

# Asymmetric size and shape variation in the Central European transect across the house mouse hybrid zone

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We studied asymmetric variation of the mandible in the Central European portion of the hybrid zone between two house mouse subspecies, *Mus musculus musculus* and *Mus musculus domesticus*. Within introgression classes, defined by the share of diagnostic allozymes, we quantified the directional and fluctuating component of asymmetric variation, as well as skewness and kurtosis of individual asymmetry distributions. Furthermore, in the same manner we re-analysed asymmetric variation of the ventral side of the skull. According to the quadratic polynomial model, the mandible shape-fluctuating asymmetry, but not size-fluctuating asymmetry, was significantly decreased in the centre of the hybrid zone (with a minimum predicted for a hybrid index of 0.41). On the contrary, the skull shape-fluctuating asymmetry non-monotonically increased towards the *musculus* side of the hybrid zone (with a peak predicted for a hybrid index of 0.86). Thus, the impact of hybridization on fluctuating asymmetry is trait-specific in this portion of the house mouse hybrid zone. The only general feature of asymmetric variation we observed was the shift towards the platykurtosis of asymmetry distributions in the centre of the hybrid zone. Taken together, we suggest genetic variability for right–left asymmetries to be generally increased, but the developmental instability of mandible shape to be decreased, by hybridization. We hypothesize the decrease of developmental instability to be caused by overdominant effects on developmental dynamics rather than by increased heterozygosity. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **101**, 13–27.

**ADDITIONAL KEYWORDS:** asymmetric variation – developmental instability – fluctuating asymmetry – mandible – *Mus musculus* – skull.

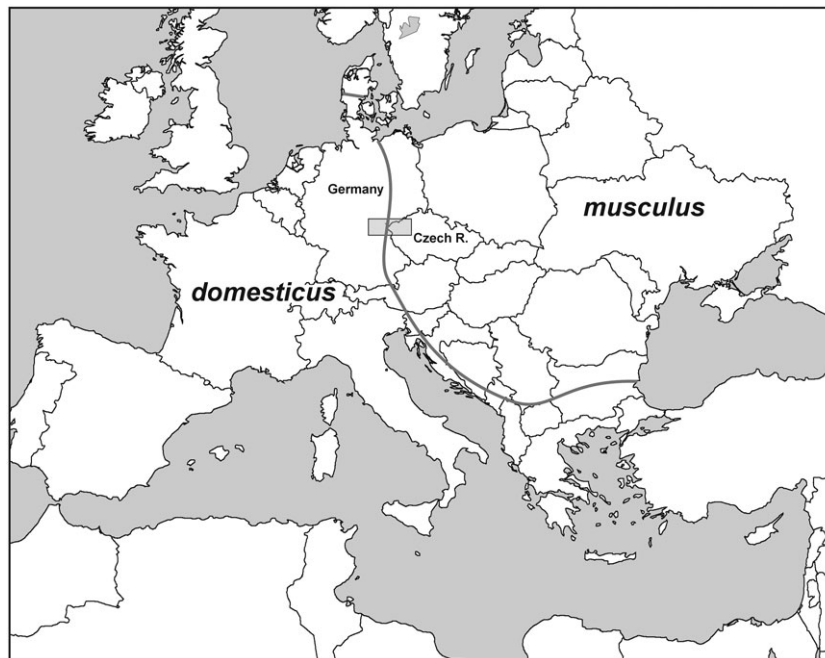
## INTRODUCTION

When related, yet divergent, populations hybridize in nature, unprecedented mixtures of genetic/epigenetic information are created. This can change patterns of phenotypic variation in hybrid offspring relative to parental populations (Rieseberg, Archer & Wayne, 1999; Albertson & Kocher, 2005; Chiba, 2005; Parnell, Hulsey & Streelman, 2008) by a disruption of developmental processes or a creation of new developmental potential.

In this respect, traits that are essentially symmetric deserve special attention. Here, we can assume that development tends to a specific right–left asymmetry

(zero or non-zero), and thus we can conceive of an ideal form, whether viewed as a target phenotype (Nijhout & Davidowitz, 2003) or a developmental attractor (Emlen, Freeman & Graham, 2003). In principle, it is therefore possible to estimate developmental instability (DI) as a deviation from such an ideal form. The variation of right–left differences is usefully decomposed into two components: one is called ‘directional asymmetry’ (DA), and corresponds to the magnitude of the mean of individual asymmetries; the second, called ‘fluctuating asymmetry’ (FA), is a measure of their variation (Van Valen, 1962; Mardia, Bookstein & Moreton, 2000). If differences between individual asymmetries are of purely stochastic origin, DI is reflected by FA (Van Dongen, 1998; Klingenberg, 2003). If not, the interpretation is more difficult, because both

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**Figure 1.** The course of the *musculus/domesticus* hybrid zone in Europe. The shaded rectangle indicates 35-km-wide and 115-km-long area sampled in this study.

