

## Permutation Tests for Difference between Two Multivariate Allometric Patterns

Tzong-Der Tzeng and Shean-Ya Yeh\*

*Institute of Oceanography, National Taiwan University, Taipei, Taiwan 106, R.O.C.*

*Tel: 886-2-23637753. Fax: 886-2-23925294.*

*(Accepted September 23, 1998)*

**Tzong-Der Tzeng and Shean-Ya Yeh (1999)** Permutation tests for difference between two multivariate allometric patterns. *Zoological Studies* 38(1): 10-18. Studies that include comparisons of multivariate allometric patterns between sexes, species, discrete growth stages, or geographic populations have gradually increased. Some statistical methods assume that compared groups share the same multivariate allometric pattern, so comparisons of multivariate allometric patterns also have to be performed before using these methods. Several methods have been used to detect the difference between 2 multivariate allometric patterns, but these methods lack an objective guide to test whether the 2 multivariate allometric patterns are the same or not. In this study, a permutation test was used to determine whether the difference of 2 patterns was significant or not. Four examples were used to explain and verify this test. The multivariate allometric pattern was estimated by the 1st eigenvector of the sample covariance matrix of the logarithmic measurement. The angle between the 2 first eigenvectors was taken as the test statistic. For each example, 5000 permutations were performed to assess the significance level. Finally, the effect of sample size difference on the permutation test was also examined. We found that all 1st eigenvalues explained the largest part of total variance and all 1st eigenvectors can satisfactorily interpret the multivariate allometric patterns. These tests can successfully detect the relationship between 2 multivariate allometric patterns in each example, so they can be a tool to test whether the difference of 2 multivariate allometric patterns is significant or not. Although this method is not sensitive to sample size differences, we still suggest that the sample size difference be as small as possible when using permutation tests to address this question.

**Key words:** First eigenvector, Angle, Allometry, Reorder.

Allometry is a method providing valuable information about evolutionary modifications of growth trajectories (Cock 1966, Klingenberg 1996). Studies on allometry trace to the pioneering work of Huxley (1932). He derived his formula of allometry,  $y = b x^\alpha$ , where  $x$  and  $y$  are trait measurements, and the constant  $\alpha$  is often called the allometric coefficient. Since generalization of allometry was proposed by Jolicoeur (1963), studies on multivariate allometric patterns of organisms have increased. Jolicoeur (1963) suggested that the 1st eigenvector extracted from the covariance matrix of logarithmic values reflects the multivariate allometric pattern. The 1st principal axis is the line passing through the greatest dimension of the concentration of data points of the multivariate

distribution (Legendre and Legendre 1983), but it need not represent the size component, except the coefficients in the 1st principal axis with the same sign (Jolicoeur and Mosiman 1960). This size component usually can represent sex, population, or species differences.

Three different levels of allometry are distinguished: static, ontogenetic, and evolutionary allometry. This classification has also been used in most comparisons between allometric levels (Cock 1966, Klingenberg 1996). Additionally, some statistical methods, e.g., Burnaby's method (Burnaby 1966) and shearing principal component analysis (PCA) for size correction (Humphries et al. 1981) or multiple groups PCA (Thorpe 1983) for ordination, assume that groups under consideration

---

\*To whom correspondence and reprint requests should be addressed.

share a common multivariate allometric pattern or parallel principal axes. Therefore, multivariate allometric patterns must be compared before using these methods.

Two methods frequently have been used to compare 2 multivariate allometric patterns. A straightforward indication of differences of 2 multivariate allometric patterns is provided by measurement of the angle between them (Gibson et al. 1984, Klingenberg 1996). Another method is based on multivariate ordination (Klingenberg and Froese 1991, Klingenberg and Spence 1993), e.g., PCA.

How far do 2 principal component scores need to be separated, or how large does the angle between the 2 first eigenvectors need to be before the difference of 2 multivariate allometric patterns is considered to be significant? Clearly, these methods lack an objective guide to judge whether 2 multivariate allometric patterns are the same or not. It would be desirable to have a statistical test for this purpose.

The permutation test involves determining the significance level of a test statistic calculated for 2 observed sets of data by comparing the distribution of values that is generated by randomly reordering the data (Manly 1997). This is a computer-intensive procedure because the permutation distribution must be determined either by enumerating all possible data orders, or by taking a large random sample from the permutation distribution.

The aim of this study is to describe a permutation procedure to test whether the difference of 2 multivariate allometric patterns is significant or not.

## MATERIALS AND METHODS

Four examples, including 7 kuruma shrimp data sets and 1 crab data set, were employed to explain and verify this test. Each example comprises 2 data sets. Except the 4th example which includes both a shrimp data set and a crab data set, the others contain 2 data sets of kuruma shrimp only.

