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Independence between developmental stability and canalization in the skull of the house mouse

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The relationship between the two components of developmental homeostasis, that is canalization and developmental stability (DS), is currently debated. To appraise this relationship, the levels and morphological patterns of interindividual variation and fluctuating asymmetry were assessed using a geometric morphometric approach applied to the skulls of laboratory samples of the house mouse. These three samples correspond to two random-bred strains of the two European subspecies of the house mouse and their F_1 hybrids. The inter- and intraindividual variation levels were found to be smaller in the hybrid group compared to the parental ones, suggesting a common heterotic effect on skull canalization and DS. Both buffering mechanisms might then depend on the same genetic condition, i.e. the level of heterozygosity. However, related morphological patterns did not exhibit any congruence. In contradiction with previous studies on insect wing traits, we therefore suggest that canalization and DS may not act on the same morphological characters. The fact that this discrepancy could be related to the functional importance of the symmetry of the characters under consideration is discussed in the light of our knowledge of the genetic bases of both components of developmental homeostasis.

Keywords: fluctuating asymmetry; morphological variation; geometric morphometrics; hybridization

1. INTRODUCTION

Waddington (1957) proposed that phenotypic constancy is ensured by two processes: canalization and developmental stability (DS). The former is thought to ensure phenotypic constancy in populations in spite of genetic and environmental variations, whereas the latter is believed to operate in given genetic and environmental conditions, i.e. in spite of random developmental errors. In populations, the easiest way to appraise canalization may be by estimating interindividual variance and DS by intraindividual variance, which is often estimated by the level of fluctuating asymmetry (FA) in bilaterally symmetrical organisms (Palmer & Strobeck 1986). Waddington (1957) suggested that canalization and DS are genetically independent and may affect morphological structures differently. Nevertheless, recent studies have reported that highly canalized characters could simultaneously present high levels of DS (Clarke 1998; Klingenberg & McIntyre 1998; Livshits *et al*. 1998; Woods *et al*. 1999), leading some authors to conclude that there is no reason to suspect canalization and DS as being distinct processes (Klingenberg & McIntyre 1998). In order to explain the congruencies between both types of variation, Clarke (1998) hypothesized that both mechanisms, even though functionally distinct, could depend on the same genetic background (i.e. genomic coadaptation and/or heterozygosity) or on the fitness value of the characters under study, that is on the intensity of the stabilizing selection acting on those characters. In fact, most studies which have reported a correlation between FA and morphological variability have considered characters for which bilateral symmetry was of critical morpho-functional importance, for example, wing traits in insects (Clarke 1993*a*,*b*, 1998; Klingenberg & McIntyre 1998).

In this study, we focused on a morphological structure, the skull of the house mouse, for which the global bilateral symmetry may not be of such crucial importance for fitness as for locomotion-related characters. In addition, we chose closely related groups presenting a different genetic background and level of inbreeding: two laboratory strains of the house mouse *Mus musculus musculus* and *Mus musculus domesticus* (slightly inbred), as well as their F_1 hybrids (outbred). Previous studies based on tooth characters have shown that intersubspecific hybrids benefit from a heterotic effect for DS and size, both in the wild (Alibert *et al*. 1994), where the two subspecies form a narrow hybrid zone across Europe (Boursot *et al*. 1993) and in laboratory conditions (Auffray *et al*. 1996; Alibert *et al*. 1997). The comparison of these closely related groups differing in their degree of DS, at least for teeth, offered a good opportunity of assessing the covariation in phenotypic variation and FA in a hybrid group which was expected to benefit from heterosis on development.

