Analysis of asymmetries in the African fruit bats *Eidolon helvum* and *Rousettus egyptiacus* (Mammalia: Megachiroptera) from the islands of the Gulf of Guinea. II. Integration and levels of multivariate fluctuating asymmetry across a geographical range

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geographical variation;
Gulf of Guinea;
*Rousettus egyptiacus*.

Abstract

Using a set of cranial morphometric characters, trends of variation in multivariate fluctuating asymmetry were evaluated and compared in populations of African fruit bats *Rousettus egyptiacus* and *Eidolon helvum* from the Gulf of Guinea islands, and the adjacent mainland. Levels of asymmetry were compared across populations and species, and significant differences were found in both comparisons. Differences coincided with species-specific patterns of morphological and genetic differentiation. Concordance of correlation matrices of asymmetry was also compared. Results were significant: concordance is hypothesized to be a by-product of developmental processes that produce the 'fox-like' morphology shared by these species. Consistency of asymmetry patterns suggests that the developmental pathway producing it is highly canalized. A prediction of the above hypothesis is that a radical change in the 'fox-like' structural pattern would result in breakage of the asymmetry parameter associated with it.

Introduction

Fluctuating asymmetry (FA) is a pattern of variation in which asymmetry values are normally distributed around a mean of zero (Palmer, 1994). Traditionally, FA has been considered the product of random ‘errors’ in development, and thus a measure of developmental stability (Palmer & Strobeck, 1986, 1992; Palmer, 1994). The biological significance of FA is a matter under debate in the current literature on asymmetry (Graham et al., 1993; Hutchinson & Cheverud, 1995; Kraak, 1997; Graham et al., 1998). The basic assumption has been that high levels of FA are associated with disruptions of genetic or environmental origin, and with a lower capacity of individuals to cope with the environment (Palmer & Strobeck, 1986; Parsons, 1990; Palmer, 1994; Markov, 1995). More recently, it has been suggested that increased FA may not be necessarily an expression of low ‘quality’, but rather another expression of phenotypic variability, specifically a bet-hedging strategy (Simons & Johnston, 1997). Notwithstanding methodological problems (Palmer & Strobeck, 1986, 1992; Palmer, 1994), there is a large body of evidence indicating that genetic or environmental variation among populations often is reflected in the amount and direction of variation between sides of bilateral characters (Palmer & Strobeck, 1986; Markov, 1995; Möller & Thornhill, 1998).

Determined or stochastic, the expression of asymmetry is ultimately the result of a genetic program translating into an integrated, functional whole, and associations with genetic, developmental or morphological patterns can be expected. Morphological patterns result from developmental processes working in concert on different
body parts to produce a functional structure. Perturbations in the developmental environment affecting specific bilateral characters in a similar way should be reflected in a significant association or integration of the asymmetries for these characters, regardless of the source of the perturbation (Soulé, 1967; Leamy, 1994). Characters that are developmentally or functionally related are expected to be more highly correlated than characters without this relationship, this being the basic hypothesis of morphological integration (Leamy, 1977, 1994; Cheverud, 1982). Concordance of FAs at the individual level indicates the presence of ‘individual asymmetry parameters’ or IAPs (Soulé, 1967; Leamy, 1984, 1994). Analogously, concordance of character FAs across populations is indicative of a ‘population asymmetry parameter’ or PAP (Soulé & Baker, 1968; Leamy, 1992, 1994). Presence of a PAP would indicate that levels of asymmetry of a set of characters in population A would bear the same relationship to one another as in population B (Soulé, 1967). Significant IAPs are rare in the literature, but significant PAPs have been detected for some populations (Soulé, 1967; Soulé & Baker, 1968; Leamy, 1994; but see Jackson, 1973). A significant PAP would suggest that whichever the mechanism controlling the levels of developmental noise within a population, it acts across the whole phenotype (or a section of it), and indirectly in all components of the gene pool (Soulé, 1967).

