GLOBAL CHANGE ECOLOGY - ORIGINAL RESEARCH

A potential role for parasites in the maintenance of color polymorphism in urban birds

L. Jacquin · C. Récapet · A.-C. Prévot-Julliard ·
G. Leboucher · P. Lenouvel · N. Erin ·
H. Corbel · A. Frantz · J. Gasparini

Abstract Urbanization is a major challenge for biodiversity conservation, yet the evolutionary processes taking place in urbanized areas remain poorly known. Human activities in cities set new selective forces in motion which need to be investigated to predict the evolutionary responses of animal species living in urban areas. In this study, we investigated the role of urbanization and parasites in the maintenance of melanin-based color polymorphism in the feral pigeon *Columba livia.* Using a correlative approach, we tested whether differently colored genotypes displayed alternative phenotypic responses to urbanization, by comparing body condition, blood parasite prevalence and parasite load between colored morphs along an urbanization gradient. Body condition did not vary with urbanization, but paler individuals had a higher body condition than darker individuals. Moreover, paler morphs were less often parasitized than darker morphs in moderately urbanized habitats, but their parasite prevalence increased with urbanization. In contrast, darker morphs had similar parasite prevalence along the urbanization gradient. This suggests that paler morphs did better than darker morphs in moderately urbanized environments but were negatively affected by increasing urbanization, while darker morphs performed equally in all environments. Thus, differently colored individuals were distributed non-randomly across the urban habitat and suffered different parasite risk according to their location (a gene-by-environment interaction). This suggests that melanin-based coloration might reflect alternative strategies to cope with urbanization via different exposure or susceptibility to parasites. Spatial variability of parasite pressures linked with urbanization may, thus, play a central role in the maintenance of plumage color polymorphism in this urban species.

Keywords Urban ecology · Host-parasite interactions · Melanin-based coloration · Avian malaria · Feral pigeon

L. Jacquin · C. Récapet · P. Lenouvel · N. Erin · H. Corbel ·
A. Frantz · J. Gasparini
Laboratoire Ecologie et Evolution (EcoEvo), Université Pierre et
Marie Curie, CNRS ENS UPMC, 7 quai St Bernard,
Paris, France

L. Jacquin (✉)
Department of Biology and Redpath Museum, McGill
University, 859 Sherbrooke Street West, Montréal, QC, Canada
e-mail: lisa.jacquin@mail.mcgill.ca

C. Récapet
Laboratoire de Biométrie et Biologie Evolutive (LBBE),
Université Claude Bernard-Lyon 1, Lyon, France

A.-C. Prévot-Julliard
Laboratoire Conservation des Espèces, Restauration et Suivi des
Populations (CERSP), Museum National d'Histoire Naturelle,
55 rue Buffon, Paris, France

G. Leboucher
Laboratoire d'Ethologie et Cognition Comparées (LECC),
Université Paris-Ouest, 200 avenue de la République,
Nanterre, France

Introduction

Most humans now live in urbanized areas, and increasing urbanization is recognized as a major challenge for biodiversity conservation (McKinney 2002; Crane and Kinzig 2005). In urban environments, spatial heterogeneity in environmental conditions as a result of human activities may create different local habitats with contrasting

selective forces (Pickett et al. 1997; Cadenasso et al. 2007; Shochat et al. 2006). For instance, urbanization is thought to affect resource availability patterns (Shochat 2004; Shochat et al. 2006) and dynamics of infectious diseases (Bradley and Altizer 2007). Variations in selective regimes driven by human activities could potentially affect the evolution of urban species. For instance, species with higher immune responses and faster life history strategies (e.g., with earlier and more frequent reproduction) are believed to be more successful in heavily urbanized habitats (Møller 2009). Within species, populations of urban birds display shorter flight distances (Møller 2008), increased boldness (Atwell et al. 2012), earlier reproduction (Partecke et al. 2004) and smaller body sizes (Liker et al. 2008) compared to their rural counterparts (reviewed in Evans 2010). Part of such phenotypic differences can be attributed to phenotypic plasticity, but there is growing evidence that genetic differences may also be involved (e.g., Liker et al. 2008), opening the possibility of a local adaptation process to urbanization (Evans 2010).

Moreover, variations in coloration between urban and rural animal populations have been reported. For instance in urban habitats, great tits *Parus major* have a duller yellow plumage (Hõrak et al. 2008), dark-eyed juncos *Junco hyemalis* have a reduced ornamental coloration (Yeh 2004), and feral pigeons display a darker melanin-based coloration (Johnston and Janiga 1995) compared to birds living in rural habitats. However, the evolutionary processes underlying such differences and their consequences are still unclear. Melanin-based coloration has been widely used to understand the adaptive value of alternative genotypes, with growing knowledge of the genetic basis of such coloration (Hubbard et al. 2010). Moreover, the study of melanin-based coloration has provided powerful tests of the role of variable selection in the emergence and maintenance of phenotypic diversity in wild populations, such as in the peppered moth *Biston betularia* (Majerus 1998), in the rock pocket mouse *Chaetodipus intermedius* (Hoekstra et al. 2005), and in several species of birds (Roulin 2004). Indeed, melanin-based coloration is often correlated with a suite of physiological, behavioral and morphological traits (Moreno and Møller 2006; Ducrest et al. 2008; McKinnon and Pierotti 2010), although, the nature of the correlation depends on environmental conditions (e.g., Piault et al. 2009; Sirkiä et al. 2010; Jacquin et al. 2012). This suggests that melanin-based coloration could reflect adaptations to alternative environments (Roulin 2004). Balancing selection in heterogeneous environments may, thus, have the potential to maintain coloration polymorphism through gene-by-environment interactions (Gillespie and Turelli 1989). For instance, there is some evidence that variations in melanin-based coloration reflect alternative strategies to cope with parasite attacks (e.g., Gasparini et al. 2011; Karell et al.

2011), so that spatio-temporal variations in parasite pressures may participate in the maintenance of coloration polymorphism (Sánchez-Guillén et al. 2013). However, it is not clear yet how human-driven perturbations and parasitism interact to shape the apparition and maintenance of alternative colored genotypes.