DA and FA may reflect genetic variability as well as DI (McKenzie & Clarke, 1988; Graham *et al.*, 1998; Kark, 2001; Stige, David & Alibert, 2006).

Two house mouse subspecies, *Mus musculus musculus* and *Mus musculus domesticus*, can serve as an example of partially reproductively isolated taxa. They hybridize in Europe along a contact zone running across the Jutland peninsula, and from northern Germany to the Black Sea coast (Fig. 1; Boursot *et al.*, 1993; Macholán, Kryštufek & Vohralík, 2003). The zone width varies from a few to about 20 km for autosomal loci (Raufaste *et al.*, 2005; Macholán *et al.*, 2007), despite hundreds or thousands of hybridizing generations (Auffray, Vanlerberghe & Britton-Davidian, 1990; Cucchi, Vigne & Auffray, 2005). The most general and significant patterns found until now in the zone are defects of spermatogenesis (Britton-Davidian *et al.*, 2005; Mihola *et al.*, 2009; Vyskočilová, Pražanová & Piálek, 2009) and the limited introgression of some X chromosome-linked markers (Tucker *et al.*, 1992; Prager *et al.*, 1993; Payseur, Krenz & Nachman, 2004; Macholán *et al.*, 2007). It is therefore likely that divergence of developmental processes, crucial for fertility, is responsible for their continuing separation.

Ontogeny may also differ between the two subspecies in more subtle aspects, and indeed differences in the skull morphology were found (Auffray *et al.*, 1996; Macholán, 1996a, b; O. Mikula, unpubl. data). Nevertheless, studies on FA yielded conflicting

results. The FA of the widths and lengths of lower molars was decreased in the centre of the Danish transect across the mouse hybrid zone (Alibert *et al.*, 1994), and this result was corroborated in laboratory hybrids between *domesticus* and *musculus* mice of Danish origin (Alibert *et al.*, 1997). In the dorsal side of the skull, however, only the shape FA was lower in hybrids, whereas the size FA remained unchanged (Debat *et al.*, 2000). Moreover, when the ventral side of the skull was studied in the Central European portion of the hybrid zone, no evidence of decreased shape FA was found (Mikula & Macholán, 2008).

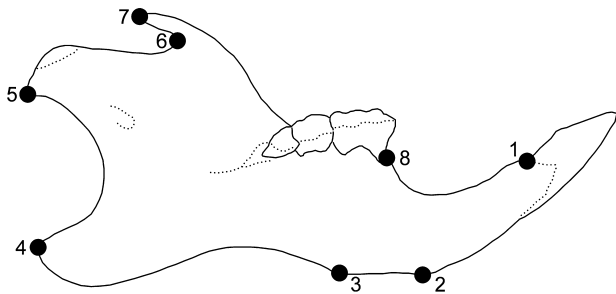
In the present study we investigated the asymmetry of mandible size and shape in the Central European transect. We examined which aspects of asymmetric variation are influenced by hybridization and, especially, whether the lack of a decrease in FA is a general phenomenon in this portion of the hybrid zone. In addition, we used the same methods to reanalyse the skull asymmetry data (Mikula & Macholán, 2008) in order to compare the asymmetric variation of both traits directly.

## MATERIAL AND METHODS

### MICE

Mice were collected along a transect running across the hybrid zone from north-eastern Bavaria (Germany) to western Bohemia (Czech Republic)

(Fig. 1). Samples were collected from 1991 to 2004 during the same season each year (September–October). In total, 785 mice were investigated. Mandibles were cleaned using dermestid beetles. They were photographed under microscope Olympus SZX12 and the positions of eight landmarks (Fig. 2) were digitized. Each mandible was digitized three times independently to reduce the measurement error.



**Figure 2.** Eight landmarks digitized on the lingual side of the mandible. Definitions according to Richtsmeier's lab (<http://www.getahead.psu.edu>): 1, superior point on mandibular symphysis; 2, inferior point on mandibular symphysis; 3, anterior edge of the coalescence of curve of masseteric ridge with post-symphyseal rugged area; 4, tip of mandibular angle; 5, most posterior point of the condyloid process on the left side of the mandible; 6, posterior base of coronoid process; 7, apex of coronoid process; 8, anterior edge of alveolar process, where first molar hits alveolus.

The genetic constitution of each individual was characterized by a hybrid index (HI), defined as the percentage of *musculus* alleles on six allozyme loci (*Es1*, *Gpd*, *Idh*, *Mpi*, *Np*, and *Sod*). According to their hybrid indices, individuals were grouped into 12 introgression classes, characterized by their average HIs (Table 1).

#### GEOMETRIC MORPHOMETRICS PROCEDURES

In geometric morphometrics, objects are represented by configurations of homologous landmarks. The size of the configurations is defined as centroid size: the square root of the summed squared distances of the landmarks from their centroid (Bookstein, 1991). The shape is represented by Procrustes shape coordinates, i.e. the full set of landmark coordinates standardized by the Procrustes superimposition (Rohlf & Slice, 1990) to have the same size, the same position, and a specific orientation. For two-dimensional objects, it means a loss of four degrees of freedom. Mandible shapes were thus described by 16 Procrustes shape coordinates, but with only 12 degrees of freedom.