The following 12 measurements were used in the 1st and 2nd examples: (1) antennal spine width, denoted ASW; (2) hepatic spine width, HSW; (3) carapace length, CL; (4) diagonal carapace length, DCL; (5) 1st abdominal segment length, FSL; (6) 1st abdominal segment width, FSW; (7) 1st abdominal segment height, FSH; (8) 2nd

abdominal segment length, SSL; (9) 6th abdominal segment height, SSH; (10) 6th abdominal segment width, SSW; (11) body length, BL; and (12) total length, TL. The characters used in the 3rd example were the same as in the 1st example, except characters BL and TL were replaced with body weight (WT) and rostrum length (RL). To ensure large differences between 2 multivariate allometric patterns, the characters for the 2 data sets in the 4th example are different and come from different species. Four characters measured in the shrimp data set were WT, ASW, HSW, and CL, but in the crab data set were: (1) the width of the posterior region of the carapace, the rear width, RW; (2) the length of the carapace along the median line, CLM; (3) the maximum width of the carapace, CW; and (4) the maximum depth of body, BD. The sample size, means, and standard deviations of every character and relative attributes of the 2 data sets in each example are summarized in Table 1.

Estimation of multivariate allometric pattern followed the recommendation of Jolicoeur (1963). The 1st eigenvector of the sample covariance matrix calculated from measured values transformed to natural logarithms was used to reflect the multivariate allometric pattern.

The angle between the 2 first eigenvectors was taken as the test statistic to assess the significance of difference of the 2 multivariate allometric patterns. The angle ( $\theta$ ) is the arc cosine of the inner product of the pair of the 1st eigenvectors,  $\theta = \arccos(b \cdot c) \cdot 180/\pi$ , where  $b$  and  $c$  are the 1st eigenvectors (Klingenberg 1996). If the difference increases, the angle will increase. Since the orientation of the PCA vector is arbitrary, the absolute value of the angle computed is used. The angles can be computed as the arc cosine of the inner product of the 2 first eigenvectors, but all possible reflections of axes must be ignored (Klingenberg 1996).

The permutation test is described in Edgington (1987), Crowley (1992), Good (1994), and Manly (1997). In this study, the permutation method required 2 assumptions: (1) the original samples were collected randomly, or observations were independent; and (2) the observations must be exchangeable among samples.

The null hypothesis that we want to test is that the 2 first eigenvectors are the same. Suppose that we have a sample size  $n_1$  from data set 1 and a sample size  $n_2$  from data set 2. The following procedure can be used to assess the significance of the observed value of the angle estimated from

the original 2 data sets. The procedure is as follows:

- Step 1. Find the 1st eigenvectors for the 2 original data sets, and compute the angle between the 2 first eigenvectors,  $\theta_0$ .
- Step 2. Combine the 2 data sets, and randomly partition total sample size  $n_1 + n_2$  into 2 new data sets, of sizes  $n_1$  and  $n_2$ . Find the 1st eigenvectors for the 2 new permuted data sets, and recompute the angle between the 2 new 1st eigenvectors,  $\theta_1$ .
- Step 3. Repeat step (2) a large number of times (N) to find a sample distribution of the test statistic,  $\theta_1$ . We chose  $N = 5000$  times.

The significance of  $\theta_0$  can be determined from its position among the ordered values of  $\theta_1$  found

by permutation (Good 1994, Manly 1997). If  $\theta_0$  is in the bottom 95% of the permutation distribution, the test is not significant; but if  $\theta_0$  is among the values in the top 5% tail of the distribution, then the test is significant at the 5% significance level. Similarly, a value in the top 1% tail is significant at the 1% level. Another way to conclude the permutation results involves calculating the proportion (P) of the observed  $\theta_1$  values that are  $> \theta_0$ . This P value can be interpreted in the same way as for conventional tests of significance: if it is  $< 5\%$  then this provides some evidence that the null hypothesis is not true, and if it is  $< 1\%$  then it provides strong evidence that the null hypothesis is not true (Good 1994, Manly 1997). Because only large positive values of the test statistic give evidence against the null hypothesis, a one-sided test is suitable to test our hypothesis (Crowley 1992).