The number of characters which can be defined on a skull is obviously high but, as the skull represents an integrated structure, one may consider the developmental pathways of these characters as not being independent of each other (Zelditch *et al*. 1992). We thus chose to assess the interindividual variation and FA of the skull using the geometric morphometric approach recently proposed by Klingenberg & McIntyre (1998). In order to test whether canalization and DS were both enhanced in hybrids, we estimated the levels of interindividual variation and FA in parental and hybrid groups for skull size and shape. Since geometric morphometrics allowed us to depict patterns of

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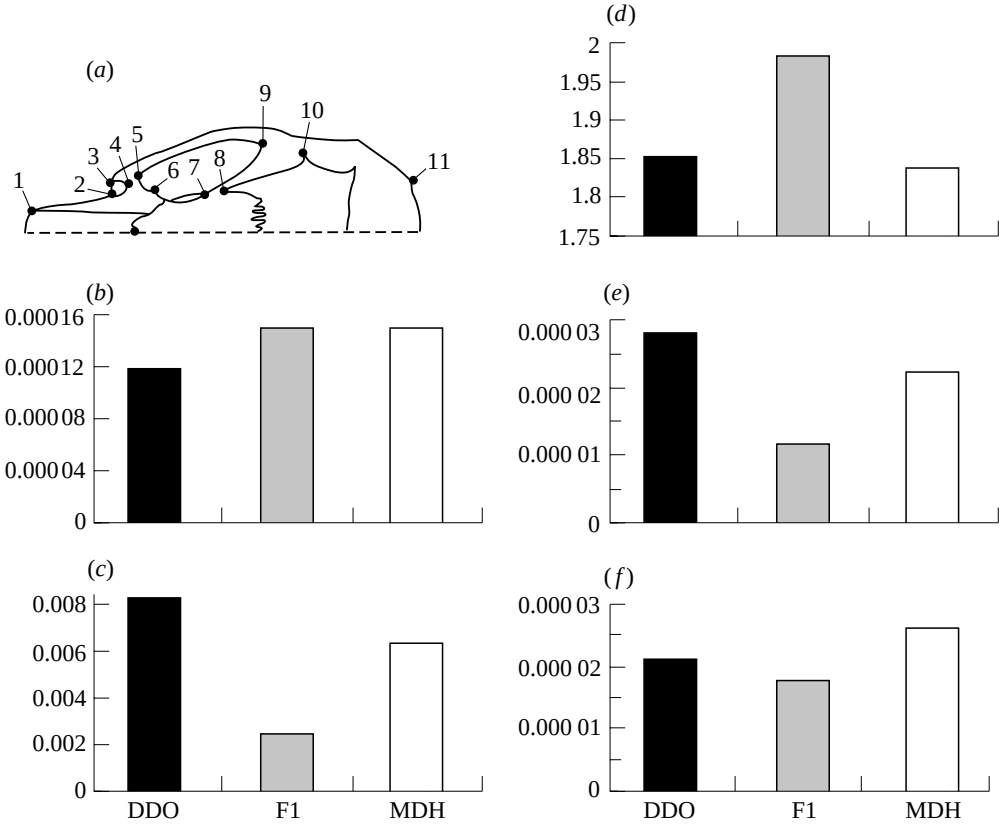

Figure 1. (*a*) Location of the landmarks on a hemi-skull. For the three samples, (*b*) size FA10, (*c*) size VAR, (*d*) mean centroid size, (*e*) shape FA10 and (*f*) shape VAR. Black bars, MDH; white bars, DDO; grey bars, hybrids.

morphological variation precisely (Bookstein 1991; Rohlf & Marcus 1993), it was possible to test for congruence between and within groups of these patterns with respect to the inter-individual and between-sides variances. A non-significant congruence between these patterns within groups would argue for independence between canalization and DS.

2. MATERIAL AND METHODS

Samples of two laboratory, random-bred strains of the European subspecies of the house mouse *M. m. musculus* (referred to as MDH, $n = 38$) and *M. m. domesticus* (referred to as DDO, $n = 44$) and their F_1 hybrids (F_1, $n = 35$) were used in this study. The crossing procedure and rearing conditions are detailed in Alibert *et al.* (1997). Both MDH and DDO are slightly inbred due to low laboratory population sizes: using pedigrees, we estimated mean *f*-values of 0.24 (MDH) and 0.25 (DDO). In contrast, F_1 individuals obtained by crossing MDH and DDO parents are therefore completely outbred. Eleven landmarks were digitized on the two-dimensional projection of the upper side of half skulls (figure 1*a*) using the Bioscan image analysis software Optimas v. 4.0. Two different measurement sessions during which the order of digitalization was randomly attributed between individuals and samples were conducted. Both sides of the skull were then digitized twice.

