

- HINTON, H. E. 1981. *Biology of Insect Eggs*, Vol. 1. Pergamon, Oxford, U.K.
- MANS, R. J., AND G. D. NOVELLI. 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. *Arch. Biochem. Biophys.* 94:48–53.
- MARKOW, T. A., AND P. F. ANKNEY. 1984. *Drosophila* males contribute to oogenesis in a multiple mating species. *Science* 224:302–303.
- MULLINS, D. E., AND C. B. KEIL. 1980. Paternal investment of urates in cockroaches. *Nature* 283:567–569.
- PARKER, G. A. 1970. Sperm competition and its evolutionary consequences in insects. *Biol. Rev.* 45: 525–567.
- PROUT, T., AND J. BUNDGAARD. 1977. The population genetics of sperm displacement. *Genetics* 85: 95–124.
- TURNER, M. E., AND W. W. ANDERSON. 1983. Multiple mating and female fitness in *Drosophila pseudoobscura*. *Evolution* 37:714–723.

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FLUCTUATING ASYMMETRY IN THE CHEETAH: METHODOLOGICAL AND INTERPRETIVE CONCERNS

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A recent paper by Wayne et al. (1986) presented the results of a morphometric analysis of four felid species with special reference to the cheetah (*Acinonyx jubatus*). The goals of that paper included: 1) a comparative analysis of asymmetry in these four species, and 2) an evaluation of the concordance between asymmetry levels and genic variability within species. We believe that their data may be insufficient and that their analyses have certain methodological and interpretive shortcomings.

Two independent treatment factors (sex and species) were recognized by the authors as potentially affecting differences in asymmetry values among individuals. Two one-way ANOVAs (one for sex, one for species) were calculated for each of the 16 mensural characters (a total of 32 one-way ANOVAs). Both Duncan's and SNK tests were performed when the ANOVA was significant. Because only two of the 16 characters yielded significant ANOVAs for sex, males and females were pooled for comparisons of species. Significant differences among species were subsequently ascertained for six of the 16 characters. A composite asymmetry index was calculated for each species, ignoring possible sex-associated differences, based on the sum of the absolute values of the log of the ratio of left and right sides of the skull for each character divided by the total number of characters. A one-way ANOVA was then utilized to evaluate possible differences among species in mean composite asymmetry index. Again, both SNK and Duncan's tests were used to identify where differences exist among taxa because the ANOVA was significant.

We believe that both the composite asymmetry index and the 32 one-way ANOVA methodologies are inappropriate for evaluating statistically significant differences among groups. If we assume that a series of ANOVAs can evaluate overall differences in asymmetry (but see below), then differences ascribed to sex

and species ought to be evaluated by 16 two-way ANOVAs rather than 16 one-way ANOVAs for sex and 16 one-way ANOVAs for species. The two-way approach has two advantages over the authors' methodology. First, the existence of a sex-by-species interaction can be evaluated. Second, the two-way approach is more powerful for detecting true main-treatment effects (either species or sex); for example, detecting a treatment effect due to species is facilitated because the residual sum of squares is reduced by controlling for sex and the sex-by-species interaction.

More important, the series of univariate ANOVAs is not the appropriate statistical test for evaluating differences in overall asymmetry when various degrees of interdependence exist among the 16 characters (for an exposition of univariate vs. multivariate analyses in biological research see Jolicoeur, 1959, 1984; Kramer, 1972; Pimentel, 1979; Baron and Jolicoeur, 1980; Jolicoeur et al., 1984; Cheverud et al., 1985). When sample sizes are small, it is not surprising to find few significant correlations among asymmetry values. It is impossible to evaluate whether most nonsignificant results obtained by Wayne et al. (1986) approached the 0.05 level of significance because the matrix of intercharacter correlations was not reported. Moreover, a joint test of significance would seem to be a more convincing test. A two-way MANOVA (species vs. sex) for all 16 characters should be the methodology of choice because it controls for the various degrees of correlation among characters in assessing overall significance. MANOVA is clearly superior to the univariate approach; in addition, it does not suffer from the shortcomings of the composite asymmetry index. Such an index, if it is to be informative, assumes a coincident distribution of character-specific asymmetry values within the groups that are being compared. A simple example, in the extreme, illustrates the problems inherent in the com-

posite asymmetry index. If four characters were examined in two taxa, one could fail to detect significance based on a one-way ANOVA with the composite asymmetry index as dependent variable, if all individuals from one taxon had character-specific asymmetry values of 0.9, 0.9, 0.0, 0.0, and the individuals in the other taxon had values of 0.0, 0.0, 0.9, 0.9. The taxa would be considered indistinguishable for asymmetry (composite asymmetry indexes for each taxon equal at 0.45), even though clear taxon-associated differences in character-specific asymmetry exist. In addition, the composite asymmetry index ignores the effects of various degrees of correlation among characters. The authors produce a matrix of 120 pair-wise comparisons of character-specific asymmetry indexes. It is unclear if this matrix was produced for the cheetah only, for all four taxa combined, or for each taxon separately. Wording in the text would seem to preclude the last option, and neither of the former two possibilities validate the use of numerous one-way ANOVAs or composite asymmetry indexes.

