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FLUCTUATING ASYMMETRY: AN EPIGENETIC MEASURE OF STRESS

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CONTENTS

I. INTRODUCTION

Under severe stress conditions imposed by the physical and biological environments there is accumulating evidence that variability tends to be high. This applies directly to quantitative traits, especially those important in determining survival, and more indirectly at the level of genes controlling protein variation. This conclusion assumes that populations have not previously been selected for phenotypic extremes for the trait in question, which could modify variability. Furthermore, the definition of the severity of a stress poses difficulties. Considering physical stresses, substantial increases in variability appear most likely when stresses are of such severity that continuous exposure is rapidly lethal. These are conditions exemplified by short bursts of extreme stresses occurring at climatic and ecological margins where just a small environmental perturbation could be lethal. Experiments at this critical boundary pose logistic difficulties not apparent in most studies in evolutionary biology involving more optimal environments (Parsons, 1987).

Recombination illustrates these dilemmas well. Within the first decade of genetical experiments on *Drosophila melanogaster*, Plough (1917, 1921) found that recombination, especially in centromeric regions of chromosomes 2 and 3, increased when the temperature at which the fly developed was increased or decreased from 25 °C, the normal laboratory culture temperature, with major effects at temperature close to lethality around 13° and 30 °C. In other words, both at high and low temperatures,

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there is a sharp escalation of recombination close to the environmental conditions at which species continuity is threatened (Parsons, 1988). Other environmental stresses of relevance in nature that increase recombination include starvation in *D. melanogaster* (Neel, 1941) and behavioural stress from overcrowding in mice (Belyaev & Borodin, 1982).

In *D. melanogaster*, structural heterozygosity due to inversions in one part of the genome tends to increase recombination in the remainder of the genome in a qualitatively similar manner to, and cumulative with, direct environmental effects especially temperature (Grell, 1978). In other words, genomic stress has an effect parallel with environmental stress. Indeed, McClintock (1984) has argued for the importance of stress within the cell and environmental stress imposed from without as triggers for rapid genomic reorganizations.

Epigenetic proceses can similarly be studied following environmental and genetic perturbations (Waddington ,1956). For example, ether applied at sublethal doses to the eggs of *D. melangaster* gives a few flies with the phenotype, bithorax, and a short period of sublethal temperature applied at a certain critical stage in pupal life causes the appearance of cross-veinless flies (Waddington, 1953). Severe stress may therefore greatly increase the variability of development. Continuous directional selection of abnormal individuals results in a rapid increase in genes favouring the abnormalities, until ultimately the specific environmental stress becomes unnecessary, at which stage genetic assimilation of the trait has occurred.

In relation to natural habitats, however, these experiments can be regarded as rather contrived. Furthermore, the phenotypes are frequently so abnormal that survival in nature would be unlikely. A trait that can be generalized more readily across taxa is needed. One epigenetic process that has attracted increasing recent attention is asymmetry of bilateral characters (Palmer & Strobeck, 1986). There are three different kinds of asymmetry (van Valen, 1962) characterized by differing combinations of right-minus-left differences (R – L). They are (1) fluctuating asymmetry (FA), with a normal distribution of R – L values around a mean of zero, (2) directional asymmetry where the mean of one side is almost always greater than that of the other, and (3) antisymmetry where there is a difference between the two sides but it cannot be predicted which will show the greater value, so giving a broad-peaked or bimodal distribution of R–L values about a mean of zero.

FA has been studied in a wide variety of organisms. Characters such as the size of paired formations, numbers of scales or chaetae, wing venation, and skeletal variants especially dentition have been used in FA studies. On several occasions, FA has been advanced as a measure of developmental stability (Thoday, 1956; Beardmore, 1960; Reeve, 1960; Parsons, 1961, 1962, van Valen, 1962; Soulé, 1967).

