Individual variation in behavioural responsiveness to humans leads to differences in breeding success and long-term population phenotypic changes

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1 **Abstract**

2 Whether human disturbance can lead to directional selection and phenotypic change
3 in behaviour in species with limited behavioural plasticity is poorly understood in
4 wild animal populations. Using a 19-year study on Montagu’s harrier, we report a
5 long-term increase in boldness towards humans during nest visits. The probability of
6 females fleeing or being passive during nest visits decreased, while defence intensity
7 steadily increased over the study period. These behavioural responses towards
8 humans were significantly repeatable. The phenotypic composition of the breeding
9 population changed throughout the study period (4-5 harrier generations), with a
10 gradual disappearance of shy individuals, leading to a greater proportion of bolder
11 ones and a more behaviourally homogeneous population. We further show that nest
12 visit frequency increased nest failure probability and reduced productivity of shy
13 females, but not of bold ones. Long-term research or conservation programmes
14 needing nest visits can therefore lead to subtle but relevant population compositional
15 changes that require further attention.

16

17 **Keywords:** aggression, boldness, fitness, long-term studies, Montagu’s harrier,
18 phenotypic change, personality, plasticity

19 INTRODUCTION

20 There is growing evidence that variations in behaviour may reflect consistent
21 differences among individuals (defined as temperament or personality traits), may
22 have a genetic basis, and be thus target of selection in natural environments (e.g. Sih
23 *et al.* 2004; Dingemanse & Réale 2005; Réale *et al.* 2007; Dingemanse *et al.* 2010;
24 Bize *et al.* 2012; Bromer & Klueen 2012; Patrick *et al.* 2013). The study of those traits
25 has thus major implications in evolutionary ecology (e.g. Réale *et al.* 2007; Quinn *et*
26 *al.* 2009; Duckworth & Sockman 2012; Wolf & Weissing 2012), but also in
27 conservation biology (McDougall *et al.* 2006; Smith & Blumstein 2013). For
28 example, maintaining variability of personalities within a population may be
29 beneficial as this may be related to the population genetic diversity and thus its
30 capacity of adaptation to environmental changes (Smith & Blumstein 2013).

31 Several studies have shown links between personality and life history traits
32 like dispersal or productivity (Réale *et al.* 2007; Biro & Stamps 2008; although these
33 relationships have been found to be minimal in some species, e.g. Quinn *et al.* 2009,
34 2011; Patrick *et al.* 2012). Life-history links of personality may be context-
35 dependent, be even reversed in different ecological conditions, or be shaped by trade-
36 offs between current and future fitness prospects (Dingemanse & de Goede 2004;
37 Dingemanse *et al.* 2004; Wolf *et al.* 2007). Therefore, variation in personality may be
38 maintained by fluctuating environmental pressures (Wolf & Weissing 2010). On the
39 other hand, changes in the relative frequency of personality types within a population
40 may appear following differential selection on personality types from environmental
41 pressures that shift in a particular direction over time, such as human exposure (Smith
42 & Blumstein 2008, 2013).

Much attention has been paid to relationships between temperament or personality traits and adaptation to human presence, especially in urban environments (e.g. Møller 2008, 2010; Bokony *et al.* 2012). Bold individuals have been shown to be better adapted for living in urban environments, where exposure to humans is high. Accordingly, various studies have found different phenotypical composition of urban populations as compared with rural ones, as well as differences in inter-individual variability in behaviour (Evans *et al.* 2010; Carrete & Tella 2011).

More recently, attention has also focused on species that experience increasing exposure to humans in non-urban areas because of recreational activities, research programs or conservation efforts (Viblanc *et al.* 2012; Geffroy *et al.* 2015; Tarjuelo *et al.* 2015). Through behavioural plasticity, animals repeatedly exposed to humans may show either habituation, reducing the intensity of the reaction (e.g. Ellenberg *et al.* 2009; Viblanc *et al.* 2012), or sensitization, showing increasingly stronger reactions (Wheeler *et al.* 2009). Alternatively, if personality effects are stronger than individual plasticity, continued human exposure may lead to directional selection of certain phenotypes, if a personality type is disproportionally affected by human presence. These latter aspects are however not well explored, despite their relevance and implications for research, management and conservation.

Using data from a raptor population monitored during 19 years, we investigated the responses of breeding females to humans during nest visits. We analysed the repeatability of female behaviour, both within and between years, accounting for environmental covariates such as food conditions and nesting stage. We show long-term changes in these behavioural responses, and assessed whether the change was compatible with long-term trends in the phenotypic composition of the female breeding population. We further evaluated whether these changes were

associated with a heterogeneous selection of personality types: specifically, we assessed interactive effects on breeding performance between personality and varying exposure to humans through nests visits (testing whether shy individuals are more susceptible to disturbance), and between personality and time since the beginning of the study (testing for differential productivity of personality types along an environmental gradient that may have changed with time, not related to disturbance). We discuss our results in relation to potential impacts for long-term research and nest protection conservation programs.

MATERIAL AND METHODS

Study site and species

The Montagu's harrier *Circus pygargus* is a medium-sized ground-nesting species that has shifted from its original grassland habitats to agricultural land during the XXth century (Arroyo *et al.* 2002). In Western Europe, >70% of pairs now breed within crops, exposing the species to losses of eggs and nestlings during crop harvest. Conservation campaigns have been developed over the last 30 years in many areas, which include locating and visiting nests before and during harvest (e.g. Santangeli *et al.* 2014; 2015). Conservation programmes are often coupled with (or arise from) research programmes, so nest visits for acquiring data from nestlings also occur.