Comparative studies of FA among populations in island–mainland systems are scarce. Isolation by distance, immigration of ‘foreign’ genes into islands, higher levels of inbreeding in insular populations, and varying degrees of habitat heterogeneity across these systems may affect coadaptation, and hence asymmetry, in island populations. Nonetheless, the evidence available is inconclusive and no general trends have been detected (Soulé, 1967; Sarre & Dean, 1991). This study focuses on patterns of variation in cranial FA across populations in two species of African fruit bats (Eidolon helvum and Rousettus aegyptiacus). Both species are widespread in continental Africa, and also occur in the islands of the Gulf of Guinea, West Africa. Previous research on these species has shown unique patterns of morphological and genetic variation across the islands (Juste & Ibáñez, 1993; Juste et al., 1996, 2000). We used a set of bilateral morphometric characters to compare multivariate patterns of FA within and between species, and with other data sets. We addressed the following specific questions: (1) Are levels of FA correlated within and across populations? (2) Are there significant differences in levels of FA among populations or between species? (3) Are there any geographical patterns of variation in FA, and if they exist, do they reflect other morphological and genetic patterns of variation in these species? (4) Are asymmetry patterns consistent across species, or have the patterns in each species evolved independently in a distinct way?

Fig. 1 Geographical setting of populations of the fruit bats Eidolon helvum and Rousettus aegyptiacus examined, and geographical distances between them. CA = Cameroon (Mount Cameroon area); RM = Rio Muni; Bi = Bioko island; Pr = Princep island; ST = Sao Tome Island; A = Annobon island. R. aegyptiacus does not occur in Annobon.

Material and methods

Study area

The islands of Bioko, Princep, Sao Tome and Annobon constitute the only insular system offshore the sub-Saharan Atlantic coast of Africa (Fig. 1). The islands are aligned along a volcanic fracture stretching NE–SW from Mont Kupe in inland Cameroon to St. Helena island (Terán, 1962; Eisenraut, 1963). Princep, Sao Tome and Annobon are typical oceanic islands, whereas Bioko is a ‘land-bridge’ island, which has been periodically connected to the mainland during past climatic cycles. As a result, Bioko’s flora and fauna are relatively species-rich and poor in endemics. The oceanic islands, in contrast, show low richness, but high levels of endemism at the specific and generic levels (Exell, 1968; Jones, 1994). The small area and relatively long distances among oceanic islands (Fig. 1) have predominantly determined colonization events from the mainland to each island, generating distinct insular communities. Following this general trend, bat communities show a high proportion of endemics on each of the oceanic islands, and only three species (Hipposideros ruber, Eidolon helvum, and Rousettus aegyptiacus) are shared by more than two of them (Juste & Ibáñez, 1994). Samples from insular populations
of the fruit bats *Eidolon helvum* and *Rousettus egyptiacus* were examined. *R. egyptiacus* occurs in all the islands except the most-distant Annobón, whereas *E. helvum* is spread throughout the system. For each species, two populations from the mainland were used as reference populations, one from the Mount Cameroon area, originated from the same volcanic fracture than the islands, and the other from Río Muni, located south-west of Cameroon, and approximately equidistant to all islands (Fig. 1).

**Data collection**

Landmark coordinates were digitized from a total of 224 skulls of *Eidolon helvum* (Cameroon, 44; Río Muni, 49; Bioko, 37; Príncipe, 38; Sáo Tomé, 29; Annobón, 27) and 241 *Rousettus egyptiacus* (Cameroon, 66; Río Muni, 54; Bioko, 44; Príncipe, 39; Sáo Tomé, 38) using a 3-D Reflex Microscope (Reflex Measurement Ltd, Butleigh, UK). From each skull, a set of 82 landmark points was taken across all areas and on three dimensions. From these landmarks, 74 pairs of bilateral distances were calculated following the protocol described in Juste et al. (2001).

