# Stress response varies with plumage colour and local habitat in feral pigeons

Hélène Corbel, Ariane Legros, Claudy Haussy, Lisa Jacquin, Julien Gasparini, Battle Karimi, Adrien Frantz

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3    Hélène Corbel[a], Ariane Legros[a], Claudy Haussy[a], Lisa Jacquin[a,b], Julien Gasparini[a], Battle

4    Karimi[a] & Adrien Frantz*[a]

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6    [a] Sorbonne Universités, UPMC Univ Paris 06, UPEC, Paris 7, CNRS, INRA, IRD, Institut

7    d'Ecologie et des Sciences de l'Environnement de Paris, F-75005, Paris, France

8    [b] Univ. Toulouse 3 Paul Sabatier, CNRS, ENFA, UMR 5174 EDB (Laboratoire Evolution and

9    Diversité Biologique), FR-31062 Toulouse, France

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12    *Corresponding author:

13    Dr. Adrien FRANTZ

14    Institute of Ecology and Environmental Sciences - Paris

15    7, quai Saint-Bernard - Bât. A 7ème étage (Case 237)

16    F-75252 Paris cedex 05

17    adrien.frantz@upmc.fr

18    Telephone number: +33144273823

19    Fax number: +33144273516

20

21    **Running title**: colouration and stress response in feral pigeons

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**Abstract**

Bird populations exposed to different extrinsic conditions often differ in the responsiveness of

the hypothalamo-pitituary-adrenal (HPA) axis and thus in corticosterone response that

individuals mount when facing stressful events. However, the contribution of genetic

variation to among-individual variability in HPA axis responsiveness across different

environmental conditions is poorly understood. Melanin-based coloured types provide reliable

phenotypic markers of alternative genotypes underlying stress coping styles. Large variations

in melanin-based colouration are heritable in feral pigeons. We tested whether melanin-based

colouration is associated with variation in corticosterone stress response in feral pigeons. To

this aim, we examined how corticosterone response varies both within and between

differently coloured individuals across different environmental conditions. Differently

coloured individuals mounted different stress-induced corticosterone levels in interaction with

environmental conditions: dark pigeons exhibited a higher corticosterone when originating

from rural habitats, while such relation was not observed in pale pigeons. This suggests that

among-population variation in stress response is higher in dark pigeons, this variation

possibly reflecting adjustment and/or (epi)genetic adaptation to environmental conditions. In

addition, corticosterone response increased with the degree of melanin-based colouration in

pigeons originating from rural habitats but not in pigeons originating from more urbanized

populations, resulting in the coexistence of alternative stress responses in some populations,

but not in others. Our results suggest that species with melanin-based variation in differently

44 urbanized populations along rural-urban gradient are potentially good candidate systems for

45 studying stress coping styles under alternative selective regimes.

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50 **Introduction**

51 When facing unpredictable and threatening events due to biotic or abiotic factors, vertebrates

52 mount a stress response which helps individuals to meet energetic demands through resource

53 allocation trade-offs. The hypothalamo-pitituary-adrenal (HPA) axis which culminates in the

54 release of glucocorticoids (GC) is one of the most important physiological mediators of stress

55 response. Thus, stress-induced increases in GC concentrations redirect behaviour and

56 physiology towards immediate survival functions through neoglucogenesis, an increase in

57 locomotor activity and the inhibition of non-vital functions such as reproduction (Dhabhar &

58 McEwen 1997; Breuner *et al.* 1998; Sapolsky *et al.* 2000). Due to its broad implication in the

59 trade-off between survival and reproduction, the HPA-axis reactivity is closely related to

60 fitness (Breuner *et al.* 2008 ; Crespi *et al.* 2013). Hence the adaptive function of the HPA-axis

61 is pervasively invoked to explain differences in stress-induced GC concentrations between

62 populations or subspecies exposed to different extrinsic conditions, with a heavy bias towards

63 bird studies (e.g. altitude: Addis *et al.* 2011; habitat quality: Busch & Hayward 2009;

64 predation risk: Thiel *et al.* 2008; urbanization level: Parteke 2006). Indeed, rising GC stress

65 response could facilitate a fast recovery of positive energy balance in contexts of great

66 energetic demands and low energy stores (McEwen & Wingfield 2003; Addis *et al.* 2011)

67 whereas lowering the response reduces the negative impacts of chronically elevated GC on

68 non-vital functions (e.g. immunity and resistance to oxidative stress; Stier *et al.* 2009; Silverin

69  1998). For instance, up- or down-modulations of stress response would be of primary

70  importance for all animals copying with frequent anthropogenic disturbances (Partecke,

71  2006), possibly facilitating the expansion of species ranges in novel urban environments as

72  suggested by persistent differences detected in GC responses between recently urbanized

73  populations of dark-eyed juncos *Junco hyemalis* and wildland populations in the ancestral

74  range (Atwell *et al.* 2012).

75  Comparing bird populations or subspecies, what drives differences in the HPA stress response

76  remains unclear. On one hand, such differences may arise because populations or subspecies

77  adjust stress response to the prevailing conditions that birds experience at the time of

78  sampling. Indeed, the HPA-axis activity is highly flexible to e.g. food availability (Schoech

79  2006; Schoech *et al.* 2007) or human disturbances (Cockrem *et al.* 2006). The HPA-axis

80  activity also varies as a function of internal state, e.g. body condition (Corbel *et al.* 2010) or

81  stage of life cycle (Romero 2002). On the other hand, stable differences between populations

82  may result from differential selective pressures favouring individuals with respective specific

83  corticosterone stress response through fitness consequences (Satterlee & Johnson 1988; Evans

84  *et al.* 2006; Wada *et al.* 2009; Almasi *et al.* 2010). Indeed, inter-individual variation in the

85  magnitude of elevated GC concentrations is repeatable and partly under genetic control

86  (Evans *et al.* 2006; Partecke *et al.* 2006; Rensel & Schoech 2011; Jenkins *et al.* 2014). The

87  heritability of the GC response to stress is supported by cross-fostering experiments in the

88  field (Jenkins *et al.* 2014) and by selection studies in captive settings (Evans *et al.* 2006 ;

89  Satterlee & Johnson 1988), which confirm that individual variation in GC stress response is

90  underlaid by an appreciable amount of standing additive genetic variation. Stable differences

91  may also arise because of environmental factors that have affected the development of the

92  HPA axis during early life and, thus, the GC stress response that adults can mount (Love &

93  Williams 2008a; Love & Williams 2008b; Wada *et al.* 2009). Thus, the degree to which

94    phenotypic adjustments to environmental stimuli and genetic differences resulting from local

95    adaptation underlie between-population differences in stress response is often unclear while

96    the interplay of both processes is likely to be significant. The maintenance of different

97    populations in common conditions is the first step for disentangling acute adjustments from

98    more stable, possibly genetically-determined, divergences in the HPA-axis reactivity

99    (Angelier *et al.* 2011; Atwell *et al.* 2012). To have a full view about the regulation of active

100   blood circulating corticosterone, we should also consider the regulation of CBG and hence of

101   free corticosterone and corticosterone bound to CBG (Almasi *et al*. 2009).

102   Large variations in the magnitude of GC response to stress also occur within populations with

103   some individuals having constantly low or constantly high GC responses across time, together

104   with a suite of correlated behavioural and physiological traits, leading to distinct, repeatable

105   stress copying styles (Cockrem 2007; Cockrem *et al.* 2009; Koolhass *et al.* 1999). Genetic

106   studies using offspring-parent regression found significant narrow-sense heritability estimates

107   of coping styles (25%, Drent *et al.* 2003 ; 22% : Dingemanse *et al.* 2002 ; reviewed in van

108   Oers *et al.* 2005). Stress-coping styles define coherent sets of behavioural and physiological

109   responses, including corticosterone release, to challenges faced by animals (Koolhaas *et al.*

110   1999). Based on the empirical findings that different coping styles influence fitness

111   parameters (Dingemanse *et al.* 2003; Dingemanse *et al.* 2004), individual variations in stress

112   responses may have major eco-evolutionary implications and consequences (Wolf &

113   Weissing 2012). First, the coexistence of coping styles occurs only under certain selective

114   regimes, presumably involving fluctuating and/or frequency-dependent selective pressures

115   (Carere *et al.* 2010; Wolf & McNamara 2012). Second, the potential for evolvability in

116   response to new selective regimes should be particularly high for populations with genetic

117   differences in copying styles (Wolf & Weissing 2012). Thus, the consideration of among-

118    individual variation while comparing stress response between populations is crucial, although

119    largely overlooked (Williams *et al.* 2008; but see Atwell *et al.* 2012).