In this study, we investigated the potential role of gene-by-environment interactions in the maintenance of melanin-based color polymorphism in feral pigeons *Columba livia* living in differently urbanized habitats. Feral pigeons stem from domesticated forms of the wild rock pigeon that have escaped and colonized the urban environment to become one of the most abundant species in urban ecosystems (Johnston and Janiga 1995). Wild rock pigeons have a pale coloration while feral populations display a high diversity in plumage color stemming from their domestic ancestors (Johnston and Janiga 1995). Part of the color variation selected during the domestication process is maintained in current urban populations (Leiss and Haag-Wackernagel 1999) but its adaptive significance remains unclear (Johnston and Janiga 1995). Variation in melanin-based coloration in feral pigeons is genetically determined (Johnston and Janiga 1995; Jacquin et al. 2013) and is associated with variable traits such as behavior, reproductive rate and parasite resistance (Johnson and Johnston 1989; Jacquin et al. 2011, 2012). Interestingly, previous studies have reported a relationship between the level of urbanization and the frequency of melanic morphs, with darker morphs found more frequently near city centers (Johnston and Janiga 1995; Johnson 2010; Obukhova 2011). These results have led to the idea that the selective pressures linked to urbanization could participate in the maintenance of coloration polymorphism. We hypothesized that a darker melanic coloration may reflect adaptations to more urbanized conditions while a paler coloration may reflect adaptations to more rural conditions through particular combinations of fitness-linked traits (Johnston and Janiga 1995).

To test this hypothesis, we investigated the distribution of feral pigeon color morphs along an urbanization gradient and tested whether different colored morphs have different phenotypic responses to urbanization (gene-by-environment interactions) using a correlative approach. We focused on body condition as a proxy of fat reserves, and on parasite load and prevalence. Body condition is often linked to survival prospects and is recognized as important fitness-related trait in birds (e.g., Monros et al. 2002). Urbanization is believed to be characterized by high food predictability but low food availability compared to rural habitats, due to high population densities and high competition levels (Shochat 2004). Since darker eumelanic birds generally have superior competitive abilities compared to paler birds (Reyer et al. 1998; Senar 2006), they

should have better access to food in densely urbanized areas. In contrast, paler vertebrates generally have a lower metabolic rate and a higher propensity to store fat compared to darker individuals (Ducrest et al. 2008), which could allow them to better withstand seasonal food depletion in rural habitats (Johnston and Janiga 1995). We thus expected coloration-by-urbanization interactions on body condition resulting in darker pigeons having a better body condition in more urbanized areas, and/or in paler pigeons having a better body condition in more rural areas. We also searched for potential differences in blood parasite prevalence and parasite load between morphs along the urbanization gradient. Indeed, parasites may represent a potent selective force acting on the evolution of animal species within the urban environment (Bradley and Altizer 2007; Delgado and French 2012). Heavily urbanized areas are characterized by high population densities and clumped resources, so that such areas could be associated with a higher parasite transmission and exposure compared to rural areas (e.g., Farnsworth et al. 2005; Wright and Gompper 2005) although the relationship between urbanization and parasite levels might be more complex for vector-borne diseases (e.g., Grégoire et al. 2002; Bradley and Altizer 2007; Fokidis et al. 2008; Evans et al. 2009). Any relationship between coloration and blood parasitism along the urbanization gradient may thus depend on several factors such as differences in host contact rates, exposure to vectors and/or host susceptibility between morphs. A darker eumelanic coloration is often associated with a higher immune capacity and parasite resistance (Roulin et al. 2001; Jacquin et al. 2011), so that we could expect darker pigeons to have a lower parasite load compared to paler conspecifics in heavily urbanized areas. Alternatively, darker pigeons are believed to live in larger groups near the center of cities compared to paler conspecifics (Johnston and Janiga 1995), so that they may be more exposed to parasites than their paler counterparts in densely urbanized areas. We thus tested how coloration and urbanization interacted to predict prevalence and intensity of avian malaria.

Materials and methods

Study sites

We captured 488 pigeons with trap cages in 14 different suburban/urban locations in Paris and the surrounding areas between February and August 2009 in collaboration with different local associations and local authorities (Table 1). Urbanization level was calculated as the proportion of surface covered by buildings and roads (impervious surface) on the total surface of circular area around each capture site. The surface of buildings and transport infrastructure was calculated using the land use inventory for the Paris area (MOS 2003, IAU Île-de-France), which describes 11 different land uses on a 25×25-m grid. For each site, urbanization levels were calculated within a radius of 1,000 m around the capture site. This value was chosen based on previous studies showing that most feral pigeons have a relatively limited home range within urban areas (mostly below 1.5-km radius; Sol and Senar 1995; Rose et al. 2006; Frantz et al. 2012). The urbanization level of capture sites varied between 67.6 and 96.2 % (semi-urban to heavily urbanized sites). No rural site could be sampled due to the high urbanization level of the Parisian suburbs, and to the difficulty of finding dense populations of feral pigeons in rural areas.

Pigeons were captured in two different habitat types: either in streets or in pigeon houses (Table 1). To control pigeon populations, pigeon houses have been set up in cities in Europe (e.g., Basel, Switzerland; Haag-Wackernagel 1995), including Paris since 2003 (Contassot 2007). Pigeon houses are artificial breeding facilities in which pigeons are fed and reproduction is limited by egg removal to control population growth. Such habitats are likely to constitute a particular environment and to influence biological traits in this species, such as reproduction phenology or parasite transmission (Jacquin et al. 2010; Gasparini et al. 2011). Coloration, body condition and parasite prevalence and intensity are thus likely to be influenced by this particular environment and we included this environmental factor in the analyses.

