

THE GENETICS AND EVOLUTION OF FLUCTUATING ASYMMETRY

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■ **Abstract** Variation in the subtle differences between right and left sides of bilateral characters, or fluctuating asymmetry (FA), has long been considered to be primarily environmental in origin, and this has promoted its use as a measure of developmental instability (DI) in populations. There is little evidence for specific genes that govern FA per se. Numerous studies show that FA levels in various characters are influenced by dominance and especially epistatic interactions among genes. An epistatic genetic basis for FA may complicate its primary use in comparisons of DI levels in outbred or wild populations subjected or not subjected to various environmental stressors. Although the heritability of FA typically is very low or zero, epistasis can generate additive genetic variation for FA that may allow it to evolve especially in populations subjected to bottlenecks, hybridizations, or periods of rapid environmental changes caused by various stresses.

INTRODUCTION

What role does genetics play in producing variation in the subtle differences between right and left sides of bilateral characters known as fluctuating asymmetry (FA)? It might be supposed that the answer to this question should be clear, because there have been a number of quantitative genetic studies of FA in various characters since the classical artificial selection experiment was conducted by Mather (1953) more than 50 years ago. Most show the heritability of FA to be quite low, but significant heritabilities have been found and therefore the extent of additive genetic variation for FA across various characters is uncertain (Fuller & Houle 2003, Leamy 1997, Markow & Clarke 1997, Palmer 2000, Palmer & Strobeck 1997). Few studies have attempted to assess the importance of nonadditive (dominance and epistatic) genetic effects in producing FA, and of those conducted, results have been mixed (reviewed in Leamy 2003). As a consequence, FA remains an elusive character whose genetic architecture is still largely unknown.

FA generally is not of interest for its own sake, but rather for what it is thought to assess: developmental instability (DI) in populations. DI results from internal or external stressors that disturb the development of structures along their normal developmental pathway in a given environment and produce developmental “noise” (Palmer 1994, Waddington 1957, Zakharov 1992). Thus, DI can be thought of as variation around the expected (target) phenotype that should be produced by a specific genotype in a specific environment. Developmental noise affects left and right sides of bilateral characters separately and therefore produces FA, but the eventual level of FA in a character depends on how successful developmental stability (DS) processes are in reducing this noise (Zakharov 1992). Although developmental noise theoretically is purely environmental in origin, DS has long been assumed to be at least partly under genetic control (Mather 1943, 1953; Palmer 1994; Waddington 1940, 1942, 1957; Zakharov 1992).

It is unfortunate that we do not yet fully understand the nature and extent of the genetic basis of FA. This kind of knowledge is essential if we are to use FA properly as a measure of DI and know, for example, whether sufficient genetic variance in DI exists so that it can respond to selection. FA in certain characters also must have genetic variability if it is used as a cue for female choice of males in “good genes” models of sexual selection (Møller & Pomiankowski 1993). Knowledge of the genetic basis of FA would help us understand whether there are organism-wide developmental mechanisms that act to ensure symmetry of multiple bilateral characters or whether DI is character-specific (Gangestad & Thornhill 1999, Leamy 1993, Polak et al. 2003). More generally, a better understanding of the genetic architecture of FA should provide a much-needed perspective for sorting out the sometimes unexpected or contradictory patterns of differences in FA levels between populations subjected or not subjected to various genetic or environmental stressors or among individuals differing in fitness.

In this review, we first explain how FA and DI are assessed, and provide a brief, genetical perspective of the relationship of FA to stress, fitness, and integration in populations. We then describe the theoretical origin of FA and its implications for the genetic basis of FA. This is followed by an assessment of genetic studies of FA. Based on inferences from the assumed origin of DI and evidence especially from recent genetic studies, we conclude that it is likely there are no genes that govern FA per se, and develop the hypothesis that FA levels in various characters are influenced by dominance and especially epistatic interactions among a number of genes affecting these or other characters. We use this hypothesized genetic architecture as a basis to discuss the implications of the use of FA in ecological and evolutionary studies and offer some final thoughts about the kinds of studies that would be appropriate to test this hypothesis.

FLUCTUATING ASYMMETRY

FA is perhaps easiest to visualize at the population level as variation of differences in left-minus-right sides ($L - R$) of a bilateral character. Individual values of FA are most often assessed by unsigned left-minus-right-side differences,

$|L - R|$, although a number of other indices of FA are in common use (Palmer & Strobeck 2003). Besides FA of various size measures, methods recently have been developed to generate shape FA measures from the results of the Procrustes procedure applied to digitized landmark points on bilateral structures such as wings or mandibles (Klingenberg et al. 2002, Klingenberg & McIntyre 1998). The precision of measurement of subtle asymmetries has improved in recent years with better technology and especially with an increasing realization of the importance of minimizing measurement error (Palmer 1994). Most investigators now repeatedly measure both left and right sides of each character in order to obtain estimates of the extent of measurement error and to test for the significance of FA.