First, the centroid size of each configuration was computed. Afterwards, configurations of right mandibles were reflected and superimposed on their left counterparts (Smith, Crespi & Bookstein, 1997; Klingenberg & McIntyre, 1998) to yield directly comparable Procrustes shape coordinates. Prior to computation of individual asymmetries, three

**Table 1.** Introgression classes for mandible data with the range of included individual hybrid indices (HI), average HIs and sample size (*N*)

Introgression class	Range of HI	Average HI	<i>N</i>	Size DA	Size FA	Size skewness	Size kurtosis	Shape DA	Shape FA
M-01	0.000	0.000	182	3.83*	8.42	0.30	0.76	0.0073*	0.0242
M-02	0.083–0.125	0.088	75	3.67*	8.25	0.19	0.78	0.0062*	0.0218
M-03	0.167	0.167	77	2.48	8.32	0.17	0.83	0.0061*	0.0225
M-04	0.200	0.200	21	0.34	7.34	0.98	3.62*	0.0083	0.0217
M-05	0.250	0.250	35	3.13	7.29	0.28	−0.15	0.0083*	0.0222
M-06	0.300–0.333	0.325	37	6.21*	8.86	0.22	−0.49	0.0080*	0.0222
M-07	0.375–0.417	0.413	22	3.88	6.72	0.07	−0.62	0.0058	0.0222
M-08	0.500–0.700	0.578	29	0.44	7.43	0.36	−0.81	0.0054	0.0227
M-09	0.750–0.800	0.771	21	7.24*	6.95	0.72	−0.27	0.0096	0.0229
M-10	0.833	0.833	37	7.11*	9.33	0.62	0.25	0.0089	0.0233
M-11	0.900–0.917	0.914	45	3.99*	8.01	0.58	0.77	0.0105*	0.0238
M-12	1.000	1.000	204	4.88*	8.06	0.13	0.92	0.0079*	0.0248

Classes are ranked according to their average HIs.

For size as well as shape, directional asymmetry (DA) and fluctuating asymmetry (FA) are listed.

For size asymmetries, skewness and kurtosis of their distributions within introgression classes (denoted as 'size skewness' and 'size kurtosis') are also listed.

\*Significant mean asymmetries (in DA) after sequential Bonferroni correction or significant deviations from normality (in skewness and kurtosis) at *P* = 0.01.

replicated measurements of each side were averaged to reduce measurement error.

#### SIZE ASYMMETRY

Individual size asymmetries were calculated as the differences between the right and the left centroid sizes, and their magnitudes as absolute values. Asymmetric variation was decomposed into two components denoted as 'size DA' and 'size FA'. The former corresponds to the magnitude of mean asymmetry; the latter was calculated as an average of absolute values of the differences between individual asymmetries and their mean (FA1 index of Palmer, 1994). In each class we tested for the significance of DA by one-factor ANOVA with 'side' as a factor. *P* values were obtained by a permutation procedure (1000 random assignments of individual right- and left-centroid sizes to body sides). Sequential Bonferroni correction (Rice, 1989) was applied, and *P* values higher than required after correction but lower than the conventional 0.05 were considered to be marginally significant.

Furthermore, in each class we quantified skewness and kurtosis of individual asymmetry distributions. Skewness was calculated as  $g_1$ , as described by Zar (1999: eq. 6.5), and the absolute value of this quantity was taken, as we were interested in the degree of any skew. Kurtosis was calculated using eq. 7 of Palmer & Strobeck (2003). Statistical tables in the cited works were used to assess null hypotheses of normality, with the significance level set to  $P = 0.01$ .

#### SHAPE ASYMMETRY

Individual shape asymmetries were calculated as differences between the right and the left vectors of Procrustes shape coordinates, and thus they are of multivariate nature. The magnitude of shape asymmetry can be quantified as the Procrustes distance between the right and the left shapes (Smith *et al.*, 1997; Klingenberg & McIntyre, 1998), which is closely approximated as the square root of the sum of squared differences between corresponding Procrustes shape coordinates (Bookstein, 1991). Thus, shape DA was expressed as a Procrustes distance between the right and the left mean shape, and shape FA was expressed as the mean of Procrustes distances between individual asymmetries, and the mean asymmetry in a class. To test for the significance of DA, we applied the  $T^2$ -test as described by Mardia *et al.* (2000), with *P* values estimated by the permutation procedure.

The skewness and kurtosis of shape asymmetry distributions were calculated, and their departure from normality was tested using Mardia's multivariate measures (Mardia, 1970) with sequential

Bonferroni correction. To increase sample sizes, we pooled mice into three broad introgression classes, delimited by HI as follows: *domesticus*-like (0.0–0.3), hybrid (0.3–0.7), and *musculus*-like (0.7–1.0). To decrease the number of dimensions of the data, we used only the first eight principal components of the original asymmetries, together explaining over 90% of the variation.

