Morphological asymmetry and interspecific hybridization: A case study using hylid frogs

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Abstract

The limited studies addressing developmental stability of interspecific hybrids suggest a positive association between the level of fluctuating asymmetry and 1) the degree of divergence between parental species, and 2) the recency of the contact zone. To evaluate these associations, we examined asymmetry in a recently-established hybrid population of treefrogs (*Hyla cinerea* and *H. gratiosa*) that show marked structural gene divergence. Fluctuating asymmetry (FA), directional asymmetry, and antisymmetry were assessed for eight paired osteometric traits in allozymically-defined parental and hybrid categories. FA levels varied considerably among traits. Nonetheless, for any given trait, the hybrid categories did *not* demonstrate elevated levels of FA compared to the parental categories, or compared to frogs from a non-hybridizing parental population. The only trait that differed statistically among categories (pterygoid length) involved a significantly lower FA value for the F1 hybrids. Thus, observed FA values do not support expectations that the hybrid categories should experience decreased developmental stability.

Introduction

The merit and popularity of fluctuating asymmetry (FA) as an evolutionary tool stem largely from its ease of measurement and its seemingly close connection to developmental stability. Any disruptions along a supposedly ideal developmental trajectory that result in nondirectional deviations among symmetric bilateral traits broadly define FA; accordingly, higher levels of FA imply reduced developmental stability. The terms "nondirectional deviation" and "symmetric bilateral traits" are central in defining FA, for they distinguish it from two other kinds of asymmetry: antisymmetry and directional asymmetry (sensu Van Valen, 1962). Only FA is of consequence in addressing developmental stability. Nonetheless, it is of utmost importance to assess antisymmetry and directional asymmetry statistically, since either form may accompany FA in a given character and therefore may distort FA values.

Over the past two decades, an extensive literature on FA has accumulated, reflecting the technique's versatility and breadth of applicability across various biological disciplines (see Palmer and Strobeck [1986] and Leary and Allendorf [1989] for reviews). Many contributions have centered on the association between FA and levels of heterozygosity, testing the assumption that heterozygosity is positively correlated with developmental stability. Populations characterized by high levels of heterozygosity often possess correspondingly low levels of FA, though this trend is not without inconsistencies as well as some degree of controversy (Palmer and Strobeck, 1986).

Far fewer investigations have used FA to address the effects of interspecific hybridization, despite the opportunity it offers to explore the heterospecific interaction of polygenic factors governing bilateral traits. Rather than implicate heterozygosity per se (indeed, interspecific hybrids represent an extreme heterozygous condition [Vrijenhoek and Lerman, 1982]), researchers examining hybrid asymmetry discuss observed levels of FA in terms of genomic coadaptation (Jackson, 1973a; Graham and Felley, 1985). Specifically, they argue that interactions between independent genomes of distinct taxa may result in decreased developmental stability since the respective genomes have not been selected for mutual coadaptation. Few reports, however, clearly demonstrate increased FA in hybrid populations (Graham and Felley, 1985; Leary et al., 1985); other studies provide equally convincing evidence for normal hybrid development (Jackson, 1973a, 1973b; Felley, 1980). Though seemingly contradictory, these investigations may actually reflect two important, interdependent factors: 1) extent of genetic divergence, and 2) age and stability of the contact (hybrid) zone.

The potential influence of genetic divergence is well illustrated in a series of interspecific crosses between centrarchid fishes (Parker et al., 1985). Parker and his co-workers noted that as genetic distance increased between species pairs, so did their progeny's developmental instability. The reports addressing FA in hybrids are somewhat similar as they also represent comparisons between taxa exhibiting different levels of genetic divergence. For example, Felley (1980) and Jackson (1973a, 1973b) examined hybridization among closely related taxa and failed to detect increased FA in a zone of intergradation between sunfish (*Lepomis*) or in a zone of introgression between lizards (*Sceloporus*), respectively. Graham and Felley (1985) noted that both contact zones are relatively old, possibly dating back to the Pleistocene. Minimal genetic divergence in these taxa coupled with the nature of their contact zones may account for observed developmental stability in each case, conceivably through *secondary* genomic coadaptation (see Graham and Felley, 1985). Conversely, increased FA has been observed in hybrid crosses between taxa with substantial genetic divergence as well as hybrid populations of recent origin (or both) (Graham and Felley, 1985; Leary et al., 1985). Thus, despite limited information on asymmetry in interspecific hybrids, there appears to be some evidence for a positive association between FA and level of divergence and/or recency of contact zone.

To explore these associations further, we evaluate developmental stability in a hybrid population of treefrogs as measured by the FA of eight osteometric traits. Certain aspects of the population in question afford an excellent opportunity to test supposed correlations between FA, genetic divergence and age of contact zones.