Beyond FA, two other kinds of asymmetries are sometimes found in bilateral characters. Directional asymmetry (DA) is fairly common and occurs when one side consistently differs from the other side, whereas antisymmetry (AS) is rarer and is characterized by a mixture of left- and right-biased individuals (Palmer & Strobeck 1986, 2003). It has been debated whether these asymmetries may reflect increased DI (Graham et al. 1993, Klingenberg 2003b), and in fact some empirical studies have shown changes from FA to either DA (Leamy et al. 1999a) or AS (McKenzie & Clarke 1988) in stressed populations. However, FA is the most commonly accepted measure of DI and we restrict our review to this type of asymmetry. Appropriate statistical procedures for adjusting for DA, AS and/or size effects on FA, and for testing for significant differences in FA among two or more groups, are discussed in some detail by Palmer & Strobeck (2003).

Because FA is calculated from only two sides (one degree of freedom) of a bilateral character in a given individual, it is a rather imprecise estimator of DI variability. Thus, sampling variation should result in many FA values at or near zero (the expected mean for FA) even in individuals with very high levels of DI. The correspondence between FA and DI has been parameterized by the hypothetical repeatability, \Re , which expresses the degree to which differences in FA reflect differences in DI. Theoretical formulas for calculating \Re that are based on variation in FA have been derived by Whitlock (1996, 1998) and Van Dongen (1998), and estimates of \Re from a number of FA studies have averaged about 0.08 (Gangestad & Thornhill 2003). This very low value serves as an important reminder of the difficulty of obtaining good estimates of DI in populations from measurement of FA in various characters.

Fluctuating Asymmetry and Stress

FA has been widely used to compare presumed DI levels in populations subjected or not subjected to a number of environmental stressors such as temperature, nutrition, radiation, chemicals, population density, noise, parasites, light conditions, predation risk, and habitat structure (reviewed in Hoffmann & Woods 2003, Møller & Swaddle 1997). The working hypothesis in such comparisons is that DI, and therefore its most easily observable outcome, FA, will be higher in the more stressed populations compared to the control or unstressed populations. In fact, this result often has been found (e.g., Graham et al. 2000, Pankakoski et al. 1992).

But for all of these studies suggesting that stress can increase FA, there are others where no or even opposite effects of stress on FA have been observed (Leamy et al. 1999a, Markow 1995, Woods et al. 1999). A number of factors such as uncertainty about the degree of stress imposed and the choice of characters and FA indices have been invoked as potential reasons for this inconsistent relationship between stress and FA (Hoffmann & Woods 2003).

In these studies it is often assumed that FA has no genetic basis and that differences in FA levels between stressed and unstressed populations are purely environmental in origin (Palmer 1994). Yet unless genetically identical (isogenic) populations have been used, FA levels may also partly reflect genotypic differences in response to stress (i.e., DS differences). In fact, environmental stresses in genetically variable populations may result in selection of certain genes that ameliorate the immediate stress but that themselves perturb developmental processes and result in increased asymmetry. An outstanding example of this effect is seen in populations of the Australian blowfly, *Lucilia cuprina*, that have developed resistance to exposure to various insecticides such as dieldrin, diazinon, or malathion but that also show increased levels of FA in bristle numbers compared to susceptible (nonexposed) populations (McKenzie 1997, 2003).

Fluctuating Asymmetry and Fitness

If FA truly measures DI, it is natural to imagine that individuals with the greatest levels of DI would be the least fit, and thus that there should be a negative relationship of FA with fitness or individual condition. Such a relationship has been reported in some studies (for example, Badyaev et al. 2000), and several meta-analyses have found a significant negative relationship, albeit a weak one, between FA and various fitness components (Møller & Thornhill 1998, Thornhill et al. 1999, Tracy et al. 2003). Some findings in the early 1990s suggesting that FA levels may provide a cue for female choice in sexual selection (Møller 1990, 1992; Møller & Høglund 1991) also generated much interest in searching for a possible connection between FA and fitness. But this connection has not always been found in subsequent studies (reviewed in Simmons et al. 1999), and thus the precise role of FA in the sexual selection process is controversial (Tomkins & Simmons 2003). Many other studies also have not discovered a link between FA and various fitness components, suggesting extreme caution in the use of FA as an overall indicator of fitness (Clarke 1995a,b, 1998a, 2003; Leung & Forbes 1996).

Fluctuating Asymmetry and Integration

Many investigators have measured FA for multiple traits, and the question therefore arises whether the asymmetries are integrated or independent among traits. There are two different contexts in which the FA of multiple traits has been studied. The majority of studies have used FA of multiple traits to quantify organism-wide DI as a measure of individual quality or exposure to stress, whereas others use FA as a tool to investigate developmental integration among traits.

