Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity?

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Abstract

Human-altered environmental conditions affect many species at the global scale. An extreme form of anthropogenic alteration is the existence and rapid increase of urban areas. A key question, then, is how species cope with urbanization. It has been suggested that rural and urban conspecifics show differences in behaviour and personality. However, (i) a generalization of this phenomenon has never been made; and (ii) it is still unclear whether differences in personality traits between rural and urban conspecifics are the result of phenotypic plasticity or of intrinsic differences. In a literature review, we show that behavioural differences between rural and urban conspecifics are common and taxonomically widespread among animals, suggesting a significant ecological impact of urbanization on animal behaviour. In order to gain insight into the mechanisms leading to behavioural differences in urban individuals, we hand-raised and kept European blackbirds (*Turdus merula*) from a rural and a nearby urban area under common-garden conditions. Using these birds, we investigated individual variation in two behavioural responses to the presence of novel objects: approach to an object in a familiar area (here defined as neophilia), and avoidance of an object in a familiar foraging context (defined as neophobia). Neophilic and neophobic behaviours were mildly correlated and repeatable even across a time period of one year, indicating stable individual behavioural strategies. Blackbirds from the urban population were more neophobic and seasonally less neophilic than blackbirds from the nearby rural area. These intrinsic differences in personality traits are likely the result of microevolutionary changes, although we cannot fully exclude early developmental influences.

Keywords: animal personalities, anthropogenic environmental change, behavioural syndromes, colonization, microevolution, neophilia, neophobia, phenotypic plasticity, urbanization

Received 8 February 2013 and accepted 18 April 2013

Introduction

Animals are frequently confronted with environmental challenges, such as adjusting to disturbances in their habitat, coping with shifts in food availability and distribution, and interacting and competing with other individuals. The way an individual reacts to such challenges can greatly affect its ability to reproduce and survive. Hence, consistent individual behavioural differences likely evolved as a response to different environmental challenges (Wilson, 1998). Animal personalities (also called behavioural syndromes or temperament) are defined by two criteria: (i) individuals differ in their predominant behavioural strategy in a stable manner across time; and (ii) various behavioural reactions tend to be correlated across contexts (Bell, 2007). The evolutionary ecology of animal personalities has recently emerged as a new field of research (Dall *et al.*, 2012). The existence of

animal personalities is taxonomically widespread, having been documented in over 100 species (Wolf *et al.*, 2008). Moreover, a genetic component for personalities has been shown in a number of cases (Van Oers *et al.*, 2004; Schielzeth *et al.*, 2011), demonstrating that personality traits can be shaped by selection. But although recent studies have attempted to identify the selection pressures that maintain polymorphisms in behavioural strategies, the mechanisms are not yet fully understood (Dingemanse *et al.*, 2004; Biro *et al.*, 2006; Quinn *et al.*, 2009; Schielzeth *et al.*, 2011; Nicolaus *et al.*, 2012).

With the rapid increase in urbanization over the last centuries, urban areas have emerged as habitats with distinct environmental conditions that could favour individuals with different personality traits. Animals colonizing urban areas might face numerous and potentially dangerous interactions with novel anthropogenic situations (Partecke *et al.*, 2006; Brearley *et al.*, 2012). On the other hand, factors such as warmer temperatures (Arnfield, 2003), access to nocturnal illuminated areas (Longcore & Rich, 2004), and potentially

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higher availability of anthropogenic food (Bateman & Fleming, 2012) might be partly beneficial for urban species. Colonization of urban areas is commonly associated with species' ecological and life history traits (Tratalos *et al.*, 2007; Evans *et al.*, 2011) and animal personalities have been related to ecological processes such as urbanization (Sih *et al.*, 2012; Wolf & Weissing, 2012). Although there is increasing evidence that the invasion of urban habitats is related to intraspecific behavioural shifts (Evans *et al.*, 2010; Scales *et al.*, 2011), information is still lacking regarding the generality, and the mechanisms underlying such differences in behaviour (Atwell *et al.*, 2012).

In order to better understand the ecology and evolution of animal species in urban areas, our aim was to answer the following questions: (i) how common and individually repeatable are shifts in behaviour in urban colonizers; and (ii) are such behavioural differences in urban animals due to habituation to local environmental conditions or to microevolution? To resolve these questions, we first reviewed the literature to assess the ubiquity of behavioural shifts between rural and urban conspecifics. In a second step, using rural and urban hand-raised European blackbirds (*Turdus merula*), we investigated the underlying mechanisms generating shifts in behaviour, or even personalities, in urban populations. Largely confined to forest areas as little as two centuries ago, the blackbird is now among the most common urban bird species in the Western Palearctic and one of the best-studied urban colonizers (Evans *et al.*, 2009). Rural and urban populations of blackbirds differ in several aspects, including song, disease risk, propensity to migrate, timing of reproduction, and stress physiology (Partecke *et al.*, 2004, 2006; Partecke & Gwinner, 2007; Geue & Partecke, 2008; Nemeth *et al.*, 2013). In our common-garden experiment with hand-raised blackbirds, we tested whether (i) two behavioural responses to novelty were consistent over time and across contexts; and (ii) differences existed between individuals from a rural and an urban population in these behaviours. Thereby, we aim to gain insight into whether behavioural differences in the urban habitat result from phenotypic plasticity or from microevolution.

