Individual variation in Hocrine systems: moving

variability, phenotypic plasticity and (reversible) phenotypic flexibility (Piersma & Drent 2003). Therefore, comparative and ecological endocrinologists are in a

six eggs

estimates can provide insight into the heritable nature of traits and their potential response to selection (Dohm 2002; see below). Despite the value of this estimate, our knowledge of repeatability of physiological traits in general, and especially endocrine traits, remains surprisingly poor (cf. Dohm 2002). A few studies have reported

consistency of individual variation in hormone titres, e.g. stress-induced corticosterone in birds (Cockrem & Silverin 2002) and fishes (Schjolden . 2005), and timing of luteinizing hormone (LH) surges, but not peak plasma LH levels in rats (Gans & McClintock 1993; although repeatability was not calculated explicitly in these studies). Several studies have also reported consistency of hormonal responses to a standardized, exogenous hormone treatment, i.e. individual variation in to an endocrine stimulus. In male dark-eyed juncos ( ), testosterone (T) release in response to a standard gonadotrophin-releasing hormone (GnRH) challenge was repeatable (=0.36), and initial (baseline) plasma LH levels predicted post-challenge LH levels (Jawor . 2006). Similarly, in non-breeding female zebra finches, inter-individual variation in plasma yolk precursor levels in response to exogenous 17β-oestradiol treatment is consistent among individuals (T.D. Williams 2004, unpublished data). Other studies have reported repeatability of inter-individual variation in putative endocrine-mediated traits, e.g. oestrogen-dependent yolk precursor production, over time scales of several months (=0.5–0.7; Challenger . 2001).

These studies, though limited in number, and the types of traits that have been investigated suggest that repeatability can vary among traits, and for the same trait in different species. More studies of repeatability and multiple measurements of the same trait within individuals would allow resolution of apparently contradictory findings. Are these systematic differences in repeatability either for the different traits or for the same traits in different species? Has natural selection maintained phenotypic plasticity or flexibility in some physiological systems but not others, and if so why? Hormone titres are an archetypal example of a phenotypically flexible trait (Piersma & Drent 2003), i.e. they show - , continuous, but variation within single individuals—yet hormone data are rarely considered in this context. Does selection favour individuals which can more rapidly up- or downregulate hormone titres or individuals which can minimize time lags for these changes (e.g. Sih . 2004)? Are there 'costs' generated by the plastic nature of hormone systems ? One further problem with ignoring repeatability of physiological trait values is that researchers cannot be sure if the physiological measurements (e.g. hormone titres) they obtain truly characterize the phenotype(s) of the sampled individual. Nevertheless, most studies this is the case and they then go on to interpret this 'phenotypic' variation functionally and to test adaptive hypotheses, an approach that is increasingly common in evolutionary endocrinology (Zera . 2007).

4. ANALYTICAL ISSUES: REPEATED MEASURES DESIGNS AND REACTION NORMS

The simplest analytical method to both deal with and take advantage of inter-individual variation is to use a repeated measures design where multiple measurements are made on the same set of individuals, e.g. during control or sham and experimental treatments. Each individual acts as its own control and data can be analysed as a in trait value relative to each individual's initial value thus controlling for any marked variability in initial values. This straightforward experimental design is still rarely used in endocrine studies and is undoubtedly complicated in certain study systems (e.g. field studies) where any individual is only

norm could also be the hormone titre itself, e.g. androgen responsiveness (AR, the ratio of breeding season maximum and baseline androgen titre, e.g. Hirschenhauser . 2003) could be treated as a reaction norm if this is calculated for individuals rather than species (see also fig. 1 in Cockrem & Silverin (2002) and fig. 1 in Angelier . (2007)). It also seems