**Statistical analyses**

Significance of AA and DA patterns were examined for each of the 74 characters prior to all multivariate analyses. A subsample of 31 *Eidolon helvum* and 30 *Rousettus egyptiacus* drawn at random from the total sample was analysed following the methodology recommended by Palmer (1994). Presence of AA in the samples was tested on the raw data (right-minus-left differences), by species, using the Shapiro–Wilk’s test for normality (SAS Institute, 1995). The subsamples were measured twice, and a mixed-model sides-by-individuals analysis (Palmer & Strobeck, 1986) was performed for each species. Significance of DA (the ‘sides’ component), as well as the magnitude of measurement error with respect to nondirectional asymmetry (the sides-by-individuals interaction component) were tested on each of the 74 variables. DA was statistically significant only for 10 characters of *E. helvum* and 10 of *R. egyptiacus*. The sides-by-individuals interaction term of the ANOVA was not significant for nine characters of *E. helvum* and 11 of *R. egyptiacus*. Additionally, correlation analysis found no significant relationship between asymmetry and size, and AA was not significant for any character. Results of univariate analyses are discussed in detail in Juste et al. (2001).

The main objective of this paper is to examine patterns of variation in multivariate fluctuating asymmetry across island populations and between species. Therefore, only variables with no significant DA, and significant interaction term in the sides-by-individuals ANOVA (Palmer, 1994) were used in further analyses (Juste et al., 2001). Thus the data set consisted of 55 variables for *E. helvum* and 57 for *R. egyptiacus*. Because no significant relationship with size was detected, data were not scaled by size, but only log-transformed to linearize possible allometric relationships. In order to keep adequate sample sizes in multivariate comparisons, missing values were estimated using the expectation-maximization algorithm of Little & Rubin (1987).

Because antisymmetry was not significant, univariate asymmetry in this sample represents ‘true’ fluctuating asymmetry *sensu* Palmer (1994), an apparently random pattern of variation between sides for a character within a population, normally distributed, and with a mean not significantly different from zero. When considering multivariate fluctuating asymmetry we extend the concept of FA to more than one variable and their correlations, with no *a priori* association of pattern to any process.

Absolute deviation of asymmetry values (log r – log l) from their mean was calculated for all individuals, by character, population and species. These transformed asymmetry values were used in all further analyses. Although the mean absolute deviation for any one character is not equivalent to a variance (mean squared deviation of asymmetry values), it is a measure of spread around the mean that allows for parametric univariate and multivariate comparisons of variances via Levene’s test (Schultz, 1985). A preliminary two-way multivariate analysis of variance (MANOVA) was carried out to test for significant differences in FA between subadults and adults (specimens with fully grown dentition and with bone sutures fused) and between sexes, pooling all populations within each species. No significant differences were detected, either for main factors or interaction term for any species (*E. helvum*: sex, $F_{65,152} = 0.82, P = 0.78$; age, $F_{65,152} = 1.29, P = 0.05$; interaction, $F_{65,152} = 1.02, P = 0.43$. *R. egyptiacus*: sex, $F_{63,172} = 0.82, P = 0.80$; age, $F_{63,172} = 1.32, P = 0.08$; interaction, $F_{63,172} = 0.9, P = 0.64$). Therefore, sexes and ages were pooled in further tests.

To estimate the degree of association (integration) of FA in the skull, Spearman’s rank correlation and its significance was estimated for all possible pairs of characters, by population. Correspondence of character asymmetries across populations was assessed by calculating the Spearman’s rank correlation of mean asymmetry values of all characters for all possible pairs of populations within a species. Significance of correlations indicates the presence of a population asymmetry parameter (PAP). Additionally, we tested for pairwise associations in multivariate FA between populations using Mantel’s tests, significance of these tests also is indicative of a PAP. MANOVA was used to test for differences in levels of multivariate FA between populations. Analyses were run by species, with populations as treatments. To obtain a visualization of the trends of asymmetry being compared, the grand mean of the asymmetry values for each character was calculated, by population and species.
For species comparisons, populations were pooled into one sample by species (excluding Annobón, in which *R. egyptiacus* does not occur). Concordance of character asymmetries was tested on the variables common to both species (48) by calculating the mean asymmetry value of each character for each species and then the rank correlation between them. Using the same set of characters, matrices of pairwise correlations of characters were compiled for each species and the significance of their association tested using Mantel’s tests. Differences in levels of asymmetry between species were tested via MANOVA (multivariate Levene’s test); a two-way analysis species-by-population was performed to control for differences between populations within each species.