The study took place within the LTER "Zone Atelier Plaine & Val de Sèvre" (west-central France, 46° 11' N, 0° 28' W), covering 450 km² of agricultural habitat: winter cereal dominates (ca. 42% of the surface in 2012), together with spring-sown crops (sunflower and corn, ca. 25%) or crops dedicated to livestock rearing (alfalfa, rye-grass or hay fields, ca.18%). From 1995 to 2013, we systematically searched for

all Montagu's harrier nests in the study area, monitoring 14-104 nests each year. Variation in numbers across years are mainly related to prey abundance: common voles (*Microtus arvalis*) are a main prey for Montagu's Harriers in the study area, fluctuate in abundance among years, and strongly influence harrier breeding numbers and performance (Millon & Bretagnolle 2008). Overall, harrier density fluctuated strongly and cyclically until 2002, and breeding numbers were relatively constant subsequently at around 70 pairs per year (Bretagnolle, unpublished data), concomitant with a dampening of the vole cycles (Barraquand et al. 2014). No long-term trend in harrier breeding density occurred during the study period (Pearson $r = 0.22$, $n = 19$, $P > 0.10$).

Montagu's harriers are sexually dimorphic in plumage (males are light grey, females are brown) and in reproductive roles: females stay at the nest during the incubation and early nesting stages, and males provide food for female and nestlings. Both sexes may defend the nest, but females are usually first to react since they spend more time at or near the nest (Arroyo *et al.* 2004).

Data collection

We obtained information on harrier behaviour for a total of 2402 visits to 833 nests over 19 years (1995-2013). This time period represented ca 4-5 harrier generations, according to estimated age of maturity (1-2 year for females, 2-3 years for males, Arroyo *et al.* 2004) and adult survival (ca. 70%; Millon & Bretagnolle 2008).

When visiting a nest, we noted the following behaviours: distance from the approaching person at which the female left the nest (hereafter "nest departure distance"); whether she stayed around the nest during the visit, or disappeared from

sight (hereafter “fleeing”); whether she circled over the person carrying out the nest visit, and at what height; whether she alarmed-called, and the rate (alarm calls / min); whether she attacked the person at the nest (i.e., whether she performed a dive, even if not touching the person; real attacks are rare towards humans or other potentially dangerous predators in this species, Arroyo *et al.* 2001), and the rate (attacks / min). For some nest visits (including all of those in 2011 and 2012), only qualitative information was noted (i.e., female circled or not, alarmed or not, attacked or not, etc.). Hence, sample sizes differ between response variables. We also noted whether the male was present or not during the nest visit. In this study we focus on female behaviour, as males were absent in 79% of nest visits (n = 2402).

Date of visit was transformed into an estimate of nesting stage (relative to the laying onset of each study pair = day 0). We also noted the visit rank (whether it was the first, second, third, etc, visit to the same nest) and duration (in min). Number of visits per nest averaged 3.1 ± 1.8 (range 1-13, n=833 nests). Higher numbers corresponded to nests included in experimental studies. Visits lasted on average 9 ± 6 min (range 0.5-70, n=1881 visits). Shorter visits aimed at quickly checking nest contents, longest ones involved the installation of fences around the nest for protection at harvest time (Santangeli *et al.* 2015).

Through nest visits, we also calculated for each pair clutch size and fledged brood size (number of chicks that reached fledging age, ranging 0-6). We also estimated lay date by backdating from hatching date, estimated in turn from nestling measurements (Arroyo 1995) or from egg density (weight divided by volume, $0.51 \times \text{length} \times \text{width}^2 / 1000$; Hoyt 1979), which decreases linearly from laying to hatching (Bretagnolle, unpubl. data). When we did not have an exact lay date (nests visited during incubation that failed before hatching and where eggs had not been measured,

n = 38) we assumed arbitrarily that relative date of the first visit was 15 (mid incubation). For the 833 reproductive events for which we had fleeing behaviour, there was no information about breeding output for 7, and we did not have estimated lay date for a further 36 (these were nests that had failed after the first visit but for which it could not be determined with certainty that failure occurred during incubation). The relationship between personality and breeding parameters was thus analysed for 790 reproductive attempts.

Vole abundance (vole captures /100 traps) was estimated through live-trapping in different crop types (cereal, alfalfa, rye-grass, rape-seed and pasture) twice a year (April and June). We calculated an annual estimate of vole abundance for the area as the average abundance obtained for the five habitat types in each trapping session, then averaging April and June values (see Barraquand *et al.* 2014 for more details).

Behavioural variables for analyses

Behavioural data included categorical variables (flee, attack, alarm) as well as continuous variables (nest departure distance, circling height, alarm rate, and attack rate). We simplified these variables and categorised female responses during each nest visit as follows: a) fled; b) stayed around surveying but neither alarmed nor attacked (hereafter called “passive presence”); c) circled over the human carrying out the nest visit, alarming and/or attacking (hereafter called “defence”). For defending females, we carried out a Principal Component Analysis on the three quantitative variables (circling height, alarm rate, attack rate). The first PC was negatively correlated with circling height, and positively with attack and alarm rate (see details in Electronic Supplementary Material), and thus described “defence intensity”. Behavioural variables for analyses thus ultimately included: i) nest departure distance; ii)

probability of fleeing, iii) probability of being passive if present, and iv) defence intensity (PC1) for non-passive individuals.

Statistical analyses

Analyses were made with R 3.2.2, except for calculations of adjusted repeatability (see below).

When analysing behavioural variables, nest departure distance and defence intensity were fitted to a normal distribution (using an identity link) and fleeing or being passive were fitted to a binomial distribution (using a logit link).