(a) *Variation in size*

The size of an individual side was estimated by the centroid size of its landmarks configuration, i.e. the square root of the sum of squared distances between each landmark to the centroid (Slice *et al.* 1996). Individual size is then represented by four values, one for each side and one for each session. Strictly following Palmer's (1994) procedure, the two-way mixed model ANOVA was used to assess the significance of potential sources of variation. An index of interindividual size variance (referred to as VAR) was computed as the added component of variance between individuals in the two-way ANOVA. The size FA index (referred to as FA10 according to Palmer's (1994) nomenclature) was computed as the added component of variance due to the interaction 'individual × side' effect (i.e. the intraindividual variance). The VAR and FA10 were both compared between groups using parametric *F*-ratios. In addition, we performed an ANOVA on centroid size to test for the difference in mean size between samples.

(b) *Variation in shape*

The method used was largely based on Klingenberg & McIntyre (1998) (see also Auffay *et al.* (1999*a*) for additional explanations). These authors modified the two-way mixed model ANOVA appropriately for application to a constrained data set corresponding to the landmark coordinates after all configurations (right and mirrored left configurations of the two sessions and for the three samples) were superimposed following the Procrustes generalized least-squares (GLS) superimposition method (Rohlf & Slice 1990).

In order to test for the difference in mean shape between groups and considering that the level of variation remained low both between and within groups (see Dryden & Mardia (1998) for the conditions of application), a MANOVA involving group as a single effect was applied to the coordinates of the superimposed configurations.

The procedure used to estimate the FA and interindividual variation levels in shape is as follows. Within each sample, the

two-way mixed model ANOVA was applied to each of the 22 coordinates after superimposition, producing the sums of squares relative to the potential sources of variation (individual, side and their interaction). For each effect, these sums of squares were summed across the *x*- and *y*-coordinates of all landmarks in order to obtain the Procrustes sums of squares. Procrustes mean squares were computed by dividing these sums of squares by the relevant degrees of freedom (which were the usual degrees of freedom multiplied by the number of coordinates minus four degrees of freedom) lost through the GLS superimposition procedure (Klingenberg & McIntyre 1998). Following Palmer & Strobeck's (1986) design, the resulting Procrustes mean squares were used to test for the significance of each effect (i.e. individual, side and their interaction) across landmarks using *F*-ratios and also to estimate the population indices of shape FA (FA10) and interindividual shape variation (VAR). The shape FA10 and VAR indices were then compared between samples using parametric *F*-ratios.

The morphological patterns of the interindividual variation, FA and digitizing error were assessed within each group according to the following procedure. Within each sample, a two-way MANOVA involving individual, side and their interaction was performed on the coordinates of the landmarks of superimposed configurations (i.e. the strictly analogous multivariate procedure of the univariate two-way ANOVA). The variance–covariance (VCV) matrix corresponding to the between-side variation was obtained by subtracting the error VCV matrix from the interaction one and dividing by the appropriate number of degrees of freedom. Similarly, the interindividual VCV matrix was calculated by subtracting the interaction one from the individual one. The congruence of interindividual and between-side patterns of variation was first assessed by the correlation between corresponding VCV matrices within samples. These correlations were tested using permutation tests designed to maintain the association between pairs of *x*- and *y*-coordinates: in order to focus on the landmarks covariation, the permutations involved pairs of rows and columns of one of the two matrices. Correlative patterns of whole shape variation are difficult to interpret: a lack of correlation is insufficient for ascertaining the independence of patterns, since a weak congruence does not imply a significant correlation but, conversely, a significant correlation would suggest a real congruence. Further investigations were therefore needed.

A principal component (PC) analysis of the VCV matrix was performed for each effect and within each group in order to depict the landmark displacements corresponding to each emerging PC and also to test the congruence of these displacements between effects (after Klingenberg & McIntyre 1998). The correlation between PCs was assessed by the cosine between their respective eigenvectors. The significance of these correlations was then assessed by comparing these observed values to a null distribution of cosines between pairs of random independent vectors. This distribution was obtained using a Monte Carlo procedure in which 100 000 pairs of 18-dimensional random vectors were generated.