Melanin-based coloration assessment

Color variation (black, red or brown) in feral pigeons is due to the deposition of two different types of melanin pigments: yellow to red pheomelanins and black eumelanins (Haase et al. 1992). In this study, we focused on eumelanic coloration because it is the most widespread coloration in feral populations (Johnston and Janiga 1995). Feral pigeons display a continuous variation in this eumelanin-based coloration from white to black, which can be divided by human eye into five main groups (Johnston and Janiga 1995; Jacquin et al. 2011). The coloration score of captured pigeons was determined from 0 to 4 by eye following the description of Johnston and Janiga (1995): (0) white or almost-white individuals ($n = 13$), (1) blue bar (gray mantle with two dark wing bars) ($n = 170$), (2) checker (checked mantle with moderate dark spots and dark surface on the wing approximately < 50 %) ($n = 160$), (3) T-pattern (dark mantle with small gray marks and dark surface on the wing approximately > 50 %) ($n = 104$), and (4) spread (completely melanic plumage) ($n = 41$). This scoring by eye method has been shown to be a reliable

Table 1 Characteristics of study sites

Site name	City (district number)	Collection date	Adults sampled	Juveniles sampled	Urbanization level (% constructed area)	Habitat type
La Plaine	Clamart (92)	27 March 2009	29	0	67.6	Pigeon house
Jussieu	Paris (V)	28 February 2009, 30 March 2009, 15 April 2009, 2 May 2009, 22 June 2009, 6 July 2009, 6 August 2009	69	29	76.8	Street
Trivaux	Clamart (92)	25 March 2009	19	2	76.8	Pigeon house
Gennevilliers	Gennevilliers (92)	18 February 2009	41	0	76.9	Street
Maison Blanche	Clamart (92)	23 March 2009	39	2	81.1	Pigeon house
Courbevoie	Courbevoie (92)	18 February 2009	38	8	82.9	Street
Pantin	Pantin (93)	1 April 2009	42	0	85.2	Pigeon house
Vanves	Paris (XIV)	9 April 2009	27	1	86.7	Pigeon house
Choisy	Paris (XIII)	23 April 2009	29	10	87.5	Street
Montreuil	Paris (XX)	16 April 2009	17	3	90.7	Pigeon house
La Roquette	Paris (XII)	30 April 2009	17	1	94.6	Street
Sacré Coeur	Paris (XVIII)	18 May 2009	18	2	95.8	Street
Saint Denis	Paris (X)	18 May 2009	12	6	96.1	Street
Lazareff	Paris (IV)	27 April 2009	24	2	96.2	Pigeon house
Total			488		85.3	

method to estimate the coloration pattern of feral pigeons (Jacquin et al. 2011). Eumelanin-based coloration patterns are highly heritable (estimated heritability 0.82 ± 0.12; Jacquin et al. 2013) and differ by the surface of dark area on the wings that corresponds to different eumelanin deposition in the feathers (Haase et al. 1992). Melanin-based coloration does not differ between sex in feral pigeons (Johnston and Janiga 1995), and patterns of melanin-based coloration are believed to be consistent throughout a pigeon's lifetime (from 6 weeks when the feather coat is complete until the end of life; Johnston and Janiga 1995).

Condition index and age determination

Captured birds were immediately weighed to the nearest gram and wing length was measured to the nearest centimeter. An index of body condition (mass scale index) was calculated using the residuals of the regression of body mass (log transformed) on wing length (log transformed) corrected following the method described in Peig and Green (2009). This body condition index has been shown to provide a reliable estimation of relative energy reserves in birds (Peig and Green 2009) which is not sensitive to sex differences (Peig and Green 2010). Sex was not recorded in this study. The age class of individuals (adults over 6 months of age versus juveniles 2–6 months old) was estimated according to the color of the iris and cere (Kautz and Seamans 1986).

Parasite prevalence and intensity assessment

Soon after capture, a blood sample was taken from the brachial vein and a drop of blood was immediately used to provide blood smears ($n = 488$). Each slide was rapidly air dried, fixed with methanol for 1 min and stained with GIEMSA (diluted 1:7) (Sigma GS128, Sigma-Aldrich, MI). Extracellular parasites such as *Trypanosoma* sp. have usually a low prevalence (Clark et al. 2009), so that we focused only on intracellular haemosporidian parasites which are responsible for the avian malaria disease. Prevalence of haemosporidian parasites (*Haemoproteus* spp. and *Plasmodium* spp.) was estimated according to the description in Hawkey and Dennet (1989) (Jacquin et al. 2011). Visual inspection of blood smears usually underestimates blood parasite prevalence compared to polymerase chain reaction screening (Fallon and Ricklefs 2008). We thus classified individuals as uninfected or infected at low intensity (no infected blood cell visually detected amongst 10,000 blood cells) versus infected individuals (at least one infected blood cell detected). For infected individuals, parasite intensity was estimated as the number of infected red blood cells amongst 10,000 cells from different microscopic fields forming a monolayer (Sol et al. 2000). Birds for whom infected blood cells were not detected were excluded from the analysis of parasite intensity. Although visual inspection of blood smears underestimates blood parasite prevalence and blood parasite intensity, it has been shown to provide reliable relative

values of prevalence and intensity (Fallon and Ricklefs 2008; Jacquin et al. 2011).

Statistical analyses

First, we tested the effects of urbanization level on coloration score using linear mixed models with capture site as a random effect and urbanization level, habitat type (pigeon house or street) and age (juvenile or adult) as fixed effects as well as their second-order interactions. The best model was chosen according to the corrected Akaike information criterion (AIC$_c$). Then, we compared the influence of urbanization level and other factors on body condition index between differently colored individuals, using linear mixed models with capture site as a random effect and urbanization level, habitat type, coloration score, age and their second-order interactions as fixed factors. Third, we compared the influence of urbanization and other factors on blood parasite prevalence (binomial distribution) and parasite intensity (log transformed) between differently colored individuals, using generalized mixed models with capture site as a random effect and urbanization level, habitat type, coloration score, age and their second-order interactions as fixed factors. Collection period (winter/early spring, i.e., February–April vs. late spring/summer, i.e., April–August) was also included as a cofactor. All variables were centered to reduce potential problems due to collinearity (correlated explanatory variables) (Aiken and West 1991). We evaluated a set of candidate models explaining our data using an information theory approach to identify the best sets of models according to several competing hypotheses (Burnham and Anderson 2002). Several models had comparable AIC$_c$ (ΔAIC$_c$ < 2) with no model having a weight >0.9 (i.e., the probability that a given submodel is the best; Burnham and Anderson 2002). We thus used a model averaging method (shrinkage method) on the best submodels (ΔAIC$_c$ < 2) (Grueber et al. 2011) using the MuMIn package of R. This enabled us to obtain parameter estimates, parameter relative importance (sum of the weights over all the submodels in which the variable appears) and 95 % confidence intervals (CIs). An explanatory variable was considered to have an effect on the response variable if the CI did not span zero (Grueber et al. 2011). To interpret interactions between age and other explanatory variables, we conducted tests separately in juveniles and adults using the same model averaging method as explained above. To interpret interactions between coloration and other variables, we conducted similar analyses separately in light-colored (coloration score < 2) and dark-colored individuals (coloration score ≥ 2). Conducting tests using the five coloration subclasses gave qualitatively similar results. All tests were conducted using the R software (R Development Core Team 2010, version 2.13.0).