120    Interestingly, melanin-based coloured morphs provide reliable phenotypic markers of

121    alternative genotypes (Theron *et al.* 2001; Mundy *et al.* 2004) that are associated with stress

122    coping styles in several taxa (Ducrest *et al.* 2008; Almasi *et al.* 2010; Kittilsen *et al.* 2009).

123    This is thought to be due to the pleiotropic effects that the genes coding for the melanocortin

124    system exert on stress response (Daynes *et al.* 1987; Racca *et al.* 2005) and suites of

125    correlated phenotypic traits (e.g. aggressiveness, Ducrest *et al.* 2008, Morgan *et al.* 2004).

126    Accordingly, differently coloured individuals differently regulate the HPA axis as reflected by

127    different stress-induced GC levels, the association between colouration and stress response

128    being heritable (Almasi *et al.* 2010). In addition, facing varying levels of stress, differently

129    coloured individuals display alternative reaction norms on energy balance and trade-offs

130    between competing physiological functions (Dreiss *et al.* 2010; Jacquin *et al.* 2012) as

131    expected if stress response is colour-specific. However, whether direction and/or magnitude

132    of the covariation between stress-induced GC levels and plumage colouration differs between

133    different populations living in different environmental conditions has not been tested yet.

134    In this study, we investigated stress response in the feral pigeon *Columba livia*. Feral pigeons

135    originate from domestic pigeons and artificially-selected variation in melanin-based

136    colouration persists in feral populations (Johnston & Janiga 1995; Sol 2008). Such variation

137    in colouration is heritable and associated with alternative physiological norms of reaction as

138    also found in other species with plumage colour variation (Jacquin *et al.* 2012; Jacquin *et al.*

139    2013). After escaping from captivity in the early XIXth century, pigeons have established in a

140    variety of habitats and undergone natural selection ever since (Johnston & Janiga 1995; Sol

141    2008). Habitats of feral pigeons markedly differ notably in their level of urbanization, ranging

142    from rural areas to city centers. Because urbanization dramatically affects both abiotic and

143  biotic environmental conditions (e.g. climate, human disturbance, population density,

144  resource availability, predation risk; Shochat *et al.* 2006; McDonnell & Hahs 2009; Niemela

145  *et al.* 2011), it can alter selection on morphological, behavioral, and physiological traits (Liker

146  *et al.* 2008; Møller 2008; Jacquin *et al.* 2013; Atwell *et al.* 2012), including stress response

147  (e.g. Partecke *et al.* 2006). Accordingly, differences in stress response between differently

148  urbanized populations have been shown in several bird species (reviewed in Bonier 2012),

149  however we crucially lack information on how this trait varies across rural-urban gradients

150  (but see Atwell *et al.* 2012). A noticeable effect of human activities is the buffering of

151  temporal changes in environmental conditions notably for those species relying on humans for

152  food resources as is the case in feral pigeons (Johnston & Janiga 1995; Shochat 2006).

153  Because instability of environment is a potent selective mechanism explaining the

154  maintenance of distinct stress copying styles within populations (Carere *et al.* 2010), the

155  magnitude of among-individual variation in stress response can be hypothesized to vary along

156  a rural-urban gradient, being lower in more stable, urban habitat.

157  In this study, we tested whether the variation in melanin-based colouration was related to

158  alternative stress responses in feral pigeons. To this aim, we examined how stress responses

159  varies both within and between differently coloured individuals in differently urbanized

160  populations. We maintained wild feral pigeons captured in three differently urbanized habitats

161  in similar environmental conditions to disentangle immediate phenotypic adjustments from

162  more stable divergences in the HPA-axis activity (Angelier *et al.* 2011). Captive feral pigeons

163  were submitted to a standard acute restraint stress to compare their corticosterone (main avian

164  GC) response. If melanin-based colouration is a phenotypic marker of stress responses in feral

165  pigeons, we expected differently coloured feral pigeons to display alternative stress responses.

166  Furthermore, if differently coloured feral pigeons display alternative stress responses

167  depending on their originating population, we expected differently coloured individuals to

168  mount different stress-induced corticosterone levels in interaction with habitat. In addition,

169  we expected to detect lower differences in corticosterone stress response between differently

170  coloured pigeons in those populations thriving with more stable environmental conditions as

171  found in urban centers.

172

173  **Materials and methods**

174  Capture and experiments were all approved by the Direction Départementale des Services

175  Vétérinaires de Seine-et-Marne (activity permit # 77-06; aviary licence # A77-431-1).

176  **Capture**

177  Feral adult pigeons were caught with trap cages at the beginning of February 2010 in three

178  different habitats (Urban center, n = 25 individuals; Urban park, n = 22 individuals; Rural

179  farm, n = 24 individuals) of the Parisian region (Table 1). Urban park and center were located

180  in the city of Paris. The farm was situated in a rural area in the Parisian suburbs. Because the

181  distance between capture sites was higher than the recently estimated distance covered by the

182  Parisian feral pigeons within their local environment (less than 800 m; Frantz *et al.* 2012), we

183  assume that these 3 habitats reflect distinct populations with restricted movements between

184  them, thus experiencing different local environmental conditions which can be described as

185  rural farm, urban park and urban center. Urbanization rate of each site was estimated using

186  land use inventory for Paris area (MOS 2003, Institut d'Aménagement et d'Urbanisme Ile-de-

187  France) and MapInfo Professional 8.5 SCP (Pitney Bowes Software Inc.). It was calculated as

188  the proportion of area covered by buildings and roads on the total circular area within a radius

189  of 1000 m around the capture site (Jacquin *et al.* 2013b; Table 1).

190  **Melanin-based colouration**

191  Feral pigeons display a continuous variation in melanin-based plumage colouration from

192  entirely white to entirely black (Johnston & Janiga 1995). The continuous variation of the

193    surface of dark area on the wings across colouration patterns corresponds to different levels of

194    melanin deposited in feathers (Haase *et al.,* 1992; Jacquin *et al.,* 2011). Melanin-based

195    colouration was assessed following Jacquin *et al.* (2011) using pictures of the upper wing

196    taken in standardized light conditions. Ligh conditions were standardized by the use of a 1 m$^2$

197    reflecting white sheet placed 50 cm in the background of the wing, and of two studio lamps

198    placed 1 m both at the left and the right sides from the wing. The wing was stretched by an

199    experimenter and pictures were taken at a resolution of 96 DPI using a camera (Sony DSC-

200    HX1) placed on tripod 1 m from the wing, being saved under JPG files. Pictures were binary

201    transformed using Gimp (GNU image manipulation program) and colouration score was

202    determined as the proportion of black pixels (%) on the upper wing using ImageJ (U.S.

203    National Institutes of Health, Bethesda, USA). For analyses purposes, pale and dark colour

204    groups were defined as pigeons with colouration scores < 50 % and > 50 % respectively

205    (Jacquin *et al.* 2013a). This colouration score has been shown to be highly heritable (82 %;

206    Jacquin *et al.* 2013a).

207    **Housing**

208    Immediately after capture, body mass was measured using a Pesola spring balance (± 5g) and

209    pigeons were brought to the Centre de Recherche en Ecologie Expérimentale et Prédictive

210    (CEREEP, Saint-Pierre-lès-Nemours, France). Pigeons from each habitat were kept in

211    separate outdoor aviaries to limit social and physiological perturbations. Indeed, behavioural

212    and immune traits such as aggressiveness levels and parasite loads often differ between

213    differently urbanized bird populations (e.g. Evans 2010; Jacquin *et al.* 2013b). Thus mixing

214    pigeons from different origins would potentially have confounded the results as both social

215    and parasite environments impact individual corticosterone release in birds (Raouf *et al.*

216    2006; Pryke *et al.* 2007). In addition, social interactions among different colour morphs can

217    generate frequency-dependent effects in the local environment, which may directly affect the

218   level of stress captive birds are exposed to (Pryke *et al.* 2007; Morgan *et al.* 2004). In this

219   study, colouration scores were similarly distributed among the different aviaries (Median test:

220   $\chi^2_2$ = 4.909, P = 0.086; Kruskal Wallis test: H = 3.671, df = 2, P = 0.160) and median score

221   was close to 50% (48.7%), thus any frequency-dependent effect of differently coloured

222   individuals within aviaries on individual corticosterone release was very unlikely. Aviaries

223   were strictly identical (3.10 m × 2 m × 2.40 m), adjacent and visually isolated from one

224   another to limit perturbations. Water and maintenance diet (a mixture of peas, corn and

225   maize) were provided *ad libitum*. This design allowed keeping pigeons in similar captive

226   conditions in terms of housing conditions, bird density and exposure to external stressors.