First, genetic distances established by electrophoretic ($D = 0.25$; Hedges [1986]), immunological ($ID = 28.5$; Maxson and Wilson [1975]) and mitochondrial DNA ($p = 0.118$; Kessler and Avise [1985]) data indicate that the parental species, *Hyla cinerea* and *H. gratiosa*, have been distinct taxa for some 5–11 million years. Second, the hybrid site is a series of farm ponds no more than 35 years old (or approximately 15 treefrog generations). We therefore hypothesize that the hybrid population should experience developmental instability through genomic disruption, resulting in high FA for the traits examined. Additionally, we use genetically defined categories (e.g., pure parental, F_1, backcross) to address questions concerning FA on a finer scale. For example, do allozymically "pure" parentals in the hybrid population exhibit greater developmental stability than the F_1 hybrids? Do any differences in FA exist between respective backcross categories, suggesting some developmental complications associated with the direction of cross? We also examine whether antisymmetry and/or directional asymmetry account for any of the variation in the chosen characters.

Materials and Methods

Fieldwork was conducted at the Auburn University Fisheries Unit (Lee Co., AL), a complex of 38 artificial ponds where *Hyla cinerea* and *H. gratiosa* have undergone extensive hybridization for over 25 years. More than 300 frogs were collected from April through August, 1984; of these, 192 specimens were used in this investigation. An additional 25 *H. cinerea* from a non-hybridizing population were captured on July 9, 1986, at McKinney's Pond (Emanuel Co., GA). McKinney's Pond is a 200+ year old mill pond adjacent to the Ogeechee River floodplain. As a permanent water source with attendant mesic vegetation, it provides optimal breeding habitat for *H. cinerea* but is effectively avoided by *H. gratiosa*, which typically breeds in temporary upland ponds. Thus it is highly unlikely that hybridization has recently, if ever, occurred at this site.

Electrophoresis

Serum and kidney samples (stored at $-80°$ C) were used for allozyme analysis. Five marker loci, known to exhibit fixed electromorphic differences between "pure" *H. cinerea* and *H. gratiosa* at the Auburn site (Schlefer et al., 1986), were assayed using the procedures of Gerhardt et al. (1980). Albumin (ALB) and phosphoglucoisomerase (PGI-2; E.C. 5.3.1.9) were resolved from serum, while kidney was used for lactate dehydrogenase (LDH-2; 1.1.1.28), malate dehydrogenase (MDH-1; 1.1.1.37) and leucyltyrosine peptidase (PEP-1; 3.4.11.1).

Morphometrics

Our survey centered on cranial osteology. Skulls were cleared and differentially stained according to Wassersug (1976). Only adult male specimens were examined.
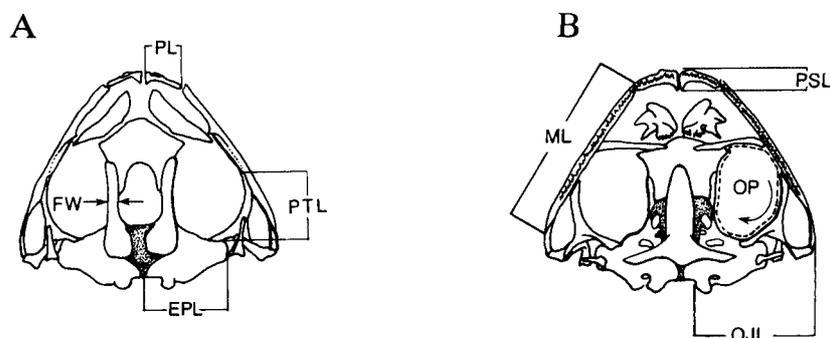
Fig. 1. Schematic drawing of a *Hyla cinerea* skull showing dorsal (A) and ventral (B) views to illustrate the bilateral traits used for analysis. Character abbreviations are defined and their measurements described in the Appendix.

Since little information is available on sexual dimorphism in hylid frogs, we felt it best to eliminate the relatively small sample of females in each population to exclude any possible morphometric complications due to sex. We selected eight paired traits with features demonstrating clear demarcation to allow unambiguous, highly repeatable measurements (Fig. 1). Both right and left sides of each trait were measured to the nearest 0.01 mm using a BioQuant Image Analysis System (R&M Biometrics, Nashville, TN). It should be noted that the actual skulls, as opposed to photographs or transparencies of skulls, were used for image analysis.

The relative contribution of measurement error to FA was assessed by repeated measurements of each side. The second set of measurements was conducted to prevent possible dependence or bias with the first set. That is, replicate measurements were not taken immediately following the original measurements; after the first set of measurements was completed on an individual, the skull was set aside and remeasured at a future time. Image analysis was conducted by D. L. Mahoney. T. Lamb assigned specimen identification numbers and generated sample order using a random numbers table, such that category designation of each skull was unknown to D. L. Mahoney at the time of measurement.