Results

Urbanization level was the most important predictor of coloration score in pigeon populations with a relative importance of 82 % (Table 3), with pigeons being darker in more urbanized areas than in less urbanized areas (Table 3; Fig. 1). Moreover, age was retained in one submodel explaining coloration, but with a lower relative importance (38 %), and with juveniles being paler than adults (Table 3). Habitat type (pigeon house or street) and urbanization level were not significantly related ($t_{12} = -0.90$, $P = 0.38$). Urbanization level and age were retained in two submodels explaining coloration score (Table 2).

Age, coloration and their interaction were retained in every submodel explaining condition index (Table 2), with darker adults in poorer body condition than paler adults (post hoc analyses, effect of coloration on condition index in adults: $n = 422$, averaged estimate ± SE = -0.10 ± 0.047, CI = -0.19, -0.009) but with no such difference in juveniles ($n = 66$, averaged estimate ± SE = 0.12 ± 0.12, CI = -0.12, 0.37). Other variables had no effects on body condition index (Table 3).

Mean prevalence (± SE) of blood parasites was of 74.4 ± 1.9 %. Coloration, urbanization level and age as well as their interactions were retained in most submodels explaining parasite prevalence (Table 2). One main predictors of parasite prevalence were the interaction between age and urbanization level (RI = 1; Table 3) Separate analyses show that parasite prevalence decreased with urbanization level in juveniles (effect of urbanization on parasite prevalence in juveniles: $n = 66$, averaged estimate ± SE = -0.78 ± 0.30, CI = -1.37, -0.19) but not in adults ($n = 422$, averaged estimate ± SE = 0.47 ± 0.26, CI = -0.031, 0.98).
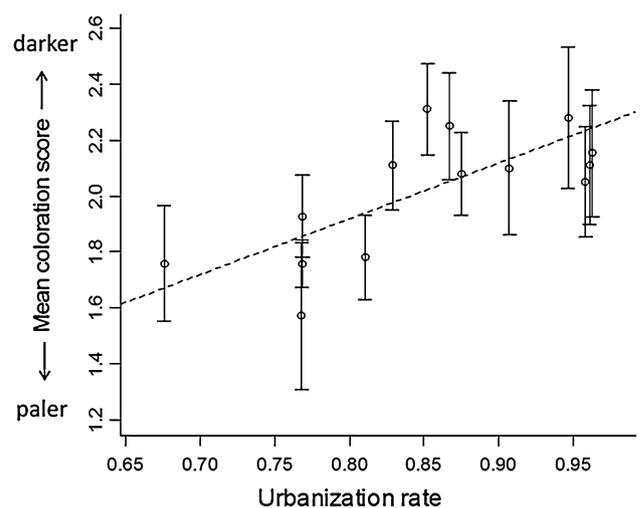
Fig. 1 Mean coloration score (±SE) increased with urbanization level (% of constructed area in a 1,000-m radius around the capture site). The *dotted line* represents the fitted values of the linear model

Table 2 Best submodels [Δ corrected Akaike's information criterion (AICc) < 2] explaining coloration and body condition index (linear mixed model with individual as random factor), blood parasite prevalence (generalized linear mixed model with a binomial distribution with capture site as a random factor) and blood parasite intensity (linear mixed model)

Model	df	Loglik.	AICc	ΔAICc	Weight
Coloration					
u	4	−690.4	1,389	0	0.338
$a + u$	5	−689.6	1,389.3	0.33	0.286
Null	3	−692.4	1,390.8	1.82	0.137
Body condition index					
$a \times c + u$	7	−662.5	1,339.2	0	0.123
$a \times c$	6	−663.9	1,340.2	0.96	0.076
$a \times c + u \times c$	8	−662.1	1,340.5	1.32	0.064
$a \times c + a \times u$	8	−662.1	1,340.6	1.36	0.062
$a \times c + u + h$	8	−662.4	1,341.2	1.96	0.046
Parasite prevalence					
$u \times c + a \times u$	7	−254.7	523.8	0	0.127
$u \times c + a \times u + h \times u$	9	−252.9	524.3	0.50	0.099
$u \times c + a \times u + a \times c$	8	−254.2	524.7	0.95	0.079
$u \times c + a \times u + a \times c + h*u$	10	−252.3	525	1.22	0.069
$a \times u$	5	−257.6	525.3	1.48	0.061
$u \times c + a \times u$	8	−254.6	525.5	1.77	0.053
Parasite intensity					
a	4	−626.46	1,261.0	0	0.15
$a + c$	5	−626.23	1,262.6	1.60	0.068
$a + u$	5	−626.24	1,262.7	1.63	0.067
$a + h$	5	−626.27	1,262.7	1.69	0.065
Null	5	−628.32	1,262.8	1.73	0.064

Collection date was not retained in any submodel

Loglik Log likelihood, *a* age, *c* coloration, *u* urbanization level, *h* habitat, *Null* null model with no fixed factor