#### TRENDS ACROSS THE HYBRID ZONE

In hybrid zones, FA was proposed to relate to the degree of introgression. It could be decreased, or conversely increased, because of antagonistic impacts of higher heterozygosity and disruption of genomic co-adaptation on DI (Lerner, 1954; Soulé, 1967; Graham, 1992; Clarke, 1993; Alibert & Auffray, 2003), or because of changes in genetic variability for a determination of the right–left body axis (Rego, Matos & Santos, 2006). Quadratic polynomial regressions on HI were therefore fitted, as they can approximate a variety of patterns characterized by monotonous change towards some middle point, or towards one edge of the hybrid zone.

The regressions were also fitted for DA as well as for skewness and kurtosis of size asymmetry distributions. DA could show trends similar to FA, as these components were suggested to be dynamically interrelated (Graham *et al.*, 1998). Skewness and kurtosis could be changed as a result of a mixture of various asymmetry distributions (Palmer & Strobeck, 1992, 2003), which is more likely in the centre of the hybrid zone. Sequential Bonferroni correction was applied to all six regressions, including FA, with the most stringent significance level being  $P = 0.008$ .

#### COMPARISON OF UNSIGNED ASYMMETRY DISTRIBUTIONS

To obtain a more graphic view on differences in asymmetric variation in the hybrid zone, we also contrasted individual asymmetry distributions via quantile–quantile plots. Instead of asymmetries themselves, however, we focused on their magnitudes (called 'unsigned asymmetries') as they are scalar quantities, which enabled us to treat univariate size asymmetry and multivariate shape asymmetry in the same way. To work with sufficiently large samples, we compared the distributions among three broad introgression classes.

#### REANALYSIS OF SKULL-ASYMMETRY DATA

In order to compare FA of two different traits along the same transect, we used the same methods to reanalyse the ventral side of the skull studied



**Table 2.** Introgression classes for skull data with a range of individual hybrid indices (HIs), average HI, sample size (*N*), shape directional asymmetry (DA) and shape fluctuating asymmetry (FA)

Introgression class	Range of HIs	Average HI	<i>N</i>	Shape DA	Shape FA
S-01	0.000–0.083	0.007	74	0.1286*	0.3998
S-02	0.100–0.200	0.163	28	0.1268	0.4180
S-03	0.250–0.333	0.287	25	0.1188	0.4161
S-04	0.375–0.417	0.413	18	0.1657*	0.4314
S-05	0.500–0.625	0.547	19	0.1268	0.4587
S-06	0.667–0.750	0.710	16	0.1315	0.4652
S-07	0.800–0.900	0.832	19	0.1157	0.4331
S-08	0.917–1.000	0.989	85	0.1257*	0.4562

\*Significant differences between sides after sequential Bonferroni correction.

earlier (Mikula & Macholán, 2008). These data were in the form of three-dimensional landmark configurations digitized in 284 specimens divided into eight introgression classes (Table 2). For analyses of the distribution of shape asymmetries, three broad introgression classes were delimited, as for the mandibles, and the first 17 principal components (90% of variation) were used instead of original data. Unlike the mandible, the skull is symmetric in itself, i.e. it has object symmetry (Mardia *et al.*, 2000). In such a case, the overall shape asymmetry is calculated as a difference between landmark configurations and their relabelled reflections. It involves differences between half-skulls as well as irregularities of their mutual arrangement.

#### SIZE DEPENDENCE

Magnitudes of individual asymmetries may be dependent on size, calculated as the average centroid size of both mandibles. If so, it may be appropriate to scale them so that they express the relative extent of deviations from perfect symmetry. We followed an approach proposed by C. P. Klingenberg (pers. comm.): natural logarithms of unsigned asymmetries are regressed on natural logarithms of centroid sizes, and if the result is significant, original asymmetries are divided by antilogarithms of residuals.

We tested for size dependence separately within each introgression class, but the correction was applied generally if the null hypothesis of overall size independence was rejected by Fisher's combined probability test. Furthermore, we calculated correlations between FAs and class mean sizes to test for size dependence among classes.

#### MEASUREMENT ERROR

Estimates of FA may be harshly influenced by measurement errors (MEs; Palmer & Strobeck, 2003). To

quantify the ME, three estimates of individual asymmetries were made from three pairs of replicated measurements, they were then averaged, and the magnitudes of their deviations from the average were calculated. The class-specific ME was estimated as an average of these deviations, and its correlation with FA was computed. The correlation was, however, non-significant for the mandible (size FA,  $r = 0.43$ ,  $P = 0.160$ ; shape FA,  $r = 0.38$ ,  $P = 0.230$ ) as well as for the skull (shape FA,  $r = 0.40$ ,  $P = 0.330$ ).