Taking recombination as a model, genetic and environmental perturbations might be expected to affect FA in an analogous way because both stress categories are imposed during development. In other words, can FA be used as a measure of perturbations upon epigenetic processes? In this way, the emphasis of this discussion differs from two major earlier reviews (Mitton & Grant, 1984; Palmer & Strobeck, 1986) which concentrated more upon the relationship between FA and heterozygosity, however, a short paper (Leary & Allendorf, 1989) published during the referee stage of the present article is convergent with it in approach. Laboratory evidence is considered first mainly from *Drosophila* and rodents, where perturbations are imposed under controlled environmental conditions. Non-experimental observations including those on our own species follow and are generally consistent with the laboratory studies. A relatively complete literature review (Palmer & Strobeck, 1986) shows that at the field level a number of studies are inconclusive. This indeterminacy is predictable since sample sizes may be inadequate, the trait invariant, or the environmental perturbation insufficiently extreme.

II. EXPERIMENTAL - INSECTS

(1) *Genetic effects*

Reeve (1960) and Beardmore (1965) found that the level of sternopleural chaeta FA in *D. melanogaster* could be increased with difficulty by directional selection. A heritability of 0·02–0·03 was obtained in Reeve's (1960) experiment, which is consistent with a progeny test on a wild stock where the genetic variance accounted for about 2% of the total phenotypic variance. This heritability is extremely low compared with conventional quantitative morphological traits in *Drosophila* for which the range is 10–60% and where most concentrate in the 20–45% range (Roff & Mousseau, 1987). For behavioural traits the range is 0–30%, which implies a greater environmental component than for morphological traits. In this context, the lack of a positive response obtained by Ehrman *et al.* (1978) in selection experiments for wing folding (left-over-right *vs.* right-over-left) and maze direction choice (left or right turns) is not surprising. Selection experiment results therefore indicate that FA contrasts with other quantitative morphological and behavioural traits, since the genotypic component is at most minimal. Even so, it is important to note that in many of these and following studies, changes in variance were assumed to be due to increased FA rather than to changes in antisymmetry (Palmer & Strobeck, 1986). Actual frequency distributions of R–L scores (McKenzie & Clarke, 1988) can provide a simple verification.

Turning to inbred strains and their hybrids, early data on sternopleural chaeta number in *D. melanogaster* established that the FA and its variance are normally reduced in hybrids (Mather, 1953; Thoday, 1956; Beardmore, 1960; Reeve, 1960). Under the normal assumption (Lerner, 1954) that hybrids are fitter than the inbreds from which they are derived, this result is consistent with FA being correlated with fitness.

Artificial selection may reach a limit, not because variability is exhausted, but because the fitness of organisms so produced is lower than unselected organisms (Mather & Harrison, 1949). Profound changes may occur under intense directional selection, but as less fit genetic combinations not previously subjected to periods of selection become exposed, deteriorating fitness is likely. Assuming FA to be associated with fitness, a rapid response to selection for a trait should be associated with greater FA, implying lowered fitness manifested by lowered developmental homeostasis. Thoday (1958) analysed FA in lines selected for high and low sternopleural chaeta number. Using the ratio

$$\frac{\Sigma(\mathrm{L}-\mathrm{R})}{\Sigma(\mathrm{L}+\mathrm{R})}=\frac{\mathrm{A}}{\mathrm{T}}$$

to correct for changes in the mean with selection, the ratio increased equivalently in the various lines, so that directional selection caused a deterioration of developmental homeostasis.

Reeve (1960) took third chromosomes of a São Paulo wild stock made homozygous in the background of an Edinburgh inbred strain. Of the fully tested lines, some were homozygous for one of two third chromosomes with recessive effects on the chaeta system, giving *polychaetoid* and *sternopleural gaps*. These homozygous lines were characterized by increased sternopleural variances and FA levels, which disappeared in crosses where the third chromosome effects were heterozygous. This means that FA may be affected by major chaeta mutants, whereby developmental homeostasis is reduced.