In order to appraise to what extent the interindividual variation as well as the FA patterns were preserved, despite morphological and genetic divergence, we also implemented these tests between samples.

(c) *Directional asymmetry, anti-symmetry and allometric effects*

Two other types of asymmetry, directional asymmetry (DA) and anti-symmetry (AS), may occur in populations and potentially bias FA estimates. Furthermore, as these types of asymmetry are at least partly genetically determined, they are considered as poorly relevant in assessing DS (Palmer & Strobeck 1986; but for a review, see Auffray *et al*. (1999*a*)). The significance of DA on size was assessed within each group using the two-way mixed model ANOVA (Palmer & Strobeck 1986; Palmer 1994), which is classically used in FA approaches (individual used as a random effect and side as a fixed effect). The occurrence of size AS was investigated within each sample using the Dallal–Wilkinson approximation of the Lilliefors test of normality (Sokal & Rohlf 1995) of the distribution of right minus left ($R_s - L_s$) values, R_s and L_s being, respectively, the centroid sizes of the left and the right sides averaged over the two sessions. Finally, the independence between size and size FA was appraised by a linear regression of ($|R_s - L_s|$) against the mean centroid size ($(R_s + L_s)/2$).

The significance of shape DA was estimated using Procrustes mean squares associated with side-effect. Shape AS was examined by scatter plots of the vectors corresponding to the right minus left differences for each landmark: a clustering would have argued for the occurrence of AS. Following the Procrustes procedure, allometric effects were tested using multiple regressions of the between-side difference vectors on centroid size (for details, see Klingenberg & McIntyre (1998)). When the *p*-values are not provided they are coded as follows: n.s., not significant, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

3. RESULTS

(a) *Size*

Preliminary tests did not reveal the presence of DA or AS for size: the side-effect in the two-way ANOVA was not significant for the three groups (table 1) and no departure from normality was detected by the Dallal–Wilkinson approximation of Lilliefors tests (D_{max}DDO = 0.115 n.s., $D_{max}F_l = 0.110$ n.s. and D_{max}MDH = 0.083 n.s.). None of the three regressions of $|R - L|$ on $(R + L)/2$ was significant (table 1), indicating an independence between size and size asymmetry. The individual and individual × side-effects were both significant for the three groups (table 1). It was then possible to compute the FA10 and VAR indices in all cases (table 1). The *F*-tests on FA10 were not significant (DDO/F_l $F_{27,27} = 0.2609$ n.s., F_l/MDH $F_{27,28} = 0.499$ n.s. and DDO/MDH $F_{27,28} = 2.757$ n.s.) (figure 1*b*), indicating that the FA levels were not different between samples. In return, the *F*-tests applied to the VAR indices showed that the variance in size was significantly lower for the hybrids than for the parental groups which did not differ significantly (DDO/F_l $F_{42,32} = 3.54^{**}$, F_l/MDH $F_{32,36} = 2.70^{**}$ and DDO/MDH $F_{42,36} = 1.31$ n.s.) (figure 1*f*). In addition, one-way ANOVAs have shown that the mean skull size of the hybrid sample was significantly larger than that of the parental ones (DDO/F_l $F_{1,314} = 230^{***}$, F_l/MDH $F_{1,290} = 337.8^{***}$ and DDO/MDH $F_{1,326} = 0.139$ n.s.) (figure 1*c*). The hybrids then appeared to be both larger and less variable than the parental samples, though they do not differ in their size FA level.

(b) *Shape*

Comparisons of the mean left and mean right configurations suggested a certain amount of DA in all groups. This was confirmed by the significance of the

Table 1. *Results of the size and shape analyses of the interindividual variations and FA*

(This detailed presentation follows the recommendations of Palmer (1994). The regression for size is a simple linear regression and that for shape is a multiple regression. MS, mean square.)