Moreover, the interaction between urbanization level and coloration was also an important predictor of parasite prevalence (RI = 0.88; Table 3). Separate analyses showed that paler morphs had a lower parasite prevalence than darker morphs in moderately urbanized areas (urbanization level < median = 82 %; post hoc analyses, effect of coloration: n = 231, averaged estimate ± SE = 0.39 ± 0.16, CI = 0.063, 0.71). In contrast, in heavily urbanized areas pale and dark morphs had similar parasite prevalence (urbanization level > median = 82 %; post hoc analyses, effect of coloration: n = 257, averaged estimate ± SE = −0.061 ± 0.23, CI = −0.51, 0.39). In addition, differently colored individuals varied in their response to urbanization. In pale morphs (coloration score < 2, n = 183), parasite prevalence increased with urbanization (effect of urbanization on parasite prevalence in pale morphs, averaged estimate ± SE = 0.53 ± 0.23, CI = 0.089, 0.98) whereas in darker morphs (coloration score ≥ 2, n = 305), there was no relationship between parasite prevalence and urbanization (averaged estimate ± SE = 0.38 ± 0.24, CI = −0.10, 0.86) (Fig. 2).

This suggests that paler morphs did better than darker morphs in less urbanized environments but were negatively affected by urbanization, whereas darker morphs performed equally in all environments.

Mean parasite intensity was 22.9 ± 2.22 infected cells for 10,000 blood cells. Age was retained in four submodels explaining blood parasite intensity, while coloration, urbanization and habitat were retained in at least one submodel (Table 2). Age had the highest predictive importance (85 %) and juveniles tended to have a higher parasite load compared to adults, although this effect was not significant (Table 3).

Discussion

This study aimed at understanding the potential effects of urbanization and parasites on the maintenance of melanin-based color polymorphism in urban pigeons. We found that feral pigeons were darker in more urbanized areas than in less urbanized areas. This confirms previous studies

Table 3 Model-averaged coefficients of best submodels (ΔAICc < 2) explaining coloration, body condition index (linear mixed model with capture site as random factor), blood parasite prevalence (generalized linear mixed model with a binomial distribution with capture site as random factor) and blood parasite intensity (linear mixed model)

	Estimate ± SE	CI	RI
Coloration			
Urbanization	0.15 ± 0.050	0.055, 0.25	0.82
Age	J: −0.26 ± 0.13	−0.52, −0.003	0.38
Body condition index			
Age	J: −0.68 ± 0.13	−0.93, −0.41	1
Habitat	P: 0.052 ± 0.18	−0.30, 0.41	0.12
Urbanization	−0.14 ± 0.084	−0.30, 0.024	0.8
Coloration	−0.10 ± 0.046	−0.19, −0.01	1
Urbanization × age	J: 0.12 ± 0.14	−0.16, 0.40	0.17
Coloration × age	J: 0.32 ± 0.13	0.055, 0.58	1
Coloration × urbanization	−0.036 ± 0.042	−0.12, 0.046	0.17
Parasite prevalence			
Age	J: −1.40 ± 0.31	−2.01, −0.79	1
Habitat	P: 0.15 ± 0.28	−0.39, 0.69	0.45
Urbanization	0.44 ± 0.23	−0.005, 0.89	1
Coloration	0.067 ± 0.12	−0.17, 0.31	0.88
Urbanization × habitat	P: −0.52 ± 0.28	−1.07, 0.038	0.34
Urbanization × age	J: −1.22 ± 0.37	−1.95, −0.50	1
Coloration × age	J: 0.37 ± 0.33	−0.28, 1.02	0.30
Coloration × urbanization	−0.26 ± 0.12	−0.48, −0.026	0.88
Parasite intensity			
Age	J: 0.50 ± 0.26	−0.008, 1.01	0.85
Coloration	−0.047 ± 0.070	−0.18, 0.091	0.16
Urbanization	0.85 ± 1.45	−1.99, 3.70	0.16
Habitat	P: −0.13 ± 0.24	−0.59, 0.33	0.16

Parameters estimates are given ± adjusted SE

CI 95 % Confidence interval, *RI* relative importance of each parameter, *J* juvenile, *P* pigeon house

conducted in Europe showing that feral pigeons tend to be darker near the center of cities compared to the periphery (Johnston and Janiga 1995; Obukhova 2011; Johnson 2010). Several processes could explain this correlation, such as random fluctuations of allelic frequencies linked to restricted gene flow and genetic drift along the urbanization gradient (Lehtonen et al. 2009). However, independent observations in different cities show the same pattern (Johnston and Janiga 1995; Johnson 2010; Obukhova 2011; this study), and previous studies on neutral markers suggest substantial gene flow in the Parisian suburbs (Jacob, Prévot-Julliard and Baudry, unpublished data). Genetic drift is thus unlikely to account for the spatial repartition of morphs observed. The existence of a spatial variability in coloration distribution despite such gene flow thus suggests a strong effect of natural selection on this trait (Kawecki and Ebert 2004). Geographical variation in color morphs may thus be linked to variation in selection processes across urban environments.

Our results partially support this hypothesis, as the mean coloration in adults is darker than the mean coloration in juveniles (see also Haag-Wackernagel et al. 2006). This suggests a higher survival of darker juveniles compared to paler juveniles. Supporting this idea, darker juvenile pigeons have a higher survival than paler juveniles in one urban population (C. Récapet et al., submitted manuscript). However, we did not detect any interaction between urbanization and age on the mean coloration of populations in this study, which suggests that selection on coloration is fine tuned by more complex environmental factors. Morph-specific reproductive rates or habitat choice could also occur and promote local adaptation to the urban environment, but this hypothesis remains to be tested.

