Stress hormone receptors change as range expansion progresses in house sparrows

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As ranges expand, individuals encounter different environments at the periphery than at the centre of the range. Previously, we have shown that glucocorticoids (GCs) vary with range expansion: individuals at the range edge release more GCs in response to restraint. Here, we measured hippocampal mRNA expression of GC receptors (mineralocorticoid, MR and glucocorticoid, GR) in eight house sparrow (Passer domesticus) populations varying in age. We found that individuals closest to the range edge had the lowest expression of MR relative to GR; in all likelihood, this relationship was driven by a marginal reduction of MR mRNA at the range edge. Reduced MR (relative to GR) might allow enhanced GC binding to GR, the lower affinity receptor that would enhance a rapid physiological and behavioural response to stressors. The insights gained from this study are not only enlightening to introduced species, but may also predict how certain species will react as their ranges shift owing to anthropogenic changes.

1. Introduction

One of the largest threats to global biodiversity is the introduction and spread of non-native species [1]. In novel habitats, individuals may face more unpredictable stressors (e.g. unknown/novel resources, predators/parasites); to cope with these stressors, vertebrates often release glucocorticoids (GCs; [2]). Significant variation in the regulation of and response to GCs exists, and how an individual physiologically and behaviourally responds to GCs dictates its fitness in certain contexts. GCs can enhance vigilance and memory consolidation [3], and mediate behaviours necessary to survive stressors (e.g. avoidance [4]), all of which may increase survival in unpredictable environments. Indeed, in a population undergoing range expansion, individuals at the range edge (where stressors and resources are potentially less known) released more GCs in response to restraint than those from more established areas [5].

Basal GCs, which respond to daily and seasonal fluctuations, are controlled by mineralocorticoid receptors (MR), whereas glucocorticoid receptors (GR) predominantly mediate physiological and behavioural changes to restore homeostasis after stressors [6]. In the hippocampus, which plays a role in GC negative feedback, MR and GR work in a coordinated and antagonistic fashion to regulate GCs (i.e. the MR/GR balance hypothesis [6]). It has been suggested that coordinated fluctuations in MR and GR allow greater physiological flexibility in response to GCs [7]. Also, when MR and GR are incubated together in vitro, they show enhanced binding to glucocorticoid response elements compared with when incubated individually; the composition of the two receptors ultimately determines binding efficiency, with the greatest binding when concentrations of GR exceed MR [8]. These authors concluded that ‘the cooperativity of MR and GR in DNA binding suggests a direct interaction between these two receptors’ [8, p.1458] and ‘when MR and GR are expressed in the same cell, their relative levels . . . will define which corticosterone receptor dimer [each homodimer versus the heterodimer] . . . is constituted [which] enables a more finely tuned regulation of corticosterone responsive genes’ [8, p.1460]. Greater
density of GR relative to MR should, therefore, facilitate enhanced resolution of stressors. Also, in rodents [9] and birds [10], the combination of MR and GR, not the concentration of either alone, dictated phenotypic effects of GCs. Although measuring absolute hormone concentrations is informative, understanding whole systems, including receptors, might further illuminate how variation arises [11].

Although we know GCs change throughout range expansion [5], we know little about how hormone receptors respond; importantly, it is hormone–receptor complexes [11] that initiate and mediate downstream effects of GCs. To address this point, we measured expression of hippocampal MR and GR mRNA throughout a range expansion. House sparrows (Passer domesticus) are expanding northwest from their most recent introduction site, Mombasa, Kenya (introduced approx. 1950 [12]). Individuals caught from areas near the range edge released more GCs in response to restraint than those from Mombasa. We predicted that mRNA expression of MR relative to GR would be lowest, closest to the range edge to facilitate stressor resolution afforded by activated GR and rapid downregulation of GCs after the stressor is resolved [6].

2. Material and methods

(a) Sampling
In February–May, 2011, house sparrows were caught from eight cities in Kenya (Mombasa, Malindi, Voi, Nairobi, Nyeri, Nakuru, Isiolo and Kakamega). We use distance from Mombasa (DFM) as a proxy for time since colonization (most recently colonized cities are furthest from Mombasa [5,13]). Between 6 and 13 adult birds (median = 9; electronic supplementary material, table S1) were caught from each city and individual sex, tarsus and mass were recorded. Within 15 min of capture, birds were deeply anaesthetized and decapitated. The hippocampus was removed immediately (by conservatively cutting around the anterior perimeter of the hippocampus and pulling from the forebrain [10] with RNase-free tools) and stored in RNAlater (Qiagen). Gastric water was used as a negative control. A housekeeping gene, 18S, was also measured to determine total RNA activity. For a detailed description of the qPCR methods, please refer to the electronic supplementary material, methods.

(b) Mineralocorticoid receptors’ and glucocorticoid receptors’ gene expression
RNA was extracted from hippocampal tissue and used to make cDNA [10]. MR and GR expression were measured using qPCR (Applied Biosystems) with approximately 300 ng cDNA and multiplexed primers and probes specific to house sparrow MR and GR [10]: a four-step standard curve was made, and ultrapure water was used as a negative control. A housekeeping gene, 18S, was also measured to determine total RNA activity. For a detailed description of the qPCR methods, please refer to the electronic supplementary material, methods.