#### SOFTWARE

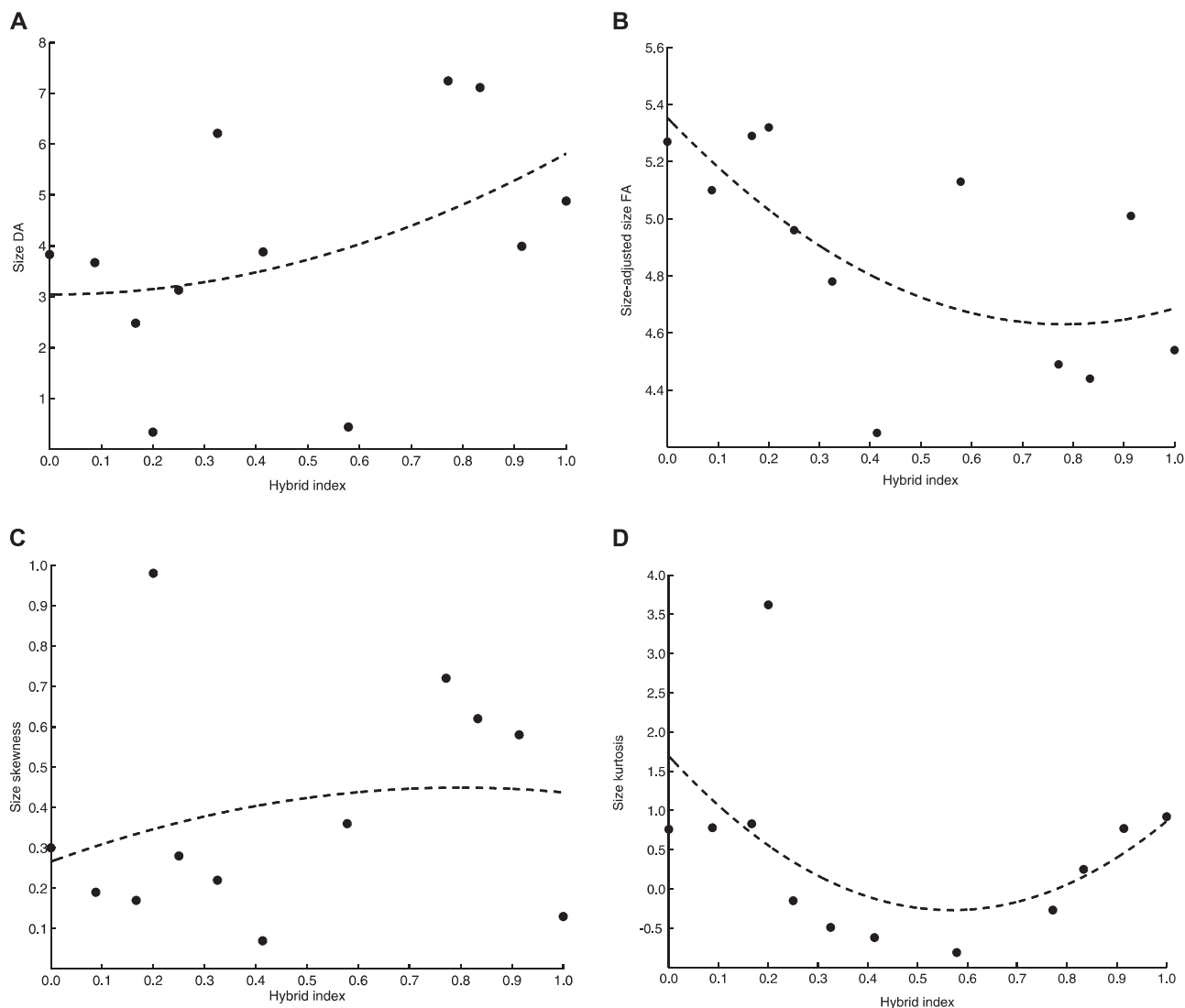
Landmark coordinates were captured in tpsDig software (Rohlf, 2004). Centroid sizes and Procrustes shape coordinates were extracted using tpsRelw (Rohlf, 2007). Statistical tests and computations were performed with PAST v1.95 (Hammer, Harper & Ryan, 2001) and MATLAB v7.4 (The MathWorks, Inc., 2007). Deformation grids were generated using the 'shapes' package (Dryden, 2009) written in R (R Development Core Team, 2009).

## RESULTS

#### MANDIBLE SIZE ASYMMETRY

Results of quadratic regressions analysing changes in size asymmetry are summarized in Figure 3 and Table 3. Quadratic regression of size DA on HI (Fig. 3A) was not significant ( $P = 0.365$ ). Size DA was only significantly different from zero in some introgression classes (Table 1), and the right mandible was the larger one in all of them.