**Table 1.** Comparisons of sample size, age group, sampling area, and species for 2 data sets in each example. YY represents the same attribute and XY is different. The means and corresponding standard errors (in parentheses) of variables in each data set are also shown

Data set	First example		Second example		Third example		Fourth example		
	1	2	1	2	1	2	1	2	
Sample size	100	100	130	130	103	120	200	200	
Age group	YY		XY		YY		XY		
Sample area	YY		YY		XY		XY		
Species	YY		YY		YY		XY		
Variable					Variable	Variable			
ASW	9.8808182 (1.0725705)	9.7847706 (1.1083576)	9.2663014 (0.8349974)	18.7613014 (2.1481014)	WT	88.2883495 (29.6528620)	87.7166667 (20.6161691)	Kuruma shrimp	
HSW	11.1252727 (1.3182778)	11.1287156 (1.3442119)	10.4179452 (0.9726621)	22.228082 (2.7633002)	RL	24.9163107 (2.3842812)	24.7725641 (2.6901610)	WT	11.5557000 (3.3540608)
CL	28.9892727 (3.3725159)	28.8536697 (3.4377514)	27.1447945 (2.5554659)	60.9826027 (8.1008562)	ASW	18.0985437 (2.0272784)	18.0128846 (1.2180381)	ASW	16.0324000 (1.7269246)
DCL	32.7263636 (3.6953511)	32.5806422 (3.8313109)	30.6796575 (2.8280646)	67.4136301 (8.6189155)	HSW	21.3718447 (2.5922985)	21.3152564 (1.7647174)	HSW	9.6805000 (1.0109117)
FSL	11.8952727 (1.4825027)	11.6673394 (1.5172579)	11.0013699 (1.1320897)	24.2271233 (2.7383728)	CL	58.4656311 (7.5688945)	57.8014103 (4.7291361)	CL	10.9260000 (1.2027431)
FSW	12.1338182 (1.4015385)	12.5924771 (1.4119714)	11.5887671 (1.0040318)	24.4244521 (2.7095833)	DCL	64.7099029 (8.0478896)	63.9925000 (5.0802882)		
FSH	13.9104545 (1.7078799)	14.1233028 (1.8401007)	13.0531507 (1.2652844)	30.6702740 (3.6966398)	FSL	23.3770874 (2.6321156)	23.4178646 (1.7831617)	Crab	
SSL	9.5242727 (1.2192617)	9.4918349 (1.1821083)	8.8774658 (0.8807644)	19.4906849 (2.0160055)	FSW	23.5875728 (2.6172132)	24.5653205 (1.7399330)	RW	15.5830000 (3.4953251)
SSH	8.9548182 (1.0588965)	8.9218349 (1.0972028)	8.4089041 (0.8480347)	18.3263014 (2.1034146)	FSH	29.4966990 (3.4783187)	29.4222436 (2.2908310)	CLM	12.7375000 (2.5727688)
SSW	6.2237273 (0.7795657)	6.3329358 (0.7891748)	5.8582192 (0.5624610)	13.2191781 (1.6343095)	SSL	18.8809709 (1.8743781)	18.9713462 (1.4487788)	CW	32.1040000 (7.1209849)
BL	104.8636364 (11.5452657)	105.2385321 (11.3691420)	98.9383562 (8.4700060)	208.9109589 (23.2693142)	SSH	17.6811650 (2.0061011)	17.7494872 (1.1930556)	BD	36.4085000 (7.8796971)
TL	121.0325455 (13.0124311)	121.5009174 (12.9865083)	114.4054110 (9.8237414)	234.0479452 (24.1524846)	SSW	12.6883495 (1.5140713)	12.7308333 (0.9670120)		

## RESULTS

The first three eigenvectors and percentages of total variances explained by the first three eigenvalues for each original data set in each example are shown in Tables 2-5. All 1st eigenvalues explained the largest part of total variance, and ranged from 83.46% to 96.71%. All 1st eigenvectors have all-positive loadings and are interpreted as representing multivariate allometric patterns, while the remaining eigenvectors have loadings of different signs and are interpreted as representing shape variation.

The frequency distributions of 5000  $\theta_1$  in the 1st to 4th examples are shown in Fig. 1A~D, respectively.  $\theta_0$  is 1.675938 degrees in the 1st example. This angle is a typical value from the permutation distribution (Fig. 1A), which is in the bottom 95% of the distribution. The value of P is 55.82%. This value is clearly larger than 1% or 5%, so the test is not significant. Therefore, we accept the null hypothesis that the 2 multivariate allometric patterns in the 1st example are the same.