227   Pigeons were acclimatized to captivity for at least three weeks before measuring their stress

228   responses until daily food intake and body weight were stabilized (Pascual *et al.* 1999).  At

229   the end of the acclimation period, body mass varied by only 2.2 ± 0.5 % (n = 71) from the

230   values at capture, this variation being four-fold lower than that associated with an alteration of

231   the HPA function in captive birds ( > 8%, Romero *et al.* 1997; Romero & Wingfield 1999;

232   Dickens *et al.* 2009). Mass changes during acclimation were not affected by pigeon origin

233   ($F_{2,68}$ = 1.319, *P* = 0.274), colour type  ($F_{1,69}$ = 0.005, *P* = 0.942) or the interaction  between

234   both ($F_{2,65}$ = 1.306, *P* = 0.278).

235   **Stress response measurement**

236   At the end of the acclimation period, all pigeons were subjected to a standard acute restraint

237   stress following a modified version of a protocol by Romero & Wingfield (2001).

238   Experiments were done from the end of February to mid-March 2010 between 9:00 h and

239   13:00 h to minimize the effects of seasonal and circadian changes in corticosterone plasma

240   levels, respectively (Breuner *et al.,* 1999; Romero 2002). The time of day was not related to

241   baseline levels of corticosterone ($R^2$ = 0.001, P = 0.845, N = 41) however it was weakly but

242   significantly related to the levels of stress-induced corticosterone ($R^2$ = 0.096, P, = 0.049 N =

243    41). Because neither did the time of day differ between the aviaries ($F_{2,38}$=1.860, P=0.170) nor

244    was it related to colouration scores ($R^2$ = 0.0003, P = 0.918, N=41), we feel confident that the

245    time of day did not interact with the outcomes of this study. Each pigeon was stressed only

246    once, and a single intervention was done per day and per aviary in order to avoid

247    physiological habituation to stress created by human intervention as demonstrated in

248    chronically stressed birds (Cyr & Romero 2009). Within each aviary, pigeons were randomly

249    sampled with respect to colour type. A rapid screening of feathers was done to ensure that no

250    bird was engaged in moult process as feather renewal may affect the HPA-axis activity

251    (Romero 2002). The onset of the stress was considered as the time at which pigeons detected

252    our presence nearby the aviary, usually 15 s before capture. Pigeons were captured with a net

253    and thereafter kept motionless in an opaque cloth bag in order to create a restraint, stressful

254    condition (Romero & Wingfield 2001). At different times along the restraint stress, blood

255    samples of 0.5 ml were taken from the brachial vein, using a 26 g needle and a 1 ml

256    heparinized syringe. A total of 13% of blood samples, corresponding to 30 pigeons could not

257    be used because of hemolysis or insufficient volume. Thus, analyses hereafter in this study

258    were done on 41 pigeons. Blood was first collected at 3 min after the onset of stress (mean

259    value: 3.30 ± 0.10 min, $n$ = 41). These samples were expected to provide baseline

260    corticosterone levels, since increase in corticosterone levels generally does not occur within 3

261    min following initiation of a stressful stimulus (Wingfield *et al.* 1982). Subsequent samples

262    were collected at 10 min (mean value: 10.71 ± 0.41 min, $n$ = 41), 20 min (mean value: 20.46

263    ± 0.20 min, $n$ = 41) and 30 min (mean value: 29.89 ± 0.10 min, $n$ = 41) after the onset of

264    stress, to obtain a time course of the stress response (Romero & Wingfield 2001). Total blood

265    volume collected from any individual did not exceed 2 ml *i.e.* less than 1 % of body mass as

266    recommended by McGuill & Rowan (1989). Immediately after restraint, pigeons were

267    weighed to the nearest 5g. Body mass at restraint was similar between individuals displaying

268    alternative colour types ($F_{1,39}$ = 1.304, $P$ = 0.260) or from different habitats ($F_{2,38}$ = 0.670, $P$ =

269    0.517) as this was also the case for body mass at capture (colour type effect: $F_{1,39}$ = 0.951, $P$ =

270    0.336; habitat effect: $F_{2,38}$ = 0.958, $P$ = 0.393). Finally, a blood smear was taken to determine

271    parasite intensity (*Haemoproteus spp.*) as the number of infected red blood cells among 10

272    000 cells from different microscopic fields forming a monolayer (Sol *et al.* 2000; Jacquin *et*

273    *al.* 2011). Parasite intensity did not vary between colour types ($F_{1,39}$ = 2.002, $P$ = 0.165) or

274    habitats ($F_{2,38}$ = 0.625, $P$ = 0.541). Blood samples were immediately centrifuged for 10

275    minutes at 14000 rpm to separate plasma from red blood cells. Plasma were then kept at $-20°$

276    C until corticosterone analyses (see below). Red blood cells were kept in a PBS/EDTA

277    anticoagulant solution and were used for molecular sex determination. Briefly, DNA was

278    extracted using a Qiagen kit (DNeasy Blood and Tissue kit, cat. 69506, Qiagen Sciences) and

279    amplified by polymerase chain reaction using the P8 and P2 primers following Griffiths *et al.*

280    (1998). Sex ratio did not differ significantly for pale and dark pigeons ($\chi_1^2$ = 0.028, $P$ = 0.867)

281    and for different habitat types ($\chi_2^2$ = 0.196, $P$ = 0.907). Because the HPA axis activity is likely

282    to be influenced by body mass, parasite intensity and sex (e.g. O'Reilly & Wingfield 2001;

283    Cockrem *et al.* 2006; Raouf *et al.* 2006), all these factors were included in the model

284    explaining the variation in corticosterone levels along restraint stress (see below).

285    **Corticosterone levels measurement**

286    Plasma corticosterone concentrations were determined with an enzyme-immunoassay

287    procedure (corticosterone EIA kit, cat. AC-14F1, IDS Ltd) using polyclonal antiserum against

288    corticosterone coated onto the inner surface of polystyrene microtitre wells. Calibrators,

289    controls and diluted (1:2) plasma samples were incubated overnight at 28 °C with peroxidase-

290    labeled corticosterone in the antibody-coated wells. Wells were then washed and a colour

291    reaction was developed using the tetramethylbenzidine chromogen. The absorbance of the

292    reaction mixtures was read in a microplate reader. Colour intensity developed is inversely

293  proportional to the concentration of corticosterone in the diluted samples. The sensitivity of

294  assays was 0.55 ng.ml$^{-1}$ and intra- and inter-assay repeatability were 87.4 % and 79.4 %,

295  respectively.

296  **Statistical analyses**

297  The aim of this study was to investigate the interacting effect of melanin-based colouration

298  with habitat on corticosterone levels. To do this, we used general linear mixed models with

299  corticosterone levels as the dependent variable, blood sampling time along with restraint (*i.e.*

300  the time since the onset of stress), habitat, colour type (pale and dark pigeons) and their

301  interactions as fixed factors, and individual nested within habitat as a random factor. Sex,

302  body mass and parasite intensity were also added as covariates in the model. The best model

303  was chosen according to the AIC$_C$ criterion (Burnham & Anderson 2002) using a descending

304  model selection procedure starting with a model containing the following predictor variables:

305  time, habitat, colour type and all second- and three-term associated interactions plus sex, body

306  mass and parasite intensity. When an interaction was significant, we conducted post-hoc

307  pairwise t-tests using a Bonferroni correction applied on α level of significance for the t-

308  statistic computed by Fishers's LSD method, to control for multiple comparisons of

309  corticosterone levels. *P*-values were adjusted so that a significant level of 0.05 was

310  consistently used (see below). The interacting effect of colour type with habitat on

311  corticosterone levels (see results) was further examined by separate analyses of regression of

312  stress-induced corticosterone levels on colouration scores (continuous variable) in pigeons

313  from each origin. All analyses were run under SPSS 20.0.0. Significance levels were set to

314  0.05 and tests were two-tailed. Means are computed with their standard errors.

315

316

317  **Results**

318    Corticosterone levels increased from $2.07 \pm 0.16$ ng.ml$^{-1}$ (at 3 min after capture) to $14.27 \pm$

319    1.38 ng.ml$^{-1}$ (at 30 min after capture; $n = 41$, $F_{3,120} = 63.422$, $P < 0.001$) across successive

320    blood sampling times, showing that restraint induced a significant stress in individuals.

321    Corticosterone levels measured at 30 minutes after capture were significantly higher than

322    baselines in all groups of pigeons as defined by each combination of colouration $\times$ habitat

323    levels (Bonferroni correction, all $P \leq 0.012$).