If FA is to be used as a measure of organism-wide DI, then clearly there should be a correspondence between the amounts of FA of different traits. Individuals with higher DI should be generally more prone to developmental noise in all their traits, and therefore, it should be possible to use a combined index for FA in multiple traits to increase the precision of the estimate of organism-wide DI (Lens et al. 2002, Leung et al. 2000). This reasoning assumes that there is a positive correlation between the amounts of FA in different traits, that is, of the unsigned asymmetries (absolute values of the trait asymmetries). This type of correlation is traditionally called the individual asymmetry parameter (IAP) (Clarke 1998b, Leamy 1993, Polak et al. 2003). In order to provide independent evidence on DI, the traits should be developmentally unrelated, which means they should be chosen from different parts of the organism. The correlations of FAs found in empirical studies are generally weak but tend to be greater than zero (Polak et al. 2003). Such a correlation, indicating organism-wide DI, is of critical importance for the use of FA as an indicator of exposure to stress, individual quality, or fitness in good genes models of selection (Lens et al. 2002, Møller & Swaddle 1997, Tomkins & Simmons 2003).

A different use of correlated FA is inferring whether there are developmental interactions between traits (Klingenberg 2003a, 2004b). Because FA is due to random perturbations in development, the signed asymmetries (signed values of the trait differences between left and right sides) of two traits will be correlated only if there is a developmental interaction that can transmit the effects of perturbations to both traits jointly. If the precursors of the traits develop separately, without signaling or other interactions among them, random perturbations cannot be transmitted between them and there will be no statistical association of the resulting asymmetries. Therefore, a correlation of the signed asymmetries between traits indicates interactions of the respective developmental pathways (Klingenberg 2003a, 2004b). Such correlations have been shown in a number of studies, and, as is to be expected, they have been limited to sets of traits that are anatomically and developmentally related (Klingenberg & Zaklan 2000; Klingenberg et al. 2001a, 2003; Leamy 1993).

This second use of correlated FA differs fundamentally from the preceding one. In studies of organism-wide DI, this second approach can be used as a preliminary step to assess whether the traits are developmentally independent, in which case the correlations between signed asymmetries should be zero.

ORIGIN OF FLUCTUATING ASYMMETRY AND BUFFERING

FA originates from small perturbations that produce a component of random noise in developmental processes (Klingenberg 2003b). At the molecular level, most cellular processes are stochastic—the way in which individual molecules bind to each other, take part in metabolic reactions, or are transported from one location to another are all in part chance events (McAdams & Arkin 1999). If a kind of

molecule exists in many copies in a cell, then the random variability will not be apparent in the behavior of the cell as a whole, because the random variability will be averaged out over the large number of molecules present. If developmentally important molecules are present only in small numbers, however, random variation will be apparent at the cell, tissue, and morphological levels.

In particular, DNA is a type of molecule that is present in cells only in small numbers. In diploid organisms, single-copy genes (i.e., those that are not duplicated) are present in only two alleles per nucleus. Therefore, understanding the stochastic component in the dynamics of gene expression is particularly relevant to the study of developmental noise (Blake et al. 2003, Cook et al. 1998, Ozbudak et al. 2002, Raser & O'Shea 2004). Because regulation of gene activity is substantially through switching between "on" and "off" states by the formation and decay of macromolecular complexes, a component of random noise is generated as part of the normal cell function (Fiering et al. 2000). Cook et al. (1998) used a simplified mathematical model of gene switching to study the effects of gene dosage on the levels of gene products and found that the loss of a copy of the gene not only decreased the average level of the gene product in the cell, but above all, that it substantially increased the variability. Experiments with yeast showed that differences in promoter sequences, upstream activating sequences, and deletions of components of chromatin-remodeling complexes produce changes in the level of stochastic variation of gene expression that are not necessarily tied to the absolute level of gene expression (Raser & O'Shea 2004). Accordingly, there can be genetic regulation of variability in gene expression, and this variability itself can therefore evolve.

Nonlinear Developmental Mapping and Developmental Instability

The molecular origin of developmental noise is just one of the components required for a full understanding of DI. A different question is how this variability is translated into morphological outcomes like FA or, in other words, how noise is transmitted through the developmental system. The link to the genetics of FA is to ask how genetic variation in the system mediates this transmission of variability.

One approach is to use models of simple developmental processes such as diffusion-threshold models in which the parameters are each controlled by a separate gene (Nijhout & Paulsen 1997) in combination with a small component of random noise that is independent of the genotype (Klingenberg & Nijhout 1999). FA was simulated by running the model twice for each genotype, with separate values for the noise component, and calculating the difference between the two resulting trait values. The differences in the behavior of the system caused by the genetically controlled variation of model parameters can amplify or dampen the effects of the random noise. Therefore, the model generates genetic variation of FA through genetically mediated expression of perturbations that are themselves strictly nongenetic (Klingenberg & Nijhout 1999).

