The Effects of Dietary Consistency on the Mandibular Form of *Peromyscus maniculatus bairdii*

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ABSTRACT

The objective of this study is to determine the impact of dietary consistency on mandibular form in the prairie deer mouse (*Peromyscus maniculatus bairdii*). Three groups of mice were fed diets that differed solely in physical consistency, not quantity or nutritional quality. One group was fed a hard pellet diet, the second was fed a gruel diet, and the third was fed a powder diet. Mice were sacrificed once they reached sexual maturity and full adult body size. Both size and shape of the jaw were analyzed, using a combination of traditional morphometric methods and geometric landmark-based methods. Diet proved to significantly affect all measurements of mandibular shape and size, with most differences in shape in the region of the angular process. Measurements of jaw height (measured between the coronoid and angular process) and the width of the condyle had the largest differences between the pellet-fed and treatment groups. Of the total variation in mandibular shape, 7.2% can be attributed to diet. These results support the idea that ecological variables, in this case dietary consistency, can alter phenotype, which may affect an organism's ability to function in nature.

INTRODUCTION

The form and function of an organism have long been viewed as intricately related, because form provides the structures required for an organism to function in nature. Ecology is also strongly related to an organism's phenotype and morphology, or its form, structure, and function. The discipline of ecomorphology studies the relationship between the role of ecology and its morphological adaptations in the environment; therefore, ecomorphology looks at the link between ecology and phenotype and an organism's ability to function and survive in its natural environment (Wainwright 1991). This interaction between ecology and structure can have an especially significant impact on plastic features because they are traits that are directly molded by ecological variables.

Plasticity, as defined by Callahan et al. (1997), is "the ability of an organism to alter its physiology, morphology or development in response to changes in its environment." Plasticity is expected when the optimal phenotype varies with the environment. In contrast, when the optimal phenotype is constant across the environments, canalization (when an organism develops along a relatively predictable pathway) is typically expected (Stearns 1989). The present study focuses on the former, because according to Wolff's law, the plasticity of the bony phenotype is a result of the load strain (Lanyon and Rubin 1985). Various studies show this to be valid in the rodent mandible (e.g., Watt and Williams 1951, Beecher and Corruccini 1981, Bouvier and Hylander 1984, Maki et al. 2002, He and Kiliaridis 2003, Langenbach et al. 2003). For these reasons, this study examines the effects of environmental variability (here dietary consistency) on morphological plasticity in the rodent mandible. Because harder diets cause a greater magnitude in strain, mandibular bone growth will be stimulated. Therefore, it is expected that animals eating harder diets will display more mandibular growth than those eating softer diets.

Prior studies have found that plasticity can be maladaptive under certain specified conditions. In particular; soft diets, such as those characteristic of modern industrial humans, have been correlated to instances of malocclusion due to a reduction in the stimulation of the masticatory muscles (e.g., Watt and Williams 1951, Beecher and Corruccini 1981, Bouvier and Hylander 1984, He and Kiliaridis 2003, Langenbach et al. 2003, Lieberman et al. 2004). Previous studies have been fascinated by the prevalence of malocclusion in human populations, leading to a focus on pathological as opposed to adaptive consequences of plasticity (Beecher and Corruccini 1981, Maki et al. 2002). In contrast, this study focuses on the role of ecological variables (diet) in shaping form to fit into function.

This study looks at the effects of dietary consistency jaw form by varying solely the consistency of the food, not either amount or nutritional quality, the same procedure used by Watt and Williams (1951), Beecher and Corruccini (1981), Bouvier and Hylander (1982, 1984) and Lieberman et al. (2004). I also took additional precautions to help improve this study, using a large sample of deer mice from the experimental population analyzed by Myers et al. (1996), which explored the relationship between dietary consistency and craniofacial morphology. My sample size (N = 268 mice) is considerably larger than in most prior studies, which increases the reliability of the results. Additionally, many studies treat individuals from the same litter as if their morphologies were independent, but they are not, and failing to take their correlations into account violates an important assumption of statistical tests. However, the major distinction between this study and prior ones is the comprehensive sampling of mandibular shape. Until recently, most analyses studying the relation of diet to jaw form relied solely on traditional distance measurements. In contrast, this study uses traditional distance measurements as well as geometric landmark-based morphometrics. This geometric approach allow for separating the

impact of diet on shape from its impact on size, and the statistical power of geometric morphometrics improves the ability to detect even slight differences in shape caused by differences in diet (Zelditch et al. 2004).