Although the authors have properly evaluated homoscedasticity for their morphological data, they did not do so before performing the ANOVA and a posteriori tests for the composite asymmetry indexes. We evaluated the homoscedasticity assumption for the data in their table 3 using the Bartlett-Box F test (SPSS program: ONEWAY; Nie et al., 1975). The results are highly significant ($F = 3.923, 0.01 < P < 0.001$), indicating that the treatment group variances are unequal. The overall ANOVA is fairly robust with respect to deviations from homoscedasticity; however, the SNK test is not reliable when variances are heteroscedastic (Sokal and Rohlf, 1981) and Duncan's test yields higher comparison-wise error rates than many multiple-range tests including the SNK test (Boardman and Moffitt, 1971). We therefore analyzed the data for mean differences in the composite asymmetry index (their table 1; note the apparent computational error in their calculation of the mean composite asymmetry index for the margay) using the Games and Howell method which permits unequal sample sizes and unequal variances (Sokal and Rohlf, 1981). Our results are not in accord with those presented by the authors. The degree of asymmetry, as measured by the composite asymmetry index, is indistinguishable in the cheetah and leopard (Fig. 1). Therefore, the relation between the composite asymmetry index and the degree of genic variation is more complex than the authors suggest.

In addition, we question the authors' use of the term "fluctuating asymmetry." Far from being a trivial point of semantics, this is crucial to their thesis. As the authors point out, it is fluctuating asymmetry *only* which has been suggested to result from poorly coadapted gene complexes (presumably including inbreeding-induced monomorphism). Although the authors cite some of the seminal work in this area, they overlook important aspects of these articles. Especially critical are the articles by Van Valen (1962) and Soulé (1967) in which the mathematical relationships of three modes of asymmetry are distinguished. Briefly, these are: 1) directional asymmetry, 2) antisymmetry, and 3) fluctuating asymmetry. Directional asymmetry in a population results in a nonzero mean of left minus right ($L - R$) values. Antisymmetry results in deviation from a normal distribution in the form of either skewness

GH				
SPECIES	C	L	M	O
\bar{y}	8.24	6.20	5.56	5.48
$S_{\bar{y}}$	0.45	0.59	0.50	0.27
n	33	21	19	23
SNK				
SPECIES	C	L	M	O

FIG. 1. Descriptive statistics (\bar{Y} : mean, $S_{\bar{Y}}$: standard error of the mean, n: sample size) for the cheetah (C), leopard (L), margay (M), and ocelot (O) are based upon the composite asymmetry index. The result from the Games and Howell method (GH) is portrayed at the top of the figure, and the result from the SNK and Duncan's tests (SNK) is illustrated at the bottom of the figure. Each focal mean, represented by a dot, is indistinguishable from other treatment-group means that lie within the range of the horizontal bar that passes through it (experiment-wise error rate = 0.05).

or kurtosis. The important point is that these two forms of asymmetry are intrinsically population parameters (each is expressed as a predictive function for the population).

To evaluate fluctuating asymmetry alone (which is the presumed objective of the authors), one must first correct the population data matrix for any directional asymmetry or antisymmetry which is detected in the population. As clearly stated by Soulé (1967), "Asymmetry is fluctuating if the signed differences between paired structures are normally distributed with mean zero." Although at first glance the authors' use of a log transformation might be thought to achieve these corrections, we would point out that their technique could only correct for skewness (and in one direction only), and that this correction would be imprecise at best. Thus, even if the authors had utilized a more appropriate statistical analysis of the asymmetry values as discussed above, their results would have had little relevance to the question at hand (i.e., the correlation of genetic monomorphism with fluctuating asymmetry) because of their conceptual confusion concerning all three types of asymmetry. Until proper statistical techniques are applied to adequate data, discussion of the potential genic correlates of asymmetry is not well founded.