Asymmetry analysis

Although eight characters were measured, high correlations between these traits could reduce the actual number of independent asymmetry estimates. Simple correlation analysis revealed no significant relationships between the left minus right differences for any pair of traits when the acceptance level was adjusted to maintain an experimentwise error rate of 0.05 (Harris 1975:76). A principal component analysis also revealed no interdependencies among the paired differences since only one mensural character loaded significantly on any component. Thus, asymmetry of each trait yields an independent estimate of developmental stability.

Antisymmetry was examined using Kolmogorov-Smirnov tests of the frequency distributions of left-right paired differences compared to an expected normal distribution. In addition, the third and fourth moments (skewness and kurtosis, respectively) of the frequency distributions were tested for deviation from their expectation in a normal distribution using t-tests (Sokal and Rohlf, 1981).

Fluctuating asymmetry, directional asymmetry, and the efficacy of size scaling were analyzed using an analysis of variance (ANOVA) procedure suggested by Leamy (1984) as modified by Palmer and Strobeck (1986). Specifically, it is a mixed model, two way ANOVA with replicated measurements of each side (Table 1a). Thus, the model is a randomized complete-block design, in which sides are a fixed effect and individuals are treated as a random subset of all possible individuals. Differences among allozymically-defined categories in directional asymmetry were tested using a modified ANOVA involving a three way analysis (sides, individuals, categories) with replicated measurements (Table 1b). The interaction of sides × category was used to assess differences in directional asymmetry among categories. Differences among categories in FA were tested using a Scheffé-Box homogeneity of variances test. The pattern of category differences in FA among

Table 1. a) Effects, expected mean squares, and components estimated for the analysis of variance procedure within genetic categories. Note that the appropriate estimate of FA from the analysis is 2 × (interaction mean square − residual error). b) Effects, expected mean squares, and components estimated for the modified analysis of variance to analyze differences among categories in directional asymmetry. For both a and b, M is the number of replicate measurements taken and thus σ_m^2 is the residual measurement error.

a)	ANOVA	
Effect	Expected Mean Square	Estimated Component
Sides (S)	$\sigma_m^2 + M\{\sigma_i^2 + [J/(S-1)]\Sigma\alpha^2\}$	directional asymmetry
Individuals (J)	$\sigma_m^2 + M(\sigma_i^2 + S\sigma_j^2)$	Size or shape variation
Interaction (I) (S × J)	$\sigma_m^2 + M\sigma_i^2$	Antisymmetry, FA/2 + measurement error
Measurements	σ_m^2	Measurement error

b)		
Sides (S)	$\sigma_m^2 + M\{\sigma_i^2 + [J/(S-1)]\Sigma\alpha^2\}$	
Category (G)	$\sigma_m^2 + M\{\sigma_i^2 + [S(\sigma_{jg}^2 + J\Sigma\beta^2)]\}$	
Individuals [Category] (J[G])	$\sigma_m^2 + M(\sigma_i^2 + S\sigma_{jg}^2)$	
Sides × Category (S × G)	$\sigma_m^2 + M\{\sigma_i^2 J\Sigma(\alpha\beta)^2\}$	directional asymmetry differences among categories
Interaction (I) (S × J[G])	$\sigma_m^2 + M\sigma_i^2$	
Measurements (residual error)	σ_m^2	

cranial traits was assessed using Kendall's rank concordance test (Sokal and Rohlf, 1981:607). Degrees of freedom for test statistics are written as subscripts in the text. Significance was accepted when the probability of the value of a test statistic being obtained by random chance was 0.05 or less.

Results

Following electrophoretic assay, frogs from the Auburn population were assigned to one of five provisional categories based on multi-locus genotype: pure *H. cinerea*, pure *H. gratiosa*, F_1 hybrid, *cinerea* backcross, and *gratiosa* backcross. Classification criteria concerning these assignments and the numbers of individuals in each category are presented in Table 2. Since category assignments are based on five diagnostic loci, which appear to be unlinked or at least not tightly linked (Lamb and Avise, 1986), misclassifications are presumably minimal. Of the five categories, the backcross groups are the most likely to contain a few misidentified individuals, primarily F_2s and later-generation hybrids. However, such individuals also may be informative in assessing the developmental stability of hybrid genotypes beyond those of the F_1 progeny. As expected, frogs from McKinney's Pond possessed only *cinerea* alleles at all five marker loci and are henceforth treated as a sixth category termed the McKinney's Pond *cinerea*.

