al.* 2000), which may affect the direction and strength of the  
380 relationship between hormones and reproductive success in males. Nevertheless, the  
381 demonstration here of rather tight relationships between individual variation in hormone

382 concentrations and reproductive performance represents an important advance in our  
383 understanding of evolutionary endocrinology.

384

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- 592

593 Figure 1. Relationships between total number of fledglings produced by individual birds during  
594 the breeding season and a) pre-breeding prolactin concentrations, b) pre-breeding baseline  
595 corticosterone concentrations (ng/ml). Females: closed symbols and solid line of best fit, males:  
596 open symbols and dashed line of best fit.

597

598 Figure 2. Correlation between residuals of total fledging number (controlled for lay date) and  
599 pre-breeding baseline corticosterone concentrations (ng/ml). Individual females above the dotted  
600 zero line had more fledglings and lower pre-breeding baseline corticosterone concentrations than  
601 females below the dotted zero line regardless of lay date. Females: closed symbols and solid line  
602 of best fit, males: open symbols.

603

604 Figure 3. The direction of the change in baseline corticosterone concentrations (breeding-pre-  
605 breeding baseline corticosterone; ng/ml) is related to total number of fledglings (n). Individuals  
606 with low pre-breeding and high breeding baseline corticosterone had the highest reproductive  
607 success. Females: closed symbols and solid line of best fit, males: open symbols and dashed line  
608 of best fit.

609

610 Fig. 4. Stress-induced corticosterone concentrations (ng/ml) during the breeding season were  
611 negatively correlated with parental provisioning rates (number of trips/hour). Females: closed  
612 symbols and solid line of best fit, males: open symbols and dashed line of best fit.

613

614

615

Table 1. Results from multiple regression model to predict total number of fledglings from variables measured during the pre-breeding season.

<b>females</b>	<b>coefficient</b>	<b>SE</b>	<b>t</b>	<b>partial r</b>	<b>p</b>
<b>lay date</b>	-1.772	0.031	-5.88	-0.78	<b>0.0001</b>
<b>cort0</b>	-1.098	0.032	-4.18	0.71	<b>0.001</b>
<b>prolactin</b>	0.941	0.064	2.40	-0.61	<b>0.030</b>
<b>body condition</b>	-2.600	0.565	-0.52	-0.25	0.609
<b>males</b>					
<b>lay date</b>	-2.230	0.558	-3.64	-0.65	<b>0.002</b>
<b>prolactin</b>	1.026	0.448	2.23	0.52	<b>0.040</b>
<b>body condition</b>	7.177	9.966	0.78	0.09	0.447

Table 2. Results from multiple regression models to predict total number of fledglings controlled for lay date from variables measured during the pre-breeding season.

<b>females</b>	<b>coefficient</b>	<b>SE</b>	<b>t</b>	<b>partial r</b>	<b>p</b>
<b>cort0</b>	-0.116	0.042	-2.74	-0.47	<b>0.014</b>
<b>prolactin</b>	0.037	0.044	0.84	0.11	0.421
<b>body condition</b>	-0.268	0.725	0.37	0.12	0.717
<b>males</b>					
<b>cort0</b>	-0.051	0.046	-1.08	-0.26	0.289
<b>prolactin</b>	0.062	0.042	1.42	0.29	0.177
<b>body condition</b>	-0.426	1.134	-0.43	-0.12	0.581