The norm of reaction in the sense of Schmalhausen (1949) is therefore upset. He considered that all really new reactions of an organism are never adaptive, and that mutations are inherited changes of the norm of reaction. There are many examples of increased variability and reduced fitness in newly arisen mutants, which over time will become ameliorated by the accumulation of modifier genes increasing the fitness of the phenotype carrying the mutant (Fisher, 1928, 1930), a phenomenon elegantly demonstrated on Danforth's short-tailed mice (Fisher & Holt, 1944). In the sheep blowfly, *Lucilia cuprina*, increased FA was found at the initial stage of development of diazinon resistance, which could be mapped to a region of chromosome III, showing an association of FA with a major gene as a pleiotropic effect (McKenzie & Clarke, 1988). In accord with Fisher (1928, 1930), FA became reduced in subsequent generations, and ultimately the resistant and susceptible flies became equivalent in their effects as predicted for a fitness associated trait.

Thoday (1958) discussed data directly bearing on fitness in a study of sternopleural chaetae in *D. melanogaster* over 30 generations at 25 °C, the normal culture temperature, and three 'novel' environments, 20°, 30 °C and one fluctuating between 20° and 30 °C in a smooth diurnal cycle. Adaptation to the new environments was shown by greater regressions of chaeta number over generations in the new environments than the old indicating that chaeta number was correlated with something of adaptive significance. In particular, chaeta number increased at 20 °C and decreased at 30 °C, a result in the same direction as the well-known observation that flies have fewer chaetae when cultured for one generation at high than at low temperatures (Parsons, 1961). The results also provided direct evidence that sternopleural FA is a trait of adaptive significance since there is no indication of significant regression over generations in the 25 °C population, but in each of the other populations (in new environments) following an initial increase, FA declined over generations, significantly so at 30 °C and in the 20°/30 °C populations, and close to significance in the 20 °C population. This is reasonable since 20 °C is not a major environmental perturbation compared with 30 °C which is at the limits of survival of *D. melanogaster*. Additional evidence for a fall in sternopleural FA over generations was presented by Bradley (1980) in *D. melanogaster*. Furthermore, wing length FA fell quite substantially over 15 generations in the adaptation of chromosomally polymorphic populations of *D. pseudoobscura* to laboratory environments (Beardmore & Levine, 1963).

(2) *Environmental effects*

Figure 1 gives the results of an experiment on an Oregon-R stock of *D. melanogaster* indicating far more FA of sternopleural chaeta number of flies grown at 30 °C than at 25 °C, especially those derived from young females when developmental stability

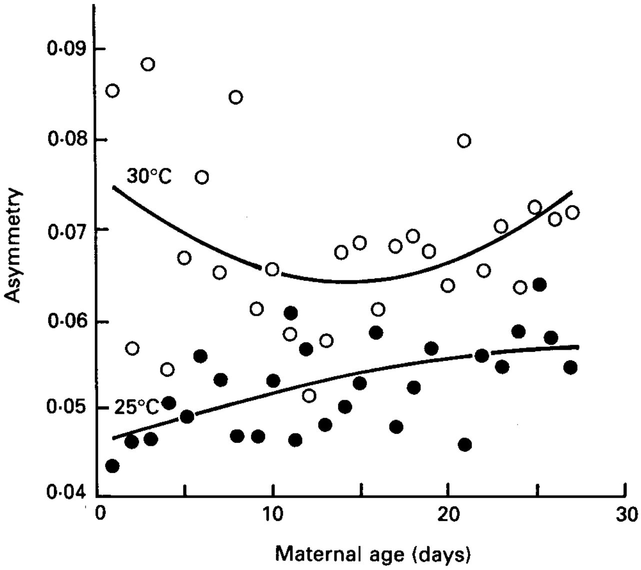

Fig. 1 FA of offspring sternopleural chaeta number expressed as A/T (see text) plotted against maternal age for the Oregon R stock of *D. melanogaster* grown at 25 °C (●) and 30 °C (○). At 25 °C the increase of FA with maternal age was significant at $P < 0{\cdot}01$ (after Parsons, 1962).

appears to be particularly high (Parsons, 1962). At 30 °C there is a great deal of variability from day to day which might be expected since this temperature is an extreme stress where slight changes in microenvironment may have major effects on developmental homeostasis. At 25 °C FA increases with maternal age; this result is consistent with many observations showing increased developmental instability with maternal age especially in mice and our own species (Parsons, 1964). The poorer developmental homeostasis at 30 °C was confirmed in another data set (Parsons, 1961) indicating in addition 10 abnormal flies with crumpled or curled wings and one with 24 sternopleural chaetae on the left side and 9 on the right side out of 315 flies classified, compared with no abnormalities out of 451 classified at 25 °C.

