


LETTER

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

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Abstract

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

Keywords

Aggression, boldness, fitness, long-term studies, Montagu's harrier, personality, phenotypic change, plasticity.

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INTRODUCTION

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

Several studies have shown links between personality and life history traits like dispersal or productivity (Réale *et al.* 2007; Biro & Stamps 2008) although these relationships have been found to be minimal in some species (e.g. Quinn *et al.* 2009, 2011; Patrick *et al.* 2012). Life history links of personality may be context-dependent, be even reversed in different ecological conditions, or be shaped by trade-offs between current and future fitness prospects (Dingemanse & de Goede 2004; Dingemanse *et al.* 2004; Wolf *et al.* 2007). Therefore,

variation in personality may be maintained by fluctuating environmental pressures (Wolf & Weissing 2010). On the other hand, changes in the relative frequency of personality types within a population may appear following differential selection on personality types from environmental pressures that shift in a particular direction over time, such as human exposure (Smith & 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 interindividual 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 programmes or conservation efforts (Viblanç *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; Viblanç *et al.* 2012), or sensitisation, showing increasingly stronger reactions (Wheeler *et al.* 2009). Alternatively, if

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personality effects are stronger than individual plasticity, continued human exposure may lead to directional selection of certain phenotypes, if a personality type is disproportionately 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 of long-term research and nest protection conservation programmes.

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 20th 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 (c. 42% of the surface in 2012), together with spring-sown crops (sunflower and corn, c. 25%) or crops dedicated to livestock rearing (alfalfa, rye-grass or hay fields, c. 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 (VB, 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 c. 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 (c. 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 alarm-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, longer ones involved the installation of fences around the nest for protection at harvest time (Santangeli *et al.* 2015).

Through nest visits, we also recorded 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 (VB, 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 Supporting Information), and thus described 'defence intensity'. Behavioural variables for analyses thus ultimately included: (1) nest departure distance; (2) probability of fleeing, (3) probability of being passive if present, and (4) 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 Generalised Linear Mixed Models (GLMM) 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 1 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 nonlinearly 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 sensitisation (Viblanco *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 GLMMs, 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 nonlinear 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 General Linear Models (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 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 individuals 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 overemphasise 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), 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 nonlinear 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 behavioural 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 > 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 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 towards 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% ($\chi^2 = 87.73$, $P < 0.0001$), whereas the proportion of females that were always passive when present decreased from 30% to $< 5\%$ in recent years ($\chi^2 = 22.29$, $P < 0.0001$; Fig. 2). The harrier population also became more behaviourally homogeneous throughout the study period (as shown by the increasingly lower coefficient of variation in 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 three 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,

Table 1 Repeatability values (\pm SE) of behavioural responses to humans during nest visits (95 confidence intervals and sample sizes are given in brackets)

	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)

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 (nonlinearly), male presence and visit rank.

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

Dependent variable	Explanatory variable	F/χ^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 0.0003
	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 0.0001
	Presence of male	0.61	0.43	0.06 \pm 0.07

The quadratic term of relative date was also included (nonlinear relationships). All variables except presence/absence of male are continuous. Models included 'nest identity' as a random term.

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 > 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, 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 towards 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, for example, 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,