MATERIALS AND METHODS

Sample

I took advantage of the specimens of *Peromyscus maniculatus bairdii* analyzed in a previous study of the impact of dietary consistency on skull shape (Myers et al. 1996). The mice are from the second-generation born in the laboratory, offspring of pregnant females caught in the wild around Ann Arbor, Michigan. After birth, litters were randomly allocated to one of three diet groups: control/pellet diet (solid Purina Mouse Chow pellets), and two soft diets: liquid/gruel (Purina Mouse Chow ground into a powder and mixed with water) and powder (Purina Mouse Chow ground into a powder). The sample had 268 specimens (60 pellet-, 109 liquid/gruel-, and 99 powder-fed). All groups were housed in clear plastic cages with pine shavings and cotton Nestlets for bedding. All groups were fed ad libitum and the nutritional content for all three groups was identical.

Mice were sacrificed at 50 days old, once they reached sexual maturity and attained full adult body size. Sacrificed mice were cleaned by dermestid beetles, soaked in ammonia, and dried. Mandibles were separated into right and left hemimandibles; only left mandibles were measured for this study. The hemimandibles were photographed in lateral view with the long axis in the photographic plane. The order that the photographs were taken was random and the photographer was unaware of the gender, family, or treatment group of the individuals being

photographed. However, the person analyzing the photographs was awre of which individual belonged to which diet group.

Shape Coordinates

Mandibles were measured using geometric landmark-based morphometrics (Rohlf 2006). Fourteen landmarks were chosen to provide complete coverage of the mandible (Fig. 1). Specimens were removed from the study if all 14 landmarks could not be easily located or if the specimens were otherwise damaged. Additional landmarks (semi-landmarks) along the curves of the incisor alveolus, the mandibular process, and the ventral ramus were used to provide information about jaw curvature and shape. Six curves were measured with 30 evenly spaced semi-landmarks per curve. The number of semi-landmarks was later reduced to 15 per curve following superimposition, leaving 104 total landmarks and semi-landmarks (Fig. 2). All landmarks and semi-landmarks were digitized using tpsDig2.1 (Rohlf 2006).

Landmarks and semi-landmarks were superimposed to remove differences unrelated to shape (position, orientation and scale, and in the case of semi-landmarks, variations in their spacing along the curve). Generalized Procrustes-based analysis was used, which superimposes all individuals to minimize the summed squared Procrustes distances of all individuals relative to the mean shape (Rohlf and Slice 1990). Since the spacing of the points along the curve is arbitrary, semi-landmarks require specialized methods of superimposition. To remove that source of non-shape variation, the tangent to the curve was approximated at each semi-landmark. Each semi-landmark was then slid toward the normal of the tangent, minimizing the distance from the reference shape. All 180 semi-landmarks were used to estimate the tangent to the curve, but half of the semi-landmarks from each curve were removed following superimposition. After

superimposition, the coordinates of semi-landmarks can be used in any method of conventional shape analysis. Semi-landmarks have only one degree of freedom apiece so the total degrees of freedom for the data is $2K + L - 4$, where K is the number of landmarks (14) and L is the number of semi-landmarks (90) (Zelditch et al. 2004).

Centroid size was used as the size measurement; centroid size is defined as the square root of the summed squared distances of all landmarks to the centroid. This is the preferred measure of size because it is the one-size variable that is uncorrelated with shape in the absence of allometry (Bookstein 1991). All tests of statistical analysis used log-transformed values of centroid size because this eliminates the dependence of the variance on the mean.

In addition to measuring overall mandible size, four inter-landmark distance measurements were selected in order to evaluate the differences between groups. These four measurements are: (1) jaw height (JH), from the highest point on the coronoid to the angular process (between landmarks 8 and 11); (2) condyle width (CW), between landmarks 9 and 12; (3) length of the molar row (ML), between landmarks 5 and 7; (4) length of the jaw (JL), measured from the posterior of the incisor to the most posterior landmark on the condyle between landmarks 2 and 12 (Fig. 4).