These conclusions do not rely on the specific nature of the particular model, but are valid for a broad range of nonlinear models. Because most developmental models are nonlinear, complex genetic behaviors like dominance and epistasis of the trait invariably emerge. These nonadditive effects are particularly important for the genetic architecture of FA in these models and can produce consequences such as an asymmetric response to selection on FA, with a greater response to selection for increased than for decreased FA (Klingenberg & Nijhout 1999).

A more general way to think of the developmental origin and genetic control of FA is in terms of a mathematical mapping from the genetic and environmental factors that influence a trait to the resulting phenotypic value, which can be called “developmental mapping” (Klingenberg 2003b, 2004a; Klingenberg & Nijhout 1999). This is related to the idea of a “genotype–phenotype map” (Wagner & Altenberg 1996) but explicitly includes nongenetic factors as well as the genotype (Rice 2002). These mapping functions will normally be nonlinear; that is, plots of a phenotypic trait against the genetic and environmental factors will be curved (if multiple factors are considered, the mappings will be curved surfaces). As a result, the slope of the mapping depends on the genetic and environmental factors. These slopes determine the change of the phenotypic value in response to a small perturbation of the factors, that is, the sensitivity to developmental noise and therefore DI. As a consequence of nonlinear developmental mapping, different genotypes can therefore have different DI and FA.

Because nonlinear developmental mapping is associated with nonadditive genetic effects, dominance and epistasis are also expected to be the prevalent features of the genetic architecture of FA (Klingenberg 2003b, 2004a; Nijhout & Klingenberg 1999).

Origins of Developmental Buffering

FA is the observable outcome of developmental noise as it has been “filtered” by the developmental system. It is possible that a substantial proportion of variability is not apparent because it has been absorbed by the action of developmental buffering.

Simulation and experimental studies have found that developmental systems are remarkably robust against perturbations (e.g., Bergman & Siegal 2003, Houchmandzadeh et al. 2002, von Dassow et al. 2000). This means that there is a considerable buffering capacity built into the networks of developmental interactions that set up morphological structures, which may be a major determinant of DS.

Moreover, from the perspective of developmental mapping, buffering and DS result from mapping functions that are relatively flat, for instance, a curve that reaches an asymptote or a surface with a plateau (Klingenberg 2003b, 2004a). Because flat mapping functions are associated with the absence of change in the mean phenotype in response to the genetic or other inputs, this perspective on buffering assigns a common origin to DS and canalization (e.g., Meiklejohn & Hartl 2002), a view that is not shared universally (e.g., Debat & David 2001). Canalization is the ability to develop the same phenotype despite genetic or environmental variability

(Klingenberg 2003b). Viewed from this perspective, genetic variation of buffering capacity is linked to genetic variation in the steepness of the developmental mapping function, which is again linked to dominance and epistasis in the genetic architecture of the phenotype under study (Klingenberg 2004a).

There have also been studies of buffering that have focused on particular mechanisms rather than on global properties of the developmental system as a whole. A specific gene that has received particular attention in this context is the heat-shock protein 90 (*Hsp90*; Queitsch et al. 2002, Rutherford 2000, Rutherford & Lindquist 1998, Sangster et al. 2004). Inhibition of *Hsp90* activity led to the regular occurrence of various morphological anomalies in various qualitative traits in *Drosophila* and *Arabidopsis* (Rutherford & Lindquist 1998, Queitsch et al. 2002). The specific anomalies produced in these experiments depended on the genetic background, and their prevalence could be increased by selection (Rutherford & Lindquist 1998), indicating an important role for genetic interactions. Further experiments yielded evidence for both genetic and epigenetic interactions of *Hsp90* with other genes (Sollars et al. 2003). *Hsp90* is an attractive candidate for a specific “buffering gene” because it encodes a molecular chaperone protein that takes part in stabilizing a variety of signal transduction and other cellular processes (Rutherford 2000). However, an empirical study of bristle counts and wing size traits in *Drosophila* found that inhibiting *Hsp90* activity pharmacologically or by mutation did not lead to increased FA or variation among individuals (Milton et al. 2003).

Further experimental studies are needed to test the generality of *Hsp90* as a potential buffering mechanism and to explore whether there are other mechanisms of this kind. In general, the relationship between the mechanisms that generate or buffer against variation within and between individuals is still largely unclear. A result emerging consistently from the available studies, however, is the central role of gene interaction in these processes.