We calculated within-year repeatability of behavioural variables (as a measure of individual consistency in that trait, Réale *et al.* 2007) from GLMM models including “nest identity” as a random variable. Some females were individually identified through wing-tags (98 study nests), 30 of which were observed in more than one year (range 2-7). We calculated between-year repeatability using GLMMs including “female identity” as a random variable, for the subset of nests with identified females and multiple observations among years. Repeatability was calculated using the rpt.binomGLMM.add (binomial variables) and rpt.mcmcLMM (normal variables) packages within rptR library (Nakagawa & Schielzeth 2010).

Adjusted repeatability (R_{adj}) is the repeatability after controlling for confounding effects that can potentially systematically bias the intensity of the trait expression (Nakagawa & Schielzeth 2010), and is considered closer to real repeatability (Díaz-Real *et al.* 2014). Risk-taking behaviour by parents in nest defence may be modulated by the value of current offspring, which usually varies non-linearly with nest age (Montgomerie & Weatherhead 1988), food abundance (as nestlings born in better environmental conditions may have higher survival probabilities; Hakkarainen &

Korpimäki 1994), and the partner's behaviour (Harrison et al. 2009; Trnka & Grim 2013). Additionally, animals exposed repeatedly to the same disturbance may show either habituation or sensitization (Viblanc *et al.* 2012). We therefore considered vole abundance, relative date (and its quadratic term), presence of the male and visit rank for R_{adj} calculations. R_{adj} of normal variables was calculated with the rptR package. However, binomial errors are not implemented in this package. Thus, we used SAS 9.2 software (SAS Institute, Cary, NC, USA) for estimating R_{adj} for binomial variables, from σ^2_{α} and σ^2_{ϵ} estimates retrieved with SOLUTION statement, and ω with the RANDOM statement (indicating "_residual_") in the GLIMMIX procedure (Díaz-Real *et al.* 2014).

We tested for temporal trends in behavioural variables using Generalised Linear Mixed Models (GLMM), with nest identity as a random factor, and year as a fixed continuous variable. We also included in these models vole abundance (log transformed), visit rank, relative date (as an indicator of nesting stage) and its quadratic term (to account for non-linear relationships between nest defence intensity and nesting stage) as continuous fixed effects, and presence/absence of the male as a categorical variable. We checked for potential collinearity and redundancy of the explanatory variables by analysing the Variable Inflation Factor (VIF). All explanatory variables had VIF values <2 , thus below the threshold suggested for eliminating them (Zuur et al. 2010).

To evaluate population compositional changes, we tested whether the proportion of monitored females that engaged in certain behaviours changed over time. We carried out GLMs, using two-vector variables, number of females that showed a particular behaviour over total number of monitored females each year

(fitted to a binomial distribution, with a log link function) as response variables, and year as a continuous fixed effect.

To test for relationships between personality and breeding parameters, we first calculated the solution to the random terms of a GLMM that analysed the probability of fleeing in relation to relative date (its quadratic term) and presence/absence of male, using “individual” as a random factor. In other words, we calculated the random intercept to the relationship between reaction and time in the breeding season and male presence (variables that affected within-year variation in fleeing probability, see results), which gives an overall comparable value of the individual variation in response among individuals, and used this value as an indicator of individual “personality” (hereafter referred to as “shyness”). We used “probability of fleeing” because information about this behaviour was available for more nest visits (as e.g. intensity of defence was not available if fled or were passive). The use of estimates of a random effect in a mixed effect model (Best Linear Unbiased Predictor or BLUP) for predicting individual reaction norm intercepts may overemphasize statistical differences despite very small effect sizes, and may not appropriately acknowledge uncertainty around the estimates (Hadfield et al. 2010). Our estimates, however, correlated significantly with raw behavioural data for each of the monitored females (see Fig. S1 in Electronic Supplementary Material), so we believe them to be biologically meaningful. Also, the number of individuals sampled, number of samples per individual, and repeatability of traits were relatively high, which may increase the accuracy of the estimates (Dingemanse & Dochtermann 2013). We subsequently implemented GLMMs (with “year” as a random categorical term to account for among-year variation in breeding parameters not related to any of the evaluated variables) for the following response variables: clutch size (fitted to a normal

distribution, identity link function), fledged brood size (fitted to a Poisson distribution, log link function) and probability of nest failure (fitted to a binomial distribution, logit link function). In all models, we included the individual estimate of “shyness” (as defined above) as a fixed effect, as well as variables known to affect breeding output (vole abundance and relative lay date). Relative lay date was calculated as the difference between lay date for a given nest and the average lay date for that particular year. We also included the frequency of visits carried out to that nest as an estimator of exposure to humans. We used nest visit frequency rather than number of visits, since total number of visits to a nest is necessarily lower in those nests that fail (as visits stop after failure). We calculated nest visit frequency as number of visits per time-length of total monitoring. For this, and considering that total incubation period is 30 days, and total nestling period is 35 days (Arroyo *et al.* 2004), we estimated that monitoring period for nests that failed during incubation was 20 days; 30 days for nests that failed during hatching; 45 days for nests failed during nestling; and 65 days for nests that did not fail. We express visit frequency as visits/month. We also initially included its quadratic term to test for non-linear relationships between breeding output and visit frequency. Additionally, we included “year” as a continuous variable to account for potential temporal trends in breeding parameters not related to the above-mentioned variables. Finally, initial models also included all two-way interactions between personality and other explanatory variables, to test for heterogeneous selection of shyness in different environmental gradients. If an interaction was not significant, we excluded it from the model and present results of the model without it. As above, we checked for potential collinearity issues, and all explanatory variables had VIF values <2.

We checked for model overdispersion for binomial and poisson models (all dispersion parameters were <1.3), and checked residuals for gaussian models (which were normally distributed in all cases).

RESULTS

Repeatability of behavioral responses

Within-year repeatability of all behavioural variables except nest departure distance was high and fairly similar across behavioural traits, ranging between 0.52 and 0.54 (Table 1). Between-year repeatabilities of those variables were also significantly greater than 0, and values were even higher for adjusted repeatabilities (Table 1).