Materials and methods

Literature review

To investigate how common are shifts in species' behaviours related to the urbanization phenomenon, we reviewed the literature on empirical studies comparing rural and urban conspecifics in aggression, alarm, escape, exploration (in novel environments), neophilic, neophobic, innovative, and risk-taking behaviours towards stimuli. We performed a search in ISI Web of Knowledge (http://www.isiknowledge.com, see data S1 of the supporting information for exact search terms and for description of included behavioural categories) and included all pertinent articles in English from relevant research areas, as well as articles resulting from backward or forward searches of the initial search. We classified all significant differences ($P < 0.05$) into 'rural > urban' or 'rural < urban' depending on average trait values. In studies with multiple species or related behaviours, we considered significant differences when these were shown in at least one species and behaviour in the same category. For the sake of clarity, we included only studies that focused both on rural and urban populations of the same species, excluding studies focusing on gradients of urbanization, or disturbance in only rural or urban environments. Nevertheless, the concept of rural and urban inevitably differs in the retrieved studies regarding area of the habitat and degree of anthropogenic disturbance.

Experimental setup

Between 21 March and 23 June 2007, we collected blackbirds at 3–11 days of age from a rural (14 males and 11 females from 9 nests) and an urban habitat (14 males and 14 females from 9 nests) in southeast Germany. Urban birds were collected in the city centre of Munich (48°07′N, 11°34′E; 518 m asl), and rural birds in a managed forest (Raisting 47°53′N, 11°04′E; 553 m asl) ca. 40 km southwest of Munich. The nestlings were hand-raised and kept under common-garden conditions, housed in individual home cages in a single room (birds could hear but not see each other) with simulated natural photoperiod, constant temperature ($20 \pm 2\ °C$), and *ad libitum* water and food (Granvit, Chemi-Vit, Quattro Castella, Italy).

Experimental procedure

We performed behavioural tests to quantify two responses to novelty: (i) object-neophilia, the approach of an individual to a novel object in a familiar environment; and (ii) object-neophobia, the avoidance of a novel object in a familiar environment (normally measured in a foraging context). Neophilia and neophobia are thought to have independent motivations and to belong to different personality dimensions (Budaev, 1997; Coleman & Wilson, 1998; Mettke-Hofmann *et al.*, 2002, 2009; Herborn *et al.*, 2010). To assess individual consistency in these behaviours, we repeated each test in three trials with different novel objects. We videotaped all trials for determination of behavioural parameters, particularly latency to approach the novel object. Whenever an individual did not approach the object during the entire trial, its latency to approach was set to the maximum duration of the trial. The different objects used and details of the cage are pictured in Figure S1 of the supporting information.

Object-neophilia tests. The object-neophilia tests took place in November 2007, June 2008, and December 2008 using different objects. Each trial was conducted for 2 h at the same

time of day (ca. 10:00 hours–12:00 hours), beginning after the replacement of a perch in the middle of the bird's home cage with a perch with the novel object mounted. Following Mettke-Hofmann *et al.* (2002), we considered the latency to approach a neutral perch with a novel object a measure of neophilia, although a certain extent of neophobic motivations cannot be completely excluded.

Object-neophobia tests. The object-neophobia tests were performed in December 2007/January 2008, August/September 2008, and November/December 2008 using different objects. Each trial included a control day and two subsequent experimental days, conducted for four hours at the same time of day (ca. 10:00 hours–14:00 hours).

To motivate individual birds to feed during the experiment, we removed the food in the evening of the day preceding each individual test, returning it at the beginning of the test. We always manipulated the perch used by birds while feeding. On the control day, we replaced this perch with an identical one (without any novel object) while reintroducing food. On the first experimental day, this procedure was repeated using a perch with a mounted novel object directly next to the feeder. We repeated this procedure on the second experimental day using the same object. Latency to approach the introduced perch was registered on each day. The control day assesses potential differences between the rural and the urban population in their reaction to disturbance unrelated to the presence of the new object. Latency to approach in the first experimental day is a measure of neophobia, while latency to approach in the second experimental day reflects avoidance after habituation to the object. The motivation to feed in the neophobia test should reduce neophilic responses to a very low level, thus individual differences in the latency to approach should be mainly due to individual differences in neophobia (Mettke-Hofmann *et al.*, 2002).