GENETICS OF DEVELOPMENTAL STABILITY

Heritability of Fluctuating Asymmetry

Most empirical studies estimating the heritability of FA have yielded very low, nonsignificant values (for example, Leamy 1999, Pelabon et al. 2004), but occasional significant heritabilities have been found as well (Polak & Starmer 2001, Santos 2002, Scheiner et al. 1991, Thornhill & Sauer 1992). In an effort to test for a potential global heritability for FA, Møller & Thornhill (1997) performed a meta-analysis of 34 studies that yielded a significant heritability of FA that averaged 0.19 over a number of characters and taxa. But the estimates of heritabilities from the studies sampled often were rather poor because of various measurement, statistical, and/or experimental difficulties, and the meta-analysis itself suffered from several errors and misinterpretations (Fuller & Houle 2003, Leamy 1997, Markow & Clarke 1997, Palmer 2000, Palmer & Strobeck 1997). Other analyses show lower FA heritability values (Gangestad & Thornhill 1999, Whitlock &

Fowler 1997); the most recent by Fuller & Houle (2003) estimated the average heritability of FA to be just 0.026. Most investigators now appear to agree that there is very little additive genetic variation for FA in most characters, although in some cases this variation may be statistically significant.

It is important to discover whether even very low heritabilities exist for FA in various characters, because they may translate into moderate to high heritabilities of DI (Gangestad & Thornhill 2003, Houle 2000). Because FA is an imprecise estimator of DI, an unbiased heritability of DI must be obtained by dividing the heritability of FA in a given character by its repeatability, \mathfrak{R} (Van Dongen 1998; Whitlock 1996, 1998). Unfortunately, heritabilities of DI estimated in this manner have not been very informative because they have varied erratically from less than 0 to well over 1.0 (Houle 1997). Fuller & Houle (2003) have suggested that the inherent variability in these estimates either is too large for precise estimates, or that the assumptions on which \mathfrak{R} has been formulated are wrong. It is likely that developmental errors are not additive and independent and therefore do not produce a normal distribution of left-minus-right-side differences as has been assumed in the standard model relating FA with DI (Klingenberg 2003b). Given that the precise relationship between FA and DI remains speculative, our present state of knowledge of the extent of additive genetic variation for DI is even less than that for FA.

Single Gene Effects

Although rare, there are a few unambiguous cases of single genes that significantly affect FA in one or more characters. The best-known examples come from the extensive studies by McKenzie and colleagues on mutants in Australian blowflies and in *Drosophila* that cause increased levels of FA in bristle number (review in McKenzie 2003). *Rop-1* and *Rdl* confer resistance to various insecticides in blowflies and act with partial or complete dominance specifically on bristle FA and not on FA levels in various wing characters (Clarke et al. 2000). The action of *Rop-1* is totally ameliorated by a modifier gene, *Scl* (scalloped wings). *Scl* is homologous to the *Notch* gene in *Drosophila* that is involved in bristle formation. Various *Notch* mutants affecting specific bristle types typically cause elevated asymmetry as well, but only in the specific bristle type that they affect (Indrasamy et al. 2000). *Notch* is not an “asymmetry” gene per se, but rather a gene that is character-specific in its action on specific bristles and that also affects the asymmetry level in these bristles. These sorts of genes represent precisely the kind of genetic basis of asymmetry predicted by the perspective of developmental mapping (Klingenberg & Nijhout 1999).

Some genes may act more generally by affecting FA levels in a suite of characters. For example, alleles at the lactose dehydrogenase (LDH) locus in killifish affect DI in a number of scale and fin ray characters (Mitton 1993). Atchley et al. (1984) found that mice with one dose of the muscular dysgenesis gene (+/*mdg*) generally produce higher FA than wild genotypes (+/+) in several different regions of the mandible, all of which are associated with skeletal muscle attachment. In addition, Leamy et al. (2001) discovered that mice carrying the *t-locus* haplotype

exhibit more DI than wild mice when assessed by a composite FA index calculated over four different skeletal characters. The *t*-locus is a complex one consisting of inversions that suppress recombination and that are thought to harbor various mutations (Silver 1985), some of which may well affect the pathways leading to bone formation.

Dominance and Heterozygosity

Historically, developmental buffering (including both canalization and stability) in organisms has been thought to depend on the presence of specific gene complexes that become coadapted over time by natural selection (Mather 1943; Waddington 1940, 1942, 1957). Early investigators such as Dobzhansky (1950) and Lerner (1954) believed that selection primarily favored heterozygotes at many loci (see Woolf & Markow 2003), and this belief stimulated a number of experimental studies that focused on testing whether FA levels were less (and thus DS greater) in heterozygotes compared with homozygotes. This comparison was especially facilitated by the development of molecular markers such as allozymes, and in fact several allozyme studies did find that reduced FA tended to be associated with heterozygosity (for example, Leary et al. 1984, Soulé 1979, Vrijenhoek & Lerman 1982). But the typically few allozyme loci used in such studies were recognized to be poor indicators of overall genomic heterozygosity (Chakraborty 1981, Mitton & Grant 1984, Mitton & Pierce 1980), suggesting instead that the specific loci themselves might be important in maintaining DS. Mitton (1993) reviewed a number of investigations showing an association between heterozygosity at certain allozyme (or protein) loci with reduced FA levels, and he has argued that these loci may be exerting their effects by regulating overall metabolism and/or other physiological processes.