We assessed the relationships of asymmetry variation with other morphological (average trait size), genetic (allozymic variation) and geographical patterns of variation across the islands. General patterns of multivariate FA were evaluated by species by calculating pair-wise Mahalanobis distances among all possible pairs of populations. From these, a distance matrix was constructed and used with a neighbour-joining algorithm (Swofford et al., 1996) to construct unrooted trees, which summarize relationships of multivariate FAs among populations for each species. In these trees, branch lengths are proportional to the distance among populations. Similar matrices of genetic distances between pairs of populations, by species, were built from allozymic data using Rogers’ (1972) distances (Juste et al., 1996, 2000). To assess variation in mean trait size, a matrix of morphological distances was generated by taking the average of the raw measurements on right and left sides of the skull (r + l/2) for each observation and calculating the Mahalanobis distances between populations for each species. Finally, a matrix of geographical distances among localities was constructed. Genetic, morphometric and geographical distance matrices were compared against multivariate FA matrices using Mantel’s tests. A sequential Bonferroni adjustment (Rice, 1989) of α (expected Type I error for a number of simultaneous hypotheses being tested) was used to decide on the statistical significance of multiple simultaneous tests at a global α level of 0.05 by set of tests and species. Unless otherwise specified, all statistical analyses were performed in Matlab for Windows ver. 4.2c (The MathWorks Inc., 1994).

**Results**

**Concordance of asymmetries**

Correlations between character FAs were not significant for most pairs of characters in the populations after Bonferroni correction (Table 1). In all cases except one, less than 1% of all possible pair-wise correlations of character asymmetries (1485 combinations for *E. helvum*, 1596 for *R. egyptiacus*) were significant. There is a low degree of integration in character asymmetry for these species, and no evidence for an individual asymmetry parameter. In contrast, mean character FAs were strongly correlated between pairs of populations; all tests of

<table>
<thead>
<tr>
<th>CA</th>
<th>RM</th>
<th>B</th>
<th>P</th>
<th>ST</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Edolon</em></td>
<td>0.930</td>
<td>0.885</td>
<td>0.883</td>
<td>0.908</td>
<td>0.840</td>
</tr>
<tr>
<td><em>helvum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM</td>
<td>0.151</td>
<td>0.912</td>
<td>0.953</td>
<td>0.932</td>
<td>0.908</td>
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<tr>
<td>B</td>
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<td>0.904</td>
<td>0.926</td>
<td>0.904</td>
</tr>
<tr>
<td>P</td>
<td>0.197</td>
<td>0.149</td>
<td>0.194</td>
<td>0.810</td>
<td>0.620</td>
</tr>
<tr>
<td>ST</td>
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<td>0.162</td>
<td>0.099</td>
<td>0.100</td>
<td>0.087</td>
</tr>
<tr>
<td>A</td>
<td>0.131</td>
<td>0.151</td>
<td>0.122</td>
<td>0.111</td>
<td>0.151</td>
</tr>
<tr>
<td><em>Rousettus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>egyptiacus</em></td>
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<td>0.949</td>
<td>0.908</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>RM</td>
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<td>0.898</td>
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<td></td>
</tr>
<tr>
<td>B</td>
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<td>0.199</td>
<td>0.918</td>
<td>0.926</td>
<td></td>
</tr>
<tr>
<td>P</td>
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<td>0.220</td>
<td>0.133</td>
<td></td>
<td>0.921</td>
</tr>
<tr>
<td>ST</td>
<td>0.183</td>
<td>0.190</td>
<td>0.198</td>
<td>0.153</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Minimum, maximum and mean values of Spearman’s rank-correlations (integration) of character multivariate FAs for all possible pair-wise comparisons of asymmetry variables, by population and species. Last row (% vars.) is the percentage of significant correlations (table-wide α = 0.05) after the Bonferroni adjustment (table-wide α = 0.05 per species) for 1484 and 1596 comparisons for *E. helvum* and *R. egyptiacus*, respectively. CA = Cameroon; RM = Río Muni; B = Bioko; P = Príncipe; ST = São Tomé; A = Annobón.