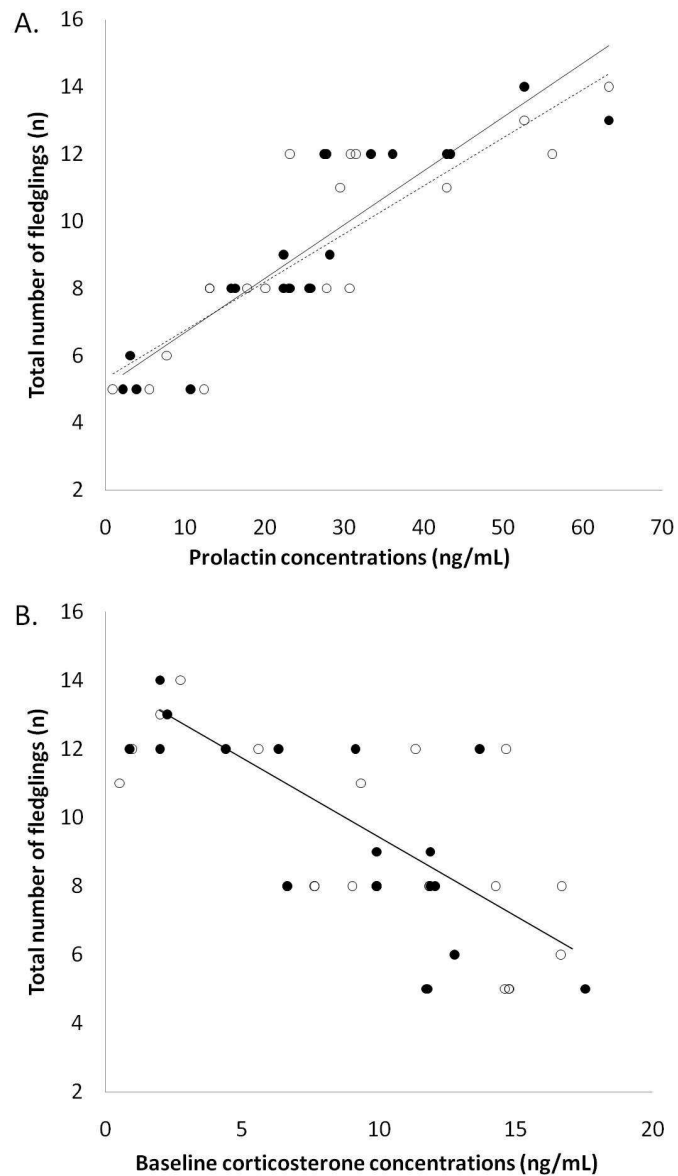


Figure 1. Relationships between total number of fledglings produced by individual birds during the breeding season and a) pre-breeding prolactin concentrations, b) pre-breeding baseline corticosterone concentrations (ng/ml). Females: closed symbols and solid line of best fit, males: open symbols and dashed line of best fit.  
186x323mm (150 x 150 DPI)

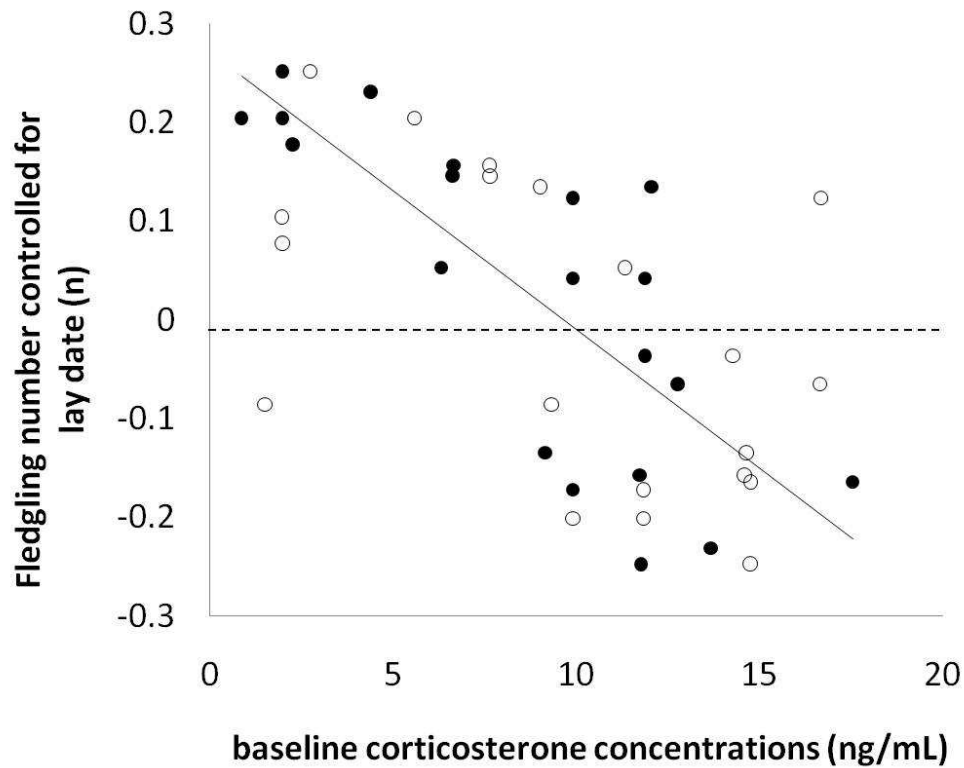


Figure 2. Correlation between residuals of total fledging number (controlled for lay date) and pre-breeding baseline corticosterone concentrations (ng/ml). Individual females above the dotted zero line had more fledglings and lower pre-breeding baseline corticosterone concentrations than females below the dotted zero line regardless of lay date. Females: closed symbols and solid line of best fit, males: open symbols.

173x144mm (150 x 150 DPI)