Statistical analyses

To assess consistency in individual behavioural responses, we calculated adjusted repeatabilities (Nakagawa & Schielzeth, 2010) and their standard errors using linear mixed-effects models (LMM). We use adjusted repeatabilities because we found pronounced differences in mean responses among different trials (see Results) that might have occurred due to seasonal variation or behavioural plasticity. Such trial effects tend to decrease similarity among observations of the same individual, but adjusted repeatabilities account for this by allowing for seasonal variation in means (Nakagawa & Schielzeth, 2010). The *P*-values for the repeatability estimates were obtained from a permutation test (1000 permutations), in which the vector of individual identities was randomized while maintaining a balanced design with respect to the fixed effect 'trial'. We investigated differences between repeatabilities in the rural and the urban population using Monte Carlo simulations performed on bootstrapping samples of the two repeatability estimates. We sampled 10 000 times with replacement from the bootstrapping samples and estimated the asymptotic two-tailed *P*-value for the difference in repeatabilities as twice the

proportion of samples for which the difference (R_{urban}–R_{rural}) was equal or smaller than zero.

For each trial, both for the overall data and for each individual population, we calculated the Spearman coefficient of correlation between latencies to perch in neophilia and neophobia (first experimental day) tests.

We fitted LMM (R package lme4, Bates *et al.*, 2011) to investigate whether trial, population (rural–Raisting or urban–Munich), and sex affected the behavioural variables of interest. We included nest ID and individual ID in the models as random intercepts to account for family effects and repeated measures of each individual. Full model estimates are shown in Table S1 of the supporting information. To simplify the interpretation of the models, we sequentially removed non-significant interaction terms from the initial model using likelihood ratio tests (R package LMERConvenienceFunctions, Tremblay, 2011). While the usual estimation of *P*-values in LMM is anticonservative for small data sets, Markov chain Monte Carlo (MCMC) methods are an alternative that works well for large and small data sets. Therefore, we approximated *P*-values based on MCMC sampling of the final models (R package languageR, Baayen, 2011). A similar model was fitted to test whether the two populations differed in body condition (scaled mass index, Peig & Green, 2009).

To achieve normality of residuals in the LMM-based analyses, data from the neophobia tests were log-transformed. Statistical analyses were performed using R 2.15.0 (R Development Core Team, 2012).

Results

Literature review

The literature review resulted in 29 empirical studies comparing conspecific rural and urban populations in aggressive, alarm, escape, neophobic, innovative, and risk-taking behaviours towards different types of stimuli (Table 1). Most of the studies were made in the wild, with only five studies made under controlled laboratory conditions and only one of these studies (Atwell *et al.*, 2012) having used individuals with limited experience of their environment. Of the 29 studies, 27 showed significant differences between rural and urban populations for at least one of the behaviours and species analyzed. Urban populations seemed to be more aggressive (5 of 6 studies), showed reduced escape behaviour (21 of 22 studies), and increased risk-taking behaviour (6 of 9 studies). For the other considered behaviours, 7 of 9 studies showed differences between rural and urban conspecific populations. Repeatability was only assessed in two studies, which found the behaviours under study to be repeatable, and only seven studies assessed correlations between different behaviours, with most of the studied behaviours found to be correlated.

Table 1 Summary of empirical studies comparing conspecific rural and urban populations in behaviours towards different types of stimuli. The third column shows how average trait values of rural individuals (R) differ from urban individuals (U); all the reported trends are significant ($P < 0.05$); non-significant results are reported as NS. The following columns indicate where the study was made (Experiment), which environment animals' experienced before the experiment (Experience), and if the studies report behavioural repeatability (Repeatability) or correlation with other behaviours (Correlation). Unavailable data are reported as NA. For details on the literature search, see methods section of the main text and Data S1 of the supporting information.