<table>
<thead>
<tr>
<th><em>Edolon</em></th>
<th>0.440</th>
<th>0.410</th>
<th>0.553</th>
<th>0.468</th>
<th>0.592</th>
<th>0.587</th>
<th>0.458</th>
<th>0.832</th>
<th>0.429</th>
<th>0.486</th>
<th>0.636</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>helvum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM</td>
<td>0.679</td>
<td>0.639</td>
<td>0.700</td>
<td>0.737</td>
<td>0.849</td>
<td>0.774</td>
<td>0.737</td>
<td>0.845</td>
<td>0.889</td>
<td>0.886</td>
<td>0.747</td>
</tr>
<tr>
<td>B</td>
<td>0.021</td>
<td>0.045</td>
<td>0.021</td>
<td>0.038</td>
<td>0.034</td>
<td>0.017</td>
<td>0.028</td>
<td>0.025</td>
<td>0.035</td>
<td>0.042</td>
<td>0.020</td>
</tr>
<tr>
<td>P</td>
<td>0.404</td>
<td>0.135</td>
<td>0.135</td>
<td>0.202</td>
<td>0.337</td>
<td>0.135</td>
<td>0.934</td>
<td>1.002</td>
<td>0.627</td>
<td>0.439</td>
<td>0.313</td>
</tr>
</tbody>
</table>

Table 2 Tests for significance of associations of asymmetries across populations. Upper matrices: values of Spearman’s rank-correlations of mean values of multivariate FAs between pairs of populations. Lower matrices: standardized Mantel’s statistics (r) for all possible pair-wise comparisons of population matrices of multivariate FA correlations. All comparisons were highly significant (P < 0.001) after Bonferroni adjustment of significance level (α = 0.05) per set of tests and species. CA = Cameroon; RM = Río Muni; B = Bioko; P = Príncipe; ST = São Tomé; A = Annobón.
association were highly significant in both species (Table 2), suggesting the presence of a population asymmetry parameter. Similarly, pair-wise comparisons of correlation matrices (Mantel's tests) across populations were highly significant (Table 2). The relationships among character FAs were similar for both species; correspondence of average asymmetries between species was also highly significant in both the rank correlation (r = 0.94, P < 0.001, n = 48) and Mantel's tests (r = 0.53, P < 0.001, n = 48).

Levels of asymmetry

Manovas for differences among populations yielded highly significant results in both species (Table 3). Annobón had higher levels of asymmetry than all other populations of E. helvum, whereas for R. egyptiacus average asymmetry was higher for São Tomé and Príncipe. Neighbour-joining trees summarize the patterns of variation in multivariate FA levels (Fig. 2). In E. helvum Annobón is clearly separated from the rest of the populations in the tree. The remaining islands cluster together and then with Cameroon and Río Muni. In R. egyptiacus a well-resolved tree emerged, in which Río Muni and Bioko (the continental island), and then Cameroon, are grouped together, whereas São Tomé and Príncipe (the oceanic islands) are separated in a different branch (Fig. 3). Average FA was higher for E. helvum (Table 3). The species-by-population two-way manova yielded highly significant differences between species (F_{48,381} = 2.95, P < 0.001). The interaction term species-by-population was nonsignificant (F_{192,1523} = 1.16, P = 0.08), indicating similar variation in levels of asymmetry across populations for both species.

Table 3 Average multivariate fluctuating asymmetry (FA) values by population and species, and results of multivariate Levene's tests for differences in FA levels among populations, by species. CA = Cameroon; RM = Río Muni; B = Bioko; P = Príncipe; ST = São Tomé; A = Annobón.