Size scaling, antisymmetry, and measurement error

Trait means and means for left-right differences for all categories are presented in Table 3. Plots of the left-right differences versus size and the main effect of individuals (genotype) in the ANOVA (Table 1a) indicated significant size effects for all characters in all categories. Therefore, all traits were size corrected as:

$$(L - R)/[(L + R)/2]$$

or

$$d/x$$

Table 2. Allozyme categories, category assignments, and classification criteria for the Auburn *Hyla* population.

Provisional Category	N	Category Criteria
Pure *H. cinerea*	55	*cinerea* alleles at all marker loci
Pure *H. gratiosa*	44	*gratiosa* alleles at all marker loci
F_1 hybrid	9	heterozygous for *cinerea/gratiosa* alleles at all marker loci
H. cinerea backcross	43	homozygous for *cinerea* alleles at 1 to 4 loci, heterozygous for *cinerea/gratiosa* alleles at remaining loci
H. gratiosa	35	homozygous for *gratiosa* alleles at 1 to 4 loci, heterozygous for *cinerea/gratiosa* alleles at remaining loci

Table 3. Means (and standard errors) of traits and left-right differences for the eight osteometric traits for the Auburn and McKinney's Pond treefrogs. Sample sizes, which vary among categories for each trait listing (due to damage of particular cranial elements), represent the number of individuals measured though actual means are calculated over both replications for each individual.

| Trait | Provisional Category | | | | | |
	Pure *H. cinerea*	Pure *H. gratiosa*	F_1 Hybrid	*H. cinerea* Backcross	*H. gratiosa* Backcross	McKinney's Pond *H. cinerea*
OP $\bar{x}$	17.27	21.54	19.27	18.20	20.16	17.07
	(0.100)	(0.138)	(0.157)	(0.157)	(0.116)	(0.132)
L − R	−0.252	−0.405	−0.348	−0.297	−0.31	−0.249
	(0.044)	(0.048)	(0.076)	(0.046)	(0.049)	(0.066)
N	51	43	19	42	34	25
ML	12.11	15.43	13.77	12.58	14.22	12.23
	(0.088)	(0.123)	(0.132)	(0.137)	(0.102)	(0.140)
	0.033	0.277	0.187	0.155	0.111	0.045
	(0.036)	(0.058)	(0.049)	(0.039)	(0.044)	(0.064)
	43	35	15	36	30	21
PTL	4.56	6.75	5.56	4.88	5.86	4.70
	(0.034)	(0.096)	(0.073)	(0.084)	(0.069)	(0.076)
	0.103	0.016	0.032	0.044	0.062	0.088
	(0.020)	(0.037)	(0.029)	(0.025)	(0.024)	(0.032)
	45	37	15	40	31	22
FW	0.57	0.71	0.61	0.59	0.67	0.57
	(0.008)	(0.016)	(0.015)	(0.011)	(0.012)	(0.014)
	−0.022	0.002	−0.026	−0.030	−0.024	−0.008
	(0.006)	(0.008)	(0.010)	(0.007)	(0.006)	(0.008)
	53	42	17	41	35	25
PSL	0.95	1.34	1.07	1.05	1.24	0.95
	(0.011)	(0.017)	(0.019)	(0.021)	(0.015)	(0.017)
	0.006	0.014	0.002	0.007	0.013	0.016
	(0.005)	(0.007)	(0.010)	(0.006)	(0.008)	(0.006)
	51	42	18	41	35	25
PL	2.36	3.00	2.63	2.47	2.81	2.25
	(0.016)	(0.021)	(0.033)	(0.027)	(0.020)	(0.024)
	−0.025	0.037	−0.028	0.002	−0.012	0.014
	(0.012)	(0.018)	(0.020)	(0.011)	(0.015)	(0.017)
	51	40	18	42	35	25
EPL	4.55	6.35	5.32	4.93	5.66	4.54
	(0.030)	(0.067)	(0.069)	(0.062)	(0.066)	(0.040)
	0.150	0.021	0.112	0.156	0.137	0.184
	(0.022)	(0.029)	(0.042)	(0.026)	(0.032)	(0.024)
	53	43	19	42	35	25
OJL	7.06	10.01	8.38	7.55	9.10	6.99
	(0.052)	(0.099)	(0.103)	(0.100)	(0.083)	(0.072)
	−0.004	0.270	0.021	0.017	0.162	0.021
	(0.024)	(0.044)	(0.046)	(0.028)	(0.044)	(0.035)
	46	38	16	39	31	23

which is simply an individual's left-right difference for a trait divided by the mean size of the trait. All further analyses were performed on size-corrected values.

