Developmental instability in relation to stress and fitness in birds and moths studied by the Laboratory of Animal Ecology of the University of Antwerp

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ABSTRACT. Evolutionary and conservation biologists are in need of simple surrogate measures of fitness and the action of stress on fitness components. Fluctuating asymmetry, an estimate of developmental instability, has been suggested to reliably reflect stress and fitness but evidence is highly heterogeneous. This heterogeneity is confirmed by the results we obtained from three different projects at the Laboratory of Animal Ecology that we review in this paper. In seven bird species inhabiting three indigenous cloud forests in Kenya, asymmetry closely related to stress and/or fitness, yet the underlying mechanisms appeared complex. On the other hand, in two moth species the link between asymmetry and both stress and fitness was less clear or even absent. These results call for more large-scale studies in order to identify factors that allow predicting if and when asymmetry reflects stress and/or fitness.

KEY WORDS: developmental instability, bilateral asymmetry, stress, fitness, review.

INTRODUCTION

Estimating fitness

Individual fitness is the result of various processes, many of which are difficult to establish directly, especially in free living organisms. Nevertheless, individual fitness takes a central place in many areas of research such as evolutionary and conservation biology. The availability of a universal measure of fitness that can be obtained easily and rapidly and that is ubiquitous across taxa and locations will facilitate many aspects of evolutionary research and the development of conservation plans. A measure that has been claimed to have these properties is fluctuating asymmetry, as an estimator of developmental instability (see below). However, a number of recent overviews of the available literature have indicated otherwise, pointing out that there are large inconsistencies in the literature (e.g. LEUNG & FORBES, 1996a; CLARKE, 1998a; BJORKSTEN et al., 2000). At present it is not clear which factors influence

the FA-fitness and FA-stress association at either the population or individual level, and this calls for further research (e.g. VAN DONGEN & LENS, 2000b).

We recently argued that there is an urgent need for comparative studies attempting to identify factors that influence the FA-stress association (VAN DONGEN & LENS, 2000b). However, given the sensitivity of FA-studies to poor statistical analyses, low statistical power due to small sample sizes and statistical properties of FA, publication bias (SIMMONS et al., 1999; PALMER, 1999), and selective reporting (CLARKE, 1998a), objective and unbiased meta-analyses are difficult to perform. In this paper we present an overview of the results of three projects that were initiated at the Laboratory of Animal Ecology (Antwerp) and discuss directions for future research.

Fluctuating asymmetry as estimator of fitness

Fluctuating asymmetry (FA) is the most commonly used measure of developmental instability (DI), and can be defined as small directionally random deviations from perfect symmetry of otherwise symmetrical bilateral traits (LUDWIG, 1932; PALMER & STROBECK, 1986; PALMER,

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1996). DI, the set of processes that are assumed to be estimated by FA, reflects the cumulative effects of mistakes during ontogeny and individual specific mechanisms that counteract these faults (PALMER & STROBECK, 1992). The underlying assumptions are that the development of any trait is disturbed by random noise, that these mistakes accumulate over time and that in the course of evolution mechanisms have evolved that attempt to correct for these mistakes. The first two processes are termed developmental noise, the latter developmental stability (DS). Thus, noise causes traits to deviate from their expected growth trajectory (as predicted by genotype and environment) whereas DS is assumed to counteract the effects of noise. DI indicates the combined effect of both noise and stability, since it is impossible to disentangle both purely from asymmetry values. When expressed in statistical terms, any trait value can be viewed as a sample from a normal distribution with mean equal to the expected value and variance being the joint and opposite action of noise and stability (LEUNG & FORBES, 1996b).

Since the expected trait value, given an individual's genotype and the environment in which it developed, is seldom known, it is impossible to estimate the variance of that distribution from an observed trait value (because a mean and variance cannot be estimated simultaneously from a single observation). This problem can be circumvented by measuring traits that have the same expected value, such as bilaterally symmetrical traits. The difference between left and right can be expected to follow a normal distribution with zero mean and variance equal to twice the developmental instability (WHITLOCK, 1996).