Temporal trends in responsiveness to humans during nest visits

Nest departure distance only varied with nesting stage (Table 2; see Fig S2 in ESM for a graphical representation of the non-linear relationships of behaviour and nesting stage). Once out of the nest, investment in nest defence was modulated by nesting stage, with lower probability of fleeing or being passive and higher defence intensity later in the breeding season (Table 1, Fig. S2). In addition, defence intensity increased, whereas probability of being passive decreased, with increasing vole abundance (Table 2). Furthermore, fleeing probability decreased when the male was present, and defence intensity was relatively weaker with repeated visits.

Accounting for these contextual factors, we also found that all variables except nest departure distance showed significant temporal trends over the last 19 years (Table 2). Females were increasingly bolder toward humans throughout the study

period (Table 2; Fig. 1): probability of fleeing declined over the study period (Fig. 1a) as did probability of being passive (Fig. 1b), while defence intensity steadily increased (Fig. 1c).

Given the repeatability of behavioural variables, observed long-term trends could be associated with a change in the phenotypic composition of the study population. Indeed, the proportion of females that never fled during nest visits increased over the years from 40% to 90% ($\text{Chi}^2 = 87.73$, $P < 0.0001$), whereas the proportion of females that were always passive when present decreased from 30% to less than 5% in recent years ($\text{Chi}^2 = 22.29$, $P < 0.0001$; Fig. 2). The harrier population also became more behaviourally homogeneous throughout the study period (as shown by the increasingly lower coefficient of variation of individual personality estimates with time, Fig. S3 in ESM).

Personality, nest visits and breeding success

Clutch size and fledged brood size increased with vole abundance and decreased with relative lay date, whereas nest failure probability decreased with vole abundance and increased with relative lay date (Table 3).

Additionally, we found a significant interaction between female shyness and nest visit frequency for both fledged brood size and nest failure probability (Table 3). In the absence of nest visits, personality had only a small effect on these parameters (Fig. 3), in accordance with the lack of effect of personality on clutch size (Table 3). On the other hand, the probability of nest failure increased with the frequency of nest visits, but this was particularly marked for “shy” individuals, which almost systematically failed when exposed to more than 3 nest visits per month (Fig. 3).

Interactions between personality and vole abundance, relative lay date or year were all not significant (all $P > 0.4$).

The number of visits to each nest slightly increased throughout the study period ($F_{1,17} = 3.56$, $P = 0.07$, slope 0.07 ± 0.04), associated with an increase in research and conservation activities for the study population. However, overall, there was no long-term trend in clutch size, productivity or nest failure probability, as shown by the non-significant effect of year on those variables (Table 3).

DISCUSSION

Behaviour during nest visits and long-term changes in population composition

Female Montagu's harriers showed phenotypic plasticity in their response towards humans during nest visits, modulating behaviour (probability of fleeing or being passive, or defence intensity) in relation to the value of current offspring (Curio 1987): as expected, females invested more in defence when the nest or brood was older, and in better environmental conditions (i.e., greater vole abundance), when productivity is greater. Results also suggest some habituation to nest visits, as defence intensity decreased with increasing number of visits during the same breeding event. Nest defence behaviour of females may also depend on their partner's behaviour, if they adjust their risk taking to that of the other pair member (Harrison *et al.* 2009). In our study, probability of fleeing by females was significantly associated with male absence, which may reflect either that females are less likely to flee if the male is already present, or that female departure from the nest area decreases the probability of males being recruited to help with defence. Otherwise, male presence did not seem to affect female behaviour towards humans during nest visits.

Despite observed plasticity, repeatability values for these behaviours (both within and between breeding seasons) were fairly high and significantly greater than 0, indicating an important degree of individual consistency in reactions toward humans, which can therefore be considered a temperament trait (Réale *et al.* 2007).

Flight initiation distance (FID), the distance at which birds flee from potential predators approaching them, is usually considered a reliable and individually consistent surrogate measure of response to predation risk (Blumstein 2006; Møller 2008; Carrete & Tella 2010; Díaz *et al.* 2013). Our results, in contrast, show that for Montagu's harriers nest departure distance varied with nesting stage, with females sitting most tightly at the nest around hatching time, but that it was not a good indicator of individual variability in reactions to nest visits by humans.

Throughout the 19 study years, we found marked temporal trends in the behaviour of monitored Montagu's harriers during nest visits (except for nest departure distance), with an overall increase in boldness and aggressive behaviour toward humans, suggesting less fear of humans. Trends remained significant when taking into account the above-mentioned modulating contextual factors, indicating that these were not the result of e.g. increased vole abundance with time, or different sampling of nests in relation to nesting stage with time. Although repeatability values were high, they were far from 1, so there is still scope for phenotypic plasticity to contribute to the observed temporal changes in behaviour. This could occur, for example, if a variable influencing within-individual variation in behaviour and not included in our analyses had also changed over time, or if we had systematically sampled in later years under conditions leading to more intense nest defence (Dingemanse & Dochtermann 2013). However, sampling schemes were similar throughout the study period, and we are not aware of any environmental factor

potentially influencing within-individual behaviour systematically changing during our study period.

This, together with the relatively high effect sizes of repeatabilities, suggest that trends may reflect a change in the phenotypic composition of the population. Indeed, our results also showed a gradual disappearance of shy individuals throughout the study period (estimated to be 4-5 harrier generations), leading to a greater proportion of bolder individuals, and overall a more behaviourally homogeneous breeding population, in more recent years.