	group					
	size			shape		
	DDO	F_1	MDH	DDO	F_1	MDH
size regression	0.060 ± 0.012	0.120 ± 0.011	0.030 ± 0.012	0.015 ± 0.011	0.022 ± 0.023	0.012 ± 0.012
F	0.180 n.s.	0.490 n.s.	0.040 n.s.	1.160 n.s.	0.001 n.s.	1.440 n.s.
individual						
d.f.	43	34	37	774	612	702
MS × 1000	332 913	96 438	254 667	1492	1040	1541
F	78.905***	270.87***	649.59***	2.296***	3.203***	3.053***
side						
d.f.	1	1	1	18	18	18
MS × 1000	27	9222	10 705	2633	911	3455
F	0.009 n.s.	2.723 n.s.	3.170 n.s.	4.052***	2.806***	6.845***
interaction						
d.f.	43	34	37	774	612	702
MS × 1000	2939	3390	3367	650	325	505
F	1.579*	9.521***	8.580***	7.074***	3.534***	8.344***
error						
d.f.	88	70	76	1584	1260	1440
MS × 1000	1861	356	392	92	92	60
VAR (× 100 000)	82.49	23.25	62.83	0.210	0.180	0.260
FA10 (× 100 000)	11.82	15.17	14.87	2.790	1.160	2.220

side-effect in the three Procrustes ANOVAs (table 1). Scatter plots of the $(R-L)$ shape vectors suggested that AS was not present, as no clustering was found. Finally, none of the multiple regressions of the between-side difference vectors on centroid size were significant, providing no evidence of a size effect on shape asymmetry (table 1).

The individual and individual × side-effects of the two-way Procrustes ANOVA were significant in each group. The VAR index was lower in the F_1 group than in the parental ones (table 1 and figure 1*f*), even though this difference remained slightly insignificant between F_1 and DDO (MDH/F_1 $F_{287,264}=1.45$ and $p=0.0014$ and DDO/F_1 $F_{207,264}=1.17$ and $p=0.11$). The difference was nearly significant between the parental groups (DDO/MDH $F_{207,287}=1.23$ and $p=0.056$), DDO being less variable than MDH. The FA10 index was significantly lower in the MDH sample than in the DDO one (DDO/MDH $F_{565,540}=1.26$ and $p=0.004$) and highly significantly lower in F_1 as compared to the parental groups (DDO/F1 $F_{565,303}=2.40$ and $p \ll 0.001$ and MDH/F1 $F_{540,303}=1.91$ and $p \ll 0.001$) (table 1 and figure 1*e*). In other words, the hybrids appeared to be less variable and less asymmetrical, in terms of shape, than the parental samples.

The permutation tests (table 2) indicated that the correlation between the VCV matrices of the individual and FA effects was never significant within samples. For DDO, the individual matrix was significantly correlated with the error one. On the other hand, within the other two groups, MDH and F_1, the error matrix was correlated with the FA one. However, considering all samples together, the results of Fisher's method for combining independent tests by pairs of matrices between the three samples (Manly 1991) showed that the individual VCV matrices were not correlated with either those of the FA ($\chi^2_6=0.313$ n.s.) or with those of the error ($\chi^2_6=7.5$ n.s.). The FA and error remained significantly correlated ($\chi^2_6=13.26^*$). This suggested that a part of the FA was due to measurement error, even though the two-way ANOVA procedure involving replicates clearly showed that the FA was more important than what would have been expected by considering the measurement error alone.

Effect by effect and, in spite of the divergence in shape between samples (MANOVA Wilks' $\lambda=0.03$, *F*-values $=86.4$, d.f. $1=44$, d.f. $2=904$ and $p<0.001$), all of the VCV matrices were strongly correlated between samples (table 3).

The Monte Carlo procedure was used to assess the statistical significance of the correlation between the three first PCs between effects within groups. The percentage of variation attached to the first two PCs are reported in figure 2. To compare two effects, nine tests were thus required within each group and 27 when the three groups were considered together. The 27 tests involving the interindividual variation and FA PCs yielded four significant correlations, none of them involving the first PC of FA. These four correlations were no longer significant after the Bonferroni procedure (Rice 1989) was applied, considering the $k=9$ tests within each group. Five correlations were significant between the FA and measurement error PCs. Among them, the first PC of FA was significantly correlated with the second one of error in F_1 and