One could postulate an association between DI (and thus FA) and fitness if the mechanisms underlying developmental stability are energetically costly. When conditions (either genetic or environmental or both) during development are optimal, individuals can be expected to be able to allocate sufficient energy into processes of developmental stability and have high expected fitness. Under unfavourable situations, less energy might be available to buffer development and an increased DI may then coincide with fitness loss. Under this hypothetical model, the association between fitness and DI can be expected to critically depend on species-, trait- or even individual-specific strategies of allocation.

Specific hypotheses

Because at present there are no specific guidelines that predict when FA can be reliably used as a predictor for fitness, for each application a number of hypotheses need to be tested:

Does FA increase with stress and is this response trait specific? For population-level analyses, the most important hypothesis to test is whether FA increases with stress (either environmental or genetic). Secondly, it should be tested if responses are trait-specific such that it depends on the chosen trait whether an association is found. In

other words, one should investigate if variation in population-level FA correlates among traits [i.e. is there evidence for a so-called population asymmetry parameter (further called PAP, CLARKE, 1998b)]. Trait-specific response, or even the absences of any association, clearly hampers the general use of FA as estimator of stress and fitness.

Are the effects of environmental and genetic stress additive? It has been hypothesised earlier that FA might only be a sensitive estimator of environmental stress in the presence of genetic stress (inbreeding, mutation or disruption of genomic co-adaptation) (PARSONS, 1992). Therefore, an interaction between the effects of environmental and genetic stress should be tested.

Does FA reflect individual fitness? At the individual level, FA and DI have been argued to reflect fitness and/or (genetic) quality. In order to reflect genetic quality, FA and DI should have a genetic basis (see hypothesis 4) and reflect at least some component of fitness. The association between FA and other factors, however, underestimates associations between these factors and DI. Since FA estimates a variance with two datapoints (i.e. left and right trait value) it is only a very crude estimator of DI (PALMER, 1994; WHITLOCK, 1996; HOULE, 1997). This results in a downward bias of observed associations, which can be corrected for using the concept of 'hypothetical repeatability' as developed by Whitlock (1996, 1998).

Is FA (and DI) genetically heritable? A genetic background is required to expect any association between individual genetic quality and FA, whereas it would complicate the use of FA at the population level since this would imply that populations could evolve and adapt to the changing environmental conditions. Therefore, although only poorly understood, the genetic background and evolutionary potential of FA and DI are crucial for interpreting observed patterns (MØLLER & THORNHILL, 1997 vs. e.g. MARKOV & CLARKE, 1997; VAN DONGEN & LENS, 2000a; VAN DONGEN, 2000b).

Which type of asymmetry occurs and how do they relate to stress? Next to FA, two other forms of asymmetry may occur. With directional asymmetry (DA) the mean value of the asymmetry differs from zero, while for antisymmetry (AS) the distribution is bimodal. It has often been argued that DA and AS have a genetic basis and do not reflect the cumulative result of developmental errors (e.g. PALMER & STROBECK, 1992). Classical examples of this are different species of fiddler crabs where either DA or AS may occur when one claw grows much larger than the other does. On the other hand, both theoretical and empirical results show an association between either DA or AS and environmental and genetic perturbations accumulated over the past decade (e.g. MCKENZIE & CLARKE, 1988; GRAHAM et al., 1993a; 1993b; LEAMY et al., 1999). At present it is not clear if and when DA and/or AS increase with perturbations and if they reflect DI. Nevertheless it is crucial to distinguish between these different forms of asymmetry in order to perform correct statistical analyses.