The value of  $\theta_0$  is 4.821436 degrees in the 2nd example. This value is extreme in magnitude

**Table 2.** The first three eigenvectors and percentages of total variances explained by the first three eigenvalues estimated from the 1st example

Variable	Data set 1			Data set 2		
	1st	The 2nd eigenvector	3rd	1st	The 2nd eigenvector	3rd
ASW	0.2698565	0.0271609	0.2013917	0.2752837	-0.133152	0.3685506
HSW	0.2912468	-0.054879	0.2226448	0.2958117	-0.072261	0.3418125
CL	0.2900797	0.0242964	0.0343438	0.2926811	-0.055731	0.2930318
DCL	0.2826561	0.0591171	0.0218885	0.2876165	-0.125303	0.2614096
FSL	0.3092059	0.1573005	-0.178208	0.3174445	-0.061021	0.1702709
FSW	0.2779283	-0.398679	-0.034629	0.2682299	0.3961776	-0.061212
FSH	0.3019271	-0.009916	0.1756737	0.3117589	-0.025592	-0.380792
SSL	0.3116326	-0.004306	-0.85951	0.2990123	0.0222653	-0.251484
SSH	0.2784844	0.8202205	0.1068703	0.2892173	-0.717229	-0.435616
SSW	0.303648	-0.277994	-0.010478	0.2971993	0.4667235	-0.390772
BL	0.2743709	-0.180786	0.2073498	0.2624248	0.1918189	0.0468468
TL	0.2686071	-0.160108	0.226079	0.2610896	0.1654335	0.0865297
Variance	94.79%	1.29%	0.86%	96.06%	1.17%	0.57%

**Table 3.** The first three eigenvectors and percentages of total variances explained by the first three eigenvalues estimated from the 2nd example

Variable	Data set 1			Data set 2		
	1st	The 2nd eigenvector	3rd	1st	The 2nd eigenvector	3rd
ASW	0.2824215	-0.109901	0.0097848	0.2800289	0.087132	-0.38575
HSW	0.2921341	-0.003678	0.1539546	0.3055523	0.0061843	-0.15747
CL	0.2977158	-0.065632	-0.025521	0.3296851	-0.017809	-0.309722
DCL	0.2910943	-0.107019	-0.016722	0.3176999	-0.05913	-0.282436
FSL	0.3211295	-0.227199	-0.222397	0.281547	-0.147052	0.1349917
FSW	0.2549327	0.5282522	0.1197418	0.2722359	-0.190878	0.4556241
FSH	0.297535	-0.011709	0.1747953	0.299173	-0.041713	-0.013151
SSL	0.2998281	-0.041368	-0.837271	0.2508687	-0.123676	0.5994495
SSH	0.2943175	-0.632239	0.372086	0.2755315	0.9231491	0.2053641
SSW	0.2891454	0.400221	0.0899792	0.3058012	-0.181732	0.0927947
BL	0.2680211	0.2068443	0.1147153	0.2779161	-0.11782	-0.096781
TL	0.2700429	0.1922016	0.1421035	0.2571109	-0.119789	-0.072263
Variance	92.00%	2.30%	1.18%	96.25%	0.84%	0.69%

compared with the angles resulting from the 2 permuted data sets (Fig. 1B). The value of P is 0% and it is smaller than 1% or 5%. It is extremely unlikely that the difference between the 2 multivariate allometric patterns derived from the 2 original data sets was due to chance alone, so we accept the alternative hypothesis that the 2 multivariate allometric patterns are different in the 2nd example.

The value of  $\theta_0$  is 3.896844 degrees in the 3rd example, which is a typical value in the permutation distribution (Fig. 1C). The value of P is 8.5%, and it is larger than 1% or 5%. The test is not significant, so we accept the null hypothesis that the 2 multivariate allometric patterns are the same in the 3rd example.

The value of  $\theta_0$  is 28.957391 degrees in the 4th example. This value is extreme in magnitude compared with the permutation distribution of

the test statistic (Fig. 1D). The value of P is 0%. This value is clearly smaller than 1% or 5%, so this test is significant. It shows that the 2 multivariate allometric patterns are distinct in the 4th example.

## DISCUSSION

The majority variance in each data set is explained by the 1st eigenvalue (Tables 2-5), which reveals a good fit of the multivariate allometric model to the data in all groups considered in this study (Bjorklund 1993, Klingenberg 1996). The principal component axes are mutually orthogonal, and the eigenvectors are normalized to a unit length, so the coefficients in the eigenvector depend on the number of variables (Klingenberg 1996). Nevertheless, using the ratio of the

**Table 4.** The first three eigenvectors and percentages of total variances explained by the first three eigenvalues estimated from the 3rd example