Statistical Methods

To determine if the mean sizes and shapes differ as a function of diet, one-way nested analyses of variance (ANOVA) were calculated for the univariate analyses and one-way nested multivariate analysis of variance (MANOVA) for the multivariate analyses. The main factors in these analyses are diet and litter, the latter of which is nested within diet because all siblings were given the same diet. Univariate ANOVAs were performed to compare the means for overall

mandible size and also for each of the four inter-landmark distances. MANOVA was used to compare shapes because shape is multidimensional (Zelditch et al. 2004).

To determine the statistical significance of diet's impact on shape, I used Goodall's F-test (Goodall 1991). Goodall's F is based on the ratio of two mean squares calculated from the sums of squared Procrustes distances, which is defined as (approximately) the sum of the squared differences between corresponding landmarks between an individual and the average shape; this is the generally accepted metric for the distance between shapes in geometric morphometrics (Bookstein 1996; Zelditch et al. 2004). Goodall's F-test is extremely powerful because, unlike conventional multivariate approximations of F that lose many degrees of freedom (df) when estimating whole variance-covariance matrices, Goodall's F does not need those estimates. For Goodall's F, the df are the product of the univariate df and the dimensions of the data. Therefore, for comparisons of mandibular shape between the pellet-fed, liquid-fed, and powder-fed mice based on 104 total landmarks and semi-landmarks, the df for the diet factor is the product of the number of groups minus one and twice the number of landmarks plus the number of semi-landmarks minus four: (3-1)((2*14)+(90-4)) = 228. For the error term, df are a function of the number of litters within diets, and the number of diets and number of landmarks. For this study, which had 51 litters, the df for the nested term is (51)((2*14)+(90-4)) = 5814 and for the error term df is (214)((2*14)+(90-4)) = 24396 (Goodall 1991).

Goodall's F assumes identical, independent error at each landmark, an assumption that is unlikely to be true, therefore permutation tests were used in order to determine the probability of obtaining an F as large as the observed one under the null hypothesis; these analyses were done in Manovaboard (Sheets 2000), a program in the IMP series. The MANOVA determines whether means are different, but it does not determine which groups differ. Therefore, pair-wise

comparisons were conducted between all groups for every distance measurement, using the Bonferroni correction to adjust P-values for each test by the total number of pair-wise tests (Holm 1979).

To find the shape variable that best discriminates among treatments, Canonical Variates Analysis (CVA) was used, which finds the set of variables along which groups most differ relative to their within-sample variance. Since CVs are equivalent to multivariate one-way analyses of variance, family effects were removed from the data prior to conducting the CVA. To assess the ability of the CVs to correctly assign groups, misclassification rates were calculated using a resampling-based test (Jackknifing). During this procedure, a single specimen is excluded from the CVA analysis at a time, and the entire analysis is repeated without that particular specimen. The excluded specimen is then assigned to one of the groups using the CVA axes derived from other specimens. This procedure is repeated sequentially for all specimens in the data set. The rate of correct assignments of excluded specimens is then calculated based on these results. This jackknife procedure may be a better estimate of the performance of the assignment procedure, since the assigned specimens are not used during the axes calculation (Nolte and Sheets 2005).

The four measurements selected for individual analysis (Fig. 4) were chosen because they are in the regions that show a large difference in shape (as determined by CVA) or where no differences in shape were found. To determine whether individual dimensions (rather than shape variables) differ, ANOVAs were used to compare these means, and that analysis was followed by pair-wise comparisons. To maintain a table-wide error rate of 5%, the Bonferroni adjustment for multiple tests was used (Holm 1979).

For the analysis of size, the magnitude of the differences is measured by the percent difference between groups. To compare a particular measurement between the pellet-fed to the liquid-fed groups, the mean value of that measurement for the liquid-fed group was subtracted from that of the pellet-fed group, divided by the mean value of the pellet-fed group, and multiplied by 100 to obtain the value as a percent. For the analyses of shape, the magnitude of the differences is determined by the Mahalanobis' distance, a statistical measure of a morphometric distance that takes into account the correlations among the variables. Calculation of the Mahalanobis' distance was done in PAST (Hammer et al. 2009).