(c) Statistical analysis
Target genes were adjusted to 18S (by dividing expression values of the target gene by that of 18S, Applied Biosystems). To generate an MR : GR ratio [6], adjusted gene expression of MR was divided by the adjusted gene expression of GR, which were then log-transformed to achieve normality. General linear models were used to analyse the effect of DFM on the ratio of MR : GR gene expression, as well as MR and GR alone. In addition, Spearman correlations were used to address within-individual covariation of MR and GR, with the expectation that high covariation would provide additional support for the use of the MR : GR ratio. Statistica v. 9.1 was used to perform all analyses, with α = 0.05. Data are deposited in Dryad [14].

3. Results
The MR : GR gene expression ratio was significantly lower in individuals from populations closest to the range expansion edge (F_{1,76} = 5.385, p = 0.023; figure 1a). Gene expression of MR alone was marginally lower in newer populations (F_{1,76} = 2.860, p = 0.095), but no differences in GR (squares; F_{1,76} = 0.435, p = 0.5114) relative to those from the site of introduction. Average log-corrected values are plotted against distance from Mombasa.

4. Discussion
GC regulation is important because GCs respond to internal and external environmental changes [11], allowing organisms to respond morphologically, physiologically and behaviourally to stressors and perturbations, and to resolve them. Both MR and GR regulate GC negative feedback in the hippocampus [6]. Physiologically, the GR, with the lower affinity of the two receptors [6], is predominantly responsible for physiological and behavioural actions of a stress response. Bound GR

Figure 1. Expression of stress steroid receptor mRNA varies along a range expansion in introduced Kenyan house sparrows. (a) House sparrows at the edge of a range (right-most points) had significantly lower hippocampal MR : GR gene expression ratios (F_{1,76} = 5.385, p = 0.023) than house sparrows at the site of introduction (left-most point). (b) House sparrows at the edge of the range had marginally lower levels of MR (triangles; F_{1,76} = 2.860, p = 0.095), but no differences in GR (squares; F_{1,76} = 0.435, p = 0.5114) relative to those from the site of introduction. Average log-corrected values are plotted against distance from Mombasa.
stimulates changes (e.g. reduced metabolism, avoidance) that promote survival of a stressor [6]. Further, MR and GR heterodimers have greater binding capacity, and thus increased potential effect when concentrations of GR are greater than MR [8]. Therefore, a greater density of GR relative to MR should facilitate a greater and/or more rapid resolution of encountered stressors; in novel habitats, a strong and rapid response should be especially favourable.

Although elevated GCs at the range edge [5] may induce changes in receptor expression [6,15], it is remarkable that such strong patterns of gene expression emerge with population age. Traditionally, it is thought that selection favours the persistence of conserved homeostatic systems, independent of environmental cues [11]; this is particularly true of the physiological components responsible for receiving, processing and signalling (internal and external) environmental information [16], such as GC receptors. This view predicts that GC receptor density would be conserved throughout the range expansion, and throughout the lifetime of an individual, regardless of environmental differences/changes. This is because changes in receptor density might alter physiological set-points, potentially leading to fitness consequences in unknown environments [16]. However, our data indicate that not only do GC receptors differ throughout a range expansion, but they also vary in a manner consistent with the range expansion itself. This variation in GC responsiveness in all likelihood permits alterations in downstream physiological and behavioural events to best respond to changing and unpredictable environments [17].

Given the likely mechanism of expansion in this population (human mediated, [13]), it is unlikely that all individuals arriving in new habitats are pre-adapted to survive there. Rather, we hypothesise that variation exists among individuals arriving at the range edge (possibly exemplified by the relatively high MR:GR ratio in Kakamega, the youngest population), but only individuals with a low MR:GR ratio, or those with the flexibility to reduce it rapidly in response to the novel environment, will survive (as exemplified by the low MR:GR ratio in Isiolo, Nyeri and Nakuru, with populations less than 10 years old; and intermediate levels in Nairobi, with populations less than 20 years old). Perpetuation of distinct phenotypes among populations might be a result of rapid evolution, developmental plasticity (early life experiences can shape GC receptor densities in the brain [18,19]) or individual flexibility responding to specific cues in the environment [20]; further, the adaptive values of these phenotypes are as yet unknown. Ultimately, however, the current experiment was not designed to elucidate the mechanism(s) of change, nor the adaptive values of those changes. In the future, experiments discerning the roles of selection, development and adaptive plasticity [21] in the GC receptor changes we describe here would be particularly interesting.

GCs have strong, but complex effects on fitness in wild animals [22], but relatively little is known regarding what role GC receptors play. We argue that regulation of the signal is just as important as the signal itself. In a world where many environments are rapidly changing, often owing to anthropogenic changes, information such as that presented here might be particularly informative in predicting population outcomes.

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References


Figure 2. Gene expression of MR was positively correlated with that of GR (r = 0.731; p < 0.001).