Variable	Data set 1			Data set 2		
	1st	The 2nd eigenvector	3rd	1st	The 2nd eigenvector	3rd
WT	0.6670503	-0.03725	-0.443142	0.691987	-0.047839	-0.642155
RL	0.053559	0.9966586	0.0139096	0.0881021	0.993497	0.0161666
ASW	0.2245822	-0.043902	0.196514	0.1987197	0.0268567	0.1585013
HSW	0.2457084	0.0133774	0.1453544	0.2491708	-0.023898	0.0541738
CL	0.2637534	0.0010789	0.1738139	0.249691	-0.016486	0.2714069
DCL	0.2530547	0.0032198	0.1332281	0.2422343	-0.015936	0.2713678
FSL	0.2306686	-0.031648	-0.012182	0.2266762	-0.050409	0.3530372
FSW	0.2243608	-0.0047	-0.169052	0.2110283	-0.030384	-0.055397
FSH	0.240059	-0.001328	0.0785836	0.2365601	-0.017968	0.1774074
SSL	0.1989035	0.003359	-0.032609	0.2223817	-0.049743	0.366809
SSH	0.2208158	-0.033631	0.8098673	0.1900293	-0.005115	0.3423953
SSW	0.241385	-0.031597	-0.062244	0.2309825	-0.050689	0.0324765
Variance	93.57%	3.49%	0.79%	83.46%	11.29%	1.64%

**Table 5.** The first three eigenvectors and percentages of total variances explained by the first three eigenvalues estimated from the 4th example

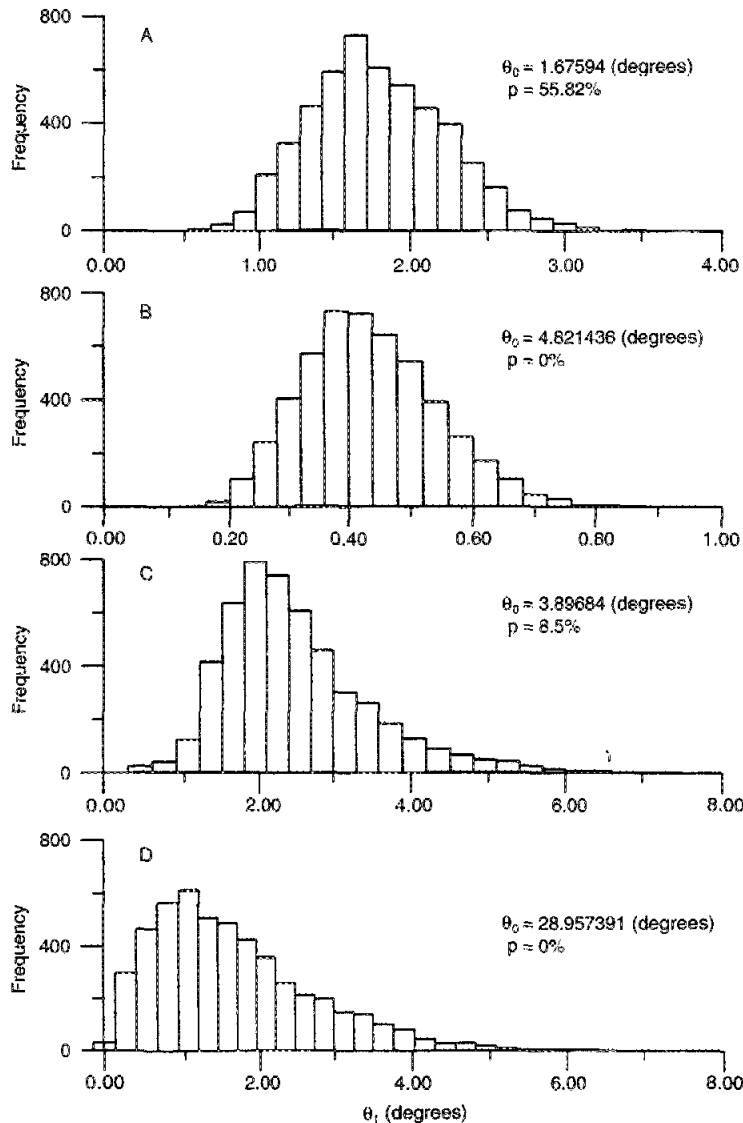
Variable	Data set 1			Variable	Data set 2		
	1st	The 2nd eigenvector	3rd		1st	The 2nd eigenvector	3rd
WT	0.8674172	-0.383448	-0.314919	RW	0.5188255	-0.203503	-0.797001
ASW	0.2715453	0.8973357	-0.347236	CLM	0.4484576	0.8898273	0.0436979
HSW	0.2855215	0.1806692	0.647113	CW	0.521372	-0.334108	0.2031651
CL	0.3038553	0.1229433	0.6012467	BD	0.507816	-0.234873	0.5671014
Variance	95.86%	2.87%	0.96%		96.71%	2.40%	0.83%

coefficients of the 1st eigenvector for 2 variables corresponding to the variable in the bivariate allometric analysis, we can translate the multivariate allometric coefficients to bivariate allometric coefficients (Huxley's  $\alpha$ ) (Jolicoeur 1963, Shea 1985).