RESULTS

Centroid size differs slightly between groups (Table 1). The reduction in size between pellet-fed and liquid-fed is merely 1.52%, but if even slight, the differences are statistically significant ($P < 0.01$). The reduction in size between liquid-fed and powder-fed is even smaller (0.9%), but also statistically significant ($P = 0.03$) even after adjusting probabilities for the multiple tests. However, the differences between the pellet-fed and powder-fed groups (0.64%) is not statistically significant ($P = 0.16$).

Jaw height (JH) is significantly affected by diet ($P < 0.01$) (Table 2). Pair-wise comparisons show that the pellet-fed mice are 6.19% larger than the liquid-fed mice and 3.38% larger than the powder-fed mice (Table 4). Differences in JH between the pellet-fed mice and the two treatment groups are statistically significant ($P < 0.01$ in both cases).

The width of the condyle (CW) is significantly affected by diet ($P < 0.01$). Pair-wise comparisons show that pellet-fed mice are 6.99% wider than liquid-fed mice and 1.11% wider

than powder-fed mice. Differences in CW between the pellet-fed mice and both treatment groups are statistically significant ($P < 0.01$ in both cases).

The length of the molar row (ML) is also significantly affected by diet ($P < 0.01$). Pair-wise comparisons show that the pellet-fed mice are 2.56% shorter than both treatment groups, which do not differ from each other. The differences in ML between the treatment groups and the pellet-fed mice are statistically significant ($P < 0.01$).

Jaw length (JL) is also significantly affected by diet ($P < 0.01$); however, this is not true between all groups. Pair-wise comparisons show that the pellet-fed mice are 1.49% larger than the liquid-fed mice ($P < 0.01$). The pellet-fed mice are only 0.87% larger than the powder-fed mice, which is not statistically significant ($P = 0.06$).

Both diet and family effects have a statistically significant impact on mandibular shape (Table 5). The variance explained by the main factor, diet is 7.2%, compared to 41.2% explained by family effects. Although diet seems to have a relatively modest impact on shape, pair-wise comparisons of shape show that differences between groups are statistically significant ($P < 0.01$ in all pair-wise comparisons); this is also reinforced by canonical variates, which reveal that the two groups do not overlap (Fig. 3). If the analysis is done without removing family effects, as many as 43.66% of specimens are misclassified (Table 7). But that misclassification rate decreases to 13.81% after removing the variation due to family effects (Table 8). Figure 3 therefore, shows the results of a CVA after removing family effects. The pellet-fed mice differ from the two soft-diet treatments primarily along CV1, whereas the two soft-diet treatments differ from each other along CV2.

Mahalanobis' distances (Table 9) show that, as expected, the pellet-fed mice differ most from the two soft-diet treatment groups, which differ little from each other. Unlike the analyses

measuring differences in size, Mahalanobis' distances show that the largest differences in shape are between the pellet- and powder-fed mice.

DISCUSSION

Diet significantly affects mandibular shape and size in *Peromyscus maniculatus bairdii* ($P < 0.01$ in all cases). Softer diets typically reduce the size of mandibular processes as well as mandibular length. The reduction in lengths of the mandibular processes is not because the entire mandible is smaller, but rather because certain areas are more affected than others. Mandibular shape as well as size, responds to dietary consistency. Overall, the variance that can be attributed to diet is 7.2 % of the total, with most of that concentrated in the region of the angular process. Size differences are typically significant statistically, even if all three groups do not always differ from each other. In particular, for jaw length (JL), the pellet-fed mice are only 0.89% larger than the powder-fed mice and the difference is not statistically significant ($P = 0.06$). Additionally, the pellet-fed mice are not the largest for all measurements; the length of the molar row (ML) is 2.56% smaller in the pellet-fed mice than in both treatment groups, which do not differ from each other. However, despite these few exceptions, the liquid- and powder-fed groups do tend to be smaller; they undergo a reduction in jaw height (JH), with the liquid- and powder-fed mice being 6.19% and 3.38% smaller than the pellet-fed mice, respectively. This measurement was analogous to that used by Maki et al. (2002), who found his pellet-fed mice to be 6.09% larger than the powder-fed mice. Maki and colleagues interpreted that decrease to result from a reduction in the coronoid process, suggesting that softer diets may slow the growth of the coronoid, possibly due to the lack of stimulation by the temporalis muscle, which inserts into the coronoid process. My results suggest that the difference is also, or even primarily, in the

angular process, suggesting that the reduction in growth is due at least as much to the stimulation by the superficial masseter.