To examine the role of urbanization and parasites on the maintenance of color polymorphism, we examined how differently colored genotypes responded to urbanization by comparing their body condition, blood parasite prevalence and parasite load along an urbanization gradient. Body condition did not vary along the urbanization gradient, which suggests that urbanization per se has no strong negative effect on pigeons (see also Bókony et al. 2012). Moreover, differently colored individuals responded similarly to urbanization regarding body condition. However, juveniles had a lower body condition than adults (Sol et al. 1998), and darker adults had a lower condition index than paler conspecifics, whatever the urbanization level of their habitat. This could be due to a poorer health status of darker individuals, but this explanation is not supported by
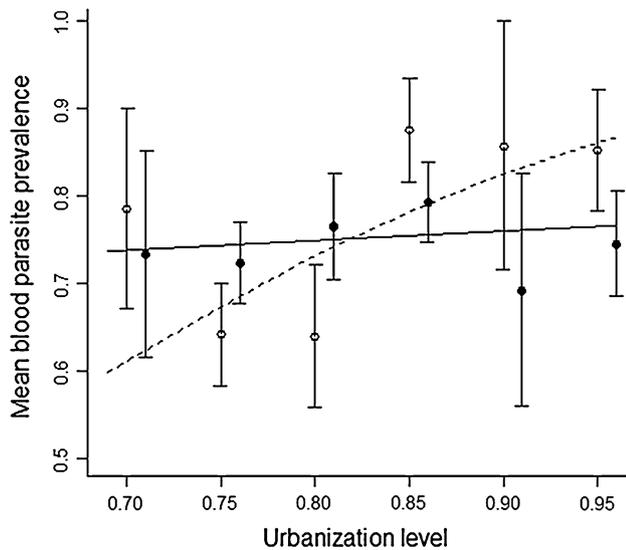
Fig. 2 Blood parasite prevalence did not depend on urbanization level in dark morphs (*filled points*: mean blood parasite prevalence ± SE for coloration score ≥ 2), while it increased with urbanization level in pale morphs (*white points*: mean prevalence ± SE for coloration score < 2) (see "Results"). *Lines* represent the fitted values of a generalized linear model of parasite prevalence as a function of urbanization level (binomial distribution) for dark morphs (*solid line*) and pale morphs (*broken line*). For better visibility, sites varying by 5 % or less in urbanization level were grouped on the graph

previous studies (Jacquin et al. 2011). Alternatively, the lower condition index of darker adults may result from their higher dominance status in urban flocks, providing them with priority access to food resources and less need to store fat (Hake 1996), or from a higher pace-of-life with a higher investment in immunity and/or reproduction at the expense of fat storage (Ducrest et al. 2008). Accordingly, previous studies showed a higher cellular immunity (Jacquin et al. 2011) and a higher egg production under food restriction in darker adult pigeons compared to paler ones (Jacquin et al. 2012). Further studies are now necessary to better understand the mechanisms underlying body condition differences between morphs in the frame of life history theories.

In addition, parasite prevalence decreased with urbanization in juveniles but not in adults. This suggests that age is a crucial factor influencing parasite prevalence along the urbanization gradient (Sol et al. 2000). However, this result should be taken with caution given the low number of juveniles sampled in each site (see Table 1), and further studies are needed to examine the interplay between age and urbanization on parasite levels. In contrast, the number of parasite found in infected individuals was not affected by age, urbanization or coloration. This contrasts with a previous study showing that infected darker morphs had a lower blood parasite load

than paler ones (Jacquin et al. 2011), maybe because of the higher number of populations sampled in the present study compared to the previous one.

Interestingly, differently colored individuals had contrasting responses to urbanization regarding blood parasite prevalence. Paler morphs were less often parasitized than darker morphs in moderately urbanized habitats, but their parasite prevalence increased with urbanization. In contrast, darker morphs had similar parasite prevalence along the urbanization gradient. This suggests that paler morphs did better than darker morphs in moderately urbanized environments but were negatively affected by increasing urbanization. In contrast, darker morphs performed equally in all environments. Such a pattern could be due to differences in parasite exposure and/or susceptibility between morphs along the urbanization gradient. For instance, paler pigeons could be less exposed to parasites compared to darker morphs in less urbanized areas because of the exploitation of different microhabitats or different behaviors that would affect exposure to malaria vectors (Hart 1997; Sol et al. 2000). Accordingly, there are some observations that paler morphs forage in smaller groups compared to darker morphs in rural areas (Johnston and Janiga 1995), which could limit parasite transmission. Increased urbanization may, however, impair their immune system via a higher susceptibility to pollutants or anthropogenic stress of paler morphs compared to darker morphs (e.g., Almasi et al. 2010). Paler morphs could thus have a higher physiological susceptibility to parasites compared to their darker counterparts when urbanization increases. Accordingly, in densely urbanized areas, paler colored pigeons have a lower proliferation ability of lymphocytes than darker individuals (Jacquin et al. 2011). Such association between immunity and melanin-based coloration could potentially be due to the pleiotropic effects of the melanocortin system, because high levels of melanocortin ligands [α-, β-melanocyte-stimulating hormone or adrenocorticotropin hormone) and receptors (for instance the melanocortin-1 receptor gene) promote both eumelanogenesis and immune activity (Ducrest et al. 2008; Roulin and Ducrest 2011). However, the effect of urbanization on the relationship between coloration and immunity remains to be investigated, for instance by comparing the immune response of differently colored individuals along an urbanization gradient.

In conclusion, the distribution of differently colored morphs of feral pigeons was strongly linked to urbanization and differently colored individuals had contrasting responses to urbanization regarding parasite prevalence (gene-by-environment interactions). This suggests that differently colored individuals might be adapted to alternative levels of urbanization via different exposure and/or susceptibility to parasites along the urbanization gradient.

Parasitic pressures linked to urbanization could thus play a role in the maintenance of color polymorphism in feral pigeons. It is, however, likely that parasites act in interaction with other selective forces such as food availability (Jacquin et al. 2012), elevated temperatures (e.g., Wolf and Walsberg 2000), or anthropogenic stress (Almasi et al. 2010) to explain the differential success of alternative morphs along the urbanization gradient. For instance, paler morphs could have a fitness advantage over darker morphs in rural areas due to a lower exposure to parasites, while darker morphs could have an advantageous combination of traits in heavily urbanized areas such as a better ability to cope with anthropogenic stress (e.g., Almasi et al. 2010) and/or a higher reproductive rate (Jacquin et al. 2012). Each morph would thus represent an optimal trait combination (e.g., Calsbeek et al. 2010) which could provide them with differential survival and/or reproductive success in alternative habitats (Jacquin 2011). Although further studies are needed to test this hypothesis and investigate whether conditions for local adaptation are met (e.g., crossing reaction norms on fitness across environments; e.g., Gillespie and Turelli 1989), this opens the interesting possibility of a balancing selection on different life history traits promoting the maintenance of melanin-based coloration diversity in the urban environment. This calls for further investigations on the role of variable selection processes on the maintenance of the intra-specific diversity in animal populations living in urban environments, since urban expansion will affect an increasing number of species in the forthcoming years.