Antisymmetry (the consistent enlargement of one side of a bilateral trait where the enlarged side varies randomly between individuals) results in deviations from normality in the frequency distribution of left-right paired differences as explained by Van Valen (1962). After adjusting the level of acceptance for an experimentwise error rate of 0.05, there were no significant deviations from normality indicated by the Kolmogorov-Smirnov tests or the t-tests of skewness and kurtosis for any of the traits in any category. Thus there is no indication of antisymmetry for the eight cranial traits.

Measurement error was assessed by an F test of interaction mean square divided by error mean square (Table 1a). Thus, we are testing whether our FA estimate is significantly larger than what we would predict given sampling error alone. Of 48 possible F tests (8 traits by 6 categories), 46 yielded p values $\leqslant 0.0001$, one had $p \leqslant 0.0002$, one had $p \leqslant 0.0008$, and the overall experimentwise error rate was $p \leqslant 0.038$. Thus, we conclude that our estimates of FA are reliable and are not mere artifacts of measurement error.

Directional asymmetry

Directional asymmetry exists when the same side of a bilateral trait is larger than its counterpart for most members of a population and results in the mean of the frequency distribution of left-right paired differences being displaced from zero. The main effect of sides in the ANOVA estimates directional asymmetry (Table 1a) and the sides × category interaction tests for differences among the groups in directional asymmetry (Table 1b).

Using acceptance levels adjusted for an experimentwise error rate of 0.05, the following patterns were observed (see Fig. 2). FW, ML, PL, PSL and PTL show no significant directional asymmetry values for any categories and no differences among categories. OP exhibits significant values of directional asymmetry in all categories from the Auburn population, but the amount of directional asymmetry does not differ significantly among categories (F test − Sides × Category MS/Interaction MS; Table 1b). EPL exhibits significant directional asymmetry for *H. cinerea*, *cinerea* backcrosses, and McKinney's pond *cinerea* as well as significant (comparisonwise) directional asymmetry differences among categories. However, the differences among categories are not significant at an experimentwise error rate of 0.05. OJL shows significant directional asymmetry for *H. gratiosa* and significant differences (comparisonwise and experimentwise) among categories. A MANOVA using all osteometric traits as dependent variables showed no significant sides × category interaction (Wilk's λ, $F_{40, 687.14} = 1.38$, $p > 0.05$).

The ANOVA procedure removes the main effect of directional asymmetry from the final estimate of FA. Thus an individual's size and directional asymmetry
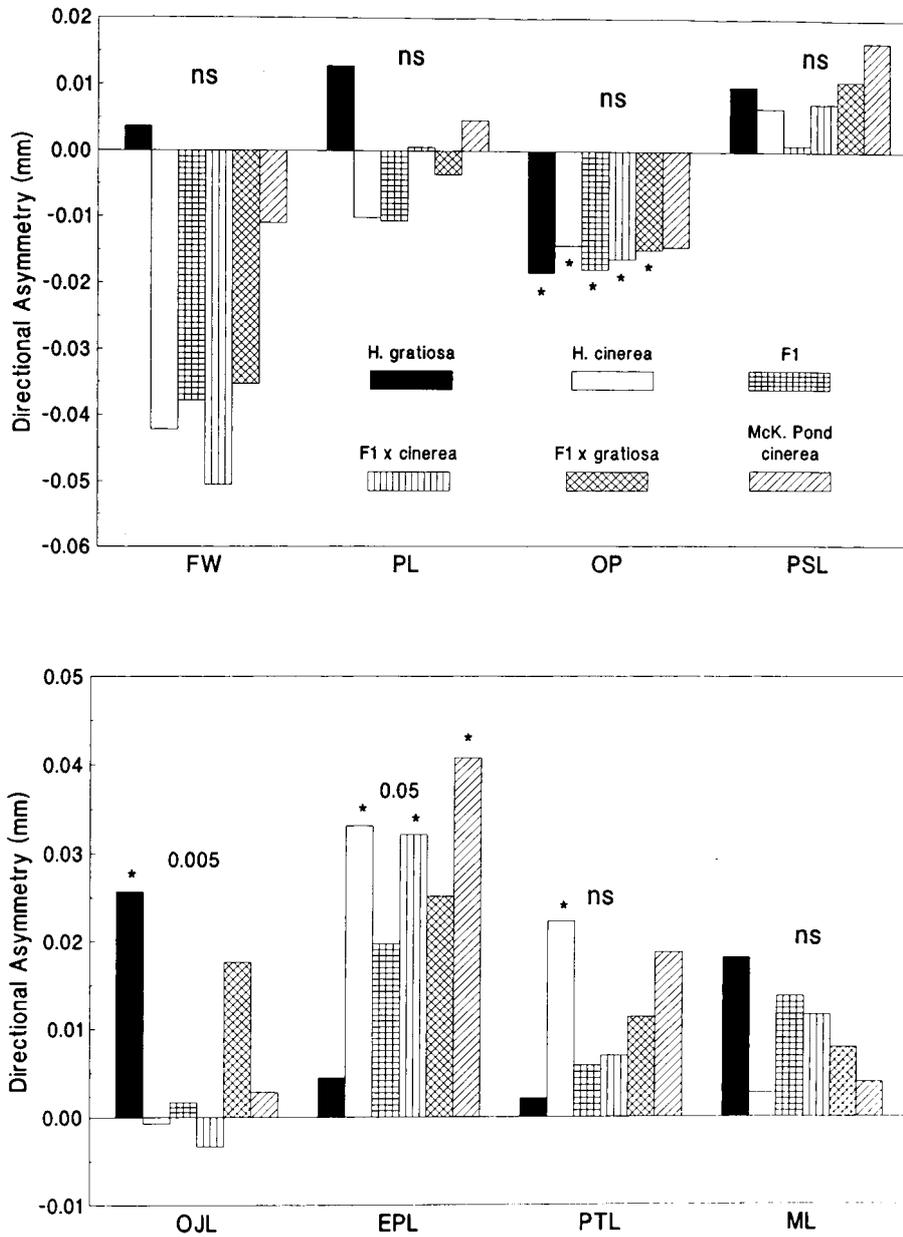
Fig. 2. Directional asymmetry estimates (least square mean differences (L R)) for the eight cranial traits. Numbers above the bars are the probability that the F value for the sides × category interaction could be expected due to random chance. Asterisks indicate that directional asymmetry was significant in a particular category for a given trait.