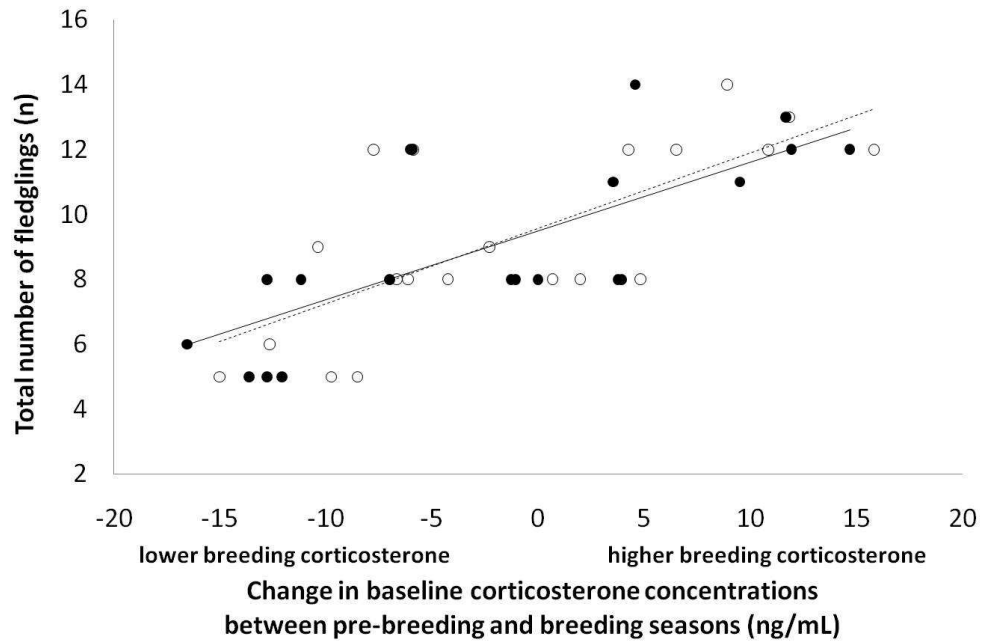


Figure 3. The direction of the change in baseline corticosterone concentrations (breeding-pre-breeding baseline corticosterone; ng/ml) is related to total number of fledglings (n). Individuals with low pre-breeding and high breeding baseline corticosterone had the highest reproductive success. Females: closed symbols and solid line of best fit, males: open symbols and dashed line of best fit. 226x152mm (150 x 150 DPI)

Only

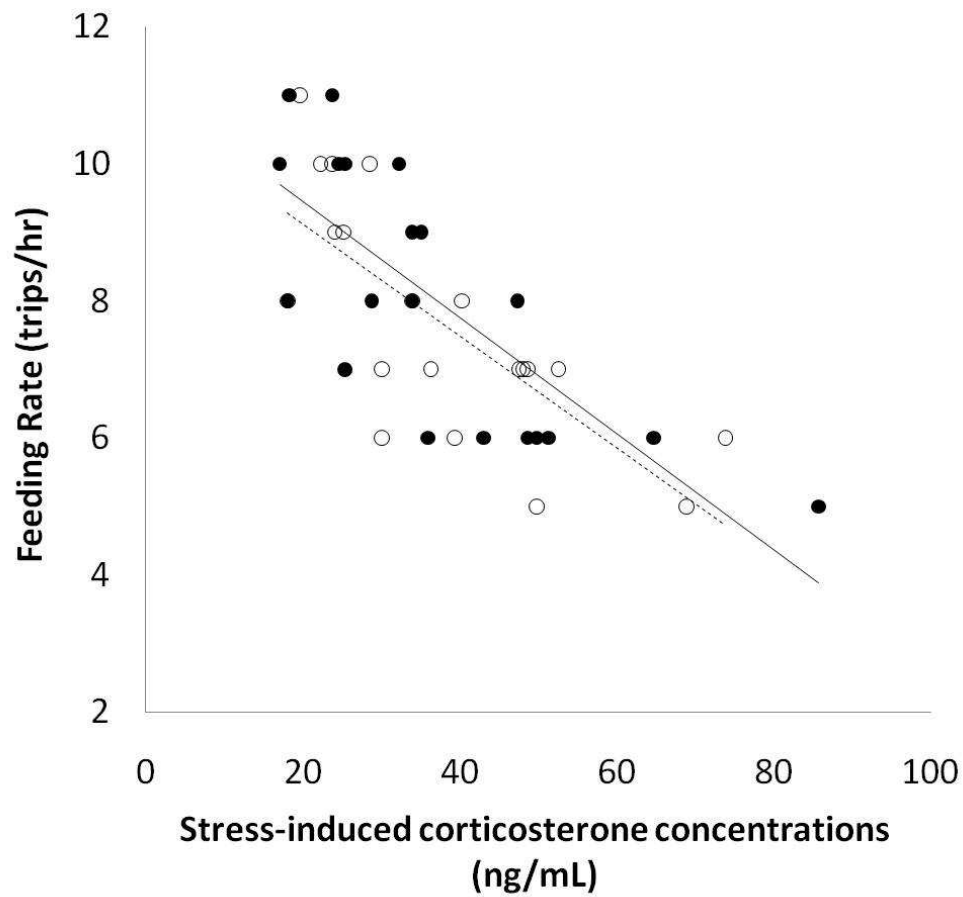


Fig. 4. Stress-induced corticosterone concentrations (ng/ml) during the breeding season were negatively correlated with parental provisioning rates (number of trips/hour). Females: closed symbols and solid line of best fit, males: open symbols and dashed line of best fit.  
167x156mm (150 x 150 DPI)