324    Changes in corticosterone across blood sampling times was significantly associated with the

325    interacting effect of colour type with habitat, as resulting from the best model (effect of

326    Sampling time x Colour type x Habitat: $P = 0.03$, AIC$_C$ = 884.31; Table 2). Corticosterone

327    levels measured in restrained individuals at 3 min and at 10 min after capture were neither

328    associated with colour type (post hoc Bonferroni correction, all $P > 0.473$) nor with habitat

329    (post hoc Bonferroni correction, all $P > 0.999$, Figure 1). By contrast, at 20 min and at 30 min

330    after capture, darker individuals reached significantly higher corticosterone levels than paler

331    individuals, this difference being detected in pigeons from rural habitat (post-hoc Bonferroni

332    correction, at 20 min: $P = 0.01$ ; at 30 min : $P < 0.001$; Figure 1), but not in pigeons from

333    other habitats (post hoc Bonferroni correction, at 20 min: all $P > 0.37$ ; at 30 min : all $P >$

334    0.08; Figure 1). In addition, corticosterone levels measured at 20 min and at 30 min after

335    capture significantly differed among habitats, being higher in pigeons from the rural habitat

336    than in pigeons from the urban center habitat, these differences being detected in darker

337    individuals (post hoc Bonferroni correction for urban center *versus* rural farm at 20 min: $P =$

338    0.046; at 30 min: $P < 0.001$; Figure 1), but not in paler individuals (post-hoc Bonferroni at 20

339    min: all $P > 0.93$; at 30 min: all $P > 0.67$; Figure 1). A positive, linear relation between stress-

340    induced corticosterone levels at 30 min and colouration scores (continuous variable) was

341    detected in pigeons from rural farm ($F_{1,11} = 7.190$, $P = 0.021$, $R^2 = 0.395$, $n = 13$; Figure 2c)

342    suggesting than darker pigeons mounted a stronger corticosterone response, but not in pigeons

343  from urban center ($F_{1,13}$ = 1.773, $P$ = 0.206, $R^2$ = 0.120, $n$ = 15; Figure 2a) or urban park ($F_{1,11}$

344  = 1.094, $P$ = 0.318, $R^2$ = 0.090, $n$ = 13; Figure 2b).

345

346

347  **Discussion**

348  The aim of this study was to compare stress response both within and between differently

349  coloured pigeons across differently urbanized habitats.

350  Our results show that dark pigeons from the rural habitat reached higher levels of

351  corticosterone levels in response to stress as compared to dark pigeons from the urban center

352  habitat and to pale pigeons from either habitat. Differences between mean corticosterone

353  levels at 30 min of restraint that are necessary for statistical tests to have an acceptable power

354  (80%) have been defined by a recent meta-analysis using the coefficients of variation

355  obtained in several bird species (Cockrem *et al.* 2009).  A rule of thumb is that the difference

356  between means must be at least 80 % and 50 % of the lowest mean for sample sizes of 5 and

357  10, respectively. In this study, mean corticosterone levels at 30 min differed by 143 % and

358  137 % of the lowest mean between differently coloured pigeons in rural habitat (9.80 ng.ml$^{-1}$

359  *versus* 23.21 ng.ml$^{-1}$, N > 6) and between dark pigeons from differently urbanized habitats

360  (9.55 ng.ml$^{-1}$ *versus* 23.21 ng.ml$^{-1}$, N ≥ 5), respectively. Thus, we were confident that the

361  significant interacting effect of colour type with habitat on stress-induced corticosterone

362  levels did not arise by sampling error.

363  Inter-individual variations in corticosterone stress response can arise from altered pituitary

364  and/or adrenal functions due to chronic stress generated by captive (Romero & Wingfield

365  1999; Dickens *et al.* 2009) or natural conditions (Walker *et al.* 2006; Cyr & Romero 2009),

366  but this possibility seems unlikely in our study. First, acclimation to captivity lasted for at

367  least three weeks (following Pascual *et al.* 1999) and resulted in low body mass variation (2

368 % only) and in corticosterone levels close to, or lower than, those of free-living pigeons

369 (baseline levels: 2 *versus* 9 ng.ml$^{-1}$; stress-induced levels; 14 *versus* 15 ng.ml$^{-1}$; Romero &

370 Wingfield 2001). This contrasts with expectations under stressful captive conditions (Romero

371 *et al.* 1997; Romero & Wingfield 1999; Dickens *et al.* 2009). Second, corticosterone baseline

372 levels did not depend on the factors of interest (colour types and habitats; Figure 1),

373 suggesting that different stress responses to restraint experiment were not due to differences

374 in physiological adjustments to captivity. Additionally, it is very unlikely that any seasonal

375 flexibility of stress response can account for differences in corticosterone levels found in our

376 work because pigeons were all synchronized for moult and were all submitted to the same

377 light, climate and feeding conditions. Finally, health parameters known to be affected by

378 chronic stress (body mass and parasite intensity; Cyr & Romero 2009) did not vary according

379 to colour types or habitats, nor did they contribute to predict variations in corticosterone

380 levels. Hence, variations in stress responses observed between pigeons in our study were

381 unlikely to result from chronic stress or associated alteration of the HPA-axis.

382 Although we found no significant difference in health marks of chronic stress between

383 differently coloured pigeons, the role for other physiological factors *e.g.* oxidative stress

384 and/or social factors affecting corticosterone levels can not be ruled out (Senar 1999;

385 Giraudau & McGraw 2014). Alternatively, the difference in stress response may rather have

386 a genetic basis. Such association between colouration and stress response may be due to the

387 pleiotropic effects of the melanocortin system (Ducrest *et al.* 2008). Melanocortins (α-, β-,

388 and γ-MSH and ACTH) of *POMC*-gene bind to the melanocortin-1 receptor (MC1-R) thereby

389 triggering melanin synthesis, but also to other receptors involved in the HPA-axis activation

390 (MC2-R and MC4-R), thus inducing some pleiotropic effects on glucocorticoid production

391 and resistance to stress (reviewed in Ducrest *et al.* 2008). Accordingly, systemic injections of

392 α-MSH in rodents induce changes in stress-induced corticosterone levels (Daynes *et al.* 1987;

393     Racca *et al.* 2005). In wild barn owls *Tyto alba*, genetic association between colouration and

394     stress response has been inferred from parent-to-offspring inheritance of covariation between

395     the degree of melanic colouration and stress-induced corticosterone levels (Almasi *et al.*

396     2010). Furthermore, in domestic rainbow trout *Oncorhynchus mykiss*, melanin pigmentation

397     patterns are associated with, and can be artificially co-selected with, the responsiveness of the

398     hypothalamus-pituitary-interrenal axis (the equivalent of HPA axis in fishes; Kittilsen *et al.*

399     2009). Here, we found that stress-induced corticosterone levels covaried with the degree of

400     melanic colouration, as reflected by black colouration scores, in one population of feral

401     pigeons. In the same species, food restriction affects the physiological trade-offs between

402     maintenance and reproduction as a function of black colouration scores in the same species

403     Indeed, under food restriction, darker eumelanic females had a higher egg production, but

404     darker adults tended to lose more body mass than paler conspecifics (Jacquin *et al.* 2012).

405     Because the HPA-axis is broadly implicated in such trade-offs (Sapolsky *et al.* 2000), it is

406     likely that a responsiveness of the HPA-axis to nutritional stress depending on plumage

407     colouration partly explains the results of Jacquin *et al.* 2012., which is in full accordance our

408     results. Overall, our results support the idea that melanin-based colouration is associated with

409     the ability to cope with stress (Fargallo *et al.* 2007; Roulin *et al.* 2008; Almasi *et al.* 2012)

410     through a differential regulation of corticosterone release (Almasi *et al.* 2010).

411         The effect of origin on the covariation between colouration and stress response could

412     reflect either an aviary effect, although the aviaries did not differ in any characteristics (size

413     or any biotic or abiotic parameter measured), and/or a habitat effect. The effect of origin on

414     the covariation between colouration and stress response could reflect either an aviary effect or

415     a habitat effect, as our design could not disentangle these factors. While the first possibility

416     cannot be ruled out, it seems unlikely because aviaries did not differ in any characteristic

417     (size, exposition, or any biotic or abiotic parameter measured). Differently coloured pigeons

418 may display different levels of expression of genes involved in the HPA axis activity in

419 relation to environmental factors (either under captive or natural conditions). These factors

420 may involve peri-natal exposure to corticosterone which has profound organizational effects

421 on the HPA axis that shape stress response later in life (Love & Williams 2008a; Love &

422 Williams 2008b; Wada *et al.* 2009). Corticosterone levels in developing birds primarily

423 depend on maternal condition, nest-site characteristics and parental provisioning, all of these

424 factors being likely affected by the level of urbanization of habitats (Crino *et al.* 2011, Bonier

425 2012). An interacting effect of melanic colouration with environment on the level of

426 melanocortins has been well identified in tawny owls *Strix aluco*. In this species, the level of

427 *POMC*-gene expression, as reflected by the circulating levels of POMC prohormone, as well

428 as the level of gene expression of convertase enzyme *PC1/3* which cleaves POMC

429 prohormone to obtain ACTH, covary with the degree of melanic colouration under favorable

430 conditions (Roulin *et al.* 2011; Emaresi *et al.* 2013). By contrast, in birds experiencing natural

431 or experimentally-induced higher levels of stress, no colour-specific difference is detected

432 potentially leading to up- and down-regulation of melanocortins acting on the HPA axis

433 activity depending on conditions (Noguchi *et al.* 2006; Roulin *et al.* 2011). Whether variation

434 in stress-induced corticosterone levels is linked to those of POMC-gene expression in feral

435 pigeons remains to be tested. Finally among-population variation in stress response could be

436 due to some genetic variation between the rural and the center-urbanized populations,

437 independently of the colour phenotype and genetic underpinning. Indeed, important genetic

438 variation exists between *C. livia* populations, for instance in the allele frequencies of

439 numerous polymorphic proteins (Johnston & Janiga, 1995). Such variations are notably

440 measured between domestic and feral populations (Johnston & Janiga, 1995). In our study, it

441 is likely that rural pigeons are genetically closer to domestic lines than are the center-

442    urbanized pigeons. Such a possibility requires future investigations examining the relationship

443    between the genetic transmission of stress response and that of plumage coloration.