Quadratic regression of FA on HI was not significant ( $P = 0.450$ ). Among introgression classes, size FA was uncorrelated with mean centroid size ( $r = -0.01$ ,  $P = 0.975$ ). Within some introgression classes, however, individual asymmetry magnitudes were significantly size dependent (Table 4), and the result of the Fisher's combined probability test ( $\chi^2 = 53.04$ ,



**Figure 3.** Variation in the mandible size asymmetry across the hybrid zone. For twelve introgression classes directional asymmetry (DA) (A), size-adjusted fluctuating asymmetry (FA) (B), skewness (C), and platykurtosis of individual asymmetry distributions (D) are plotted against hybrid indices. Quadratic polynomial fits are depicted as dashed lines.

$P = 0.001$ ) legitimated applying the scaling generally. The adjusted size FA appeared to decrease towards the *musculus* side (Fig. 3B), but the quadratic regression was still non-significant ( $R^2 = 0.43$ ,  $F = 3.45$ ,  $P = 0.077$ ).

Skewness of individual asymmetry distributions did not show any clear pattern along the transect (Fig. 3C). Their kurtosis, however, decreased smoothly towards the centre of the hybrid zone, except for unusually leptokurtic distribution in M-04 (Fig. 3D). When this introgression class was omitted, the quadratic regression became highly significant ( $R^2 = 0.82$ ,  $F = 18.46$ ,  $P = 0.001$ ).

#### MANDIBLE SHAPE ASYMMETRY

Results of quadratic regressions are presented in Figure 4 and Table 3. DA seemed to be higher on the *musculus* side (Fig. 4A), but the quadratic model was not adequate ( $P = 0.188$ ). To gain an additional insight into the observed pattern, we explored the mean shape asymmetries by principal components analysis. Introgression classes with a major genomic contribution of particular subspecies were separated along the first axis (Fig. 5A). However, the pattern was more complex: note the anomalous position of M-08, and the proximity of the M-01 and M-12 classes. Anyway, the mean right–left differences were

very subtle, as can be seen on the thin-plate splines deformation grids (Fig. 5B). They involve a slight compression of the lower edge of the mandible and reshaping of the coronoid process. In the permutation  $T^2$ -tests, shape DA was significant only in some introgression classes (Table 1).

The quadratic regression of shape FA on HI was significant ( $P = 0.002$ ), and predicted that FA would be lower in the centre of the hybrid zone (Fig. 4B),

**Table 3.** Results of quadratic polynomial regressions of asymmetry distribution parameters on hybrid indices

Trait	Parameter	$R^2$	$F$	$P$
Mandible	Size DA	0.20	1.13	0.365
	Size FA	0.16	0.87	0.450
	Size skewness	0.05	0.24	0.793
	Size kurtosis	0.23	1.37	0.303
	Shape DA	0.31	2.02	0.188
	Shape FA	0.76	13.97	0.002*
Skull	Shape DA	0.14	0.40	0.693
	Shape FA	0.75	7.37	0.032

\*Significant result after sequential Bonferroni correction applied separately to the mandible and the skull.

DA, directional asymmetry; FA, fluctuating asymmetry (FA).

with minimum value at HI = 0.41. On the *domesticus* side, however, the predicted U-shape trend was driven by a single introgression class: M-01.

The correlation of shape FA with mean centroid sizes was not significant ( $r = -0.26$ ,  $P = 0.418$ ), but a significant linear relationship ( $r = -0.77$ ,  $P = 0.009$ ) appeared when two pure classes were excluded. A marginally significant size dependency was observed within class M-01, but the null hypothesis of general size independence was not rejected by a Fisher's combined probability test ( $\chi^2 = 33.56$ ,  $P = 0.093$ ).

Distributions of shape asymmetries in two outer broad introgression classes (*domesticus*-like and *musculus*-like) were significantly skewed and leptokurtic, whereas for the hybrid class the null hypothesis of multivariate normality was not rejected (Table 4).