Behavioural trait	Species	Trend	Experiment	Experience	Repeatability	Correlation	Ref.
Aggression							
	15 bird species	R < U	Wild	Wild	NA	NA	Møller & Ibáñez-Álamo, 2012;
	American crow (*Corvus brachyrhynchos*)	NS	Wild	Wild	NA	NA	Knight *et al.*, 1987;
	Noisy miner (*Manorina melanocephala*)	R < U	Wild	Wild	NA	NA	Lowry *et al.*, 2011;
	Mute swan (*Cygnus olor*)	R < U	Wild	Wild	NA	NA	Józkowicz & Górska-Klęk, 1996;
	Song sparrow (*Melospiza melodia*)	R < U	Wild	Wild	NA	Yes	Evans *et al.*, 2010;
	Song sparrow (*Melospiza melodia*)	R < U	Wild	Wild	Yes	Yes	Scales *et al.*, 2011;
Alarm							
	15 bird species	R > U	Wild	Wild	NA	NA	Møller & Ibáñez-Álamo, 2012;
	American crow (*Corvus brachyrhynchos*)	R > U (from a distance) R < U (close distance)	Wild	Wild	NA	NA	Knight *et al.*, 1987;
	Black-tailed prairie dog (*Cynomys ludovicianus*)	R > U	Wild	Wild	NA	NA	Adams *et al.*, 1987;
	Black-tailed prairie dog (*Cynomys ludovicianus*)	NS	Wild	Wild	NA	NA	Magle *et al.*, 2005;
	Eastern grey squirrel (*Sciurus carolinensis*)	R < U	Wild	Wild	NA	NA	Partan *et al.*, 2010;
Escape							
	5 bird species	R > U	Wild	Wild	NA	NA	Clucas & Marzluff, 2012;
	10 bird species	R > U (3 species NS)	Wild	Wild	NA	NA	Cooke, 1980;
	42 bird species	R > U	Wild	Wild	NA	NA	Carrete & Tella, 2011;
	44 bird species	R > U	Wild	Wild	NA	NA	Møller, 2008;
	American crow (*Corvus brachyrhynchos*)	R > U	Wild	Wild	NA	NA	Knight *et al.*, 1987;
	Black-tailed prairie dog (*Cynomys ludovicianus*)	R > U	Wild	Wild	NA	NA	Adams *et al.*, 1987;
	Black-tailed prairie dog (*Cynomys ludovicianus*)	R > U	Wild	Wild	NA	NA	Magle *et al.*, 2005;
	Black-billed magpies (*Pica pica*)	R > U	Wild	Wild	NA	NA	Kenney & Knight, 1992;
	Cape Ground squirrel (*Xerus inauris*)	R > U	Wild	Wild	NA	NA	Chapman *et al.*, 2012;
	Dark-eyed junco (*Junco hyemalis*)	R > U	Wild	Wild	NA	NA	Atwell *et al.*, 2012;
	Eastern grey squirrel (*Sciurus carolinensis*)	R > U	Wild	Wild	NA	No	Engelhardt & Weladji, 2011;
	Fox squirrel (*Sciurus niger*)	R > U (adults*)	Wild	Wild	NA	NA	Mccleery, 2009;
	Garden skink (*Lampropholis guichenoti*)	R < U	Wild	Wild	NA	NA	Prosser *et al.*, 2006;
	House finch (*Carpodacus mexicanus*)	R < U	Wild	Wild	NA	Yes	Valcarcel & Fernandez-Juricic, 2009;
	Magpie-lark (*Grallina cyanoleuca*)	R > U	Wild	Wild	NA	NA	Kitchen *et al.*, 2010;

Table 1 (continued)

Behavioural trait	Species	Trend	Experiment	Experience	Repeatability	Correlation	Ref.
	Noisy miner (*Manorina melanocephala*)	R > U	Wild	Wild	NA	NA	Lowry *et al.*, 2011;
	Song sparrow (*Melospiza melodia*)	R > U	Wild	Wild	Yes	Yes	Evans *et al.*, 2010;
	Song sparrow (*Melospiza melodia*)	R > U	Wild	Wild	Yes	Yes	Scales *et al.*, 2011;
	Two-banded plover (*Charadrius falklandicus*)	R > U	Wild	Wild	NA	NA	St Clair *et al.*, 2010;
	White-fronted plover (*Charadrius marginatus*)	R > U	Wild	Wild	NA	NA	Baudains & Lloyd, 2007;
	Woodchuck (*Marmota monax*)	NS	Wild	Wild	NA	Yes	Lehrer *et al.*, 2012;
	Woodlouse (*Porcellio laevis*)	R > U	Lab	Wild	NA	NA	Houghtaling & Kight, 2006;
Exploration	Dark-eyed junco (*Junco hyemalis*),	R < U	Lab	Lab since juveniles	NA	NA	Atwell *et al.*, 2012;
Innovation							
Neophobia	House sparrow (*Passer domesticus*)	R < U	Lab	Wild	NA	No	Liker & Bókony, 2009;
	Eastern grey squirrel (*Sciurus carolinensis*)	R > U	Wild	Wild	NA	NA	Bowers & Breland, 1996;
	House sparrow (*Passer domesticus*)	NS	Lab	Wild	NA	No	Liker & Bókony, 2009;
	House sparrow (*Passer domesticus*)	NS	Lab	Wild	NA	Yes	Bókony *et al.*, 2012;
Risk-taking	American crow (*Corvus brachyrhynchos*)	R < U	Wild	Wild	NA	NA	Knight *et al.*, 1987;
	Black-tailed prairie dog (*Cynomys ludovicianus*)	R < U	Wild	Wild	NA	NA	Adams *et al.*, 1987;
	Cape ground squirrel (*Xerus inauris*)	R < U	Wild	Wild	NA	NA	Chapman *et al.*, 2012;
	Eastern grey squirrel (*Sciurus carolinensis*)	NS	Wild	Wild	NA	No	Engelhardt & Weladji, 2011;
	Garden skink (*Lampropholis guichenoti*)	R < U	Wild	Wild	NA	NA	Prosser *et al.*, 2006;
	House sparrow (*Passer domesticus*)	R < U (juveniles) R > U (adults)	Lab	Wild	NA	NA	Seress *et al.*, 2011;
	House sparrow (*Passer domesticus*)	NS	Lab	Wild	NA	Yes	Bókony *et al.*, 2012;
	White-fronted plover (*Charadrius marginatus*)	R < U	Wild	Wild	NA	NA	Baudains & Lloyd, 2007;
	Woodchuck (*Marmota monax*)	NS	Wild	Wild	NA	Yes	Lehrer *et al.*, 2012