444         Alternatively, our results may reflect genetically-based, colour-specific divergences in

445    stress response between differently urbanized populations due to selective forces associated

446    with the urban environment. Such divergence despite the relatively recent history of urban

447    colonization in feral pigeons would imply that evolutionary processes act quickly to shape the

448    corticosterone response to stress, as also suggested in other bird species (Partecke *et al.* 2006;

449    Angelier *et al.* 2011; Atwell *et al.* 2012). Rationales for strong selection on stress response is

450    that it is genetically determined and heritable (Evans *et al.* 2006; Jenkins *et al.* 2014) and it

451    has important fitness consequences (Breuner *et al.* 2008). Among the possible mechanisms of

452    inheritance of stress response in polymorphic species, a role for imprinting on the genes

453    coding for the receptors to regulate the HPA axis has been proposed (Almasi *et al.* 2010;

454    Chong *et al.* 2007). Such genomic imprinting would occur in darker individuals and impair

455    under some circumstances the pleiotropically-mediated genetic association between stress

456    response and melanin-based colouration (Almasi *et al.* 2010). A similar mechanism may

457    operate in dark feral pigeons established in highly urbanized areas, potentially conferring

458    offspring with selective advantages as imprinted genes are targeted by selection and allow for

459    rapid adaptive evolution (Danchin *et al.* 2011). So far, two studies have examined the genetic

460    underpinning of differences in stress responses between differently urbanized populations of

461    birds. Both studies found that urban birds raised in a common garden showed an attenuated

462    corticosterone response to a 30-min restaint stress when compared with their wildland

463    counterparts, the difference between corticosterone levels being highly comparable to that

464    observed in dark feral pigeons (mean stress-induced corticosterone levels in less urbanized

465    versus more urbanized populations: European blackbirds *Turdus merula* : 30 *versus* 15 ng.ml$^{-}$

466    $^{1}$, Partecke *et al.* 2006; dark-eyed junco *Junco hyemalis* : 20 *versus* 12 ng.ml$^{-1}$, Atwell *et al.*

467    2012; feral pigeons: 23 versus 10 ng.ml$^{-1}$, this study). Thus our findings further extend the

468    idea of urban selective pressures driving genetically-determined attenuated HPA

469    responsiveness. This hypothesis should be further examined using replicates of habitats for

470    each level of urbanization.

471         Independently of their exact underpinnings, a fundamental aspect of our results is

472    that variation in stress response across different environmental conditions was observed in

473    dark, but not in pale pigeons. This suggests that the genetic basis of stress response in darker

474    pigeons allows for a greater plasticity or flexibility to environmental conditions (genotype-by-

475    environment effect) and/or is more easily targeted by selection. Jacquin *et al.* (2013a) recently

476    showed that feral pigeons are darker in more urbanized areas of the Parisian agglomeration

477    despite important gene flux between populations (Jacob, Prévot-Julliard and Baudry,

478    unpublished), suggesting strong effect of selection on plumage colouration (Kawecki and

479    Ebert 2004, Antoniazza *et al.* 2010). Whether any modulation of the HPA responsiveness

480    accounts for some processes of local adaptation in dark pigeons requires extended field

481    studies measuring the relation between stress physiology and fitness (reproductive success

482    and survival) in differently coloured and differently urbanized individuals. The precise urban

483    selective pressures involved in such local adaptation remain unknown, but may involve any

484    factor associated with urban environments including trace metals (Chatelain *et al.* 2014) and

485    human presence (Møller 2008).

486         Another important finding is that the covariation between melanin-based colouration

487    and stress-induced corticosterone levels was observed in rural pigeons but not in more

488    urbanized ones. This result opens the possibility that the genetic, pleiotropically-mediated

489    association between colouration and stress response may be disrupted for some reasons and

490    under some circumstances, resulting in the coexistence of alternative stress responses in some

491    populations with plumage colour variation, but not in others. Theoretical models predict that

492 the instability of environment is a potent selective mechanisms explaining individual

493 variations in stress coping styles within populations (Dingemanse *et al.* 2004, Dingemanse &

494 Réale 2005; Carere *et al.* 2010) and more generally, that distinct personalities should emerge

495 only under moderately favourable ecological conditions (*i.e.* under neither unfavourable nor

496 highly favourable conditions; Luttbeg & Sih 2010). Our results support the above predictions

497 as the coexistence of colour-specific stress responses occurs in rural but not in urbanized areas

498 which are characterized by a hampering of environmental variability (e.g. the seasonality of

499 resource availability and climate) and likely constitute highly favourable ecological

500 conditions for feral pigeons (Sacchi *et al.* 2002; Shochat *et al.* 2006). Another selective factor

501 on the diversity of coping styles is the relative frequency of occurrence of similarly or

502 differently behaving individuals (Wolf & McNamara 2012), which likely varies between

503 differently urbanized populations of feral pigeons (Jacquin *et al.* 2013b). We are aware that

504 measuring stress response only once within individuals and only in captive birds limits the

505 inferences that can be made, both with respect to the repeatability and the generalization

506 under natural conditions. However, our results suggest that habitats along rural-urban gradient

507 are potentially good candidates for alternative selective regimes acting on stress coping styles

508 and more generally personalities. This hypothesis has already been partially validated by

509 recent studies in birds (Evans *et al.* 2010; Atwell *et al.* 2012; Bokony *et al.* 2012; Miranda *et*

510 *al.* 2013; Mueller *et al.* 2013) and humans (Malan *et al.* 2012). Research efforts are now

511 needed towards the measurements of suites of behavioural and physiological traits along the

512 proactive-reactive coping style axis in urban versus rural populations of pigeons and other

513 species with plumage colour variation.

514        To conclude, we show that in a bird species with plumage colour variation, the

515 degree of melanin-based colouration is associated with the ability to modulate stress response

516 as a function of environmental conditions. The extent to which phenotypic adjustments,

517 (epi)genetic divergences and the interaction between both processes explain differences in

518 colour-specific stress responses across environmental conditions requires further studies. The

519 issue could be addressed using common garden studies examining the reaction norms on

520 corticosterone release in differently coloured individuals from distinct habitats. Whatever the

521 exact cause of variation, the changes in stress response across environments being likely

522 genetically associated with colouration provides promising perspectives for the evolvability of

523 the HPA-axis in species with plumage colour variation. Thus our results suggest the

524 suitability of biological models with melanin-based variation in questioning the evolvability

525 of the HPA axis and perhaps that of copying styles under different selective regimes, at a time

526 when flourishing theoretical models explaining the evolution of personalities urgently call for

527 empirical tests (Wolf *et al.* 2007; Dingemanse & Wolf 2010; Dingemanse *et al.* 2010; Luttbeg

528 & Sih 2010; Carere *et al.* 2010; Wolf & McNamara 2012).

**Conflicts of interest**

The authors declare that they have no conflict of interest.

**Literature cited**

546

547    Addis E.A., Jason E.D., Brooks, E.M. & Wingfield, J.C. (2011). Variation in circulating

548    corticosterone levels is associated with altitudinal range expansion in a passerine bird.

549    *Oecologia*, 167: 369-378.

550    Almasi B., Jenni L., Jenni-Eiermann S. & Roulin A. (2010). Regulation of stress

551    response is heritable and functionally linked to melanin-based coloration. *Journal of*

552    *Evolutionary Biology*, 23: 987-996.

553    Almasi B., Roulin A., Jenni-Eiermann S., Breuner C.W. & Jenni L. (2009). Regulation

554    of free corticosterone and CBG capacity under different environmental conditions in altricial

555    nestlings. *General and Comparative Endocrinology*, 164: 117-124.

556    Almasi B., Roulin A., Korner-Nievergelt F., Jenni-Eiermann S. & Jenni. L. (2012).

557    Coloration signals the ability to cope with elevated stress hormones: effects of corticosterone

558    on growth of barn owls are associated with melanism. *Journal of Evolutionary Biology*, 25:

559    1189-1199.