The multivariate allometric coefficients in the 1st and 2nd examples have similar magnitudes (Tables 2, 3). Thus, in allometric plots with TL as the independent variable, all the other variables would be isometric with a slope of about 1. The variable of body weight (WT) was also included and analyzed in the 3rd example, and in the 1st data set of the 4th example; the coefficients of WT

are about 3 times those of the other variables in magnitude, except RL (Tables 4, 5). The coefficients of RL in the 3rd example are clearly smaller than the other coefficients (Table 4), so we may say the correlations between RL and the remaining variables are small. The relationships between TL, WT, or RL and the other variables have been proven by many shrimp studies (Chu et al. 1995, Chiu 1996) and our studies (data not shown). Therefore, all the 1st eigenvectors estimated in this study can satisfactorily reflect the multivariate allometric patterns of organisms.

For some statistics we are not able to know their distribution since they are not standard test



**Fig. 1.** Frequency distribution of 5000 angles ( $\theta_1$ ) estimated from the 2 data sets permuted in each example: (A) 1st example, (B) 2nd example, (C) 3rd example, (D) 4th example.  $\theta_0$  stands for the angle between the 2 first eigenvectors estimated from the original 2 data sets. P is the proportion of  $\theta_1$  larger than  $\theta_0$  among 5000 permutations.

statistics; therefore standard parametric methods can not be applied. Non-parametric methods, like the permutation test, can be used to solve such problems. Additionally, permutation tests can more easily take into account the peculiarities of the situation of interest and use non-standard test statistics than can standard statistical tests (Manly 1997). They are usually almost as powerful as the corresponding unbiased parametric tests even for small samples (Edgington 1987). The major reason permutation tests have been applied to various fields is that they only require 1 or 2 relatively weak assumptions (Good 1994).

For determining the exact level of significance and the real permutation distribution of a test statistic, all possible permutations of the observations must be listed and the test statistic computed for each permutation (Good 1994). However, sometimes the large number of possible

data permutations makes a complete enumeration extremely difficult or computationally unfeasible. In that case, the permutation distribution of the test statistic can be estimated by considering a larger number of random permutations of observations, and this test is described as a sampled permutation test or approximate randomization test (Noreen 1989). The significance level of taking a large sample from the complete permutation distribution can be expected to give the same result gained from a complete enumeration (Manly 1997). However, it is important that the significance level estimated from permutations be close to the level that would be obtained from considering all possible data rearrangements (Solow 1990). Therefore, a certain minimum number of permutations should be performed. Marriott (1979) and Edgington (1987) suggested that 1000 permutations are a reasonable minimum for a test at

**Table 6.** Means, standard deviations, and ranges of 5000  $\theta_1$  values and numbers (N) and proportions (P) of  $\theta_1$  larger than  $\theta_0$  among 5000 permutations for various data dimensions expected with the same multivariate allometric patterns.  $\theta_0$  is the angle derived from the 2 data sets before permuting.  $\theta_1$  is the angle estimated from the 2 permuted data sets

Sample size		Number of permutations	$\theta_1$				$\theta_0$	N	P
n1	n2		Mean	Std. Dev.	Minimum	Maximum			
200	30	5000	2.8023950	0.8251290	0.8780792	7.3065774	2.67171	2520	50.4%
200	40	5000	2.4080969	0.6693691	0.5875269	5.1949542	2.60687	1782	35.64%
200	50	5000	2.1502399	0.6099446	0.5055280	5.7594434	3.07310	354	7.08%
200	60	5000	1.9375528	0.5347191	0.5683803	5.1075401	1.54437	3789	75.75%
200	70	5000	1.8655150	0.5045791	0.5650887	4.2896967	0.95099	4915	98.3%
200	80	5000	1.7096402	0.4511045	0.4446091	3.8985048	1.39534	3719	74.38%
200	90	5000	1.5700116	0.4130168	0.5190032	3.5378335	1.15916	4212	84.24%
200	100	5000	1.3003858	0.3462046	0.3937044	3.0201632	0.85849	4546	90.92%
200	150	5000	1.1906324	0.3308836	0.3985607	2.5360473	1.22607	2110	42.2%

**Table 7.** Means, standard deviations, and ranges of 5000  $\theta_1$  values and numbers (N) and proportions (P) of  $\theta_1$  larger than  $\theta_0$  among 5000 permutations for various data dimensions expected with 2 different multivariate allometric patterns.  $\theta_0$  is the angle derived from the 2 data sets before permuting.  $\theta_1$  is the angle derived from the 2 permuted data sets