This is one of the first studies to measure the curvature of the angular process, where I found the largest difference in shape. This difference can be explained by the fact that the angular process is the location where one of the major rodent jaw muscles, the superficial masseter, inserts. Therefore, muscle activity most likely has a strong impact on the formation, maintenance, and growth of the angular process. Pellet-fed mice, for example have an increased load and higher muscle activity, which in turn increases activity and curvature of the angular process. This indicates that diet affects shape as well as size, and this is an area where more research needs to be concentrated.

The pellet- and liquid-fed groups significantly differed in both centroid size and jaw length ($P < 0.01$ in both cases). In both measurements, the pellet-fed mice were approximately 1.5% larger than the liquid-fed mice. The same trend occurred with the pellet- and powder-fed mice, but the differences in means were smaller and not statistically significant. However, the opposite trend was seen between groups in another measurement of the length that looked at the molar row. No differences were detected between treatment groups, and the pellet-fed mice were 2.56% smaller than both treatment groups. This difference was slight, but unlike differences detected in previous studies, significant ($P < 0.01$). Although an increased hardness in diet increased the overall length of the mandible, this was not the case for the length of the molar row. This unexpected result is difficult to explain given the data of this study.

The condyle has been the main focus of many studies because it is believed to be especially plastic (e.g., Watt and Williams 1951, Barber et al. 1963, Moore 1965, Beecher and Corruccini 1981, Bouvier and Hylander 1984, and Kiliaridis 1986). The results of this study

show that the pellet-fed mice are 6.99% wider than the liquid-fed mice ($P < 0.01$) and 1.11% larger than the powder-fed mice ($P < 0.01$). Bouvier and Hylander (1984) found the same trend, with their hard-diet mice being 12.82% larger than the soft-diet mice. What these results show is that the condyle is a plastic feature is susceptible to alteration by environmental variability. The condyle is believed to be one of the major growth centers of the mandible and needs constant stimulation to continue growing (Landesberg et al. 1994). In the cases of the soft-diet groups, it is possible that the condyle did not receive sufficient stimulation, causing a reduction in the growth and development of the condyle.

Typically, soft-diets are found to reduce the mandibular processes; however, there is little agreement among studies about how large the effect is or where it is localized. A reason for this is that many of the methods for studying the effects of dietary consistency on mandibular form have yet be standardized. Therefore, it is difficult to compare results across studies. The animals in this study were given one of three diets (pellet, liquid, or powder). Most studies typically use a hard- and soft-diet group, but the type of soft-diet group usually varies. Despite this variation, studies show that soft-diets reduce the mandibular process, but dietary inconsistencies make it difficult to agree on the degree of the effect. Another area of discrepancy is the quantity and quality of measurement. Not all studies focus on the same areas of the jaw, and the methods of measuring greatly differ.

When statistically significant differences were detected in this study, the largest differences were always found between the pellet- and liquid-fed mice. This suggests that any sort of chewing activity, even if slight as with the powder-fed group, stimulates the muscles necessary for jaw development. Since the liquid-fed group receives very little stimulation, the jaw is typically underdeveloped, and with the exception of ML, results in smaller measurements

for the liquid-fed mice, which may, in turn, affect form. To enhance further studies in this area, a more comprehensive study of the mandible should be performed, which includes additional measurements in size, such as the ramus, the incisors, and additional measurements of the coronoid and condyle. These additional measurements would provide more information on the location and magnitude of the effects of dietary consistency on mandibular form.