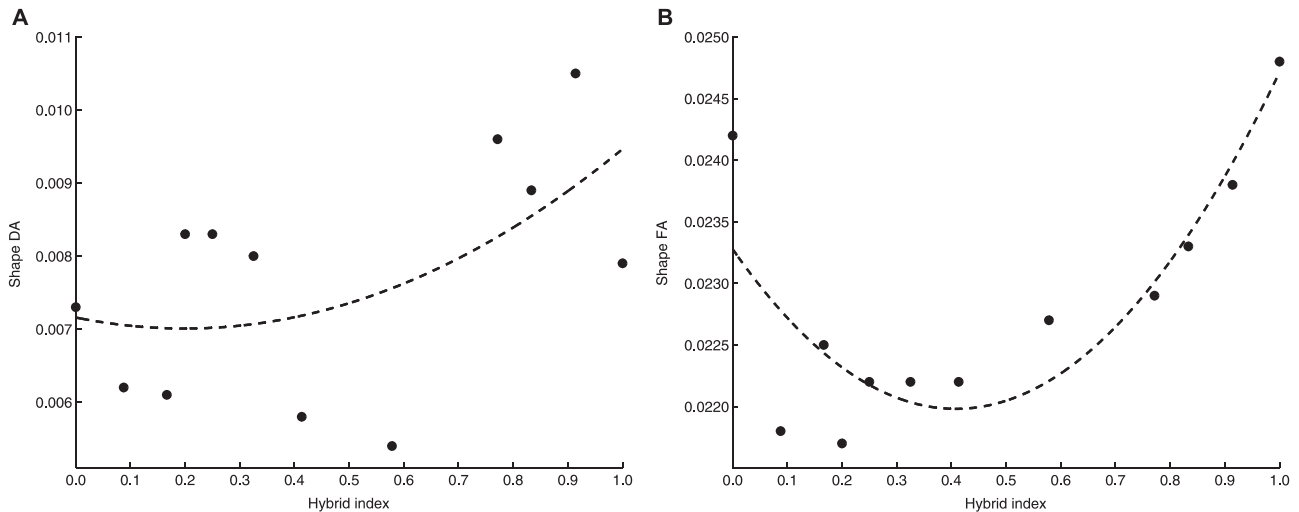
#### SKULL SHAPE ASYMMETRY

Skull shape DA did not show any distinct pattern along the transect (Fig. 6A), and its quadratic regression on HI was not significant. It was significant in three introgression classes only (Table 2). FA was higher on the *musculus* side (Fig. 6B), with the peak being predicted by the quadratic regression at HI = 0.86, but the trend was only marginally sig-

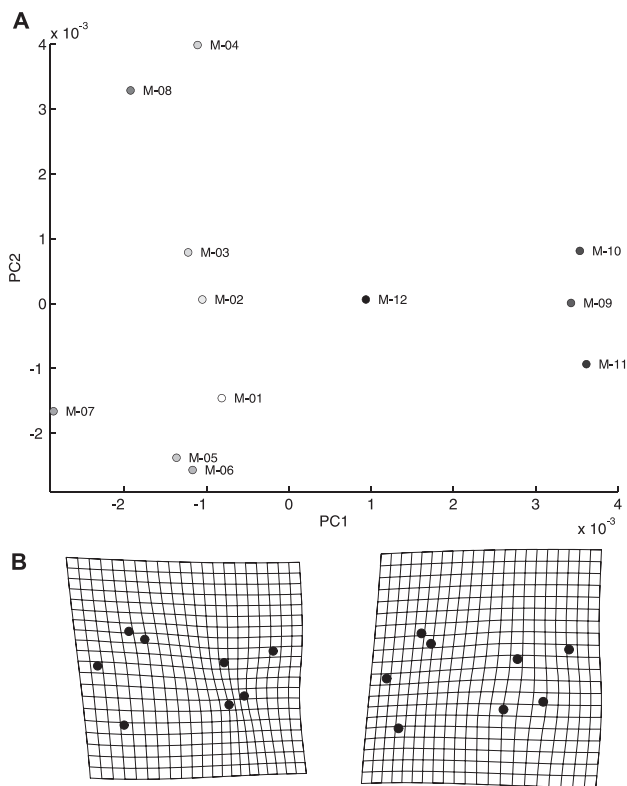
**Table 4.** Results of tests for size dependence of individual asymmetries within introgression classes

Trait	Introgression class	Size			Shape		
		$R^2$	$F$	$P$	$R^2$	$F$	$P$
Mandible	M-01	0.06	11.65	0.001*	0.04	6.67	0.011
	M-02	0.00	0.29	0.590	0.04	2.89	0.093
	M-03	0.00	0.35	0.558	0.00	0.00	0.981
	M-04	0.09	1.84	0.191	0.04	0.84	0.371
	M-05	0.01	0.50	0.486	0.00	0.02	0.888
	M-06	0.02	0.84	0.366	0.07	2.65	0.113
	M-07	0.01	0.11	0.748	0.00	0.10	0.759
	M-08	0.16	4.96	0.035	0.00	0.02	0.876
	M-09	0.05	0.95	0.343	0.14	3.09	0.095
	M-10	0.30	15.39	< 0.001*	0.03	1.14	0.292
	M-11	0.04	1.80	0.187	0.00	0.22	0.645
	M-12	0.00	0.36	0.552	0.01	2.43	0.121
Skull	S-01				0.05	4.13	0.046
	S-02				0.00	0.00	0.995
	S-03				0.17	4.76	0.040
	S-04				0.01	0.19	0.669
	S-05				0.19	3.97	0.063
	S-06				0.00	0.05	0.831
	S-07				0.01	0.11	0.743
	S-08				0.01	0.71	0.401

\*Significant results after sequential Bonferroni correction applied for each combination of trait and type of asymmetry.



**Figure 4.** Variation in the mandible shape asymmetry across the hybrid zone. For twelve introgression classes directional asymmetry (DA) (A) and fluctuating asymmetry (FA) (B) are plotted against hybrid indices. Quadratic polynomial fits are depicted as dashed lines.