In recent years, the presumed link between allozyme/protein heterozygosity and FA has been increasingly questioned (see Clarke 1993). Partly this has been because most studies purporting to show such a link suffer from the same difficulties as those testing for FA/fitness associations. Perhaps worse, a number of studies that have been conducted with various allozyme or protein loci have not shown a significant FA/heterozygosity association (see Clarke 1993, Vollestad et al. 1997). L. Leamy, E.J. Routman & J.M. Cheverud (unpublished data) also did not find a significant relationship between FA of molar size and shape and the percentage of heterozygous microsatellite markers in a population of mice. In addition, in studies with various haplo-diploid insects (Clarke 1997, Clarke et al. 1992), the loss of heterozygosity from inbreeding did not appear to affect FA levels in various characters. Thus, though there may well be a potential role for dominance of alleles at loci influencing FA (see below), a universal heterozygosity/FA association does not appear to be supported.

Epistasis

One factor that complicates virtually every test for the effects of dominance or heterozygosity on FA in genetically variable populations is epistasis. A given

locus *A*, for example, may interact with another locus *B* so that the effects of *A* (the differences among individuals with genotypes *AA*, *Aa*, and *aa*) depend on whether the genotype at locus *B* is *BB*, *Bb*, or *bb*. This is clearly seen in Australian sheep blowflies homozygous or heterozygous for the mutant allele *Rop-1* that confers resistance to the insecticide diazinon. This mutation also increases FA in bristle number over that of wild types (+/+), but only if a dominant modifier allele at another locus is absent. Over time and with continuous exposure to diazinon, this modifier allele typically increases in frequency and renders FA levels for all three genotypes at the *Rop-1* locus virtually identical (McKenzie 2003). Thus, the *Rop-1* allele acts as a complete dominant in its effect on FA in one genetic environment but has no effect at all in another genetic environment. Unambiguous tests for dominance effects at single loci can be done only in co-isogenic populations where all loci other than the one being tested are homozygous (see Leamy 1981), although in these cases the results are valid only for the genetic background used.

Beyond allozyme and protein studies, epistasis is a particularly important confounding factor in experiments intended to assess the effects of heterozygosity on FA in inbred parents versus their hybrid offspring (Alibert & Auffray 2003, Clarke 1993). Generally, we expect that hybrids produced from crossing inbred lines should show positive heterosis and have lower FA levels than the mean of their inbred parents because the inbreeding process tends to fix deleterious recessive alleles whose harmful effects are masked by dominant alleles in the hybrids. But even in those cases where the parents are homozygous at all loci (as in fully inbred laboratory strains) and only the hybrid progeny contain some heterozygous loci, differences in FA between inbreds and hybrids may be the result not of heterozygosity but rather of the epistatic interaction of one or more of these newly formed heterozygous loci with various other loci. Lower FA levels in hybrids than in either parent (for example, Leamy 1984, Mather 1953) are especially likely to be due to epistatic effects unless we assume that underdominance of individual loci for FA is pervasive.

Hybrids generated from two different species or subspecies, taxa more distinct than inbred laboratory strains, often show outbreeding depression characterized by FA levels that are greater than those in their parents (reviews in Alibert & Auffray 2003, Graham 1992). Given that the hybrids are expected to be more heterozygous than their parents, heterozygosity is not acting to reduce FA in these crosses (Clarke 1993). Instead, the conventional explanation for such results is that selection has produced coadapted gene complexes (epistatic interactions) unique to each taxon that are broken down once the hybrids are formed (Clarke 1993). If so, the greater the differences in these gene complexes between the parental taxa, the greater should be the probability that DI (as measured by FA levels) will be higher rather than lower in the hybrids compared with that in the parents. This concept appears to be supported by the results of a survey done by Alibert & Auffray (2003, table 8.3) in which the number of studies showing hybrids with increased or decreased DI compared to their parents was 17/1 from crosses of different genera or species, but 8/10 from crosses of subspecies, races, or lines ($\chi^2_1 = 10.6; P < 0.01$). Such results

provide indirect evidence that epistatic effects can be important in controlling FA levels.