CONCLUSION

Dietary consistency significantly alters mandibular form of *Peromyscus maniculatus bairdii*, affecting both size and shape. Of the total sample variation, 7.2% can be attributed to diet. With the exception of the length of the molar row, treatment groups had smaller measurements in size than those detected in the pellet-fed mice. The largest differences in shape occurred at the angular process, and the largest differences of size were those of jaw height (measured from the coronoid to the angular process) and the width of the condyle. These general trends were similar to those found in prior studies. Size measurements of the powder-fed mice were intermediate to those of the pellet- and liquid-fed mice (except for the measurement of the molar row), indicating that even minimal muscle stimulation allows for growth. The results of this study support the idea that mandibular is a plastic feature, and its form is affected by environmental variability (in this case the physical consistency of food), which in turn affects mandibular form and function in nature.

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FIGURE LEGENDS

Fig. 1. Landmark sampling scheme, shown on a photograph of the mandible of *Peromyscus maniculatus bairdii*.

Fig. 2. Schematic of the mandible indicating landmarks (larger points) and semi-landmarks (smaller points).

Fig. 3. Scores of individuals on CVs. CV1 is the dimension along which the groups most differ, whereas CV2 explains the next greatest dimension of inter-group differences. The black circles indicate the pellet-fed mice, blue X's indicate the liquid-fed mice, and the red stars indicate the powder-fed mice. Below each axis, the deformation grid shows the difference along that dimension in the direction of increasing scores (towards the pellet-fed group along CV1, towards the powder-fed group along CV2).

Fig. 4. Schematic of the mandible showing the inter-landmark distances that were measured for each individual. JH is a measure of jaw height from the coronoid to the angular process, CW is the width of the condyle, ML is the length of the molar row, and JL is a measurement of jaw length from the posterior of the incisor to the condyle.

Table 1. Average mandible size (measured by centroid size, CS) for each group.

Diet	**CS**
Pellet	15.74
Liquid	15.50
Powder	15.64

Table 2. Univariate analysis of variance (one-way ANOVA) of inter-landmark distances between treatment groups. SS denotes sums of squares, df the degrees of freedom, and MS the mean square. JH is a measure of jaw height from the coronoid to the angular process, CW is the width of the condyle, ML is the length of the molar row, and JL is a measurement of jaw length from the posterior of the incisor to the condyle.

JH					
Source	**SS**	**df**	**MS**	**F**	**P**
Diet	0.13	2	0.06	36.17	< 0.01
Litter(Diet)	0.33	51	0.00	3.77	< 0.01
Error	0.37	214	0.00		
CW					
Source	**SS**	**df**	**MS**	**F**	**P**
Diet	0.25	2	0.12	18.22	< 0.01
Litter(Diet)	0.86	51	0.02	2.49	< 0.01
Error	1.45	214	0.00		
ML					
Source	**SS**	**df**	**MS**	**F**	**P**
Diet	0.04	2	0.02	12.91	< 0.01
Litter(Diet)	0.30	51	0.00	4.19	< 0.01
Error	0.30	214	0.00		
JL					

Source	**SS**	**df**	**MS**	**F**	**P**
Diet	0.00	2	0.00	4.91	< 0.01
Litter(Diet)	0.08	51	0.00	2.31	< 0.01
Error	0.14	214	0.00		

Table 3. Means of inter-landmark distances (Std. error = standard error and Standard Dev. = standard deviation from the mean).

PELLET-FED				
	JH	**CW**	**ML**	**JL**
Mean	4.85	1.44	2.33	12.03
Std. Error	0.04	0.02	0.02	0.04
Standard Dev.	0.28	0.13	0.14	0.34
LIQUID-FED				
	JH	**CW**	**ML**	**JL**
Mean	4.55	4.55	2.39	11.85
Std. Error	0.02	0.02	0.01	0.03
Standard Dev.	0.24	0.24	0.11	0.36
POWDER-FED				
	JH	**CW**	**ML**	**JL**
Mean	4.69	1.42	2.39	11.92
Std. Error	0.02	0.01	0.01	0.03
Standard Dev.	0.22	0.14	0.04	0.34

Table 4. Matrix of pair-wise differences between means of inter-landmark distances based on the percent change from the pellet-fed group (row 1) to the liquid-fed group (row 2) and powder-fed group (row 3). 1 = pellet-fed, 2 = liquid-fed, 3 = powder-fed, JH = jaw height, CW = condylar width, ML = length of the molar, and JL = jaw length.