corrected value may be written as:

$$(L - R)/[(L + R)/2] - \{\Sigma[(L - R)/(L + R)/2]\}/n - 1$$

or

$$d/x - [\Sigma(d/x)/n - 1]$$

This has the effect of correcting the distribution of differences back to a mean of zero. If an individual's values are needed, it is important that corrections for directional asymmetry be applied before further analyses are performed.

Fluctuating asymmetry

FA (random size deviations between bilaterally paired traits) results in a frequency distribution of left-right differences with a mean of zero, variance greater than zero, and skewness and kurtosis of zero. Thus, this definition makes it clear that FA should be viewed as a variance measure. The ANOVA procedure estimates FA as two times the quantity of the interaction mean square $(\sigma_m^2 + m\sigma_i^2)$ minus measurement error (σ_m^2). Individual FA estimates may be obtained by subtracting the residuals obtained by fitting a model in which the interaction term is included from the residuals obtained from a model in which the interaction term is omitted.

As noted earlier, all FA values were significantly greater than the variance expected due to measurement error alone. The absolute values of FA varied considerably (over a range of 1.5 orders of magnitude) from trait to trait. Nonetheless, no significant differences in FA among categories were detected for seven of the eight osteometric traits (Fig. 3). For the eighth trait (PTL) the F_1 hybrids exhibited significantly lower FA compared to the other categories (Fig. 3). Moreover, when the acceptance level for an individual test was adjusted to maintain an experimentwise error rate of 0.05, none of the traits exhibited significant heterogeneity of estimated FA levels among categories. Finally, Kendall's rank concordance test failed to detect consistent ranking of the categories among the traits ($W_s = 0.104$, $p > 0.50$).

In light of these results, we collapsed the pure *H. cinerea* and *H. gratiosa* categories into a single "parental" category, and the F_1 and backcross categories into a single "hybrid" category. The analysis for FA was then repeated for a "parental" vs. "hybrid" comparison. The results of this alternative procedure were essentially identical to the multiple category comparisons: no significant differences in FA were detected for any of the eight traits.