<table>
<thead>
<tr>
<th></th>
<th>Eidolon helvum</th>
<th>Rousettus egyptiacus</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>0.0307</td>
<td>0.0280</td>
</tr>
<tr>
<td>RM</td>
<td>0.0333</td>
<td>0.0308</td>
</tr>
<tr>
<td>B</td>
<td>0.0341</td>
<td>0.0309</td>
</tr>
<tr>
<td>P</td>
<td>0.0353</td>
<td>0.0335</td>
</tr>
<tr>
<td>ST</td>
<td>0.0386</td>
<td>0.0327</td>
</tr>
<tr>
<td>A</td>
<td>0.0384</td>
<td>-</td>
</tr>
<tr>
<td>d.f.</td>
<td>5/275/822.8</td>
<td>4/228/721.6</td>
</tr>
<tr>
<td>λ</td>
<td>0.1873</td>
<td>0.25038</td>
</tr>
<tr>
<td>F</td>
<td>1.1940</td>
<td>1.93118</td>
</tr>
<tr>
<td>P</td>
<td>0.032</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Fluctuating asymmetry and other sources of variation

Association between FA and morphological matrices was highly significant for R. egyptiacus. Concordance becomes evident when the neighbour-joining morphological tree and the FA tree are compared (Fig. 3). The former shows little differences between mainland populations and Bioko, whereas the oceanic islands cluster separately and, within this cluster, São Tomé diverges from Príncipe. The remaining comparisons between data sets are marginally significant for R. egyptiacus and nonsignificant for E. helvum, but all become nonsignificant after Bonferroni adjustment (Table 4). Nonetheless, the morphological tree for E. helvum is well resolved. It consists of a cluster including the mainland populations and Bioko; the remaining populations join this cluster following the order of their geographical positions. The Annobón

![Fig. 2](image)

(A) Mahalanobis $D^2$ distances on character fluctuating asymmetries (FAs). (B) Mahalanobis $D^2$ distances on morphological variables. (C) Wagner tree based on Rogers' (1972) genetic distances; data from Juste et al. (2000). CA = Cameroon; RM = Río Muni; B = Bioko; P = Príncipe; ST = São Tomé; A = Annobón.
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Fig. 3 Neighbour-joining dendrograms showing patterns of variation in the populations of *Rousettus egyptiacus* studied. (A) Mahalanobis $D^2$ distances on character fluctuating asymmetries (FAs). (B) Mahalanobis $D^2$ distances on morphological variables. (C) Wagner tree based on Rogers' (1972) genetic distance; data from Juste et al. (1996). CA = Cameroon; RM = Rio Muni; B = Bioko; P = Principe; ST = São Tomé.

Table 4 Mantel's tests for concordance of the following matrices of distances between populations, by species: FA: Matrix of Mahalanobis $D^2$ distances of FAs. MOR: Matrix of Mahalanobis $D^2$ on 74 average skull variables ($r + 1/2$). GEN: Matrix of Rogers' (1972) genetic distances (population from Cameroon missing) based on data from Juste et al. (1996) for *R. egyptiacus* and from Juste et al. (2000) for *E. helvum*. GEO: Matrix of geographical distances using (great-circle) arc distances (RM is not considered because it is equidistant to all the islands).

<table>
<thead>
<tr>
<th></th>
<th><em>Eidolon helvum</em></th>
<th><em>Rousettus egyptiacus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>FA-MOR</td>
<td>0.597</td>
<td>0.048</td>
</tr>
<tr>
<td>FA-GEN</td>
<td>0.455</td>
<td>0.105</td>
</tr>
<tr>
<td>FA-GEO</td>
<td>0.180</td>
<td>0.275</td>
</tr>
</tbody>
</table>

*Significant concordance after a sequential Bonferroni adjustment of table-wise $P$-values to $\alpha = 0.05$, by species.

population diverges from the rest, in agreement with the FA tree (Fig. 3).