In these latter experiments, a chemical stress, phenyl-thio urea (PTU), was also studied. In contrast with 30 °C, PTU reduced fly weight substantially and increased its variability, delayed emergence time and increased its variability, but had little effect upon bristle number variability and FA. Hence PTU is a stress affecting fly weight but not the variability of the morphological structure of the fly, so has little effect upon developmental stability in contrast with the extreme temperature, 30 °C. The contrast presumably arises because temperature is a generalized stress affecting many metabolic pathways, and at certain limits the basic physiology of the organism will become restrictive with multiple consequences manifested by poorer developmental/morphological homeostasis, whereas PTU is a specific chemical inhibitor involving far fewer metabolic pathways than temperature. This of course does not imply that all chemicals are specific rather than generalized stresses (Hoffmann & Parsons, 1989*a*, *b*), and indicates a need for substantial detailed experimental work.

While the increase in FA at 30 °C in Fig. 1 is substantial, not all genotypes are affected equivalently since in a similar comparison of inbred strains and hybrids, FA was increased far more in the inbred strains (Thoday, 1956). This genotype–environment interaction is a reflection of the common observation that the fitness difference between inbred strains and hybrids tends to be maximized under conditions of environmental stress in the direction of enhancing the relative advantage of hybrids (Parsons, 1983, 1987, for references).

III. EXPERIMENTAL – VERTEBRATES

In studies of the FA of three mandibular molar widths in mice, Bader (1965) found that the bilateral variance of the hybrids was lowest for all three teeth, and they exhibited less variance than their inbred parents. For 10 paired osteometric traits in inbred and hybrid mice, Leamy (1984) found heritabilities of FA to range between 0 and 0·11 averaging about 0·02 in inbreds and 0·04 in hybrids. The inbred strains showed more FA than the hybrids which was statistically significant. In assessing these results it is important to note that the mice were of uniform age and rearing conditions, which would minimize uncontrollable environmental variation.

In rats, selection for body weight was more successful in the up lines than in the down lines (Atchley, Rutledge & Cowley, 1982), and it was in the up lines that seven of 11 FA values based upon bilateral osteometric traits were greater than comparable values in the control lines (Leamy & Atchley, 1985). The rather small selection response for low body weight was associated with low FA values.

In summary, experimental data on mice and rats show good consistency with the comparable *Drosophila* results. They confirm that FA can be used as a measure of developmental homeostasis. It follows that FA should be a good indicator of the effect of stress upon development.

Using Wistar rats under controlled conditions, Sciulli *et al.* (179) studied six stress combinations: cold, audiogenic stress, protein deprivation, cold + protein deprivation, cold + audiogenic stress, and audiogenic stress + protein deprivation for effects upon FA of the mandibular and maxillary first molars. All protocols produced higher FA than controls, especially the combination audiogenic stress + protein deprivation which produced an additive stressor effect. In some groups, litter size was reduced which is a direct indicator of stress. The increase in variances, especially for the maxillary dimensions, shows this to be a particularly useful system for examining FA (see also Siegel & Smookler, 1973; Siegel & Doyle, 1975) under an array of stresses including heat stress of 33 °C which also increased FA (Siegel, Doyle & Kelley, 1975). Later work showing increased FA due to prenatal stress (cold, heat, noise) is given by Mooney, Siegel & Gest (1985).

In fish, Valentine and Soulé (1973) increased pectoral ray FA in fry of the grunion, *Leuresthes tenuis*, by exposure to DDT such that the increase was concentration dependent.