idea that hormone titres can be functionally uninformative since endocrine regulation occurs mainly through variation in binding globulin action, hormone receptor expression, density or affinity, or intracellular signalling pathways (Norris 1997; Ball & Balthazart 2008); this view is reinforced by increasingly reductionist thinking with a focus on cellular and molecular mechanism. This contrasts with the fact that so much effort in vertebrate endocrinology continues to be directed towards measurement of hormone titres and, interestingly, this also contrasts with invertebrate studies where a predominant focus on hormone titres is the consequence of a large body of evidence implicating regulation of phenotypic trait expression by changes in circulating hormone levels (e.g. growth, polymorphisms; Zera . 2007). Yet it remains unclear to what extent receptors or other components of endocrine signalling modulate, contribute to, or override hormone titres in determining hormone-dependent phenotypic trait variation. Selection studies selecting directly (and solely) on hormone levels have demonstrated correlated responses to selection in putative hormone-mediated traits, confirming the functional significance of hormone titres . In Japanese quail (*C* , ,), selection for low or high stress-induced corticosterone leads to changes in behavioural phenotype: greater avoidance and more fear-related behaviour in high-selection lines (Jones . 1994). Zebra finches (*T*) selected for high stress-induced corticosterone levels (Evans . 2006) showed reduced spatial ability and lower hippocampal mineralocorticoid-receptor mRNA expression compared with control lines (Hodgson . 2007). Other studies

9.0096 Tc[415electipoefl7 TD-557.94514.9to255.7(()]TJ0 0 0.s-indu6992.3(o) TDD[(.)-255.3(206.1742171 Tc[(.)9n7.68pr

between hormone titres and phenotypic variation in hormone-dependent traits? For example, for the hypothalamic-pituitary-gonadal axis, is there a systematic relationship between the marked (10-fold) inter-individual variation in plasma oestrogen levels and variation in either the hormonal stimulus for E2

correlations are often weak, i.e. there is large, unexplained residual variance. In some systems (e.g. E2-dependent yolk formation), individuals with very high circulating hormone levels appear to derive no functional benefit in terms of increased expression of hormone-dependent traits. Why then do some individuals maintain much higher hormone titres than other individuals? Here, I suggest three possible, non-mutually exclusive, explanations that deserve further consideration.

(a) *H* *a* *b*

'Direct' costs of hormone production, e.g. the energy cost of biosynthesis, are generally thought to be small and inconsequential to the evolution of hormonal variation (though this appears never to have been quantified). However, hormones can have both beneficial (positive) or costly (negative) pleiotropic effects and individual hormone titres should reflect a trade-off between costs and benefits of these multiple physiological effects. For example, although corticosterone is essential in regulating routine metabolism, energy management and adaptive responses to acute stressors, even moderate chronic elevation of corticosterone can have negative effects on growth and immune function (Charmandari . 2005). Much attention has focused on the role of T in mediating various trade-offs based on the pleiotropic costs and benefits of this hormone. For example, T is thought to mediate a trade-off between mating effort and parental care (Wingfield . 1990), and this central concept has survived relatively well in the face of experimental study (at least in birds; Hirschenhauser . 2003; Lynn . 2005). In contrast, it has also been proposed that T mediates a trade-off between expression of sexual signalling traits and immune function (Folstad & Karter 1992): full signal expression requires high levels of T but this carries a cost due to the pleiotropic, immunosuppressive effects of T. Here, despite a very large number of experimental studies, there is at best only equivocal support for the central assumption of this trade-off: that T is immunosuppressive (Roberts . 2004). Furthermore, even modifications of this hypothesis, e.g. that T interacts with corticosterone to mediated the trade-off between signalling and immune function, have produced inconsistent or contradictory results (Roberts . 2007). Thus, attempts to understand variation in hormone levels in a cost–benefit framework have been limited to one or a few hormones, and they have met with mixed success, but from the perspective of this paper they have so far been restricted to interspecific differences.

Within such a cost–benefit framework, large-scale inter-individual variation presents a further paradox. If there are costs of high hormone levels, selection should generate a match between physiological capacity (hormone level) and functional demand (the amount of hormone required for physiological function; Diamond & Hammond 1992) and this should reduce inter-individual variation. A possible explanation for this paradox is that individuals have different sensitivities to specific circulating hormone levels, such that in different individuals very different hormone titres are required to support the same level of physiological function. The

pleiotropy regulates trade-offs among life-history traits. This will require endocrinologists to embrace the raw

Peters, A. 2000 Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *P . R. S . B* **267**, 883–889. (doi:10.1098/rspb.2000.1085)

Piersma, T. & Drent, J. 2003 Phenotypic flexibility and the evolution of organismal design. *T , E . E .* **18**, 228–233. (