560    Angelier F., Ballentine B., Holberton R.L., Marra P.P. & Greenberg R. (2011). What

561    drives variation in the corticosterone stress response between subspecies? A common garden

562    experiment of swamp sparrows (*Melospiza georgiana*). *Journal of Evolutionary Biology*, 24:

563    1274-1283.

564    Antoniazza S., Burri R., Fumagalli L., Goudet J. & Roulin A. (2010). Local adaptation

565    maintains clinal variation in melanin-based coloration of European barn owls (*Tyto alba*).

566    *Evolution*, 64: 1944–1954.

567    Atwell J.W., Cardoso G.C., Whittaker D.J., Campbell-Nelson S., Robertson K.W. &

568    Ketterson E.D. (2012). Boldness behavior and stress physiology in a novel urban environment

569    suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, 23: 960-969.

570      Bokony V., Kulcsar A., Toth Z. & Liker A. (2012). Personality Traits and Behavioral

571      Syndromes in Differently Urbanized Populations of House Sparrows (*Passer domesticus*).

572      *PLoS One*: 7 (5): e36639.

573      Bonier F. (2012). Hormones in the city: endocrine ecology of urban birds. *Hormones*

574      *and Behavior*, 61: 763-772.

575      Breuner C.W., Greenberg A.L. & Wingfield J.C. (1998). Noninvasive corticosterone

576      treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia*

577      *leucophrys gambelii*). *General and Comparative Endocrinology*, 111: 386-394.

578      Breuner C.W., Wingfield J.C. & Romero L.M. (1999). Diel rhythms of basal and stress-

579      induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow.

580      *Journal of Experimental Zoology*, 284: 334–342.

581      Breuner C.W., Patterson S.H. & Hahn T.P. (2008). In search of relationships between

582      the acute adrenocortical response and fitness. *General and Comparative Endocrinology*,

583      157:288-295.

584      Burnham K.P. & Anderson D.R. (2002) *Model selection and multimodel inference: a*

585      *practical information-theoretic approach*. Springer, New York.

586      Busch D.S. & Hayward L.S. (2009). Stress in a conservation context: A discussion of

587      glucocorticoid actions and how levels change with conservation-relevant variables. *Biological*

588      *Conservation*, 142: 2844-2853.

589      Carere C., Caramaschi D. & Fawcett T.W. (2010). Covariation between personalities

590      and individual differences in coping with stress: converging evidence and hypotheses.

591      *Current Zoology*, 56: 728-740.

592      Chatelain M., Gasparini J., Jacquin L. & Frantz A. (2014). The adaptive function of

593      melanin-based plumage coloration to trace metals. *Biology Letters*, 10: 20140164.

594    Chong S.Y., Vickaryous N., Ashe A., Zamudio N., Youngson N., Hemley S., Stopka T.,

595    Skoultchi A., Matthews J., Scott H.S., de Kretser D., O'Bryan M., Blewitt M. & Whitelaw E.

596    (2007). Modifiers of epigenetic reprogramming show paternal effects in the mouse. *Nat.*

597    *Genet.,* 39: 614–622.

598    Cockrem J.F. (2007). Stress, corticosterone responses and avian personalities. *Journal*

599    *of Ornithology*, 148: S169–S178.

600    Cockrem J.F., Potter M.A. & Candy E.J. (2006). Corticosterone in relation to body mass

601    in Adelie penguins (*Pygoscelis adeliae*) affected by unusual sea ice conditions at Ross Island,

602    Antarctica. *General and Comparative Endocrinology*, 149: 244-252.

603    Cockrem J.F., Barrett D.P., Candy E.J. & Potter M.A. (2009). Corticosterone responses

604    in birds: Individual variation and repeatability in Adelie penguins (*Pygoscelis adeliae*) and

605    other species, and the use of power analysis to determine sample sizes. *General and*

606    *Comparative Endocrinology*, 163: 158–168.

607    Corbel H., Geiger S. & Groscolas R. (2010). Preparing to fledge: the adrenocortical and

608    metabolic responses to stress in king penguin chicks. *Functional Ecology*: 24, 82-92.

609    Crespi E.J., Williams T.D., Jessop T.S. & Delehanty B. (2013). Life history and the

610    ecology of stress: how do glucocorticoid hormones influence life-history variation in animals?

611    *Functional Ecology*, 27: 93-106.

612    Crino O.L., Klaassen Van Oorschot B., Johnson E.E., Malisch J.L. & Breuner C.W.

613    (2011). Proximity to a high traffic road: Glucocorticoid and life history consequences for

614    nestling white-crowned sparrows. *General and Comparative Endocrinology*, 173: 323-332.

615    Cyr N.E. & Romero L.M. (2009). Identifying hormonal habituation in field studies of

616    stress. *General and Comparative Endocrinology*, 161: 295-303.

617        Danchin E., Charmantier A., Champagne F.A., Mesoudi A., Pujol B. & Blanchet S.

618 (2011). Beyond DNA: integrating inclusive inheritance into an extended theory of evolution.

619 *Nat. Rev. Genet.*, 12: 475-486.

620        Daynes R.A., Robertson B.A., Cho B.H., Burnham D.K. & Newton R. (1987) Alpha-

621 melanocyte-stimulating hormone exhibits target cell selectivity in its capacity to affect

622 interleukin 1-inducible responses in vivo and in vitro. *Journal of Immunolology,* 139: 103-

623 109.

624        Dhabhar F.S. & McEwen B.S. (1997). Acute stress enhances while chronic stress

625 suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. *Brain*

626 *Behavior and Immunity*, 11: 286-306.

627        Dickens M.J., Kristen A., Earle L. & Romero L.M. (2009). Initial transference of wild

628 birds to captivity alters stress physiology. *General and Comparative Endocrinology*, 160: 76-

629 83.

630        Dingemanse N.J., Both C., Drent P.J., van Oers K. & van Noordwijk A.J. (2002).

631 Repeatability and heritability of exploratory behaviour in wild great tits. *Anim. Behav.*, 64:

632 929-937.

633        Dingemanse N.J. & Réale D. (2005). Natural selection and animal personality.

634 *Behaviour*, 142: 1159-1184.

635        Dingemanse N.J. & Wolf M. (2010). Recent models for adaptive personality

636 differences: a review. *Philosopical Transactions of the Royal Society B-Biological Sciences*,

637 365: 3947-3958.

638        Dingemanse N.J., Both C., van Noordwijk A.J., Rutten A.L. & Drent P.J. (2003). Natal

639 dispersal and personalities in great tits (Parus major). *Proceedings of the Royal Society B-*

640 *Biological Sciences*, 270: 741-747.

641         Dingemanse N.J., Both C., Drent P.J. & Tinbergen J.M. (2004). Fitness consequences

642 of avian personalities in a fluctuating environment. *Proceedings of the Royal Society B-*

643 *Biological Sciences*, 271: 847-852.

644         Dingemanse N.J., Kazem A.J.N., Réale D. & Wright J. (2010). Behavioural reaction

645 norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, 25: 81-

646 89.

647         Dreiss A.N., Antoniazza S., Burri R., Fumagalli L., Sonnay C., Frey, C. Goudet J. &

648 Roulin A. (2012). Local adaptation and matching habitat choice in female barn owls with

649 respect to melanic coloration. *Journal of Evolutionary Biology*, 25: 103-114.

650         Drent, P.J., van Oers K. & van Noordwijk A.J. (2003). Realized heritability of

651 personalities in the great tit (Parus major). *Proc. R. Soc. Lond. B,* 270: 45-51.

652         van Oers, K. et al. (2005) Contribution of genetics to the study of animal personalities: a

653 review of case studies. *Behaviour* 142, 1185–1206.

654         Ducrest A.L., Keller L. & Roulin A. (2008). Pleiotropy in the melanocortin system,

655 coloration and behavioural syndromes. *Trends in Ecology and Evolution*, 23: 502-510.

656         Emaresi G., Ducrest A.L., Bize P., Richter H., Simon C. & Roulin A. (2013). Pleiotropy

657 in the melanocortin system: expression levels of this system are associated with

658 melanogenesis and pigmentation in the tawny owl (*Strix aluco*). *Molecular Ecology*, 22:

659 4915-4930.

660         Evans M.R., Roberts M.L., Buchanan K.L. & Goldsmith A.R. (2006). Heritability of

661 corticosterone response and changes in life history traits during selection in the zebra finch.

662 *Journal of Evolutionary Biology*, 19: 343–352.