Discussion

Patterns of concordance of FAs

Pair-wise correlations of FA among cranial characters suggest that there is no integration of FA within populations, i.e. there is no evidence for an individual asymmetry parameter (IAP) *sensu* Soulé (1967) and Leamy (1994). IAPs are seldom detected (e.g. Lens & van Dongen, 1999). The reason may be a weak signal, or failure to look at the right characters (Leamy, 1994), but also, absence of an IAP may be the normal condition if FA reflects fluctuations consequent on the normal developmental processes of bilateral structures, as well as developmental noise (Graham et al., 1993). Under this model, genetic or environmental stress may increase the levels of noise (asymmetry), but even in the absence of perturbations, a certain level of FA is to be expected for any particular developmental path. Small disturbances during the process may quickly lead to deviations from the symmetrical condition due to cascading effects. Negative feedback among growing cells on each side, or between right and left structures, would keep bilateral structures from randomly drifting in relative growth (Emlen et al., 1993). Developmental errors occurring at any further step during the process would be buffered at that level. As development proceeds, new ‘errors’ are expected to occur de novo and independently from those in other structures or generated at early stages in the process. The net result would be an asymmetry pattern in which levels of FA on one character are not necessarily correlated with levels of FA in other characters.

Under the idea of a normal or ‘base’ level of asymmetry associated with a developmental process, we would expect that, on average, individuals in a population had similar levels of FA for a given character. All characters measured in the same subset of individuals would have an associated level of asymmetry, so that a vector of average FAs for a set of characters would reflect the developmental process that gives rise to a morphological pattern. We found a significant population asymmetry parameter (PAP) in each species examined, which furthermore is the same for both species studied. These results suggest the presence of a skull-wide pattern of character FAs, which is preserved across populations and, beyond that, across species.

*E. helvum* and *R. egyptiacus* share a similar, ‘fox-like’ skull morphology, characterized by having the craniofacial axis almost horizontal, and the braincase slightly vaulted.
and raised above the face line (Andersen, 1912). This similarity led previous authors to classify Roussettus and Eidolon as sister genera (Andersen, 1912; Bergmans, 1997). However, molecular data indicate that they are phylogenetically separated (Hollard & Springer, 1997; Juste et al., 1999), and that morphological resemblance is due to a very conservative, non-specialized, primitive structural pattern for the family Pteropodidae (Kirsch et al., 1995; Álvarez et al., 1999). There may exist a general asymmetry parameter common to all ‘fox-like populations’ in Pteropodidae that is a property of the developmental process that produces that structural pattern. Under different genetic or environmental conditions, levels of FA may change (see below), but the general asymmetry pattern would persist as long as the morphological pattern to which it is associated persists. Because a morphological pattern is the product of genetic as well as epigenetic processes, organism-wide FA patterns may ultimately have a genetic origin, as implied by Soulé (1967). However, the pattern would not necessarily appear as a co-ordinated response to increased disturbance or decreased buffering, but would rather it be a product of the developmental system.

Differences in fluctuating asymmetry levels

Levels of multivariate FA were significantly different among populations within species, and between species. Patterns of multivariate FA based on Mahalanobis distances mostly reflect differences in magnitude of asymmetry. For E. helvum, the FA tree based on Mahalanobis distances identified the Annobón population as being most distinct, whereas the remaining islands cluster with the mainland populations. Cluster analysis based on genetic distances showed a pattern in which São Tomé and Príncipe clustered together, Rio Muni clustered with these two in a major group, and Annobón is segregated from the rest (Fig. 2). Morphological evidence also reflects this trend; E. helvum from Annobón is distinguishable from the remaining conspecific populations by its smaller overall size, to such extent that subspecific status is recognized for this population (Juste et al., 2000).

For R. egyptiacus, levels of multivariate FA were also significantly different. The tree based on Mahalanobis distances of FA values clearly distinguished the oceanic islands from the mainland and Bioko populations. The pattern of FA corresponded to that of genetic, morphological and geographical distances. Lack of significance in tests of association with geographical and genetic distances may reflect a reduction in power due to the exclusion of the population from Cameroon in these comparisons. Allozymic data placed Bioko closer to Río Muni, whereas Príncipe and São Tomé clustered together and separately from them (Fig. 3). Based on morphometric and qualitative external characters, three subspecies of R. egyptiacus are distinguished in this area, one for the Central African mainland and Bioko (Eisen- traut, 1959), one endemic to Príncipe, and one from São Tomé (Juste & Ibáñez, 1993). Cluster analysis of cranial morphometric data (Fig. 3) reflected these relationships among populations.