A final example from rhesus macaques concerns diabetes induced by streptozotocin, which is an artificial prenatal stress causing increased dental FA for a number of measures (Kohn & Bennett, 1986).

In summary, FA has potential for monitoring stress levels in vertebrates especially those applied prenatally. Combined with the field observations to follow, experimental possibilities emerge for studying stresses, singly and in combination.

Table 1. *Mean percentage of asymmetric characters for homozygotes and heterozygotes at eight loci in rainbow trout (after Leary, Allendorf & Knudsen,* 1983)

Locus	Homozygotes	Heterozygotes	Difference
Idh 2	36·7	29·1	+7·6
Idh 3, 4	42·7	32·5	+10·2*
Ldh 4	34·7	41·3	−6·6
Mdh 3, 4	36·7	33·3	+3·4
Pgm 1 − t	38·2	27·9	+10·3*
Pgm 2	35·8	32·5	+3·3
Sdh	36·2	30·7	+5·5
Sod	35·7	34·2	+1·5

*$P < 0·05$.

IV. NON-EXPERIMENTAL

(1) *Asymmetry and heterozygosity*

For many years, it has been accepted especially in the applied context, that heterozygosity strongly influences both vigour and stability (Lerner, 1954; Mitton & Grant, 1984). The FA comparisons between inbred strains and hybrids in *Drosophila* and rodents are consistent with this generalization. In honey bees (*Apis mellifera*), heterozygous workers, hemizygous drones and inbred workers with high degrees of homozygosity were compared for asymmetry with the expected result that homeostasis and heterozygosity are associated in haplo-diploid systems (Bruckner, 1976) which generalizes the result further.

In a survey of 15 populations of the side-blotched lizard, *Uta stansburiana*, on islands of the Gulf of California, populations with higher heterozygosity based upon 18 protein loci had lower FA using four independent scale characters (Soulé, 1979). Similarly, geographically peripheral populations of two species of fresh water bivalves (*Elliptio complanata* and *Lampsilis radiata*) had lower heterozygosity associated with higher FA (Kat, 1982). In the rainbow trout, *Salmo gairdneri*, a significant negative correlation was found between the proportion of heterozygous loci (based upon 13 polymorphic loci) and the proportion of asymmetric characters (based upon five bilateral characters) whereby the number of heterozygous loci was negatively correlated with both the number of asymmetric characters and the magnitude of the asymmetry (Table 1). Thus developmental stability is associated with both heterozygosity at specific loci and protein heterozygosity in general (Leary, Allendorft & Knudsen, 1983).

In the sexually diploid fish species, *Poeciliopsis monacha*, a gradient of heterozygosity levels (based upon 25 protein loci) was negatively correlated with FA (based upon 8 paired characters) using populations subdivided from population crashes, barriers to migration, and recolonizations (Vrijenhoek & Lerman, 1982). A hybrid, clonally reproducing biotype, *P.* 2. *monacha-lucida*, did not mirror this gradient, even though it coexists with the sexual form and so encounters the same environmental perturbations upon development. Indeed, the clonal form can be regarded as an internal standard, being a genetic constant replicated in a heterogeneous environment.

In summary, FA is minimized in highly heterozygous individuals. Whether this is due to generally superior heterozygous gene combinations (the overdominance theory) or the increased expression of deleterious recessive alleles (the dominance theory) in less heterozygous individuals, or both, remains under debate (Wright, 1977).

An exception to this generalization has been found in hybrid populations of recent origin between two sunfish species, *Enneacanthus obesus* and *E. gloriosus*, where hybridization apparently follows man-made disturbance and where FA is higher than in the parent populations (Graham & Felley, 1985). The hybrid populations are thought to be too recent in origin to permit independently evolved coadaptation within them so that the higher FA is presumably a consequence of poorer coadaptation in the hybrids due to genomic disruption, i.e. genomic stress. It is relevant that Graham & Felley (1985) cite a number of examples where developmental homeostasis is not lowered in hybrids, but these examples represent relatively old zones of contact.