JH			
	1	**2**	**3**
1	0	6.19	3.38
2		0	-2.99
3			0
CW			
	1	**2**	**3**
1	0	6.99	1.11
2		0	-6.32
3			0
ML			
	1	**2**	**3**
1	0	-2.56	-2.56
2		0	0.00
3			0
JL			
	1	**2**	**3**

1	0	1.49	0.87
2		0	-0.63
3			0

Table 5. Multivariate analysis of variance (MANOVA) for the main and nested factors. Sums of squares (SS), the degrees of freedom (df, a function of the number of landmarks, groups, and individuals), the F-ratio, and the P-value were calculated for both factors.

Factor	**SS**	**df**	**F**	**P**
Main	0.01	228	14.93	< 0.01
Nested	0.08	5814	3.35	< 0.01
Error	0.10	24396		
Total	0.20			

Table 6. Pair-wise comparisons for shape using a two-way nested MANOVA (SS = sums of squares and df = degrees of freedom).

PELLET-LIQUID				
Factor	**SS**	**df**	**F**	**P**
Main	0.01	228	22.54	< 0.01
Nested	0.05	5814	3.26	< 0.01
Error	0.07	24396		
Total	0.13			
PELLET-POWDER				
Factor	**SS**	**df**	**F**	**P**
Main	0.00	228	10.64	< 0.01
Nested	0.05	5814	3.64	< 0.01
Error	0.06	24396		
Total	0.11			
LIQUID-POWDER				
Factor	**SS**	**df**	**F**	**P**
Main	0.00	228	10.87	< 0.01
Nested	0.06	5814	2.80	< 0.01
Error	0.09	24396		
Total	0.15			

Table 7. Classification of specimens based on the canonical variates (CV) before the removal of family effects. Original groups are presented along rows (1 = pellet-fed, 2 = liquid-fed, 3 = powder-fed) and CV groups are presented down the columns. Discrepancies between the original and assigned groups are used to estimate the misclassification rates.

0	**1**	**2**	**3**
1	37	13	10
2	18	56	35
3	17	24	58

Table 8. Classification of specimens based on the CVs after the removal of family effects. Original groups are presented along rows (1 = pellet-fed, 2 = liquid-fed, 3 = powder-fed) and CV groups are presented down the columns. Discrepancies between the original and assigned groups are used to estimate the misclassification rates.

0	**1**	**2**	**3**
1	57	1	2
2	3	96	10
3	5	16	78

Table 9. Mahalanobis' distances between groups (1 = pellet-fed, 2 = liquid-fed, 3 = powder-fed).