663         Evans K.L. (2010). *Individual species and urbanization*. In *Urban ecology*. (ed Gaston,

664 K.J), pp. 53-87. Cambridge, UK: Cambridge University Press.

665    Fargallo J.A., Laaksonen T., Korpimaki E. & Wakamatsu K. (2007). A melanin-based

666    trait reflects environmental growth conditions of nestling male Eurasian kestrels.

667    *Evolutionary Ecology*, 21:157–171.

668    Frantz A., Pottier M.A., Karimi B., Corbel H., Aubry E., Haussy C., Gasparini J. &

669    Castrec-Rouelle M. (2012). Contrasting levels of heavy metals in the feathers of urban

670    pigeons from close habitats suggest limited movements at a restricted scale. *Environmental*

671    *Pollution*, 168: 23-28.

672    Giraudeau M. & McGraw K.J. (2014). Physiological correlated of urbanization in a

673    desert songbird. *Integrative and Comparative Biology*, 54 : 622-632.

674    Griffiths R., Double M.C., Orr K. & Dawson R.J.G. (1998). A DNA test to sex most

675    birds. *Molecular Ecology*, 7: 1071–1075.

676    Haase E., Ito S., Sell A. & Wakamatsu K. (1992). Melanin concentrations in feathers

677    from wild and domestic pigeons. *Journal of Heredity*, 83: 64-67.

678    Jacquin L., Lenouvel P., Haussy C., Ducatez S. & Gasparini J. (2011). Melanin-based

679    coloration is related to parasite intensity and cellular immune response in an urban free living

680    bird: the feral pigeon *Columba livia*. *Journal of Avian Biology*, 42: 11-15.

681    Jacquin L., Récapet C., Bouche P., Leboucher G. & Gasparini J. (2012). Melanin-based

682    coloration reflects alternative strategies to cope with food limitation in pigeons. *Behavioral*

683    *Ecology,* 23: 907-915.

684    Jacquin L., Haussy C., Bertin C., Laroucau K. & Gasparini J. (2013a). Darker female

685    pigeons transmit more specific antibodies to their eggs than do paler ones. *Biological Journal*

686    *of the Linnean Society*, 108: 647-657.

687    Jacquin L., Récapet C., Prévot-Julliard A.C., Leboucher G., Lenouvel P., Erin N.,

688    Frantz A., Corbel H., Gasparini J. (2013b). A potential role for parasites in the maintenance of

689    bird color polymorphism in cities. *Oecologia*, 173: 1089-1099.

690 Jenkins B.R., Vitousek M.N., Hubbard J.K. & Safran R.J. (2014). An experimental

691 analysis of the heritability of variation in glucocorticoid concentrations in a wild avian

692 population. *Proc. R. Soc. Lond.*, 281: 0141302. http://dx.doi.org/10.1098/rspb.2014.1302

693 Johnston R.F. & Janiga M. 1995. *Feral pigeons.* (eds). Oxford University Press,

694 Oxford.

695 Kawecki T.J. and Ebert D. (2004). Conceptual issues in local adaptation. *Ecology*

696 *Letters*, 7: 1225-1241.

697 Kittilsen S., Schjolden J., Beitnes-Johansen I., Shaw J.C., Pottinger T.G., Sorensen C.,

698 Braastad B.O., Bakken M. & Overli O. (2009). Melanin-based skin spots reflect stress

699 responsiveness in salmonid fish. *Hormones and Behavior*, 56: 292-298.

700 Koolhaas J.M., Korte S.M., De Boer S.F., Van Der Vegt B.J., Van Reenen C.G.,

701 Hopster H., De Jong I.C., Ruis M.A. & Blokhuis H.J. (1999). Coping styles in animals:

702 current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, 23:

703 925-35.

704 Liker A., Papp Z., Bokony V. & Lendvai A. Z. 2008. Lean birds in the city: body size

705 and condition of house sparrows along the urbanization gradient. *Journal of Animal Ecology*,

706 77: 789-795.

707 Luttbeg B. & Sih A. (2010). Risk, resources and state- dependent adaptive behavioural

708 syndromes. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 365:

709 3977-3990.

710 Love O.P. & Williams T.D. (2008a). Plasticity in the adrenocortical response of a free-

711 living vertebrate: The role of pre- and post-natal developmental stress. *Hormones and*

712 *Behavior*, 54: 496-505.

713      Love O.P. & Williams T.D. (2008b). The adaptive value of stress-induced phenotypes:

714      Effects of maternally derived corticosterone on sex-biased investment, cost of reproduction,

715      and maternal fitness. *The American Naturalist*, 172: E135-E149.

716      Malan L., Hamer M., Reimann M., Huisman H., Van Rooyen J., Schutte A., Schutte R.,

717      Potgieter J., Wissing M., Steyn F., Seedat Y. & Malan N. (2012). Defensive coping,

718      urbanization, and neuroendocrine function in Black Africans: the THUSA study.

719      *Psychophysiology*, 49: 807-814.

720      McDonnell M.J. & Hahs A.K. (2009). Comparative ecology of cities and towns: past,

721      present and future. In *Ecology of cities and towns: a comparative approach* (eds M.J.

722      McDonnell, A.K. Hahs, J. Breuste), pp. 71–89. Cambridge, UK: Cambridge University Press.

723      McEwen B. & Wingfield J. (2003). The concept of allostasis in biology and

724      biomedicine. *Horm. Behav.*, 43: 2–15.

725      McGuill, M.W. & Rowan, A.N. 1989. Biological effects of blood loss: implications for

726      sampling volumes and techniques. *ILAR News*, 31: 5-18.

727      Miranda A.C., Schielzeth H., Sonntag T. & Partecke J. (2013). Urbanization and its

728      effects on personality traits: a result of microevolution or phenotypic plasticity? *Global*

729      *Change Biology*, 19: 2634-2644.

730      Møller A.P. (2008). Flight distance of urban birds, predation, and selection for urban

731      life. *Behavioral Ecology and Sociobiology*, 63: 63-75.

732      Morgan C., Thomas R.E. & Cone R.D. (2004). Melanocortin-5 receptor deficiency

733      promotes defensive behavior in male mice. *Hormones and Behavior*, 45: 58-63.

734      Mueller J.C., Partecke J., Hatchwell B.J., Gaston K.J. & Evans K.L. (2013). Candidate

735      gene polymorphisms for behavioural adaptations during urbanization in blackbirds. *Molecular*

736      *Ecology*, 22: 3629-3637.

737      Mundy N.I. (2005). A window on the genetics of evolution: MC1R and plumage
738      colouration in birds. *Proceedings of The Royal Society B: Biological Sciences*, 272: 1633-
739      1640.

740      Mundy N.I., Badcock N.S., Hart T., Scribner K., Janssen K. & Nadeau N.J. (2004).
741      Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science*, 303:
742      1870–1873.

743      Niemelä J., Breuste J.H., Guntenspergen G., McIntyre N.E., Elmqvist T. & James P.
744      (2011). *Urban Ecology: Patterns, Processes, and Applications*. 392 pages. Oxford University
745      Press.

746      Noguchi T., Makino S., Maruyama H. & Hashimoto K. (2006). Regulation of
747      proopiomelanocortin gene transcription during single and repeated immobilization stress.
748      *Neuroendocrinology*, 84: 21–30.

749      O'Reilly, K.M. & Wingfield, J.C. (2001). Ecological factors underlying the
750      adrenocortical response to capture stress in arctic-breeding shorebirds. *General and*
751      *Comparative Endocrinology,* 124: 1-11.

752      Partecke J., Schwabl I. & Gwinner E. (2006). Stress and the city: urbanization and its
753      effects on the stress physiology in European Blackbirds. *Ecology*, 87: 1945-1952.

754      Pascual J.A., Fryday S.L. & Hart A.D.M. (1999). Effects of Food Restriction on Food
755      Avoidance and Risk of Acute Poisoning of Captive Feral Pigeons from Fonofos-Treated
756      Seeds. *Archives of Environmental Contamination and Toxicology*, 37: 115–124.

757      Pryke S.R., Astheimer L.B., Buttemer W.A. & Griffith S.C. (2007). Frequency-
758      dependent physiological trade-offs between competing colour morphs. *Biology Letters*, 3:
759      494-497.

760      Racca S., Spaccamiglio A., Esculapio P., Abbadessa G., Cangemi L., DiCarlo F. &
761      Portaleone P. (2005). Effects of swim stress and [alpha]-MSH acute pre-treatment on brain 5-

762  HT transporter and corticosterone receptor. *Pharmacology Biochemistry & Behavior,* 81: 894-

763  900.

764  Raouf S.A., Smith L.C., Brown M.B., Wingfield J.C. & Brown C.R. (2006).

765  Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows.

766  *Animal Behaviour*, 71: 39-48.

767  Rensel M.A. & Schoech S.J. (2011). Repeatability of baseline and stress-induced

768  corticosterone levels across early life stages in the Florida scrub-jay (Aphelocoma

769  coerulescens). *Hormones and Behavior*, 59: 497-502.