MATERIAL AND METHODS

Three projects

Taita Hills project: Bilateral trait asymmetry was studied in one to three traits of seven forest bird species (listed in Table 1) in the Taita Hills of southeast. In addition, a subset of 237 *Turdus helleri* was genotyped with six polymorphic microsatellite-DNA markers, and individual inbreeding coefficients were calculated following Ritland (1996) (details in GALBUSERA et al., 2000; LENS et al., 2000). Indigenous cloud forest currently covers less than 400 ha in 12 forest patches, eight of which are smaller than 5 ha, in Kenya (map and details in BROOKS et al., 1998; GALBUSERA et al., 2000). Levels of FA and inbreeding were studied in bird populations inhabiting the three largest of the Taita forest fragments. Based on congruency in the patterns of habitat disturbance (WILDER et al., 2000) and avian survival rates (LENS et al., 2000), levels of 'environmental stress' were considered highest in fragment CH (50 ha), intermediate in fragment NG (90 ha), and lowest in fragment MB (200 ha).

Winter moth project: The study of the population structure of the winter moth (*Operophtera brumata* L.) in relation to habitat fragmentation and local adaptation to individual host trees was initiated in 1991. During the course of this research project, analyses of population- and individual-level fitness in relation to tibia asymmetry, were performed at various stages. We investigated the effect of habitat fragmentation and individual heterozygosity at five allozyme loci in one large area (>200 ha) and two small fragments (<2ha) near Antwerp (VAN DONGEN, 1997), estimated the heritability of FA and DI in a full-sib breeding experiment (VAN DONGEN et al., 1999d) and investigated the association between individual FA and several fitness components in a series of experiments (VAN DONGEN et al., 1999d; 1999e). We refer the reader to the individual studies for more details.

Indian meal moth project: Effects of environmental stress on FA were studied in a laboratory culture of the Indian meal moth (*Plodia interpunctella*). Using a full sib breeding design, offspring from a total of 30 couples were distributed over a total of nine different combinations of two treatments (i.e. three different food qualities and three different densities). Front leg tibia asymmetry was measured for the surviving offspring, together with an independent measure of fitness (i.e. body length). In this way, effects of presumed environmental stress, of genotype and possible genotype¥environment interactions could be tested for both FA and two independent measures of fitness (body size and survival probability).

Statistical analyses

Although we refer to the particular case studies for details on the performed analyses, in each case three steps were carefully carried out. Lens and Van Dongen (2001) and Van Dongen et al. (2001a) give an overview of the different methods.

Firstly, we obtained unbiased estimates of FA at the individual and/or population level. This was achieved by a mixed regression approach (VAN DONGEN et al., 1999a; VAN DONGEN, 2000a). These estimates were then compared across populations (by likelihood ratio test or Levene's test) or individual estimates were used in subsequent analyses. Secondly, between-trait correlations in FA and heritabilities of FA were transformed into (upper bounds of) patterns in DI following Whitlock (1996, 1998) and Van Dongen (1998). Finally, blends of different forms of asymmetry were distinguished by mixture analysis (VAN DONGEN et al., 1999b).

RESULTS AND DISCUSSION

Table 1 summarises tests of the above listed hypotheses. Associations between FA and both stress and fitness appear very heterogeneous. Roughly, in the Taita Hills project most hypotheses were confirmed and FA appeared to reflect stress and fitness at both the individual and population level, regardless of the trait studied. Most importantly, the different traits studied developed at different stages during the life of these birds emphasising that the FA-fitness associations hold throughout large parts of their lifespan. The results also suggest that the underlying mechanisms are relatively complex. For *Turdus helleri* environmental stress and inbreeding interacted with each other such that more inbred individuals showed an increase in asymmetry in the most disturbed area only. This association was weaker but still statistically significant in the intermediately disturbed area and absent in the relatively undisturbed area (LENS et al., 2000). Likewise, the association between individual asymmetry and fitness was influenced by the degree of habitat disturbance. In the most disturbed area a relatively strong negative association between individual FA and survival was found, while no association occurred in the two other areas. In addition, directional asymmetry appeared to occur more frequently in more disturbed areas for at least four species (Table 1; LENS & VAN DONGEN, 2000). Although such shifts in types of asymmetry have been observed in other species (see above), its relationship with DI is not entirely clear (but see GRAHAM et al., 1993a).