Quantitative Trait Loci

In the past few years, the discovery of molecular markers such as microsatellites has made it possible to perform whole genome searches for genes (quantitative trait loci = QTLs) affecting quantitative characters (Erickson et al. 2004, Lynch & Walsh 1998). The approach typically starts with a cross of two inbred lines phenotypically divergent for some character of interest, such as body weight, and genotypically divergent for a number of polymorphic molecular markers. The F_1 hybrid individuals produced from the intercross of the inbred lines are themselves crossed to produce the F_2 individuals, which are phenotyped (for body weight, weighed) and genotyped for sufficient numbers of molecular markers to ensure uniform coverage of all chromosomes throughout the genome. This breeding scheme creates maximum linkage disequilibrium among all loci on each chromosome (Lynch & Walsh 1998). Because of this, significant differences in the mean of the character among the three genotypes for any marker on a given chromosome suggest that a QTL for the character exists at or near the position of that marker. Various statistical techniques have been developed to test appropriately the strength of phenotypic-genotypic associations along the length of each chromosome (including in intervals between markers) and, where a QTL is found, to estimate its additive and dominance effects on the character (Haley & Knott 1992, Lynch & Walsh 1998).

Using this approach, Leamy and colleagues searched for QTLs for FA in various morphometric characters in F_2 mice generated from an original cross of the Large (LG/J) and Small (SM/J) inbred strains. In the first such analysis, Leamy et al. (1997) discovered 11 QTLs affecting FA in 10 mandible dimensions, but 9.5 QTLs were expected by chance alone so it is difficult to say if any of these QTLs are real. Interestingly, 9 of the 11 putative QTLs exhibited significant dominance effects (Leamy et al. 1997), whereas QTLs for most characters generally show predominantly additive effects (for example, Workman et al. 2002). In a follow-up study, Klingenberg et al. (2001b) calculated mandible size and shape measures in these same mice but found only 1 QTL for FA of size and 1 for shape, neither of which were well-supported statistically. On the other hand, Leamy et al. (1998) found 13 QTLs for FA in 6 discrete skeletal characters in this same population of mice, this number being greater than the approximately 6 QTLs expected by chance alone. Again, dominance effects predominated in these QTLs (Leamy et al. 1998). Finally, no QTLs were found for FA in 15 mandible characters in a backcross mouse population created from a cross of the F_1 between a wild strain (CAST/Ei) and a strain (M16i) selected for rapid growth rate (Leamy et al. 2000). In general, therefore, these results suggest that there is little evidence for individual QTLs affecting FA in the morphometric characters used in these two populations of mice.

QTL analyses also allow tests for significant interactions among pairs of QTLs (Cheverud 2000a, Cheverud & Routman 1995, Leamy 2003, Routman & Cheverud

1995). Two QTL studies tested for epistasis for FA, both again making use of the F₂ population of mice generated from an original intercross of the Large and Small inbred strains previously described. In a full-genome scan, Leamy et al. (2002) found an abundance of epistasis for FA in mandible size, despite the fact that only one QTL of marginal significance was identified for this character. More recently, Leamy et al. (2005) conducted a follow-up study with these mice to test if epistasis might be present for FA in molar size and shape as well, and if so, whether the loci involved would be common for FA in the mandible and molar characters. Results of the single-gene analysis showed no individual QTLs for FA of molar size and just two significant QTLs for FA of molar shape, both of marginal significance and jointly contributing less than 5% of the total variance. However, numerous pair-wise combinations of QTLs again exhibited significant epistasis for FA in both molar size and shape. The (unknown) QTLs involved in these interactions differed for FA in the two molar characters (and from those for FA in mandible size), but their contribution to the total variance was nearly the same (about 20%) for FA in both molar characters. These results suggest that the genetic basis of FA in the molar characters consists almost entirely of character-specific epistatic effects whose contribution to the total variance is considerable.

A HYPOTHESIS

From all these results, it seems reasonable to hypothesize that FA has a predominantly nonadditive genetic basis with substantial dominance and especially epistasis. The genes participating in these epistatic interactions influencing FA in a given bilateral character most likely will be character-specific, and perhaps involved in some way with the formation of the bilateral character itself rather than being genes for FA per se. Occasional genes may be found in some populations whose single-locus effects on FA may generate small amounts of additive genetic variation, and where this is the case, these genes might be expected to exhibit dominance.

IMPLICATIONS OF THE HYPOTHESIS

An important implication of an epistatic genetic basis for FA is that it may complicate the primary use of FA in comparisons of DI levels in outbred or wild populations subjected or not subjected to various environmental stressors. It has been a tacit assumption that there is little if any additive genetic variation to confound such comparisons, but a potential confounding role for epistasis has generally not been considered. The situation with the *Rop-1* gene in blowflies previously described provides an example. If FA levels are measured in populations of blowflies exposed (stressed) and not exposed to diazinon (control), elevated asymmetry levels in bristle number would faithfully reflect the expected increased DI in populations first

exposed to diazinon, but not some generations later after the *Sc1* locus has exerted its modifying effects (McKenzie 2003). This suggests that the failure to detect FA differences between stressed versus nonstressed populations may sometimes be a consequence of epistatic adaptation that has occurred in the stressed population.