770  Romero L.M. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-

771  living vertebrates, *General and Comparative Endocrinology*, 128: 1-24.

772  Romero L.M., Ramenofsky M. & Wingfield J.C. (1997). Season and migration alters

773  the corticosterone response to capture and handling in an arctic migrant, the white-crowned

774  sparrow (*Zonotrichia leucophrys gambelii* ). *Comparative Biochemistry and Physiology Part*

775  *C: Pharmacology, Toxicology and Endocrinology*, 116: 171–777.

776  Romero L.M. & Wingfield J.C. (1999). Alterations in hypothalamic-pituitary-adrenal

777  function associated with captivity in Gambel's white-crowned sparrows (*Zonotrichia*

778  *leucophrys gambelii*). *Journal of Comparative Physiology Part B: Biochemistry and*

779  *Molecular Biology*, 122: 13-20.

780  Romero L.M. & Wingfield J.C. (2001). Regulation of the hypothalamic-pituitary-

781  adrenal axis in free-living pigeons. *Journal of Comparative Physiology Part B: Biochemistry*

782  *and Molecular Biology*, 171: 231-235.

783  Roulin A., Emaresi G., Bize P., Gasparini J., Piault R. & Ducrest A.L. (2011). Pale and

784  dark reddish melanic tawny owls differentially regulate the level of blood circulating POMC

785  prohormone in relation to environmental conditions. *Oecologia,* 166: 913-921.

786    Roulin A., Gasparini J., Bize P., Ritschard M. & Richner H. (2008). Melanin-based

787    colorations signal strategies to cope with poor and rich environments. *Behavioral Ecology*

788    *and Sociobiology,* 62: 507-519.

789    Sacchi R., Gentilli A., Razzetti E. & Barbieri F. (2002). Effects of building features on

790    density and flock distribution of feral pigeons *Columba livia var. domestica* in an urban

791    environment. *Canadian Journal of Zoology*, 80:48-54.

792    Sapolsky R.M., Romero L.M. & Munck A.U. (2000). How do glucocorticoids Influence

793    stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions.

794    *Endocrine Reviews*, 21: 55-89.

795    Satterlee D.G. & Johnson W.A. (1988). Selection of japanese quail for contrasting

796    blood corticosterone response to immobilization. *Poultry Science,* 67: 25-32.

797    Schoech S.J., Bowman R., Bridge E.S. & Boughtona R.K. (2007). Baseline and acute

798    levels of corticosterone in Florida Scrub-Jays (Aphelocoma coerulescens): Effects of food

799    supplementation, suburban habitat, and year. *General and Comparative Endocrinology*, 154:

800    150-160.

801    Schoech S. (2006). Food availability and timing of reproduction: Are high-latitude

802    species less flexible in responding to supplementary cues? *Journal of Ornithology*, 147: 70-

803    70.

804    Senar J. C. (1999). Plumage coloration as a signal of social status. *Proceedings of the*

805    *International Ornithological Congress,* 22: 1669-1686.

806    Shochat E., Warren P.C., Faeth S.H. & McIntyre N.E. (2006). From patterns to

807    emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, 21:186–

808    191

809    Silverin B. (1998). Stress responses in birds. *Poultry and Avian Biology Reviews*, 9:

810    153-168.

811    Sol D. (2008). Artificial selection, naturalization, and fitness: Darwin's pigeons
812    revisited. *Biological Journal of the Linnean Society*, 93: 657-665.

813    Sol D., Jovani R. & Torres J. (2000). Geographical variation in blood parasites in feral
814    pigeons: the role of vectors. *Ecography*, 23; 307-314.

815    Stier K.S., Almasi B., Gasparini J., Piault R., Roulin A. & L. Jenni. (2009). Effects of
816    corticosterone on innate and humoral immune functions and oxidative stress in barn owl
817    nestlings. *The Journal of Experimental Biology*, 212: 2085-2091.

818    Theron E., Hawkins K., Bermingham E., Ricklefs R. & Mundy N. I. (2001). The
819    molecular basis of an avian plumage polymorphism in the wild: a point mutation in the
820    melanocortin-1 receptor is perfectly associated with melanism in the bananaquit (*Coereba
821    flaveola*). *Current Biology*, 11: 550-557.

822    Thiel D., Jenni-Eiermann S., Braunisch V., Palme R. & Jenni L. (2008). Ski tourism
823    affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*:
824    a new methodological approach. *Journal of Applied Ecology*, 45: 845-853.

825    Wada H., Salvante K.G., Wagner E., Williams T.D. & Breuner C.W. (2009).  Ontogeny
826    and Individual Variation in the Adrenocortical Response of Zebra Finch (*Taeniopygia
827    guttata*) Nestlings. *Physiological and Biochemical Zoology*, 82: 325-331.

828    Walker, B.G., Boersma, P.D. & Wingfield, J.C. (2006). Habituation of adult magellanic
829    penguins to human visitation as expressed through behavior and corticosterone secretion.
830    *Conservation Biology*, 20: 146-154.

831    Williams C.T., Kitaysky A.S., Kettle A.B. & Buck C.L. (2008). Corticosterone levels
832    vary with breeding stage, body condition index, and reproductive performance in tufted
833    puffins. *General and Comparative Endocrinology*, 158: 29-35.

834    Wingfield J.C., Smith J.P. & Farner D.S. (1982). Endocrine responses of white-crowned
835    sparrows to environmental stress. *Condor*, 84: 399-409.

836    Wolf M., van Doorn G.S., Leimar O. & Weissing F.J. (2007). Evolution of animal

837    personalities. *Nature,* 450: E5-E6.

838    Wolf M. & Weissing F.J. (2012). Animal personalities: consequences for ecology and

839    evolution. *Trends in Ecology and Evolution*, 27: 452-461.

840    Wolf M. & McNamara J. (2012). On the evolution of personalities via frequency-

841    dependent selection. *The American Naturalist*, 179: 679-692.

842    Table 1

843    Characteristics of habitats

| Site | Capture date | Site name | City (district number) | GPS coordinates | Urbanization rate (% construced area) | Habitat type |
|---|---|---|---|---|---|---|
| A | 02/03/2010 | Porte Saint-Denis | Paris (X) | 48°52'11.194"N 2°21'9.342"E | 97 | Urban center |
| B | 04/02/2010 | Arènes de Lutèce | Paris (V) | 48°50'42.269"N 2°21'8.614"E | 79 | Urban park |
| C | 11/02/2010 | Mandres-Les-Roses | Mandres-Les-Roses (94) | 48°42'23.536"N 2°33'15.557"E | 47 | Rural farm |

844

845    Table 2

846    Effects of blood sampling time, plumage colour type and habitat on corticosterone levels ($n$ =

847    164 measurements) along restraint stress in feral pigeons ($n$ = 41 pigeons).

848

| Effect | *df* | *F* | *P* |
|---|---|---|---|
| Sex | 1,34 | 5.52 | **0.03** |
| Sampling time | 3,105 | 64.06 | **< 0.001** |
| Colour type | 1,34 | 3.96 | 0.05 |
| Habitat | 2,34 | 1.37 | 0.27 |
| Sampling time × Colour type | 3,105 | 2.40 | 0.07 |
| Sampling time × Habitat | 6,105 | 0.76 | 0.61 |
| Colour type × Habitat | 2,34 | 3.01 | 0.06 |
| Sampling time × Colour type × Habitat | 6,105 | 2.53 | **0.03** |

849    Significant *P*-values (< 0.05) are in bold

850    **Figure captions**

851

852    **Fig. 1** Corticosterone levels (CORT) measured at 3 min (1$^{st}$ blood sample, baselines), 10 min

853    (2$^{nd}$ blood sample), 20 min (3$^{rd}$ blood sample) and 30 min (4$^{th}$ blood sample) after capture in

854    pale (white circles) and dark (black circles) restrained feral pigeons from 3 habitats (urban

855    center: pale, $n = 10$, dark, $n = 5$; urban park: pale, $n = 5$, dark, $n = 8$; rural area: pale, $n = 7$,

856    dark, $n = 6$). Values are means ± SE. Different superscript letters represent significant

857    differences ($P < 0.05$) between habitats within each colour type morph and each sampling

858    time following post hoc tests. The letters can not be compared within habitats. Asterisks

859    represent significant differences ($P < 0.05$) between colour type morphs within each habitat

860    following post hoc tests

861

862    **Fig. 2** Relation between stress-induced corticosterone levels (CORT) measured at 30 min

863    after captures and black colouration scores in feral pigeons from 3 sites corresponding to a)

864    urban center (N = 15), b) urban park (N = 13), and c) rural area (N = 15). The line is for the

865    significant regression of CORT on colouration scores ($P = 0.021$, see results).