*Juveniles possibly with no difference.

Behavioural differences between rural and urban hand-raised European blackbirds

In the neophilia test, the latency to approach the perch with the novel object increased across trials (Fig. 1; Table 2). An overall population effect was not apparent, but the interaction between population and the second trial was significant (Table 2). In fact, in the second trial, the urban population was on average 35 min slower than the rural population to approach the perch with the novel object. A similar but non-significant trend was found in the first trial, but not in the third (Fig. 1; Table 2). Neophilia did not significantly differ between sexes (Table 2).

In the object-neophobia test, trial, population, and sex did not significantly affect latency to perch near the feeder in the control day. When first confronted with the novel object near the feeder (first experimental day), individuals from the urban population avoided it more than rural individuals. This effect was consistent in the three trials, with individuals from the urban population taking on average 30 min longer to perch near the object (Fig. 2; Table 2). A trial effect was present, revealing less avoidance in trial 2 when compared with trials 1 and 3 (Fig. 2; Table 2).

Similar to neophilia, neophobia did not significantly differ between sexes (Table 2). Avoidance on the second experimental day was unrelated to sex or trial. However, the effect of population closely approached significance (Table 2), reflecting that individuals from the urban population took on average 16 min longer to perch near the object (Fig. 2, Table 2). Extreme neophobic reactions, resulting in a complete avoidance of the perch with the object during the 4 h of the experimental days, were only observed in individuals from the urban population (for all trials $N = 6$ on first experimental day and $N = 3$ on second experimental day).
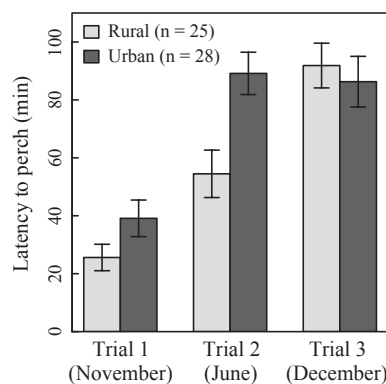
The rural and the urban population did not significantly differ in their body condition (treatment contrast Munich vs. Raisting: 2.488 ± 3.021, $P = 0.304$).

Individual behavioural consistency over time in hand-raised European blackbirds

In our common-garden experiment using hand-raised European blackbirds, neophilic and neophobic behaviours were repeatable across trials (neophilia: $R = 0.39 \pm 0.08$, $P \leq 0.001$; neophobia first experimental day: $R = 0.40 \pm 0.08$, $P \leq 0.001$). Response to disturbance unrelated to the novel object was also repeatable (neophobia control day: $R = 0.30 \pm 0.10$, $P \leq 0.001$), as well as avoidance of the object after habituation (neophobia second experimental day: $R = 0.30 \pm 0.09$, $P \leq 0.001$).

Performing the repeatability estimation separately for each population consistently resulted in higher point estimates of repeatabilities and higher statistical significances for the urban (neophilia: $R = 0.50 \pm 0.11$, $P \leq 0.001$; neophobia control day: $R = 0.35 \pm 0.13$, $P = 0.002$; neophobia first experimental day: $R = 0.45 \pm 0.11$, $P \leq 0.001$; neophobia day 3: $R = 0.31 \pm 0.13$, $P = 0.006$) than for the rural population (neophilia: $R = 0.32 \pm 0.14$, $P = 0.004$; neophobia control day: $R = 0.23 \pm 0.15$, $P = 0.021$; neophobia first experimental day: $R = 0.26 \pm 0.15$, $P = 0.018$; neophobia second experimental day: $R = 0.19 \pm 0.14$, $P = 0.053$). Nevertheless, such differences in repeatability between the two populations were not statistically significant (neophilia: $P = 0.278$; neophobia control day: $P = 0.516$; neophobia first experimental day: $P = 0.237$; neophobia second experimental day: $P = 0.524$).