Sample Size		Number of permutations	$\theta_1$				$\theta_0$	N	P
n1	n2		Mean	Std. Dev.	Minimum	Maximum			
200	30	5000	13.4104259	11.1472425	0.1140383	44.8392150	52.52610	0	0%
200	40	5000	13.0637343	10.1847369	0.1712086	49.6559040	50.48860	0	0%
200	50	5000	6.5047684	5.9651623	0.1063969	49.8005490	53.03401	0	0%
200	60	5000	4.5638548	3.4541577	0.0923533	47.4963080	49.90202	0	0%
200	70	5000	4.9087284	3.6814842	0.0453292	46.0601290	56.15404	0	0%
200	80	5000	4.0518363	2.7980301	0.1462960	27.2607240	53.51972	0	0%
200	90	5000	3.1107067	2.0321123	0.0550736	17.3698500	52.52478	0	0%
200	100	5000	2.3771073	1.4812705	0.0362931	9.5908504	53.82837	0	0%
200	150	5000	2.0969540	1.2784028	0.0483770	7.8940469	52.01624	0	0%

the 5% level of significance, while 5000 are a reasonable minimum at the 1% level. In general, the larger the sample size is, the more permutations need. When the sample size is small, the number of permutations used should not exceed the maximum possible orders for the data.

The permutation tests showed that the 2 multivariate allometric patterns in the 1st example are the same. That is because the 2 data sets have the same data characteristics. Although the 2 data sets in the 3rd example come from different fishing areas, the 2 multivariate allometric patterns show no significant different. That may be because the 2 data sets have a similar age composition and share the same gene pool. Two different multivariate allometric patterns were expected in the 2nd and 4th examples, the growth rate in younger organisms is faster than that in older groups, and the 2 data sets come from different species, respectively. We also found that the frequency distributions of 5000  $\theta_1$  values in the 1st to the 4th examples were not centered on the theoretical value (0 degrees) (Fig. 1). That may have resulted from sampling error in the process of permutation.

The permutation test is relatively sensitive to sample size differences in some studies (Romano 1989, Crowley 1992, Hudson et al. 1992). Two cases which separately have 9 data dimensions of  $200 \times 30$ ,  $200 \times 40$ ,  $200 \times 50$ ,  $200 \times 60$ ,  $200 \times 70$ ,  $200 \times 80$ ,  $200 \times 90$ ,  $200 \times 100$ , and  $200 \times 150$  were generated to investigate this effect. The observations in the 1st example in this study were combined as the reference data set and the 1st data set (with 200 observations), and then we randomly generated the corresponding size (30, 40, 50, 60, 70, 80, 90, 100, and 150) from the reference data set as the 2nd data set. We also took the shrimp data set in the 4th example as the 1st data set (with 200 observations); but the crab data set was used as the reference data set to generate the 2nd data set of corresponding size. These 2 cases have different allometric characteristics: the 1st one was expected to have the same multivariate allometric patterns, while the 2nd was expected to have different allometric patterns.

Permutation tests were performed using 9 data dimensions in each case. These results are shown in Tables 6 and 7, respectively. All permutation tests of the 9 data dimensions in the 1st case were not significant (Table 6). This indicates that the 2 multivariate allometric patterns derived from each data dimension are the same. All permutation tests of the 9 data dimensions in the 2nd case were

significant. This indicates that the 2 multivariate allometric patterns derived from each data dimension are different. Therefore, the permutation test may be not sensitive to sample size differences. However, the value of P is not very stable (Table 6), so we suggest that the sample size difference be as small as possible when using permutation tests to address this question.

**Acknowledgments:** The authors thank Mr. P.W. Shi, Miss J.J. Wu, and Y.C. Chen for their assistance in collecting samples and preparing the drawings. We would like to thank Mr. L.K. Lee and an anonymous referee for commenting on and revising the manuscript.