References

Aiken L, West S (1991) Multiple regressions: testing and interpreting interactions. Sage, Thousand Oaks

Almasi B, Jenni L, Jenni-Eiermann S, Roulin A (2010) Regulation of stress response is heritable and functionally linked to melanin-based coloration. J Evol Biol 23:987–996

Atwell JW, Cardoso GC, Whittaker DJ, Campbell-Nelson S, Robertson KW, Ketterson ED (2012) Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. Behav Ecol. doi:10.1093/beheco/ars059

Bradley CA, Altizer S (2007) Urbanization and the ecology of wildlife diseases. Trends Ecol Evol 22:95–102

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Bókony V, Seress G, Nagy S, Lendvai AZ, Liker A (2012) Multiple indices of body condition reveal no negative effect of urbanization in adult house sparrows. Landsc Urban Plan 104:75–84

Cadenasso L, Pickett S, Schwarz K (2007) Spatial heterogeneity in urban ecosystems. Reconceptualizing land cover and a framework for classification. Front Ecol Env 5:80–88

Calsbeek B, Hasselquist D, Clobert J (2010) Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral color morphs. J Evol Biol 23:1138–1147

Clark P, Boardman W, Raidal S (2009) Atlas of clinical avian hematology. Wiley-Blackwell, Chichester

Contassot Y (2007) La politique de la ville: pour une gestion durable des pigeons à Paris. Bien vivre avec les animaux à Paris, le guide de l'animal en ville. Mairie de Paris, Paris

Crane P, Kinzig A (2005) Nature in the metropolis. Science 308:1225

Delgado CA, French K (2012) Parasite-bird interactions in urban areas: current evidence and emerging questions. Landsc Urban Plan 105:5–14

Ducrest AL, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol Evol 23:502–510

Evans KL (2010) Individual species and urbanization. In: Gaston KJ (ed) Urban ecology. Cambridge University Press, Cambridge, pp 53–87

Evans KL, Gaston K, Sharp S, McGowan A, Simeoni M, Hatchwell BJ (2009) Effects of urbanization on disease prevalence and age structure in blackbird *Turdus merula* populations. Oikos 118:774–782

Fallon SM, Ricklefs RE (2008) Parasitemia in PCR-detected *Plasmodium* and *Haemoproteus* infections in birds. J Avian Biol 39:514–522

Farnsworth ML, Wolfe LL, Hobbs NT, Burnham KP, Willimas ES, Theobald DM, Conner MM, Miller MW (2005) Human land use influences chronic wasting disease prevalence in mule deer. Ecol Appl 15:119–126

Fokidis HB, Greiner EC, Deviche P (2008) Interspecific variation in avian blood parasites and haematology associated with urbanization in a desert habitat. J Avian Biol 39:300–310

Frantz A, Pottier MA, Karimi B, Corbel H, Aubry E, Haussy C, Gasparini J, Castrec-Rouelle M (2012) Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale. Environ Pollut 168:23–28

Gasparini J, Erin N, Bertin C, Jacquin L, Vorimore F, Frantz A, Lemouvel P, Laroucau K (2011) Impact of urban environment and host phenotype on the epidemiology of Chlamydiaceae in feral pigeons (*Columba livia*). Environ Microbiol 13:3186–3193

Gillespie JH, Turelli M (1989) Genotype-by-environment interactions and the maintenance of polygenic variations. Genetics 114:333–343

Grégoire A et al (2002) A comparison of infestation patterns by *Ixodes* ticks in urban and rural populations of the common blackbirds *Turdus merula*. Ibis 144:640–645

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Haag-Wackernagel D (1995) Regulation of the street pigeon in Basel. Wildl Soc Bull 23:256–260

Haag-Wackernagel D, Heeb P, Leiss A (2006) Phenotype-dependent selection of juvenile urban feral pigeons *Columba livia*. Bird Study 53:153–170

Haase E, Ito S, Sell A, Wakamatsu K (1992) Melanin concentrations in feathers from wild and domestic pigeons. J Hered 83:64–67

Hake M (1996) Fattening strategies in dominance-structured greenfinch flocks in winter. Behav Ecol Sociobiol 39:71–76

Hart BL (1997) Behavioural defence. In: Clayton DH, Moore J (eds) Host-parasite evolution: general principle and avian models. Oxford University Press, Oxford

Hawkey CM, Dennet TB (1989) A colour atlas of comparative veterinary haematology. Wolfe, London

Hoekstra HE, Krentz JG, Nachman NW (2005) Local adaptation in the rock pocket mouse *Chaetodipus intermedius*: natural selection and phylogenetic history of populations. Heredity 94:217–228

Hõrak P, Vellau Ots I, Moller AP (2008) Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. Naturwissenschaften 87:460–464

Hubbard JK, Uy JA, Hauber M, Hoekstra H, Safran R (2010) Vertebrate pigmentation: from underlying genes to adaptive function. Tr Genet 26(231):239

Jacquin L 2011 Coloration mélanique et stratégies d'histoire de vie chez le pigeon biset urbain. PhD thesis, Université Pierre et Marie Curie, Paris

Jacquin L, Cazelles B, Julliard AC, Leboucher G, Gasparini J (2010) Reproduction management affects breeding ecology and reproduction costs in urban feral pigeons *Columba livia*. Can J Zool 88:781–787

Jacquin L, Lenouvel P, Haussy C, Ducatez S, Gasparini J (2011) Melanin-based coloration is related to parasite intensity and cellular immune response in an urban free living bird: the feral pigeon. J Avian Biol 42:11–15