Correlation between neophilia and neophobia in hand-raised European blackbirds

Latency to perch in neophilia and neophobia (first experimental day) tests were correlated between trials conducted within a few weeks (trial 1: $\rho = 0.35$, $P = 0.011$; trial 2: $\rho = 0.36$, $P = 0.009$; trial 3: $\rho = 0.46$, $P < 0.001$). Performing the same analysis separately for each population (and hence with a halved sample size) did not always retrieve significant results for the first (rural: $\rho = 0.33$, $P = 0.108$; urban: $\rho = 0.33$, $P = 0.084$), second (rural: $\rho = 0.43$, $P = 0.033$; urban: $\rho = 0.21$, $P = 0.291$), and third (rural: $\rho = 0.29$, $P = 0.167$; urban: $\rho = 0.62$, $P < 0.001$) trials.

Correlations between neophilia and neophobia across larger time spans were also mostly statistically significant or close to significance (trial 1 vs. trial 2: $\rho = 0.39$, $P = 0.004$; trial 1 vs. trial 3: $\rho = 0.38$, $P = 0.006$; trial 2 vs. trial 1: $\rho = 0.40$, $P = 0.003$; trial 2 vs. trial 3: $\rho = 0.24$,

Fig. 1 Latency to perch near novel objects in each trial of the neophilia test for rural (light grey) and urban (dark grey) individuals. Means ± standard errors are shown.

Table 2 Slope estimates and Markov chain Monte Carlo *P*-values for fixed effects in linear mixed-effects models for neophilia and neophobia (control day, first experimental day, and second experimental day) after backward model simplification. The intercept represents rural females in trial 3 (reference levels), while other estimates represent treatment contrasts

Parameter	Neophilia β ± SE	P	Neophobia control β ± SE	P	Neophobia 1st day β ± SE	P	Neophobia 2nd day β ± SE	P
Intercept	5355.2 ± 587.2	<0.001	5.07 ± 0.21	<0.001	7.57 ± 0.26	<0.001	5.78 ± 0.27	<0.001
Urban	−490.0 ± 729.2	0.504	0.21 ± 0.23	0.318	0.49 ± 0.28	0.041	0.51 ± 0.29	0.058
Trial 1	−3975.9 ± 483.1	<0.001	0.02 ± 0.15	0.865	0.07 ± 0.17	0.719	−0.07 ± 0.20	0.771
Trial 2	−2242.8 ± 483.1	<0.001	0.26 ± 0.15	0.113	−0.85 ± 0.17	<0.001	−0.17 ± 0.20	0.436
Male	222.0 ± 470.0	0.503	0.02 ± 0.17	0.991	0.08 ± 0.23	0.640	−0.14 ± 0.22	0.461
Urban × trial 1	1215.3 ± 668.5	0.122	–	–	–	–	–	–
Urban × trial 2	2484.1 ± 668.5	0.002	–	–	–	–	–	–
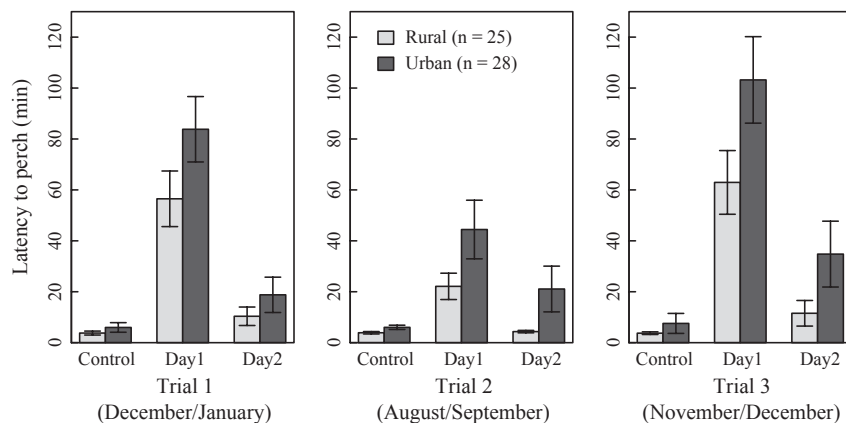
Fig. 2 Latencies to perch near novel object in each trial of the neophobia test for rural (light grey) and urban (dark grey) individuals. After a control day, we replaced the perch next to the feeder with a perch containing a novel mounted object for the first and second experimental days. Means ± standard errors are shown.