Mechanisms and drivers of behavioural phenotypic changes

Our results also point that a likely driver of the observed changes is the increasing exposure to humans through nest visits. Nest visit frequency affected negatively breeding performance, but this effect was not equal among individuals, being most marked for shy individuals, more prone to flee rather than defend their nest. This represents a clear example, based on an ecologically realistic context, that personality-fitness relationships are context-dependent. Non-anthropogenic causes of failure in the species include mainly predation and starvation (Arroyo et. al. 2004). It is thus possible that, when disturbed, shy individuals leave the nest unattended and are thus more prone to predation, nestling death due to inclement weather or poor provisioning. Further studies should address whether there is a relationship between female personality, disturbance and causes of failure.

Long-term trends in compositional changes of the population could have arisen from heterogeneous selection of phenotypes under environmental conditions that have changed over time. For example, reduced persecution following full protection of raptors in France (1972) as well as elsewhere in Europe could have

implied that fitness of shy individuals was higher when persecution occurred, as those personality types may be less likely to be detected and killed, whereas in the absence of persecution bold personalities may have higher fitness (if, for example, predation risk was lower for bold individuals because they defend better their brood against predators). Similarly, if predator density had increased over time, this could imply increasingly higher success for bold than shy individuals associated with the higher predation risk. This would imply that the relationship between personality and fitness changed over time, but the interaction between year and shyness was not significant.

Focusing on the observed differential nest failure of shy individuals when disturbed, two different (and not mutually exclusive) mechanisms may potentially account for the observed compositional changes in the population throughout time. If personality is heritable (e.g. Réale *et al.* 2007; Bize *et al.* 2012; Bromer & Klun 2012; Patrick *et al.* 2013) and natal dispersal is low, a higher proportion of bold individuals in the population over the study period could simply arise through the relative contribution to future generations of different phenotypes. Natal dispersal in our study species is high (Limiñana *et al.* 2012), so this mechanism could only lead to observed compositional changes in the study area if the increased human disturbance and the concomitant lower contribution of shy individuals to future generations had occurred simultaneously at a much larger geographical scale over the study period. Indeed, nest monitoring and protection programs (which include nest visits) for this vulnerable species occur throughout France, affecting more than 1000 nests per year (ca. a third of total breeding population, Santangeli *et al.* 2015). Increases in farming or leisure activities in Montagu's harrier habitats may have also occurred.

Additionally, individual variability in reaction to humans within a population may affect habitat selection of breeders and thus spatial segregation of different

phenotypes (Carrete & Tella 2010). Montagu's harriers also have a relatively high breeding dispersal capacity (Arroyo *et al.* 2004) which, as in other species (e.g. Serrano *et al.* 2001) is modulated by previous nesting experience. In fact, a study with marked adults in northern France showed that 30% of females bred further away than 20 km from their previous nesting site following a failure (Thierry Printemps, unpubl. data). A change in phenotypic composition of populations may thus be observed at a local scale, regardless of the heritability of the trait, if human exposure modifies habitat quality only for certain individuals, and this leads to a differential settlement in relation to perceived stressors. In this case, the large-scale current distribution of different personality types should vary in relation to the degree of human exposure, something that could be tested in future studies. Evaluation of heritability of these behavioural traits and of the spatial distribution of different phenotypes at larger scales could thus shed further light on mechanisms leading to observed trends.

One way or another, our results imply that nest visits or increased contacts with humans in non-urban environments may lead to a directional selection of bold and aggressive phenotypes, and a subsequent homogenization of behaviour in disturbed areas toward bolder responses. This is consistent with the increased boldness of urban rather than rural birds, although in that case it seems more likely that this arises not from tame (bold) individuals being overrepresented among urban invaders (Carrete & Tella 2011).

In-situ conservation programmes frequently involve locating and visiting the nests (Santangeli *et al.* 2014, 2015). Our results could thus be interpreted as suggesting that nests visits should be minimized even in a conservation context. However, the global and large positive effect of nest protection programs for the

vulnerable Montagu's harriers in Western Europe (Santangeli *et al.* 2014, 2015) overruns any potential negative consequences of an increase in boldness as a consequence of nest visits. Indeed, at the population level, the increased nest visit frequency throughout time in our study population did not translate into population changes in nesting success or productivity, but rather in subtle changes in the phenotypic composition of the population. On the other hand, these results highlight that long-term population monitoring programs may have potential unexpected consequences (see also Viblanc *et al.* 2012). If boldness towards humans is associated to other behavioural traits (e.g. intraspecific aggression), the observed compositional change could have other indirect effects, for instance on the social structuring of species that have facultative colonial breeding such as the Montagu's harrier (Arroyo *et al.* 2001). This may imply that the population under study is varying throughout the study period, but that these changes may go unnoticed.

Human activities in nature are increasingly frequent and widespread. Our findings stress out that further research on how human-animal interactions influence animal behaviour, and on the interactions between animal temperament and evolutionary population changes is critically needed to understand the likely influences of human activities (including research) on wild animal populations (McDougall *et al.* 2006).

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REFERENCES

- Arroyo, B.E. (1995). Breeding ecology and nest dispersion of Montagu's Harrier *Circus pygargus* in central Spain. PhD thesis. University of Oxford, UK.
- Arroyo, B.E., Mougeot, F. & Bretagnolle, V. (2001). Colonial breeding and nest defence in Montagu's harrier (*Circus pygargus*). *Behav. Ecol. Sociobiol.*, 50, 109-115.
- Arroyo, B.E., García, J.T. & Bretagnolle, V. (2002). Conservation of the Montagu's Harrier (*Circus pygargus*) in agricultural areas. *Anim. Conserv.*, 5, 283-290.
- Arroyo, B.E., Garcia, J.T. & Bretagnolle, V. (2004). Montagu's Harrier. *Bwp update*, 6, 41-55.
- Barraquand, F., Pinot, A., Yoccoz, N.G. & Bretagnolle, V. (2014). Overcompensation and phase effects in a cyclic vole population: between first and second order cycles. *J. Anim. Ecol.*, 83, 1367-1378.
- Biro, P.A. & Stamps, J.A. (2008). Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.*, 23, 361-368.
- Bize, P., Díaz, C. & Lindstrom, J. (2012). Experimental evidence that adult antipredator behaviour is heritable and not influenced by behavioural copying in a wild bird. *P. Roy. Soc. B-Biol. Sci.*, 279, 1380-1388.