Table 2. *Results of the permutation tests used to appraise the VCV matrices correlations: within group analysis of the correlations between VCV matrices of landmark displacements attached to interindividual variations, FA and error*

group	effects	correlation	*p*
DDO	individual/FA	0.030	0.930
	individual/error	0.470	0.008
	FA/error	0.400	0.259
F_1	individual/FA	0.230	0.872
	individual/error	0.460	0.402
	FA/error	0.670	0.009
MDH	individual/FA	0.105	0.860
	individual/error	0.460	0.055
	FA/error	0.570	< 0.000 01

Table 3. *Results of the permutation tests used to appraise the VCV matrices correlations: between group analysis of the correlations between VCV matrices of landmark displacements attached to each effect*

effect	samples	correlation	*p*
individual	DDO/F_1	0.62	< 0.0001
	MDH/F_1	0.72	< 0.0001
	DDO/MDH	0.77	< 0.0001
FA	DDO/F_1	0.81	< 0.0001
	MDH/F_1	0.85	< 0.0001
	DDO/MDH	0.84	< 0.0001
error	DDO/F_1	0.86	< 0.0001
	MDH/F_1	0.83	< 0.0001
	DDO/MDH	0.84	< 0.0001

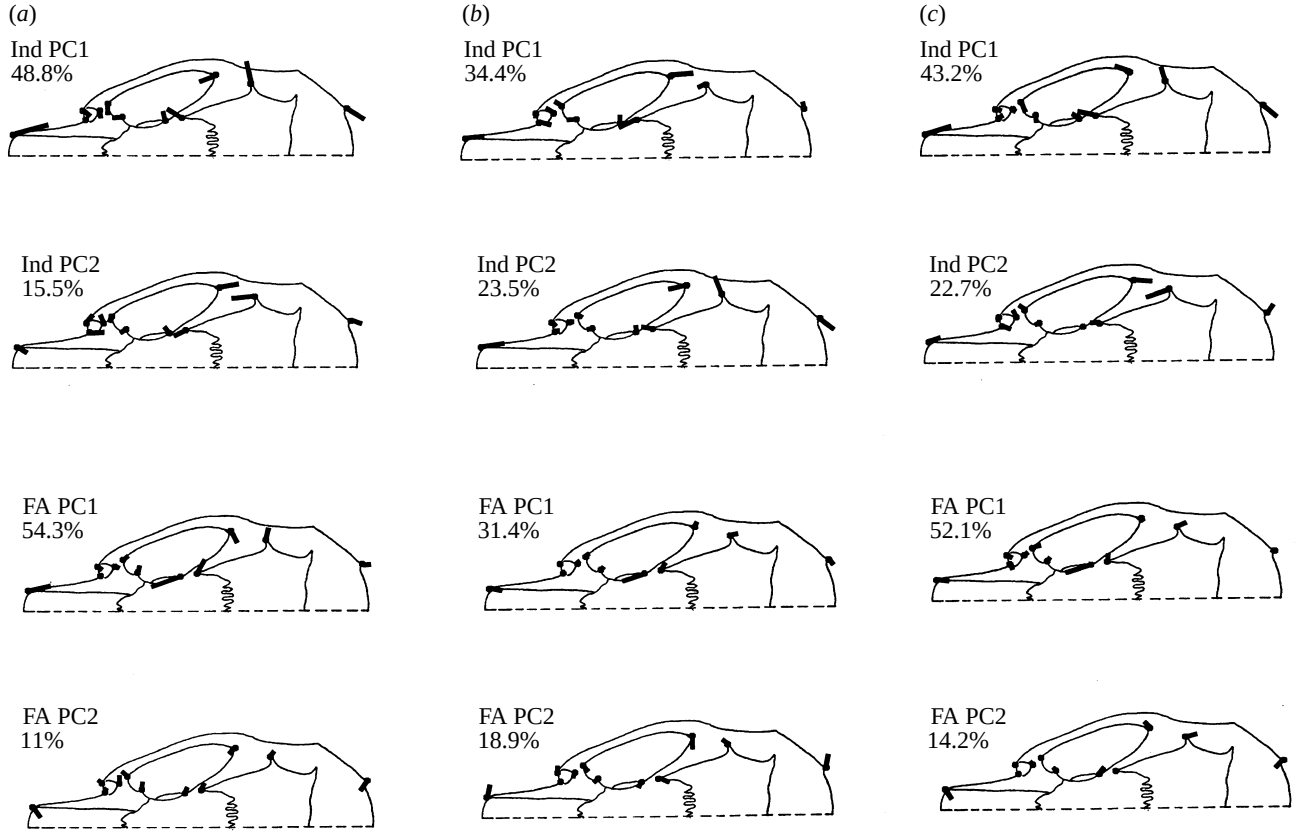

Figure 2. Vectors of the landmark displacements corresponding to the first two axes of the inter- (Ind PCs) and intraindividual, (FA PCs) variations in the three samples. (*a*) DDO, (*b*) F_1 and (*c*) MDH.

MDH. However, only the latter remained significant after having applied the Bonferroni procedure (cosine = 0.74^{**}). None of the four significant correlations between the interindividual variation and measurement error PCs remained significant. Consequently, the PCs related to each effect are poorly correlated with each other within groups (see figure 2).

For the interindividual variation and FA, the correlation between the first three PCs were tested between groups. To compare two groups, nine tests were required and $k = 9$ was used when applying the Bonferroni procedure. Considering the interindividual variation, the first and second PCs were highly correlated between the two parental groups (PCls cosine = 0.86^{***} and PC2s cosine = 0.77^{***}). The PCl and PC2 of F_1 were highly correlated, respectively, with the PC2s and PCls of both parental groups ($0.76^{***} <$ cosine $< 0.87^{***}$). Concerning FA, the PCls were correlated between all groups ($0.89^{***} <$ cosine $< 0.94^{***}$) as well as the PC2s ($-0.50^{*} <$ cosine $< 0.69^{**}$). Another significant correlation was found between the PC3 of DDO and the PC2 of MDH (cosine = -0.58^{*}). These results suggest that the interindividual patterns of variation as well as those of the FA are highly similar between the three groups (figure 2).

4. DISCUSSION

(a) *Heterosis*