This example also suggests a way in which DI may evolve in spite of the low to zero additive genetic variation typically found for FA. Mutations such as *Rop-1* that are selected because of rapid environmental change clearly can alter DI levels in populations, and in this process, generate additive genetic variation (Cheverud & Routman 1995). Cheverud & Routman (1996) have shown how additive-by-additive (*aa*), additive-by dominance (*ad*), and dominance-by-dominance (*dd*) epistatic types all can contribute to additive genetic variation, especially as populations pass through bottlenecks. The *dd* epistatic type that exerted the greatest effect on FA in molar size (Leamy et al. 2005) produces the maximum additive genetic variance when an allele is fixed at one locus and the frequency of alleles at the other epistatic locus is either 0.15 or 0.85 (Cheverud & Routman 1996). However, the consistently low levels of heritability of FA (Fuller & Houle 2003) suggest any additive genetic variation generated by epistasis is continually eroded, perhaps by selection that favors fixation of alleles at most individual loci affecting FA. Except for unusual population events such as rapid environmental changes caused by various stresses, bottlenecks, and/or hybridizations, epistasis may generally act to suppress additive genetic variation in FA (Leamy et al. 2002).

Although DI may be capable of evolving, another implication of our hypothesis is that this evolution is character-specific, and may be detectable by change in FA levels only in certain characters. Because many genes appear to control only certain characters or character complexes (Cheverud 2000b, Leamy et al. 1999b), it would not be surprising to find that epistatic interactions affecting FA levels also are unique to specific characters. In support of this hypothesis, Leamy et al. (2005) discovered that entirely different QTLs were involved in the epistatic combinations that significantly affected FA levels in mandible size, molar size, and molar shape in mice. Depending on the type, magnitude, and sign of the epistatic effects, FA levels in these characters would be expected to respond differently (increase, decrease, or show no change) to various stressors such as inbreeding or selection (Leamy et al. 2005). Clearly the choice of characters in FA analyses is critical (Indrasamy et al. 2000, Woods et al. 1999) and may account for many ambiguous results in FA studies, including those cases in which no differences in FA levels have been detected between various parental races or subspecies and their hybrid offspring (Alibert & Auffray 2003, Schneider et al. 2003).

A slightly heritable, predominantly epistatic genetic architecture of FA parallels that of fitness components such as litter size and maternal performance (Peripato et al. 2002, 2004) rather than characters such as body size and skeletal size and shape that are affected by many single-locus QTLs each with generally small effects (Cheverud et al. 1996, Klingenberg et al. 2001b, Leamy et al. 1999b, Workman et al. 2002). Further, Gangestad & Thornhill (2003) analyzed the repeatabilities for FA in a number of studies and found them to be consistent with a coefficient of

variation of DI that is within the range for typical fitness characters (Houle 1992). Although neither of these results is definitive, they do suggest that FA may have some kind of role as a fitness indicator. But we need better estimates than typically have been made of potential associations between FA levels, fitness parameters, and epistatic effects within populations. Until we obtain such improved estimates, we should be extremely cautious in the use of FA as an indicator of fitness.

Final Thoughts

Our hypothesis of an epistatic genetical basis of FA amounts to a modern version of some of the basic ideas first promoted by Waddington (1957), Lerner (1954), and other early researchers in this area. The major implication of this finding is that we cannot view FA as a purely environmentally determined character because in many cases epistatic interactions of genes may be important as well. This may well explain why we do not always obtain consistent increases of FA in various characters in populations subjected to various stressors. Perhaps we need to consider performing more experiments using isogenic strains to avoid these sorts of effects. This is practical with laboratory organisms such as mice and *Drosophila*, and may even be possible in wild populations (Kristensen et al. 2004).

All implications of our hypothesis apply only if the hypothesis holds up with additional testing. We need more, and better, tests of the genetical basis of FA. For tests of epistasis, QTL analyses should be especially useful because they are more powerful statistically than line crosses, diallel crosses, or other comparable approaches (see Leamy 2003). QTL analyses also allow us to scan the entire genome of an organism to search for individual genes affecting FA in a character, and if these are found, to test for additive and dominance effects for such genes. Traditional studies estimating the heritability of FA by parent–offspring regression, for example, cannot provide this kind of information and, in fact, are poorly suited even to detect significant additive genetic variation in FA (Fuller & Houle 2003). However, QTL studies are inherently difficult and require substantial amounts of effort and resources, particularly those searching for dominance and epistasis (Carlborg & Haley 2004, Erickson et al. 2004, Lynch & Walsh 1998). Moreover, it is a further substantial challenge to go from a QTL to specific candidate genes (e.g., Flint & Mott 2001). But only when we know the nature of the genes participating in single-locus or two-locus epistatic effects on FA will we be able to test whether they are involved in the development of the bilateral character itself and learn precisely how they affect the overall level of FA.

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