(2) *Environmental stress*

Because relatively severe stress is needed to detect FA alterations under experimental conditions, the detection of environmental effects under field conditions is expected to be quite difficult. A reasonable initial approach is to seek out ecologically marginal conditions. For a set of six characters in the butterfly, *Coenonympha tullia*, Soulé & Baker (1968) found highest FA at high altitudes in Rocky Mountain populations, where the environment can be assumed to be the most extreme. Such work requires a knowledge of the physical factors determining distribution and abundance. This information is now being acquired in some *Drosophila* species (Parsons, 1983), so that comparisons of flies from ecologically benign and stressful habitats would appear feasible for FA.

In the muskrat, *Ondatra zibethicus*, Pankakoski (1985) used a growth index, where the speed of individual growth is used as a measure of habitat suitability. Using nerve foramen numbers of the skull the correlation between FA and the growth index came to -0.94 ($P < 0.001$) indicating a clear relationship between morphogenetic homeostasis and habitat suitability. This confirms the importance of investigating populations from habitats that can be defined in terms of varying stress at the ecological level.

In three species of fish from southern California, FA increased approaching heavily populated areas from the north and the south, and with time within this region (Valentine, Soulé & Samollow, 1973; Valentine & Soulé, 1973). An explanation is that FA increases with level of exposure to environmental toxicants suggesting that FA can be used to monitor pollution stress. Later fish studies suggested higher FA under the influence of mercury (Ames, Felley & Smith, 1979). In acidified lakes, however, FA was increased only weakly, which was rather surprising since low environmental pH causes deformities, pathological conditions and lowered reproduction (Jagoe & Haines, 1985). Similarly, Wiener & Rago (1987) found that FA in adult bluegills, *Lepomis macrochirus* Rafinesque, was judged to be insensitive as a potential measure of pH related stress. Interestingly, Ames, Felley & Smith (1979) did not find an effect on FA when comparing heated and ambient temperature locations. Clearly, there is a major difficulty in not knowing how severe a stress really is at the biological level under field conditions.

In our own species, dental asymmetry was used as an indicator of stress in four populations assayed for environmental favourableness judged from ethnographic and medical data (Bailit *et al.*, 1970). In order of increasing favourableness, the ranking was

Tristan da Cunha, Kwaio, Nasioi, and Boston children, and FA decreased in the same rank order. In two populations (Kwaio, Nasioi) heritability estimates of 0·02–0·05 were obtained from geneological information but were not significantly different from zero. Using the same measures, Townsend & Brown (1980) found FA in an Australian Aboriginal population to be similar to the Kwaio, a population living on the island of Malaita in the Solomon Islands under conditions described as harsh. In another study, FA of prehistoric hunters of north America was greater than later aboriginal farmers and a modern cadaver population (Perzigian, 1977); within the prehistoric hunters, the taller and presumably best nourished had less FA than shorter individuals suggesting growth disturbance mediated by severe nutritional stress. In the Lengua Indians of Paraguay, odontometric FA is lower in individuals increasingly exposed to Western foodstuffs and medicine (Kieser, Groeneveld & Preston, 1986). It is finally important to note that where possible it is desirable to compare groups of equivalent ages, given that Ruff and Jones (1981) found that FA of tibiae and humeri decreased with age in an archaeological sample from A.D 500–1000 in California.

Consistent with the above results is a study of stress in preterm infants whereby FA of eight morphological traits gave a significant inverse correlation with gestational age and with the health status of infants and mothers, showing that FA is a good measure of disturbances of developmental homeostasis following stress (Livshits *et al.* 1988). It is not then surprising that mentally retarded individuals show greater anthropometric asymmetry than normal individuals. This was found in a study of 202 normal and 202 mentally retarded males for bilateral measures of bone, soft tissue and strength, with significant differences among aetiological categories whereby those individuals with neurological impairment show the greatest structural asymmetry (Malina & Buschang, 1984). Since mental retardation has a genetical component, the data on our own species therefore demonstrate that stress due to both environmental and genetic variation leads to epigenetic stress assessible by FA.