P = 0.081; trial 3 vs. trial 1: ρ = 0.21, *P* = 0.130; trial 3 vs. trial 2: ρ = 0.26, *P* = 0.068), demonstrating that the correlation between neophilia and neophobia was not transient, but rather stable across time.

Discussion

Our literature review shows that behavioural shifts related to the colonization of urban areas are a common, taxonomically widespread phenomenon. In a common-garden experiment, we tested whether neophilia and neophobia differed between a rural and an urban population of blackbirds raised under identical conditions. The behavioural traits showed long-term individual consistency and differences in means between the rural and the urban population. Since subjects had never experienced their natal environment outside the nest, our results suggest intrinsic rather than learned differences between the two populations. These results corroborate the hypothesis that behavio-

ural shifts in urban populations might be a result of microevolution rather than phenotypic plasticity.

Behavioural differences between rural and urban individuals

In our literature review, 27 of 29 publications indicate differences between conspecific rural and urban populations in behaviours towards different stimuli. Although a publication bias is possible, the strong predominance of behavioural differences in the literature review corroborates the idea that behavioural shifts are a common by-product of urban life in wild animals.

Our study with hand-raised blackbirds also revealed differences in personality traits between the rural and the urban population: neophilia was lower in the urban population in the summer trial, while neophobia was consistently higher in the urban population (Figs 1 and 2; Table 2). Why would increased caution be beneficial

in the urban environment? Increased neophobia and reduced neophilia might be advantageous for established urban animals, to which resources tend to be more stable (e.g., anthropogenic food and warmer temperatures), while contact with novel and potentially dangerous situations is higher. This would agree with the hypothesis by Greenberg & Mettke-Hoffman (2001) that neophilia decreases when information about resources is less valuable, and neophobia increases in more dangerous habitats. In line with our present findings, neophobia was also found to increase with the degree of urbanization for wild eared doves (*Zenaida auriculata*) and house sparrows (*Passer domesticus*) (Echeverría & Vassallo, 2008). However, in the few existing studies on this subject, non-significant or opposite trends were also found regarding neophobia (see Table 2 for detailed information). An alternative explanation would be that urban animals are forced to reduce their neophobia and increase their neophilia in order to explore new anthropogenic food resources and maintain stasis in the face of many novel stressors. Clearly, more studies on neophilia and neophobia in rural and urban populations are needed to assess this issue. In studies in the wild, it is difficult to assess reactions to novel or dangerous situations, as animals may become habituated to humans, to anthropogenic structures and objects, and to certain predictable disturbance regimes (e.g., road traffic consistently follows the path of the road). Thus, caution should be applied when interpreting the lower escape distance and higher frequency of risk-taking and aggressive behaviours of wild urban populations (see Table 1) as opposed to our results of lower object-neophilia and higher object-neophobia in an urban population. The assessed behaviours are intrinsically different: studies in the wild focused mostly on behavioural reactions towards humans (to whose presence urban animals are habituated), while our study focused on reactions towards novel objects.

In our study, neophobia and neophilia did not significantly differ between sexes, similar to other studies on personality traits (e.g., Schielzeth *et al.*, 2011). However, we found trial effects in the expression of neophilia and neophobia (Figs 1 and 2, and Table 2), the causes of which are open to speculation. The decrease in neophilia across trials could be due to increased experience with objects, or to age effects (Greenberg & Mettke-Hofmann, 2001; Mettke-Hofmann *et al.*, 2006; Biondi *et al.*, 2010). On the other hand, the non-linear change in neophobia (lower in summer than in winter) indicates a seasonal effect. In the wild, the spring and summer months are characterized by dense foliage that provides adequate cover, along with greater abundance of food resources.

Although neophobia is generally reduced in animals less exposed when foraging or in the presence of reduced competition (Greenberg & Mettke-Hofmann, 2001), we cannot exclude the hypothesis of differences in the strength of the stimulus objects or seasonal changes in hormone expression (such as testosterone or corticosterone concentrations).

Behavioural consistency and correlations

The few studies in our literature review that investigate the existence of behavioural repeatability and/or cross-context correlations are suggestive of the existence of animal personalities that differ between rural and urban areas.