LITERATURE CITED

- BARON, G., AND P. JOLICOEUR. 1980. Brain structure in Chiroptera: Some multivariate trends. *Evolution* 34:386-393.
- BOARDMAN, T. J., AND D. R. MOFFITT. 1971. Graphical Monte Carlo type I error rates for multiple comparison procedures. *Biometrics* 27:738-744.
- CHEVERUD, J. M., M. M. DOW, AND W. LEUTENEGGER. 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: Sexual dimorphism in body weight among primates. *Evolution* 39:1335-1352.
- JOLICOEUR, P. 1959. Multivariate geographical vari-

- ation in the wolf, *Canis lupus* L. *Evolution* 13:283–299.
- . 1984. Principal components, factor analysis, and multivariate allometry: A small-sample direction test. *Biometrics* 40:685–690.
- JOLICOEUR, P., P. PIRLOT, G. BARON, AND H. STEPHAN. 1984. Brain structure and correlation patterns in Insectivora, Chiroptera, and Primates. *Syst. Zool.* 33:14–29.
- KRAMER, C. Y. 1972. *A First Course in Methods of Multivariate Analysis*. Kramer, Blackburg, VA.
- NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. *SPSS: Statistical Package for the Social Sciences*. McGraw-Hill, N.Y.
- PIMENTEL, R. A. 1979. *Morphometrics*. Kendall/Hunt, Dubuque, IA.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*. Freeman, San Francisco, CA.
- SOULÉ, M. E. 1967. Phenetics of natural populations. II. Asymmetry and evolution in a lizard. *Amer. Natur.* 101:141–160.
- VAN VALEN, L. 1962. A study of fluctuating asymmetry. *Evolution* 16:125–142.
- WAYNE, R. K., W. S. MODI, AND S. J. O'BRIEN. 1986. Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*), a genetically uniform species. *Evolution* 40:78–85.

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ANALYSIS OF FLUCTUATING ASYMMETRY IN CHEETAHS

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Willig and Owen (1987) have raised methodological objections to our recent report which estimated the extent of fluctuating asymmetry in the genetically monomorphic cheetah (Wayne et al., 1986). We reported an increased incidence of fluctuating asymmetry based on 16 morphologic measurements when the cheetah was compared to three other genetically variable cat species (leopard, margay, and ocelot). Willig and Owen raise a series of statistical objections which are actually either inaccurate or inappropriate in each case. We refute each of the criticisms they raise and stand by our interpretations and conclusions. The specific difficulties inherent in their critique are discussed below.

First, they object to our utilization of two separate one-way analyses of variance (ANOVAs) as opposed to a single two-way ANOVA for analyzing sex and species effects. The primary thrust of our paper was to test for species differences in asymmetry values. We carried out a preliminary test for sex effects even though some specimens were unidentified with respect to sex and therefore were not included (see *Specimens Examined* section, p. 80). This analysis demonstrated nonsignificance and enabled us to increase our sample sizes in the subsequent species-level analyses by pooling specimens regardless of sex. Although we recognize the value of partitioning total variance as meaningfully as possible by considering all main effects and their interaction in an appropriate design, Willig and Owens' suggested usage of a two-way analysis (sex-by-species) would have required exclusion of specimens of unknown sex (about 25% of the total number available). Thus, by pooling specimens we had a more robust estimate of interspecific differences, which was the objective of the study.

Second, they feel that a multivariate analysis of variance (MANOVA) would have been more appropriate than our ANOVAs, since the former approach considers intercharacter correlations whereas the latter does not, and because the shortcomings inherent in a composite asymmetry index are circumvented (discussed below). We disagree with their comments for three reasons. First, independence among characters is apparent when correlation matrices are calculated separately for each species or when all four species are pooled. We provided results on this latter analysis. Second, as indicated in tables 1 and 2, missing values are present for various characters among many specimens. The calculation of a multivariate test statistic, such as Wilks' lambda, requires the generation of dispersion matrices which include covariance terms. In order to calculate such covariances, it would have been necessary to have included only specimens without missing data. Such complete skulls were very rare due to damage incurred during collection and preservation. Alternatively, we could have estimated missing values using any of a number of schemes. The former option was unacceptable since we did not wish to reduce the size of our data set; the latter was not exercised because results based on estimated data are often difficult to interpret. This problem in interpretation would have been particularly exacerbated in our case since the average number of missing values differs between species, such that biases imposed by using estimated data would be unequal between species. Finally, although a MANOVA provides a robust estimate of overall differences, it provides no information regarding which particular characters contribute to such significance, whereas the univariate approach does. We clearly stated, "Asymmetry was not equivalent for all skull measurements.