- Blumstein, D.T. (2006). Developing an evolutionary ecology of fear: How life history and natural history traits affect disturbance tolerance in birds. *Anim. Behav.*, 71, 389–399.
- Bokony, V., Kulcsar A., Toth, Z, & Liker, A. (2012). Personality traits and behavioural syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). *PLOS One*, 7, e36639.
- Bromer, J.E. & Klueen, E. (2012). Exploring the genetics of nestling personality traits in a wild passerine bird: testing the phenotypic gambit. *Ecol. Evol.*, 2, 3032-3044.
- Carrete, M. & Tella, J.L. (2010). Individual consistency in flight initiation distances in burrowing owls: a new hypothesis on disturbance-induced habitat selection. *Biol. Lett.*, 6, 167-170.
- Carrete, M. & Tella, J.L. (2011). Inter-individual variability in fear of humans and relative brain size of the species are related to contemporary urban invasion in birds. *PLOS One* 6(4): e18859.
- Curio, E. (1987). Animal decision-making and the concorde fallacy. *Trends Ecol. Evol.*, 2, 148-152.
- Díaz, M., Moller, A.P., Flensted-Jensen, E., Grim,T., Ibanez-Alamo, J.D., Jokimaki, J., *et al.* (2013). The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. *PLOS ONE*, 8, e64634.
- Díaz-Real, J., Serrano, D., Pérez-Tris, J., Fernández-González, S., Bermejo, A., Calleja, J.A., *et al.* (2014). Repeatability of feather mite prevalence and intensity in passerine birds. *PLOS ONE*, 9, e107341.
- Dingemanse, N.J., & de Goede, P. (2004). The relation between dominance and exploratory behaviour is context-dependent in wild great tits. *Behav. Ecol.*, 15, 1023-1030.

509 Dingemanse, N.J., Both, C., Drent, P.J. & Tinbergen, J.M. (2004). Fitness
 510 consequences of avian personalities in a fluctuating environment. *P. Roy. Soc. B-*
 511 *Biol. Sci.*, 271, 847-852.

512 Dingemanse, N. J., Kazem, A. J. N., Réale, D. & Wright, J. (2010) Behavioural
 513 reaction norms: animal personality meets individual plasticity. *Trends Ecol. Evol.*
 514 25, 81–89.

515 Dingemanse, N. J., & Réale, D. (2005). Natural selection and animal personality.
 516 *Behaviour*, 142, 1159–1184.

517 Dingemanse, N.J. & Dochtermann, N.A. (2013). Quantifying individual variation in
 518 behaviour: mixed-effect modelling approaches. *J. Anim. Ecol.*, 82: 39-54

519 Duckworth R.A. & Sockman K.W. (2012). Proximate mechanisms of behavioural
 520 inflexibility: implications for the evolution of personality traits. *Funct. Ecol.*, 26,
 521 559-566.

522 Ellenberg, U., Mattern, T. & Seddon, P.J. (2009). Habituation potential of yellow-
 523 eyed penguins depends on sex, character and previous experience with humans.
 524 *Anim. Behav.*, 77, 289-296.

525 Evans, J., Boudreau, K. & Hyman, J. (2010). Behavioural syndromes in urban and
 526 rural populations of song sparrows. *Ethology*, 116, 588-595.

527 Geffroy, B., Samia, D.S.M., Bessa, E. & Blumstein, D.T. (2015). How nature-based
 528 tourism might increase prey vulnerability to predators. *Trends Ecol. Evol.*, 30:
 529 755-765

530 Hadfield, J.D., Wilson, A.J., Garant, D., Sheldon, B.C. & Kruuk, L.E.B. (2010) The
 531 misuse of BLUP in ecology and evolution. *Am. Nat.*, 175: 116-125

532 Hakkarainen, H. & Korpimäki, E. (1994). Does feeding effort of Tengmalm owls
 533 reflect offspring survival prospects in cyclic food conditions? *Oecologia*, 9, 209-
 534 214.

535 Harrison, F., Barta, Z., Cuthill, I. & Székely, T. (2009). How is sexual conflict over
 536 parental care resolved? A meta-analysis. *J Evol Biol.*, 22:1800–1812.

537 Hoyt, D.F. (1979). Practical methods of estimating volume and fresh weight of bird
 538 eggs. *Auk*, 96: 73-77.

539 Limiñana, R., García J.T., Guerrero A., González J.M., Lavedán J., Moreno J.D. *et al.*
 540 (2012). Phylopatry and natal dispersal of Montagu's harriers *Circus pygargus*
 541 breeding in Spain: a review of existing data. *Eur. J. Wildlife Res.*, 58: 549-555

542 McDougall, P. T., Réale, D., Sol, D. & Reader, S. M. (2006). Wildlife conservation
 543 and animal temperament: causes and consequences of evolutionary change for
 544 captive, reintroduced, and wild populations. *Anim. Conserv.*, 9, 39–48.

545 Millon, A. & Bretagnolle, V. (2008). Predator population dynamics under a cyclic
 546 prey regime: numerical responses, demographic parameters and growth rates.
 547 *Oikos*, 117, 1500-1510.