Using traditional measurements, Alibert *et al*. (1994, 1997) reported that tooth development benefited from a heterotic effect in house mouse hybrids. This was shown by a higher DS and larger size of molars in the hybrid sample as compared to the parental ones and a similar result was obtained by Auffray *et al*. (1996) using a geometric morphometric method on tooth shape. Alibert *et al*. (1994, 1997) discussed this unexpected heterotic effect since the genomic coadaptation of genetic systems involved in reproduction and immune response to parasites appeared to be disrupted in wild hybrid populations (Moulia *et al*. 1991, 1993; Dod *et al*. 1993). In the present study, the significantly lower shape FA and higher size of skulls in the hybrids suggested that the development of the whole skull also benefited from a heterotic effect. Given previous results, such an effect was not unexpected, even more so considering the slight inbreeding level of the laboratory parental samples used. This interesting result, which was not the principal aim of this study, indicates that the heterotic effect on development may not only concern molars but could concern the whole body. If shape FA clearly exhibited a lower level for hybrids, FA estimates based on size were not significantly different between the parental and hybrid groups (figure 1*b*). FA occurring at random, subtle differences in location, from one side to the other, of a high number of landmarks due to developmental noise, could induce a compensation of their distances to the centroid of their respective sides, preventing an accurate estimation of FA based on centroid size. However, even though these displacements are minimized by the GLS procedure (Klingenberg & McIntyre 1998), the shape component of asymmetry could be underestimated but could be better preserved than the size one.

Moreover, the hybrids could have been expected to have a higher variance than the parental strains, since they result from crosses between two morphologically differentiated groups. Hence, the decrease in the interindividual variation in terms of both size and shape in the hybrid group as compared to the parental ones strongly suggests that canalization also benefits from a heterotic effect in hybrids.

(b) *Fluctuating asymmetry and interindividual variation patterns*

In order to test whether this similarity between canalization and DS levels reflected a single mechanistic (and therefore genetic) process, the examination and appraisal of the congruence of their respective patterns of morphological expression within groups (namely DDO, MDH and F_1) was required. The lack of a significant correlation within these groups between the VCV matrices associated with the interindividual variations and FA strongly supports the hypothesis which states that canalization and DS are distinct mechanisms: if a single mechanism were present, the patterns of expression of both sources of morphological variation would probably have been congruent. Additionally, within groups, the vectors expressing the intraindividual variation were not correlated with those expressing the interindividual variation, indicating that canalization and DS do not act on the same components of shape (figure 2).