Studies of genetic and phenotypic variation in island-mainland systems usually concern the purported genetic and morphological changes that could result from isolation. Island populations might be under genetic stress produced by loss of heterozygosity and polymorphism, or environmental stress produced by significantly harsher environments on the islands. The higher the level of stress, the lower the capacity of the organism to ‘correct’ for errors, and the higher the FA in the population (Van Valen, 1962; Palmer & Strobeck, 1986). There is no evidence of decreased or increased heterozygosity in the islands for either species. Levels of genetic polymorphism are slightly lower only in E. helvum from Annobón, whereas in R. egyptiacus polymorphism is higher in the islands than in the mainland (Juste et al., 1996, 2000). Moreover, both species are relatively abundant in the islands (Juste & Ibáñez, 1994), indicating large effective population sizes. For these two species, variation in levels of asymmetry does not seem to be the product of stress due to genetic disruptions. Overall environmental conditions may be more extreme in the islands (Puster, 1955), for instance, availability of resources may be diminished in the islands as compared to the mainland (Exell, 1968). On the other hand, however, insular fruit bat populations may benefit from lower competition in much reduced communities of bats and birds as compared to the mainland (Jones, 1994; Juste & Ibáñez, 1994). Observed asymmetry patterns, particularly that of Eidolon helvum, do not correspond to what would be expected if the insular condition was the main determinant of the FA levels. In fact, for this species, only Annobón population branches off while the other oceanic islands cluster together with the mainland populations (Fig. 2). Additionally, increased environmental stress does not affect FA levels in recent experimental studies (David et al., 1998; Bjorksten et al., 2000).

We propose an alternative explanation for the observed differences in levels of FA based on the main assumption that in both species a process of genetic and morphological differentiation is occurring or has recently occurred in the island system. This assumption is sustained by the short genetic distances found among these populations (Juste et al., 1996, 2000). We suggest that higher levels of FA in these populations are the result of a relaxation of canalization associated with the process of differentiation. This relaxation is not the product of environmental pressures, but a property of the developmental system, which may facilitate the changes occurring in the island populations, or may be the consequence of them. In either case, it would mean less control of the mechanisms that regulate levels of
asymmetry during development, resulting in an increase in asymmetry in the differentiating populations.

Observed patterns are more consistent with this prediction. In *E. helvum*, a large, vagile bat, differentiation occurs only in Annobón, the population with significantly higher levels of FA. *R. eurypterus* is a smaller bat with specific roosting requirements and lower vigilance; isolation of the oceanic populations is therefore more effective (Juste *et al.*, 1996), and populations on each of the oceanic islands are following their own evolutionary paths. Accordingly, higher levels of FA were observed in Príncipe and São Tomé. FA is usually viewed as a measure of fitness; nonetheless, an increment in the phenotypic but not necessarily the genotypic variance could confer island populations a higher probability of survival in the new environmental conditions (Simons & Johnston, 1997). Although increased asymmetry may represent ‘low quality’, it may also be an advantage in new environments, or even be irrelevant to the survival of organisms.

**General trends across islands**

Two general trends of asymmetry variation across islands are discernible from this analysis. First, skull-wide FA patterns remain consistent across populations in both species, and across species. Second, multivariate levels of asymmetry differ significantly across populations; differences correspond to the pattern of morphological divergence among populations, and they also are associated with observed patterns of genetic distances.

Differences in magnitude of FA may be associated with unique environmental pressures or genetic change (differentiation or stress), whereas the skull-wide asymmetry pattern seems to be a by-product of the development of the skull, and will persist as long as the cranial structural pattern does. Patterns of average FA seem to be as canalized as the basic cranial morphology; nonetheless, canalization may break down when the ontogeny switches from one developmental pathway to another, as some threshold value of genotypic or environmental effects is attained (Zakharov, 1992; Schlichting & Pigliucci, 1998). For the species analysed, a prediction of this hypothesis is that a radical change in the ‘fox-like’ pattern would result in the breakage of the symmetry parameter associated with that morphology. It remains to be investigated how radical and of what nature the change would have to be to produce a different skull-wide asymmetry pattern. Also, we may want to ask what kind of asymmetry patterns would be expected if we could generate similar morphologies through different developmental processes.

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