548 Møller, A.P. (2008). Flight distance of urban birds, predation, and selection for urban
 549 life. *Behav. Ecol. Sociobiol.*, 63, 63–75.

550 Møller, A.P. (2010). Interspecific variation in fear responses predicts urbanization in
 551 birds. *Behav. Ecol.*, 21, 365–371.

552 Montgomerie, R.D. & Weatherhead, P.J. (1988). Risks and rewards of nest defence
 553 by parent birds. *Q. Rev. Biol.*, 63: 167-187

554 Nakagawa, N. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian
 555 data: a practical guide for biologists. *Biol. Rev.*, 85, 935-956

556 Patrick, S.C., Charmantier, A. & Weimerskirch, H. (2013). Differences in boldness
 557 are repeatable and heritable in a long-lived marine predator. *Ecol. Evol.*, 13,
 558 4291-4299.

559 Patrick, S.C., Chapman, J.R., Dugdale, H.L., Quinn, J.L. & Sheldon, B. (2012).
 560 Promiscuity, paternity and personality in the great tit. *Proc. R. Soc. B.*, 279: 1724-
 561 1730.

562 Quinn, J.L., Cole, E.F., Patrick, S.C. & Sheldon, B.C. (2011). Scale and state
 563 dependence of the relationship between personality and dispersal in a great tit
 564 population. *J. Anim. Ecol.*, 2011, 80: 918-928

565 Quinn, J.L., Patrick, S.C., Bouwhuis, S., Wilkin, T.A. & Sheldon, B.C. (2009).
 566 Heterogeneous selection on a heritable temperament trait in a variable
 567 environment. *J. Anim. Ecol.*, 78: 1203-1215

568 Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007).
 569 Integrating animal temperament within ecology and evolution. *Biol. Rev.*, 82, 1-
 570 28.

571 Santangeli, A., Di Minin, E. & Arroyo, B. (2014). Bridging the research
 572 implementation gap – Identifying cost-effective protection measures for
 573 Montagu's harrier nests in Spanish farmlands. *Biol. Conserv.*, 177: 126-133

574 Santangeli, A., Arroyo, B., Millon, A. & Bretagnolle, V. (2015). Identifying effective
 575 actions to guide volunteer-based and nation-wide conservation efforts for a
 576 ground-nesting farmland bird. *J. Appl. Ecol.*, 52: 1082-1091.

577 Serrano, D., Tella, J.L., Forero, M.G. & Donazar, J.A. (2001). Factors affecting
 578 breeding dispersal in the facultatively colonial lesser kestrel: individual
 579 experience vs. conspecific cues. *J. Anim. Ecol.*, 70: 568-578

- Sih, A., Bell, A.M, Johnson, J.C. & Ziemba, R.E. (2004). Behavioral syndromes: an integrative overview. *Quart. Rev. Biol.*, 79, 241-277.
- Smith, B.R. & Blumstein, D.T. (2008). Fitness consequences of personality: a meta-analysis. *Behav. Ecol.*, 19, 448-455.
- Smith, B.R. & Blumstein, D.T. (2013). Animal personalities and conservation biology: the importance of behavioral diversity. Pp. 379-411. In: *Animal personalities: behavior, physiology, and evolution* (C. Carere and D. Maestripieri, eds.), University of Chicago Press, Chicago, IL.
- Tarjuelo, R., Barja, I., Morales, M.B., Traba, J., Benitez-Lopez, A., Casas, F., *et al.* (2015). Effects of human activity on physiological and behavioral responses of an endangered steppe bird. *Behav. Ecol.*, 26: 828–838.
- Trnka, A. & Grim, T. (2012). To compensate or not to compensate: testing the negotiation model in the context of nest defense. *Behav. Ecol.*, 24: 223-228
- Viblanc, V.A., Smith, A.D., Gineste, B., & Groscolas, R. (2012). Coping with continuous human disturbance in the wild : insights from penguin heart rate response to various stressors. *BMC Ecol.*, 12, 10
- Wheeler, M., de Villiers, M.S. & Majiedt, P.A. (2009). The effect of frequency and nature of pedestrian approaches on the behaviour of wandering albatrosses at sub-Antarctic Marion Island. *Polar Biol.*, 32, 197-205.
- Wolf, M. & Weissing, F.J. (2012). Animal personalities: consequences for ecology and evolution. *Trends Ecol. Evol.*, 27: 452-461
- Wolf, M., & Weissing, F. J. 2010. An explanatory framework for adaptive personality differences. *Phil. Trans. Royal Soc. London B*, 365, 3959–3968.
- Wolf, M., Van Doorn, S., Leimar, O., & Weissing, F.J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, 447:581-584.

605 Zuur, A.F., Ieno E.N. & Elphick, C.S. (2010) A protocol for data exploration to avoid
606 common statistical problems. *Methods Ecol. Evol.*, 1: 3-14.

Table 1. Repeatability values ($\pm$ SE) of behavioural responses to humans during nest visits (95 confidence intervals and sample sizes are given in brackets). Nest departure distance and defence intensity are continuous variables. Fleeing probability and passive if present are binomial. Adjusted repeatability values (R_{adj}) are those controlling for the effects of vole abundance, nesting stage (non-linearly), male presence and visit rank.