	1	**2**	**3**
1	0	0.24	0.25
2		0	0.19
3			0

Figure 1

Figure 2

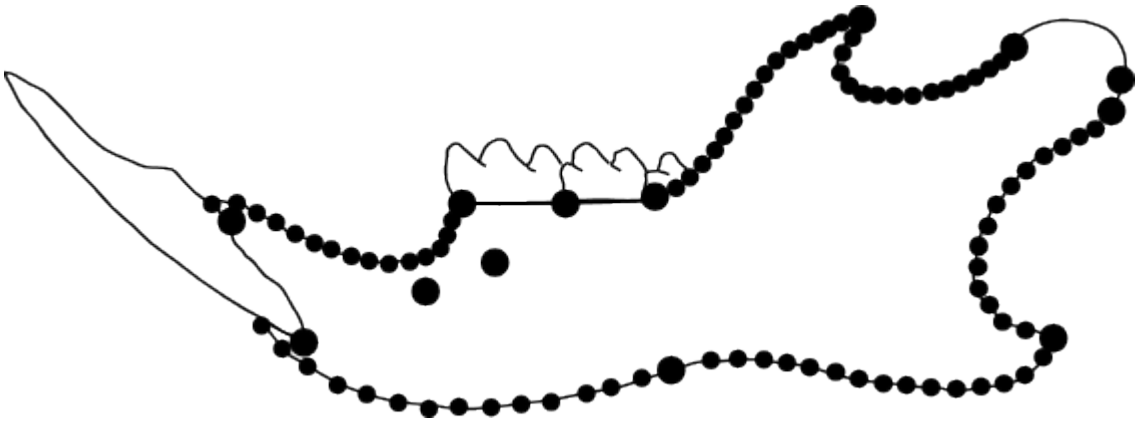

Figure 3

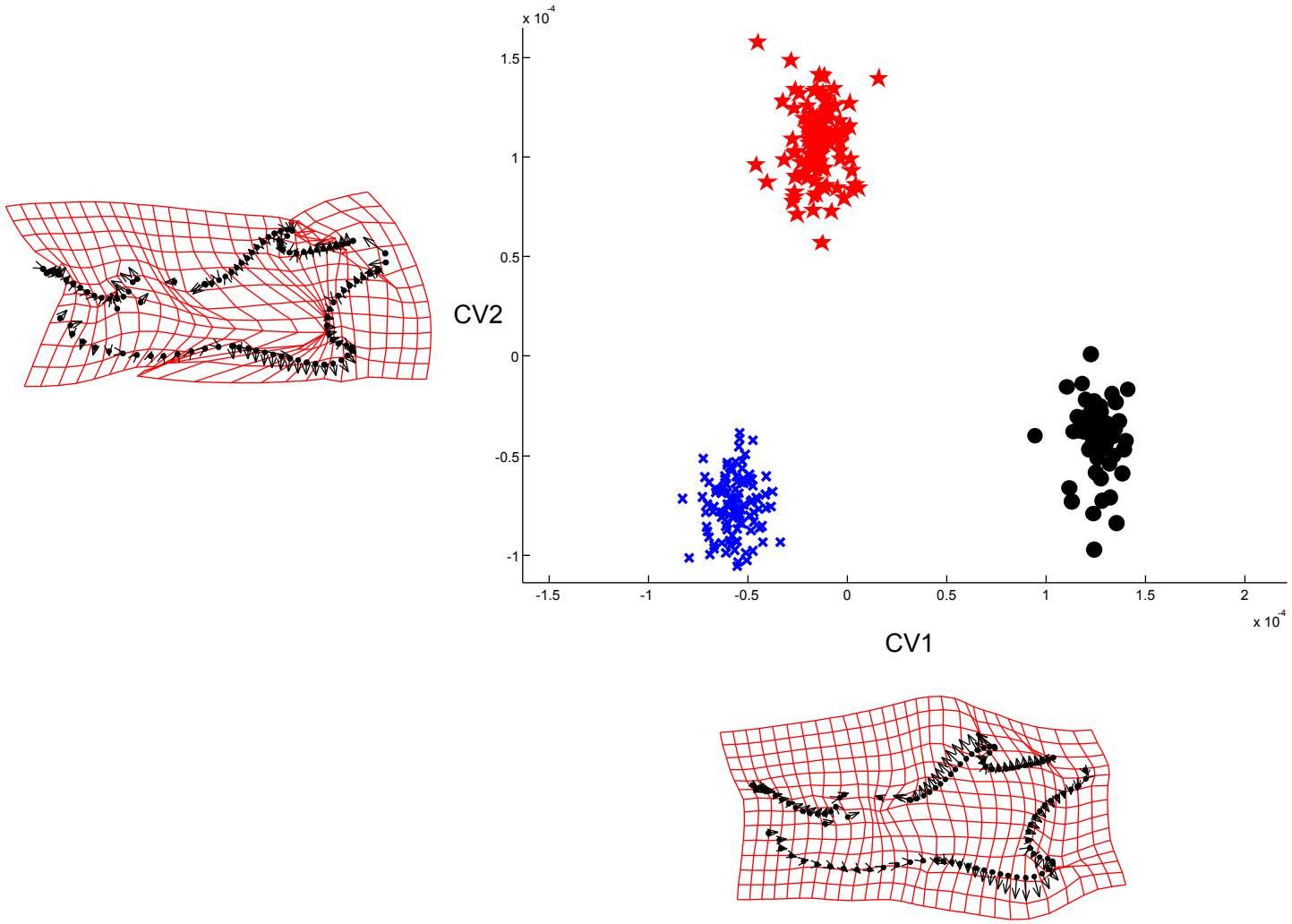

Figure 4