## REFERENCES

- Bjorklund M. 1993. Phenotypic variation of growth trajectories in finches. *Evolution* **47**: 1506-1514.
- Burnaby TP. 1966. Growth-invariant discriminant functions and generalized distance. *Biometrics* **22**: 96-110.
- Chiu CS. 1996. Age and growth of red-spot prawn (*Metapenaeopsis barbata*) from the waters off I-Lan and the comparative morphometric studies of the species collected from waters surrounding Taiwan. Master's thesis, National Taiwan Univ., Taipei, Taiwan.
- Chu KH, QC Chen, LM Hunag, CK Wong. 1995. Morphometric analysis of commercially important penaeid shrimps from the Zhujiang estuary, China. *Fish. Res.* **23**: 83-93.
- Cock AG. 1966. Genetical aspects of metrical growth and form in animals. *Quart. Rev. Biol.* **41**: 131-190.
- Crowley PH. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. *Ann. Rev. Ecol. Syst.* **23**: 405-407.
- Edgington ES. 1987. Randomization tests. New York: Marcel Dekker.
- Gibson AR, AJ Baker, A Moesd. 1984. Morphometric variation in introduced populations of the common myna (*Acridotheres tristis*): an application of the jackknife to principal component analysis. *Syst. Zool.* **33**: 408-421.
- Good P. 1994. Permutation tests: a practical guide to resampling methods for testing hypotheses. New York: Springer-Verlag.
- Hudson RR, DB Dennis, LK Norman. 1992. A statistical test for detecting geographic subdivision. *Mol. Biol. Evol.* **9**: 138-151.
- Humphries JM, FL Bookstein, B Chernoff, GR Smith, RL Elder, SG Poss. 1981. Multivariate discrimination by shape in relation to size. *Syst. Zool.* **30**: 291-308.
- Huxley JS. 1932. Problems of relative growth. New York: Dial Press.
- Jolicoeur P. 1963. The multivariate generalization of the allometry equation. *Biometrics* **19**: 497-499.
- Jolicoeur R, JE Mosiman. 1960. Size and shape variation in the painted turtle, a principal component analysis. *Growth* **24**: 339-354.
- Klingenberg CP. 1996. Multivariate allometry. In FL Marcus, M Corti, A Loy, GJP Naylor, DE Slice, eds. *Advances in*

- morphometrics. New York and London: Plenum, pp. 23-50.
- Klingenberg CP, R Froese. 1991. A multivariate comparison of allometric growth patterns. *Syst. Zool.* **40**: 410-419.
- Klingenberg CP, JR Spence. 1993. Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. *Evolution* **47**: 1834-1853.
- Legendre L, P Legendre. 1983. Numerical ecology. New York: Elsevier.
- Manly BFJ. 1997. Randomization and Monte Carlo methods in biology. London: Chapman and Hall.
- Marriott FHC. 1979. Barnard's Monte Carlo tests: how many simulations? *Appl. Stat.* **28**: 75-77.
- Noreen EW. 1989. Computer-intensive methods for testing hypotheses. New York: J. Wiley.
- Romano JP. 1989. Bootstrap and randomization tests of some nonparametric hypothesis. *Ann. Stat.* **17**: 141-159.
- Shea BT. 1985. Bivariate and multivariate growth allometry: statistical and biological considerations. *J. Zool.* **206**: 367-390.
- Solow AR. 1990. A randomization test for misclassification probability in discriminant analysis. *Ecology* **71**: 2379-2382.
- Thorpe RS. 1983. A biometric study of the effects of growth on the analysis of geographical variation: tooth number in green geckos (Reptilia: Phelsuma). *J. Zool.* **201**: 13-26.

## 利用置換排列法檢定兩多變量異速成長型式間之差異

曾宗德<sup>1</sup> 葉顯楹<sup>1</sup>

研究不同性別、不同種、同種不同成長階段或不同地理族群間之多變量異速成長的比較漸漸增加。某些多變量統計方法假設被分析的各群具有相同之異速成長型態。因此在無檢視或比較各群之異速成長型式是否相同前，不應使用這類之方法。目前已有多個方法被使用來比較兩異速成長型式間之關係，但這些方法皆缺少一客觀之理論判定兩多變量異速成長型式是否相同或相異。本研究引用置換排列法以檢定兩多變量異速成長形式間之差異是否具有統計之顯著性，並引用四個例子加以解釋及測試本法之能力。多變量異速成長型式係以變數經對數轉換後之共變方矩陣為資料，再以主成份分析所得之第一特徵向量估計而得。利用兩第一主成份向量間之角度，當檢定的統計值。每一例子，執行 5000 次之隨機排列分析，藉以評估其顯著水準。最後並檢驗兩樣本數目間之差異是否會對此方法造成影響。結果顯示，第一主成份特徵值皆能解釋絕大部分的變異；第一主成份向量亦皆能充分描述其多變量異速成長型式。四個例子中，不論異速成長型式是相同或有差異皆可被本法成功測出。因此，重複排列分析法可客觀之判定兩多變量異速成長形式差異之顯著性。雖然此方法對樣本數目間之差異並不敏感，但我們仍建議當使用這方法時，儘量將兩樣本數的差異減至最小。

**關鍵詞：**第一主成份向量，角度，異速成長，重新排列。

<sup>1</sup> 國立臺灣大學海洋研究所