	Within-year	Within-year R_{adj}	Between-year	Between-year R_{adj}
Nest departure distance	0.001 $\pm$ 0.023 (0-0.072, n = 1662)	0.03 (0-0.07)	0.001 $\pm$ 0.017 (0-0.043, n = 197)	0.001 (0-0.05)
Fleeing probability	0.541 $\pm$ 0.049 (0.48-0.65, n = 2399)	0.95	0.341 $\pm$ 0.135 (0.07-0.59, n = 299)	0.65
Passive if present	0.520 $\pm$ 0.059 (0.38-0.61, n = 1844)	0.87	0.522 $\pm$ 0.194 (0.11-0.83, n = 252)	0.88
Defence intensity (PCA)	0.518 $\pm$ 0.033 (0.45-0.57, n = 1014)	0.53 (0.51-0.55)	0.131 $\pm$ 0.073 (0.05-0.33, n = 180)	0.27 (0.15-0.39)

Table 2. Type III results of GLMM analyses testing the simultaneous effect of year, vole abundance (log transformed), visit number, relative date (in relation to lay date = day 0), and presence/absence of the male on behavioural responses of female Montagu's harriers during nest visits. The quadratic term of relative date was also included (non-linear relationships). All variables except presence/absence of male are continuous. Models included "nest identity" as a random term.

Dependent variable	Explanatory variable	F/Chi ²	P	Parameter estimate $\pm$ SE
Nest departure distance				
	Year	1.09	0.30	0.09 $\pm$ 0.09
	Vole abundance	1.07	0.29	-0.54 $\pm$ 0.52
	Visit rank	0.97	0.32	0.46 $\pm$ 0.47
	Relative date	59.95	0.001	-0.85 $\pm$ 0.11
	Relative date ²	84.24	0.001	0.02 $\pm$ 0.002
	Presence of male	1.43	0.23	-1.34 $\pm$ 1.12
Fleeing Probability				
	Year	65.41	0.001	-0.20 $\pm$ 0.02
	Vole abundance	0.23	0.63	-0.06 $\pm$ 0.12
	Visit rank	0.01	0.91	-0.01 $\pm$ 0.10
	Relative date	0.004	0.94	0.001 $\pm$ 0.02
	Relative date ²	9.48	0.002	-0.0009 $\pm$.0003
	Presence of male	19.13	0.001	-1.34 $\pm$ 0.31
Passive if present				
	Year	25.07	0.001	-0.19 $\pm$ 0.03
	Vole abundance	12.80	0.001	-0.69 $\pm$ 0.19
	Visit rank	2.46	0.12	0.18 $\pm$ 0.11
	Relative date	19.44	0.001	-0.14 $\pm$ 0.03
	Relative date ²	8.74	0.003	0.001 $\pm$ 0.0004
	Presence of male	1.02	0.31	0.28 $\pm$ 0.27
Defence intensity				
	Year	35.12	0.001	0.10 $\pm$ 0.01
	Vole abundance	6.64	0.01	0.20 $\pm$ 0.07
	Visit rank	6.88	0.008	-0.08 $\pm$ 0.03
	Relative date	38.79	0.001	0.04 $\pm$ 0.007
	Relative date ²	25.12	0.003	-0.0005 $\pm$ 0.000
	Presence of male	0.61	0.43	0.06 $\pm$ 0.07

Table 3. Type III results of GLMM analyses of breeding parameters in relation to vole abundance (log transformed), relative lay date (relative to mean annual lay date), frequency of nest visits (per month), year, female “shyness” and their interactions. All explanatory variables are continuous. “Year” was also fitted as a random categorical term. The initial model also included the quadratic term of visit frequency, to test for possible non-linear relationships (see Fig. 3). Non-significant interaction terms were removed from the model.

Dependent	Explanatory	Chi	P	Parameter estimate $\pm$ SE
Fledged brood size				
	Vole abundance	7.42	0.006	0.22 ± 0.08
	Year	0.14	0.70	-0.005 ± 0.013
	Relative lay date	56.77	0.0001	-0.021 ± 0.003
	Shyness	4.80	0.028	0.049 ± 0.022
	Visit frequency	35.60	0.0001	-0.32 ± 0.05
	Shyness*visit frequency	10.89	0.0009	-0.04 ± 0.01
Nest failure				
	Vole abundance	0.82	0.33	-0.14 ± 0.16
	Year	0.05	0.81	0.006 ± 0.02
	Relative lay date	26.62	0.0001	0.042 ± 0.008
	Shyness	3.72	0.054	-0.15 ± 0.08
	Visit frequency	26.19	0.0001	0.88 ± 0.17
	Shyness*visit frequency	8.01	0.004	0.11 ± 0.04
Clutch size				
	Vole abundance	16.47	0.0001	0.28 ± 0.06
	Year	0.07	0.79	-0.003 ± 0.011
	Relative lay date	162.76	0.0001	-0.041 ± 0.003
	Shyness	0.61	0.44	-0.01 ± 0.01
	Visit frequency	0.0002	0.98	0.0004 ± 0.03

Figure 1. Changes over time (1995-2013) in the behavioural responses of female Montagu's harrier during nest visits: (a) mean ($\pm$ SE) probability of fleeing during the visit; b) mean ($\pm$ SE) probability of being passive, if present; c) mean ($\pm$ SE) defence intensity (higher values indicate lower circling height, and greater alarm call and attack rates).

Figure 2. Changes over time (1995-2013) in the proportion of breeding females that never fled (red dots), or that were always passive if present (blue triangles) during nest visits. Numbers refer to sample sizes (number of monitored breeding females each year).

Figure 3. Relationships between female personality ("shyness"), nest visit frequency and breeding performance of Montagu's harriers. (a, c) Nesting success (probability of fledging at least one young); (b,d) Productivity (number of young fledged). Top graphs show raw data according to personality type (a,b; mean $\pm$ SE, sample size above/below bars refer to breeding events); visit frequency and personality types were categorized for visualization purposes (shy: random intercepts > 1 ; bold: random intercepts < 1). Bottom graphs show model output predictions (c,d; Table 3) for a relative lay date of 0 (population average) and average vole abundance for the study period.

Fig. 1

Fig. 2

Fig. 3