Our common-garden experiment also revealed individual consistency in neophilic and neophobic behaviours. Both being behavioural responses to novelty, doubts can arise regarding the independence of neophilia and neophobia. It has been suggested that these behaviours have independent motivations and belong to different personality dimensions (Budaev, 1997; Coleman & Wilson, 1998; Mettke-Hofmann *et al.*, 2002, 2009; Herborn *et al.*, 2010). In particular, similar to the present study, Mettke-Hofmann *et al.* (2002) measured neophilia and neophobia in captivity by placing novel objects respectively in a neutral location of the birds' familiar environment or associated with food. This study, similar to others, found no correlation between neophilia and neophobia in the 62 parrot species studied. Our mild but significant correlation between neophilia and neophobia suggests that these might be different personality traits integrated as part of a behavioural syndrome or personality.

Even though the test for differences in repeatabilities between the rural and the urban population lacked statistical power, the agreement across different traits suggests that individuals from the urban population have higher individual behavioural consistency in neophobic and neophilic responses, indicated by higher and more significant repeatabilities than the rural population. When analyzed separately for each population, the two behaviours are not always significantly correlated, possibly due to the limited sample size. A recent study on song sparrows (*Melospiza melodia*) in the wild also shows significant differences in repeatabilities between rural and urban populations (Scales *et al.*, 2011). Unlike our results, this study shows that the urban populations, in contrast to the rural one, lack correlation between boldness and aggression. The authors suggest that both behaviours could be selected for in the urban habitat, but that they can vary independently due to a potentially higher quality of the habitat.

Microevolution or phenotypic plasticity?

We show in our literature review that changes in personality traits are a common phenomenon in populations of a variety of species that colonize urban environments. In addition, the empirical part of our study reveals intrinsic differences in personality traits between rural and urban conspecifics that never experienced the natural habitat outside their nests. Personality traits have been shown to have a genetic component (Van Oers *et al.*, 2004, 2005; Schielzeth *et al.*, 2011), and variation in behavioural responses may result from the individuals' reaction to its environment or from genetic adaptation (Charmantier *et al.*, 2008; Hendry *et al.*, 2008; Dingemanse *et al.*, 2010; Sih *et al.*, 2011; Van Buskirk, 2012). Our common-garden experiment suggests that the intrinsic behavioural differences between rural and urban populations are genetically driven and we consider it likely that these differences are the result of adaptive microevolutionary changes. A recent comparative study on several rural and urban European blackbird populations across Europe using candidate genes for behavioural traits seems to support this idea (Mueller *et al.*, 2013). In the mentioned study, a candidate gene for harm avoidance behaviour (the SERT gene) exhibited a significant association with habitat type. Two notes should be made here. First, even though the existing data suggest that urbanization causes microevolutionary changes in animal personalities, a generalization can only be made through common-garden studies focusing on multiple rural–urban population pairs. Second, although the results of our common-garden experiment argue in favour of a genetic basis for behavioural shifts in urban individuals, early experience in the nest environment or pre- and perinatal maternal effects cannot be ruled out. Nevertheless, our study rules out the effects of plasticity to the environment from the moment birds leave their nests, as the birds were collected from their nests before fledging. Supporting the idea of intrinsic changes in behavioural traits in animals colonizing urban areas, a recent study also found differences in one behavioural trait between rural and urban dark-eyed juncos (*Junco hyemalis*) kept since juveniles (ca. 40 days) under a common-garden setup (Atwell *et al.*, 2012).

Intrinsic differences in personality traits between rural and urban populations could be achieved via two different evolutionary mechanisms: (i) microevolution of personality traits in response to new selection regimes where there are new optimal behaviours (post-colonization adaptation); or (ii) microevolution of personality traits by non-random gene flow, where the colonization of the urban habitat is personality-dependent (precolonization adaptation, Edelaar &

Bolnick, 2012). It is also conceivable that the two mechanisms act simultaneously, with different behavioural optima potentially selected at different stages of the colonization process. In fact, one study suggests that recent colonizers of urban habitats show higher neophilia than long established populations (Martin & Fitzgerald, 2005). To test these hypotheses, future studies should focus on the measurement of selection in rural and urban environments.

This work opens up the exciting possibility of studying, in common-garden setups, urbanization-driven evolutionary changes in animal personalities. Such studies will be an important step to understand how animals cope with our urbanizing world.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Literature search terms and description of included behavioural categories

Figure S1. (a) Novel objects used for each trial of the neophilia tests; (b) novel objects used for each trial of the neophobia tests; (c) an individual home cage: the novel objects were placed on a perch in the middle of the cage (A) for the neophilia test and on a perch in front of the feeder (B) for the neophobia test.

Table S1. Full model slope estimates and Markov chain Monte Carlo based *P*-values for the fixed effects in linear mixed-effects models for neophilia and neophobia (control day, first experimental day, and second experimental day). The intercept represents rural females in trial 3 (the reference levels), while the other estimates represent treatment contrasts. T1, T2, and ♂ represent trial 1, trial 2, and males respectively.