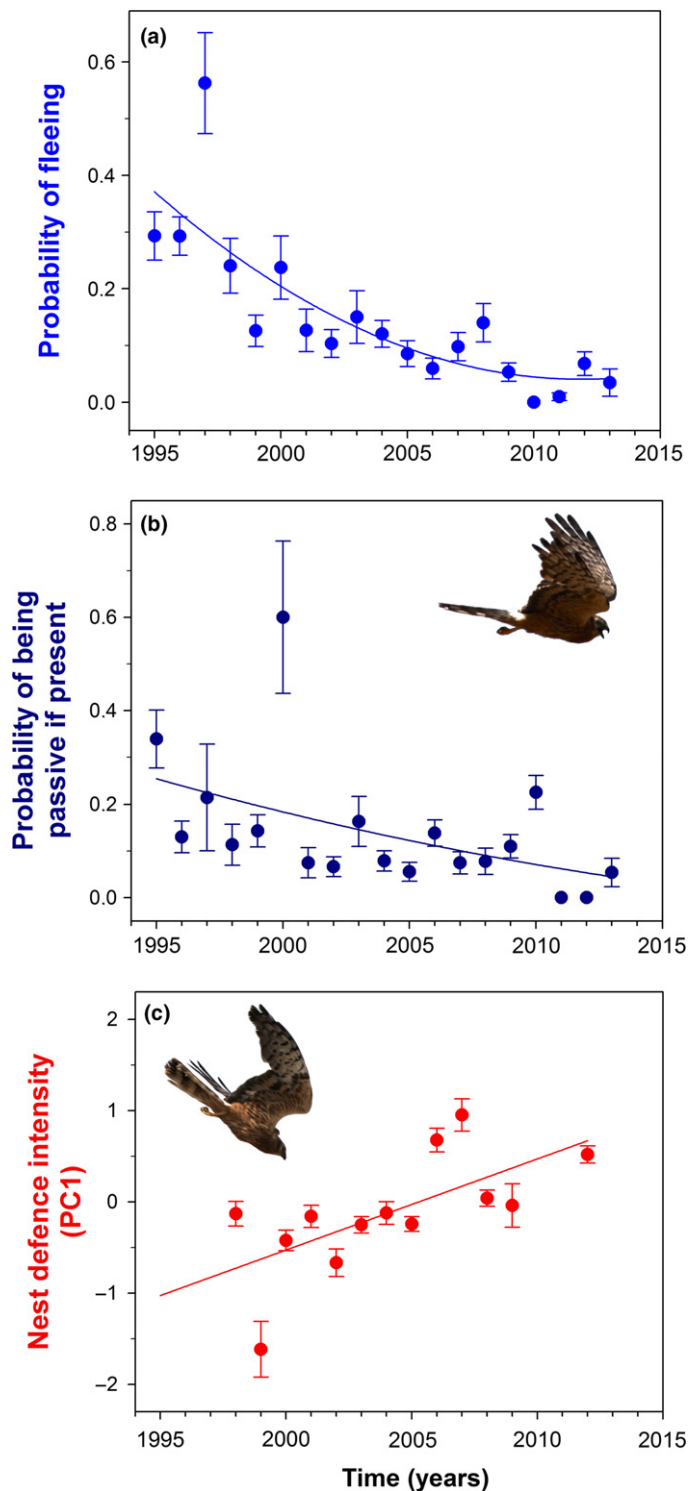


Figure 1 Changes over time (1995–2013) in the behavioural responses of female Montagu's harrier during nest visits: (a) mean (\pm SE) probability of fleeing during the visit; (b) mean (\pm SE) probability of being passive, if present; (c) mean (\pm SE) defence intensity (higher values indicate lower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

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.

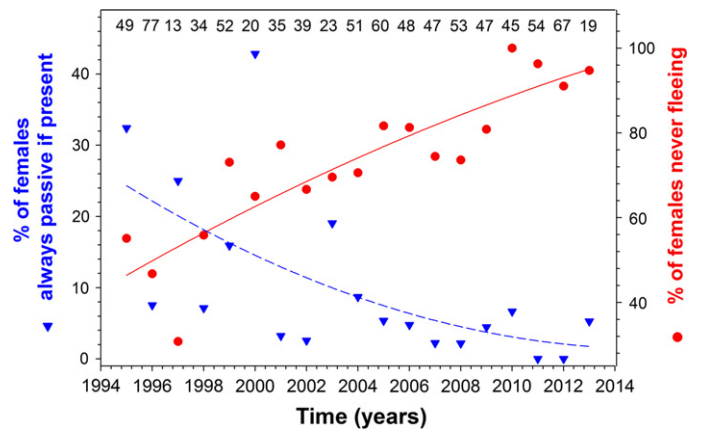


Figure 2 Changes over time (1995–2013) in the proportion of breeding females that never fled (red dots), or that were always passive if present (blue triangles) during nest visits. Numbers refer to sample sizes (number of monitored breeding females each year).

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

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

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 nonlinear relationships (see Fig. 3). Non-significant interaction terms were removed from the model.

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.

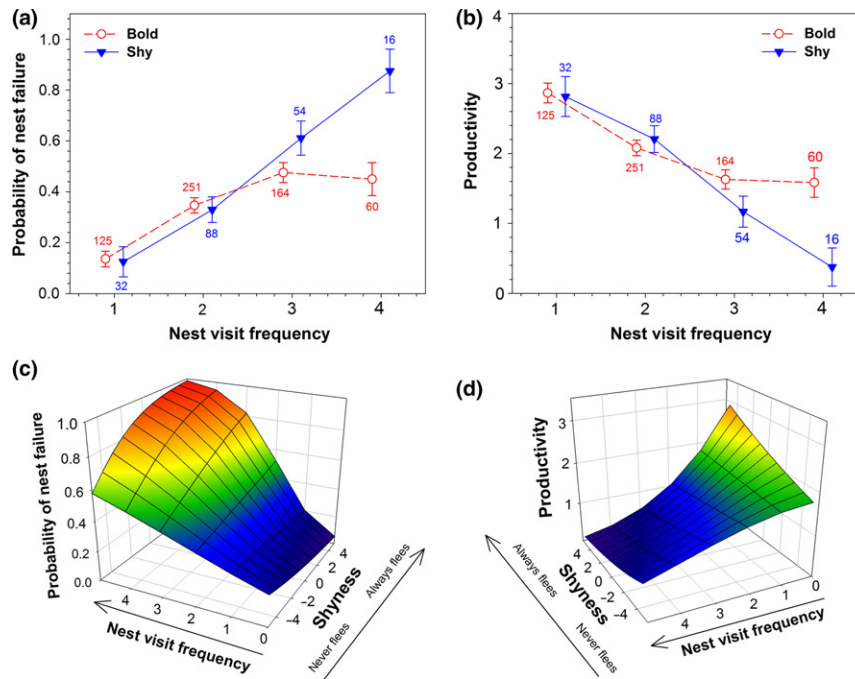


Figure 3 Relationships between female personality ('shyness'), nest visit frequency and breeding performance of Montagu's harriers. (a and c) Nesting success (probability of fledging at least one young); (b and d) Productivity (number of young fledged). Top graphs show raw data according to personality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

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 breeding performance negatively 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 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, e.g. predation risk was lower for bold individuals because they defend their brood better 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 & Kluehn 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 programmes (which include nest visits) for this vulnerable species occur throughout France, affecting more than 1000 nests per year (c. 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 (Printemps, T., 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 homogenisation of behaviour in disturbed areas towards 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 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 minimised even in a conservation context. However, the global and large positive effect of nest protection programmes 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 programmes 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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AUTHORSHIP

All authors conceptualised the study. BA conducted analyses with inputs from FM. BA wrote the first draft of the manuscript, and FM and VB contributed substantially to revisions. VB designed and coordinated long-term data collection, and secured associated funding.

DATA ACCESSIBILITY

Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository, and the data DOI will be included at the end of the article.

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