Discussion

Our hypothesis that the Auburn *Hyla* hybrids should experience decreased developmental stability through disruption of genomic coadaptation is not supported by observed FA values. These results were somewhat surprising, given the
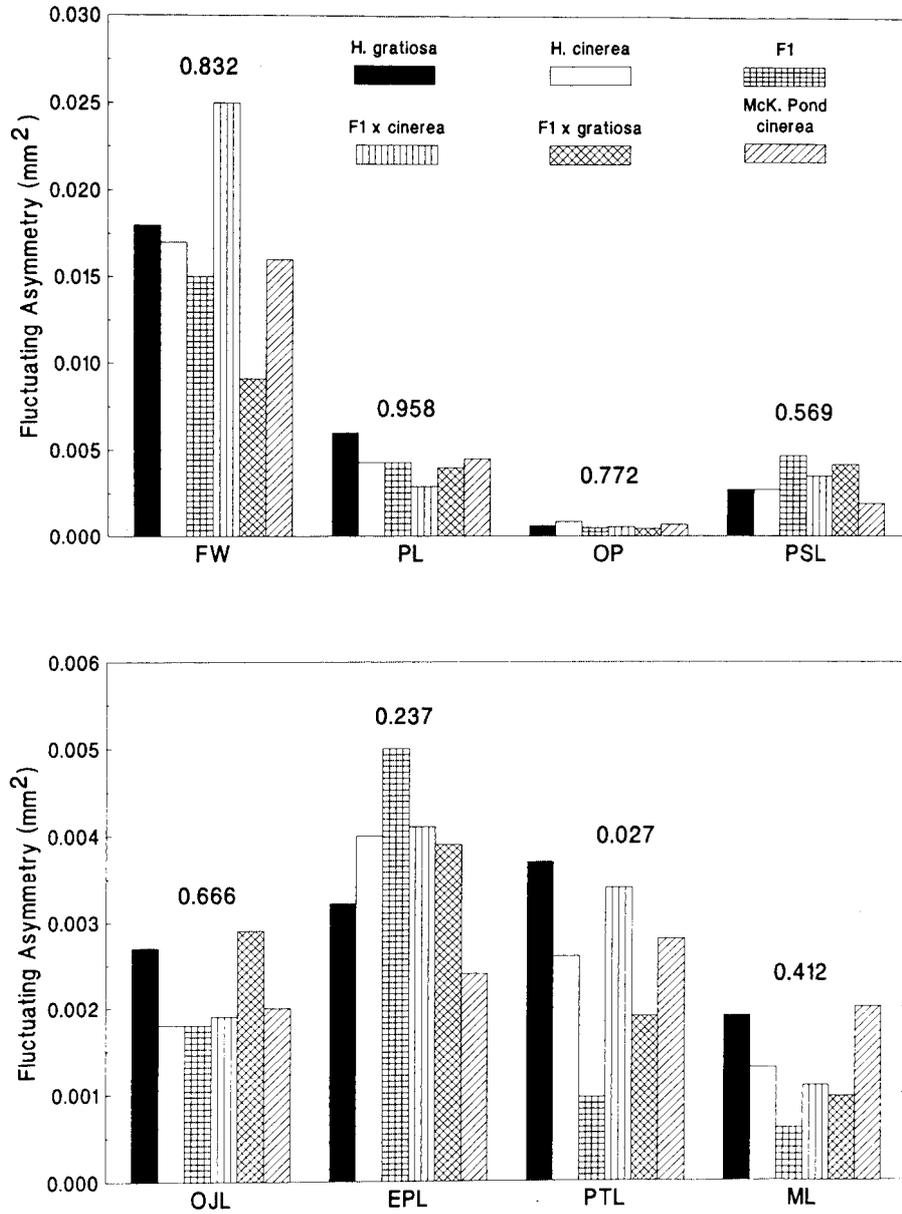
Fig. 3. Fluctuating asymmetry estimates for the eight cranial traits. Numbers above the bars are the probability that fluctuating asymmetry differs among categories using the Scheffé-Box test for homogeneity of variances.

relatively high level of genetic divergence between the parental species and the recency of hybridization. Furthermore, previous genetic and physiological data suggest some degree of selective disadvantage in the Auburn hybrids (Schlefer et al., 1986). Although the absence of higher FA values for the hybrid categories across all characters is open to broad interpretation, we address three tenable explanations.

Prefatory to other possible considerations are the character choices themselves: are these traits so highly canalized that they are not capable of revealing differences in developmental stability? We do not believe so in this particular case. Cranial traits have proved informative in numerous FA studies, especially for mammals (Soulé and Couzin-Roudy, 1982; Leamy, 1984; McGrath et al., 1984; Pankakoski, 1985; Wayne et al., 1986). Unlike mammals, most anurans possess highly fenestrate skulls in which many articulations consist of narrow, kinetic sutures. Furthermore, some bones (and portions of others) are suspended in a cartilaginous matrix. Thus, anuran development may not require the precision needed for suture formation in compact mammalian skulls. In support of this view, our results do demonstrate FA for all characters assayed, including relatively high levels for FW. Therefore, we believe that higher FA values in hybrids would be realized in the given character set if substantive developmental problems existed.