The effects of major genetic changes have been investigated by dental asymmetry. Individuals with congenital cleft lip have increased FA of their molar teeth (Adams & Niswander, 1967; Sofaer, 1979), as well as for dermatoglyphic traits indicating generalized developmental stress during the foetal stage (Woolf & Gianas, 1976, 1977). Increased dental FA occurs in individuals with Down's syndrome (Garn, Cohen & Geciauskas, 1970; Townsend, 1983) and Johnston and Penrose (1966) found marked bilateral differences in sole dermatoglyphics in some individuals with limb hemihypertrophy of varied unknown aetiology. Therefore major genetic changes as for major mutants in *Drosophila*, may impose a level of stress assessible by FA. Dermatoglyphic studies appear useful in this regard since suggestive evidence of greater FA with increasing maternal age (Parsons, 1973) also suggests sensitivity to environmental effects.

V. DOMESTICATION

Considering animal skeletons, increased strength reduces the probability of fracture. This usually involves greater mass and increased energy costs. There is therefore an expectation of an optimum strength for each skeletal arrangement (Alexander, 1981). Furthermore, the symmetry of the mechanical properties of paired long bones can provide an indication of the precision of formation of animal skeletons. Alexander *et al.*

(1984) tested homologous limb bones of three bird species to failure in bending, and load at fracture and work to fracture were measured. Gulls (*Larus fuscus*) showed much lower FA than the domesticated birds, pigeons (*Columbia livia*) and battery hens (*Gallus gallus*), as well as compared with other published reports in the literature on domesticated animals including man. In the wild, it is clearly adaptive to maximize symmetry especially in relation to minimizing variability of load. Domestication therefore reduces the selective advantage of symmetry. For example, the need to seek out food competitively is reduced, which would reduce the need for efficient flight and movement, so that the selective pressure for symmetry on limb bones would be lowered. Direct selection of this nature at the phenotypic level seems far more plausible than higher FA as a consequence of the lowered heterozygosity which could occasionally occur under domestication.

Since domestication can be regarded as an environmental stress occurring within boundary conditions selected by our own species it has potential as a model for studying stress (Kohane & Parsons, 1988). FA assessment would be useful in monitoring the stress of introduction into the domesticated environment, which may be severe as shown by long-term experiments on domestication in foxes (Belyaev, 1979; Belyaev & Borodin, 1982). The amount of genetical data specifically devoted to the study of domestication in progress is, however, rather limited.

VI. DISCUSSION

In a study of vertebral fusions of 6764 herring skeletons, Ford & Bull (1926) found most to have vertebra numbers between 55 and 57. Of the 1·43 % of fish with numbers outside these limits, many developed abnormal structures; in addition out of 95 fish with abnormal structures, 59·6 % were in this group. Therefore extreme individuals are less stable developmentally for this low variability trait. Later work showed that one way abnormal structures of this type could be increased was by environmental stress, for example chilling of embryos of garter snakes, *Thamnophis elegans terrestris*, affected scutellation patterns especially by increasing variability (Fox, Gordon & Fox, 1961). Nightly chilling of embryos during early critical periods of morphogenesis may be an important teratogenic factor, because under natural conditions, there is an unusually wide range of scutellation patterns. In the snake, *Natrix fasciata*, vertebral number and the frequency of gross abnormalities increased at both high and low temperatures for experiments carried out over a developmental temperature range of 18–32 °C (Osgood, 1978). Assuming that embryos developing in the region of their optimum temperature have counts closer to the wild population than embryos developing at abnormal temperatures, an optimal temperature of 25°–27 °C emerges which is consistent with the thermoregulatory behaviour of gravid females. These results have analogies with FA levels in novel environments. Unfavourable temperatures and most mutant genes affect morphogenesis by altering the rate of reaction velocities and the sequence of normal developmental processes (Goldschmidt, 1945). Indeed, as the environment becomes increasingly extreme, the quantitative changes tend to be replaced by qualitative changes, or phenocopies (Waddington, 1953, 1956).