Jacquin L, Récapet C, Bouche P, Leboucher G, Gasparini J (2012) Melanin-based coloration reflects alternative strategies to cope with food limitation in feral pigeons. Behav Ecol 23:907–915

Jacquin L, Haussy C, Bertin C, Laroucau K, Gasparini J (2013) Darker females transmit more specific antibodies to their eggs than paler ones in feral pigeons. J Linn Soc 108:647–657

Johnson SL (2010) Relationship between feral pigeon (*Columba livia*) plumage morphs and urban habitat types in greater Manchester. MSc report, University of Salford, Manchester

Johnson SG, Johnston RF (1989) A multifactorial study of variation in inter clutch interval and annual reproductive success in the feral pigeon, *Columba livia*. Oecologia 80:87–92

Johnston RF, Janiga M (1995) Feral pigeons. Oxford University Press, Oxford

Karell P, Ahola K, Karstinen T, Kolunen H, Siitari H, Brommer JE (2011) Blood parasites mediate morph-specific maintenance costs in a colour polymorphic wild bird. J Evol Biol 24:1783–1792

Kautz JR, Seamans TW (1986) Estimating age of nestling and juvenile feral rock doves. J Wildl Manage 50:544–547

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241

Lehtonen PK, Laaksonen T, Artemyev A, Belksii E et al (2009) Geographic patterns of genetic differentiation and plumage color variation are different in the pied flycatcher. Mol Ecol 18:4463–4476

Leiss A, Haag-Wackernagel D (1999) Variability and determination of the plumage coloration of the feral pigeon *Columba livia*. Ecol Birds 21:331–363

Liker A, Pape Z, Bokoni V, Landvai AZ (2008) Lean birds in the city: body size and condition of house sparrows along the urbanization gradient. J Anim Ecol 77:789–795

Majerus MEN (1998) Melanism: evolution in action. Oxford, Oxford

McKinney ML (2002) Urbanization, biodiversity, and conservation. Bioscience 52:883–890

McKinnon JS, Pierotti ME (2010) Colour polymorphism and correlated characters: genetic mechanisms and evolution. Mol Ecol 19:511–5125

Møller AP (2008) Flight distance of urban birds, predation, and selection for urban life. Behav Ecol Sociobiol 63:63–75

Møller AP (2009) Successful city dwellers: a comparative study of the ecological characteristics of urban birds in the Western Palearctic. Oecologia 159:849–858

Monros JS, Belda EJ, Barba E (2002) Post-fledging survival of individual great tits: the effects of hatching date and fledging mass. Oikos 99:481–488

Moreno J, Møller AP (2006) Are melanin ornaments signals of antioxidant and immune capacity in birds? Acta Zool Sin 52:202–208

Obukhova NY (2011) Dynamics of balanced polymorphism morphs in Blue Rock Pigeon *Columbia livia*. Russ J Genet 47:83–89

Partecke J, van't Hof T, Gwinner E (2004) Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences? Proc R Soc Lond B 271:1995–2001

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891

Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Funct Ecol 24:1323–1332

Piault R, Gasparini J, Bize P, Jenni-Eiermann S, Roulin A (2009) Pheomelanin-based coloration and the ability to cope with variation in food supply and parasitism. Am Nat 174:548–556

Pickett ST, Burch W, Dalton S, Grive JM, Rowntree R (1997) A conceptual framework for the study of human ecosystems. Urban Ecosyst 1:185–199

Reyer HU, Fischer W, Steck P, Nabulon T, Kessler P (1998) Sex-specific nest defense in house sparrows (*Passer domesticus*) varies with badge size of males. Behav Ecol Sociobiol 42:93–99

Rose E, Nagel P, Haag-Wackernagel D (2006) Spatio-temporal use of the urban habitat by feral pigeons (*Columba livia*). Behav Ecol Sociobiol 60:242–254

Roulin A (2004) The evolution, maintenance and adaptive significance of genetic colour polymorphism. Biol Rev 79:815–848

Roulin A, Ducrest A-L (2011) Association between melanism, physiology and behavior: a role for the melanocortin system. Eur J Pharmacol 660:226–233

Roulin A, Riols C, Dijkstra C, Ducrest AL (2001) Female plumage spottiness and parasite resistance in the barn owl *Tyto alba*. Behav Ecol 12:103–110

Sánchez-Guillén RA, Martínez-Zamilpa SM, Jiménez-Cortés JG, Forbes MR, Córdoba-Aguilar A (2013) Maintenance of polymorphic females: do parasites play a role? Oecologia 171:105–113

Senar JC (2006) Bird colors as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ (eds) Bird coloration. II. Harvard University Press, Cambridge

Shochat E (2004) Credit or debit? Resource input changes population dynamics of city-slicker birds. Oikos 106:622–626

Shochat E, Warren P, Faeth S, McIntyre N, Hope D (2006) From patterns to emerging processes in mechanistic urban ecology. Trends Ecol Evol 21:186–191

Sirkiä PM, Virolainen M, Laaksonen T (2010) Melanin has temperature-dependent effects on breeding performance that may maintain phenotypic variation in a passerine bird. J Evol Biol 23:2385–2396

Sol D, Senar JC (1995) Urban pigeon populations: stability, home range, and the effect of removing individuals. Can J Zool 73:1154–1160

Sol D, Santos D, Garcia J, Cuadrado M (1998) Competition for food in urban pigeons, the cost of being juvenile. Condor 100:298–304

Sol D, Jovani R, Torres J (2000) Geographical variation in blood parasites in feral pigeons: the role of vectors. Ecography 23:307–314

Wolf BO, Walsberg GE (2000) The role of the plumage in heat transfer processes of birds. Am Zool 40:575–584

Wright A, Gompper ME (2005) Altered parasite assemblage in raccoons in response to manipulated resource availability. Oecologia 144:148–156

Yeh PJ (2004) Rapid evolution of a sexually selected trait following population establishment in a novel habitat. Evolution 58: 166–174