Between groups, highly significant correlations between the VCV matrices of the interindividual variation as well as the congruence between related eigenvectors suggested that the control of morphological variation by canalization is highly similar among the three samples (figure 2). Similarly, the concordance of the intraindividual variation features between groups indicated that the morphological asymmetry induced by developmental perturbations, even if exhibiting a lower amplitude in the hybrids, presents a very similar morphological pattern between groups. This result suggests, as for canalization, a DS process being shared by all groups. The fact that canalization and DS, respectively, control the inter- and intraindividual morphological variability by similar processes between groups could be related to the close phylogenetic relationship between these groups: developmental buffering mechanisms inherited from a common ancestor would have been preserved during the evolutionary divergence.

(c) *Genetic bases and selective constraints*

The genetic bases of DS and canalization are for the most part unknown even though an important number of studies have focused on this subject in the last few decades, for example, Leamy & Thorpe (1984), Leamy (1993), Graham (1992) and Clarke (1993*b*) for DS and Wagner *et al*. (1997) and Eshel & Matessi (1998) for canalization. Except in the case of *Lucila cuprina* studies, which have stressed the role of a modifier gene in DS regulation (e.g. McKenzie 1994; Clarke 1997), no genetic mechanism for DS has been convincingly proposed, nor experimentally confirmed (but see Klingenberg & Nijhout (1999) for a discussion). However, it is generally admitted that DS efficiency results from a balance between genomic coadaptation and global heterozygosity (e.g. Graham 1992; Auffray *et al*. 1999*b*; Chatti *et al*. 1999), which represent genetic conditions rather than genetic bases (Clarke 1998).

In contrast, different hypotheses involving genetic and molecular mechanisms of canalization have already been suggested and in some cases tested. Thoday (1958) proposed that modifier genes could be responsible for the maintenance of canalization of a trait. This was also suggested by Scharloo (1991) and recent findings on heat-shock protein HSP-90 mutants in *Drosophila* have provided the first molecular evidence for such a mechanism (Rutherford & Lindquist 1998). Redundancy involving paralogous genes was also proposed as a possible buffering mechanism by Wilkins (1997). Moreover, Lerner (1954) suggested that heterozygosity could be responsible for stabilizing the phenotype and several studies have reported a negative association between heterozygosity and the amount of morphological variation (reviewed in Mitton & Grant 1984).

Unfortunately, both concepts of canalization and DS have been widely misused and confounded (for example, Mitton & Grant (1984) indifferently used both the amount of morphological variation and FA to measure DS and, conversely, Kieser *et al*. (1986) used asymmetry to assess canalization) until recent papers by Zakharov (1989, 1992). For this reason, papers dealing with

heterozygosity and phenotypic variation should be considered with caution where developmental homeostasis is concerned, i.e. the general concept including both canalization and DS (Waddington 1957; Zakharov 1989).

Waddington (1957) stressed that canalization and DS are genetically and functionally distinct and our results support such a hypothesis. Furthermore, we demonstrate that, even though independent, both buffering systems can be similarly affected by some genetic conditions (namely heterosis) as suggested by Clarke (1998).

Studying the tsetse fly *Glossina palpalis gambiensis*, Klingenberg & McIntyre (1998) concluded that there was no need for a specific mechanism for FA regulation, since they obtained strongly congruent patterns between the inter- and intraindividual variation. However, since the symmetry of the characters used in their study (i.e. wing size and shape) is most likely involved in individual fitness (and this also may be the case in Clarke (1998) and Livshits *et al*. (1998)), such a congruence could then be explained by Clarke's (1998) alternative hypothesis which emphasizes the relationship between character importance in terms of fitness and its developmental homeostasis. There is no evidence that the size and shape symmetry of the skull may have such a crucial importance in terms of individual fitness. This could explain the difference between our study and the one by Klingenberg & McIntyre (1998): selective constraints may override the differences in the developmental mechanisms which control canalization and DS.

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