Phenotypic extremes should therefore show more FA than individuals central in a distribution. In a study of 26 traits (16 vertebrate, 10 insects) Soulé & Cuzin-Roudy (1982) found evidence for this effect for wing length of houseflies, all four traits involving sparrow wing bone lengths, and the auditory meatus of the kangaroo rat. At the genetical level, Soulé (1982) provided evidence that these tend to be traits of high heritability and low coefficient of variation, so that the phenotypic extremes are characterized by combinations of underlying additive genes. It is, however, important to note that increased FA of phenotypic extremes was recorded for a minority of traits, and that in many cases larger sample sizes would be useful.

When studying FA from the point of view of an epigenetic assessment of genomic and environmental stress, it appears desirable to avoid traits for which stabilizing selection is strong, simply because a consequence of this form of selection is low variability due to selection against extremes. In *D. melanogaster*, this means that sternopleural chaeta number is more useful than scutellar chaeta number which is strongly canalized to four chaetae in wild flies and in selection experiments to 0, 2, 6, 8,...chaetae (Rendel, 1967; MacBean, McKenzie & Parsons, 1972). Similarly, minor discrete skeletal variants in mice and our own species show a major deficiency of asymmetrical animals e.g. animals having an extra element on one side are more likely to have asymmetrical extra element on the other than would be expected due to chance given the frequency of variants in the population as a whole. This is a form of stabilizing selection association with homeostasis during epigenesis (Parsons & Howe, 1967; Howe & Parsons, 1968), and means that quantitative metrical traits not showing strong canalization may be more useful for FA studies, although discrete traits do tend to be largely independent of body size (Palmer & Strobeck, 1986).

For quantitative traits, FA increases away from an optimum especially at stress levels approaching lethality. Since recombination tends to be high under these conditions, normal developmental appears to be upset at the molecular, chromosomal and epigenetic levels. The cost of this process is almost certainly manifested in terms of an increased metabolic energy requirement for adaptation to the stress (Hoffman & Parsons, 1989*a*, *b*). Even so, adaptation in a new environment is possible as shown in *D. melanogaster* by a progressive fall in FA over time in a relatively extreme environment under experimental conditions (Thoday, 1958). However, at such levels the metabolic cost is likely to involve alterations of cellular metabolism necessary to produce stress-induced proteins which could act as protective agents against a wide array of stresses as lethality is approached (Ananthan, Goldberg & Voellmy, 1986). Indeed, while it is inferred that stress levels needed to produce major effects upon FA are at levels approaching lethality in many cases, this needs to be investigated further.

Changes in FA may therefore provide a monitor of the disruption of homeostasis at an array of integrative levels. The precipitating stress may be genotypic, or environmental, or an interaction between the two. In an era characterized by substantial environmental stress at the global level, it will be important to define the conditions under which FA increases in both the laboratory and field. In this way FA should become an increasingly useful monitor of change in a wide range of taxa.

VII. SUMMARY

(1) Fluctuating asymmetry (FA) is a useful trait for monitoring stress in the laboratory and in natural environments.

(2) Both genomic and environmental changes can increase FA which represents a deterioration in developmental homeostasis apparent in adult morphology. Genetic

perturbations include intense directional selection and certain specific genes. Environmental perturbations include temperature extremes in particular, protein deprivation, audiogenic stress, and exposure to pollutants.

(3) There is a negative association between FA and heterozygosity in a range of taxa especially fish, a result consistent with FA being a measure of fitness.

(4) Scattered reports on non-experimental populations are consistent with experiments under controlled laboratory conditions. FA tends to increase as habitats become ecologically marginal; this includes exposure to environmental toxicants.

(5) In our own species, FA of an increasing range of traits has been related to both environmental and genomic stress.

(6) Domestication increases FA of the strength of homologous long bones of vertebrate species due to a relaxation of natural selection.

(7) FA levels are paralleled by the incidence of skeletal abnormalities in stressful environments.

(8) Increased FA is a reflection of poorer developmental homeostasis at the molecular, chromosomal and epigenetic levels.

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