A second possible explanation for equivalent FA values among hybrid and parental groups involves selective larval mortality. Differences in genetic composition between larval and adult hybrids could be created by selective forces eliminating developmentally unstable phenotypes during larval and metamorphic stages. As a result, adult hybrids would represent a nonrandom subset of genotypic combinations that are minimally perturbed through hybridization. Mecham (1960) conducted a series of 22 cinerea-gratiosa crosses, including reciprocal crosses between the parentals as well as various backcrosses, and found no clear reduction in development of cleaved eggs. Larvae from all 22 pairings underwent metamorphosis without any evidence of abnormalities. Mecham's results offer little evidence that differential larval survival is associated with levels of compatibility among various parental-hybrid combinations. Furthermore, egg and embryo predation form a large component of premetamorphic mortality in anurans without parental care (see Howard, 1978); at these stages, genotypic contributions to differential survivorship are essentially negligible. Potentially great genotypic disparities would likely be obfuscated by these aspects of larval mortality. Given the proportion of F_1 and backcross individuals in our sample ($N = 96$; 50%), it seems improbable that such a large hybrid contingent results from a few "favored" genotypes.

As a third alternative, the FA comparisons may indicate a reasonably high level of genomic integration between the parental species, such that F_1 and backcross individuals do not experience greatly reduced developmental stability. Normal sperm production in hybrids (Fortman and Altig, 1974) and the parental-hybrid cross experiments of Mecham (1960) lend support to this view. Though the mechanisms allowing compatibility are unknown, certain theoretical possibilities bear consideration. First, amphibians as a group show a very slow loss of interspecific hybridization potential with increasing genetic divergence, presumably due to conserved chromosomal and/or regulatory evolution (Wilson et al., 1974).

Such may be the case with *H. cinerea* and *H. gratiosa*. Stable hybrid zones have been documented for several species pairs showing comparable if not greater genetic distance values than those observed in *H. cinerea* and *H. gratiosa*, e.g., Arntzen (1978), Uzzell (1979), Maxson (1981), and Szymura (1983). Not surprisingly, reproductive isolation often entails effective premating mechanisms in amphibians and is based largely on behavior in the form of visual, acoustic or pheromonal cues.

Second, developmental compatibility may also reflect the idiosyncratic nature of the *cinerea-gratiosa* pairing itself. For example, Parker et al. (1985) noted that even though a negative trend exists between structural gene divergence and hybrid developmental success in centrarchid fish, there are exceptions to this pattern. Specifically, the genetic distance between the largemouth bass, *Micropterus salmoides*, and the redear sunfish, *Lepomis microlophus*, (Nei's $D = 0.648$) is greater than all other *Micropterus-Lepomis* comparisons ($D = 0.364 - 0.540$), yet largemouth-redear hybrids experience much greater developmental success than do progeny from any of the other *Micropterus-Lepomis* crosses. Such an "uncoupling" of structural and regulatory evolution underscores the independent changes occurring in species, as well as the idiosyncrasies of hybridization potential and developmental stability in species pairs.

In closing, although we provide evidence for stable development in certain *cinerea-gratiosa* progeny, we do not infer that this "stability" extends to overall hybrid fitness or to all possible classes of hybrids. For example, F_2 hybrids may be more likely to experience developmental complications than F_1s or backcrosses, since polygenic complexes are prone to be broken up by recombination in F_2s. As mentioned previously, the number of later generation hybrids at Auburn may be limited (Schlefer et al., 1986), and the population has an excess of pure parental genotypes relative to Hardy-Weinberg expectations (Lamb and Avise, 1986). Possible selective disadvantages may not be apparent during morphogenesis but could operate instead through some combination of behavioral, ecological, and/or physiological factors. Finally, it is not our intent to refute the view that developmental instability should be higher in recently established hybrid populations compared to relatively old contact zones. Nonetheless, our results, in conjunction with previous surveys cited herein, indicate that the degree FA expressed in interspecific hybrids is likely to be highly dependent on the particular situation analyzed. We hope that these findings will elicit future investigations of FA variation in hybrid populations and its bearing on genomic coadaptation.

Acknowledgements

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Appendix

OP: orbital perimeter; ventral aspect of the orbital foramen formed by the palatine, maxilla, pterygoid and frontoparietal margins.

ML: maxilla length; along the lateral curvature of the maxilla from maxilla-premaxilla juncture to the posterior-most aspect to the maxilla-quadratojugal juncture.

PTL: pterygoid length; ventrally, straight-line distance from the maxilla-pterygoid juncture to posterior-most aspect of the quadratojugal-pterygoid juncture.

FW: frontoparietal width; dorsally, at narrowest point midway along frontoparietal.

PSL: premaxilla spur length; ventrally, straight-line distance from the anterior-most aspect of the premaxilla to the posterior tip of the medial spur.

PL: premaxilla length; straight-line distance from medial aspect of premaxilla to the maxilla-premaxilla juncture.

EPL: exoccipital-prootic length; straight-line distance from foramen magnum to prootic-squamosal juncture.

OJL: occipital condyle-jaw length; straight-line distance from medial aspect of occipital condyle to posterolateral aspect of quadratojugal.