In the two moth studies, associations between asymmetry and both stress and fitness are less obvious or even absent (Table 1). The only association between FA and fitness was detected in the winter moth, where females were more likely to re-mate when the first mating occurred with an asymmetric male. The more asymmetric males are thus likely to loose paternity. However, this association was observed in a laboratory experiment, and re-mating is considered very unlikely in the field (VAN DONGEN et al., 1999e). The absence of an FA-fitness association could not be attributed to small sample sizes or inappropriate statistical analyses. Furthermore, significant heritability

TABLE 1

Overview of results obtained at the Laboratory of Animal Ecology of the University of Antwerp, testing eight specific hypotheses. An * indicates that the hypothesis is confirmed, NS that it is not supported by the data. Specific hypotheses are: (i) does environmental stress increase FA and thus DI; (ii) are different traits affected in a similar way [i.e. is there evidence for a Population Asymmetry Parameter (PAP)]; (iii) do FA and DI increase with inbreeding or decrease with heterozygosity; (iv) is there evidence for an interaction between environmental and genetic stress; (v) are FA and DI heritable; (vi) is individual fitness related to FA and DI; (vii) is FA correlated among different traits [i.e. is there evidence for an individual asymmetry parameter (IAP)]; (viii) Do other forms of asymmetry occur more frequently with increasing stress.

Species:	(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)
Taita hills project:								
Turdus helleri (Gmelin) [1]	*	*	NS	*	-	*	*	-[6]
Nectarinia olivacea (Smith)[1]	*	*	-	-	-	-	*	*
Andropadus milanjensis (Shelley)[1]	*	*	-	-	-	-	*	-[6]
Zosterops silvanus (Heuglin)[1]	*	*	-	-	-	-	*	*
Pogonocichla stellata (Vieillot)[1]	*	*	-	-	-	-	*	*
Phylloscopus ruficapillus (Sundevall)[1]	*	-	-	-	-	-	-	-
Phyllastrephus cabanisi (Sharpe)[1]	*	-	-	-	-	-	-	*
Winter moth project:								
Operophtera brumata (L.)[2]	*	(*)[4]	NS	NS	*	(*)[5]	(*)[4]	NS
Indian meal moth project:								
Plodia interpunctella (Hubner)[3]	NS	-	-	-	NS	NS	(*)[4]	NS

[1] Details in LENS et al. (1999; 2000); LENS & VAN DONGEN (1999; 2000) and unpublished results

[2] Details in VAN DONGEN (1997) and VAN DONGEN et al. (1999c; 1999d; 1999e)

[3] Details in VAN DONGEN et al. (2001b)

[4] Correlations between traits are likely to be confounded with common developmental processes (VAN DONGEN et al., 1999c)

[5] Although significant under laboratory conditions, in the field it is considered unlikely to occur (VAN DONGEN et al., 1999e).

[6] Insufficient data from the most disturbed area were available in this analysis (LENS & VAN DONGEN, 1999)

of FA, as observed for the winter moth, could not explain the weaker associations, since FA was not heritable in the Indian meal moth in which no indication of any FA-fitness association was found. One possible explanation could be that we did not measure the appropriate trait. We measured the tibias of the three pairs of legs, but these may not be considered as independent growth events (VAN DONGEN et al., 1999c). Since in *Turdus helleri* an association between FA and fitness was only found in the most disturbed area, one could argue that the environmental stress was not sufficient in the two moth species for any effect to be detectable. Nevertheless, for both species the most severe treatment led to at least 50% mortality (VAN DONGEN et al., 1999d; 1999e; 2001b).

In summary, studies performed by us show that at least in some cases FA may provide a reliable surrogate measure of fitness and stress, but also that associations may be less straightforward or even completely absent. However, even in situations where FA-stress association 'works, the underlying mechanism relating to this association appears relatively complex through an interaction between environmental and genetic stress, a differential expression of FA-fitness associations with habitat disturbance, and the occurrence of other forms of asymmetry. Developing guidelines that predict if an when FA is expected to relate to stress requires that the underlying developmental mechanisms and related biochemical pathways that lead to asymmetry are unravelled first.

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