**Figure 5.** A, mean mandible-shape asymmetries of twelve introgression classes projected into the subspace of the first two principal components comprising 33% and 26% of the asymmetric shape variation, respectively. Point darkness expresses hybrid indices of the classes. B, asymmetric shapes corresponding to the extremes of the first principal component, magnified 20 times for better visibility.

nificant ( $P = 0.032$ ). In the hybrid introgression class the distribution of shape asymmetries was more platykurtic than expected under multivariate normality. Overall, skewness and kurtosis of shape asymmetry distributions were more similar in hybrid and *musculus*-like introgression classes (Table 5).

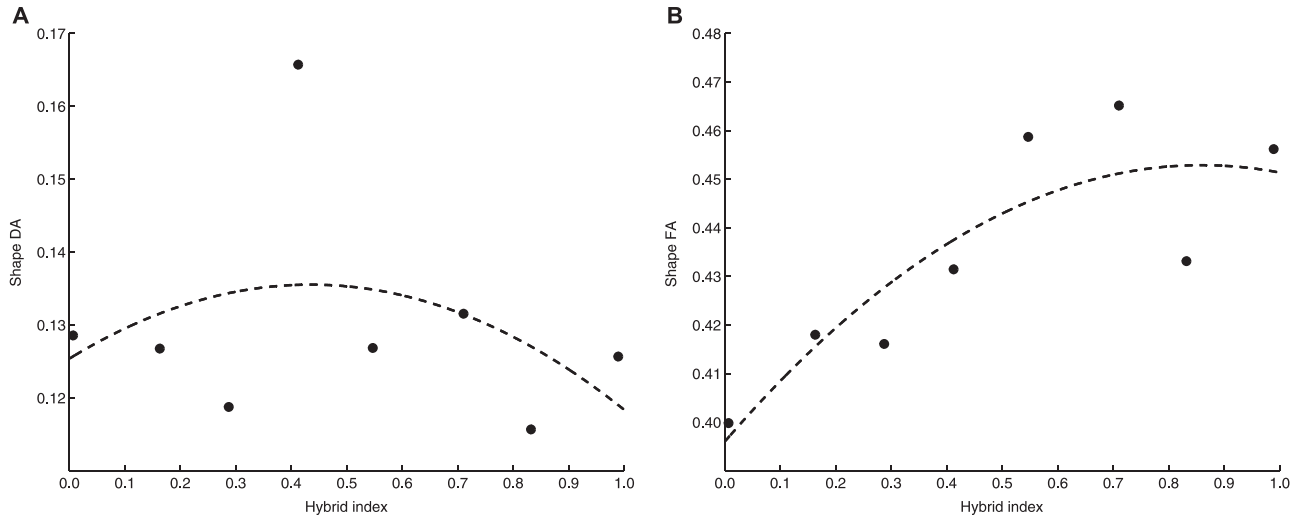
No size dependence of shape FA was observed among the introgression classes ( $r = 0.12$ ,  $P = 0.76$ ). Within them, the size dependence of unsigned shape asymmetries was only marginally significant in two classes (Table 4), and the Fisher's combined probability test was not significant ( $P = 0.151$ ).

#### DIFFERENCES AMONG UNSIGNED ASYMMETRY DISTRIBUTIONS

The quantile–quantile plots of unsigned asymmetry distributions are presented in Figure 7. In the mandible-size asymmetries, the relative excess of moderate magnitudes is apparent in the hybrid class (Fig. 7B, C). This is consistent with increased platykurtosis of the underlying distribution of signed asymmetries towards hybrid groups (Fig. 3D). In the mandible-shape asymmetries, the most remarkable feature is a much lighter right tail of the distribution in the hybrid class (Fig. 7E, F) expressing the decrease of FA in the hybrid zone centre.

In the skull-shape asymmetries, the patterns are more complex. In comparison with both outer classes, hybrids present an excess of higher magnitudes apparent as two bumps near to the right tails of the distributions (Fig. 7H, I). The mutual comparison of two outer classes reveals an overall bias to higher asymmetries in the *musculus*-like class, but no bumps





**Figure 6.** Variation in the skull shape asymmetry across the hybrid zone. For twelve introgression classes directional asymmetry (DA) (A) and fluctuating asymmetry (FA) (B) are plotted against hybrid indices. Quadratic polynomial fits are depicted as dashed lines.

**Table 5.** Skewness and kurtosis of distributions of shape asymmetries ('shape skewness' and 'shape kurtosis') in broad introgression classes, delimited for both traits as follows: *domesticus*-like (0.0–0.3), hybrid (0.3–0.7), and *musculus*-like (0.7–1.0)

Introgression class	N	Shape skewness	Shape kurtosis
M <sub>DOM</sub>	389	284*	20.03*
M <sub>HYB</sub>	88	125	0.33
M <sub>MUS</sub>	308	398*	17.84*
S <sub>DOM</sub>	116	1083*	1.496
S <sub>HYB</sub>	57	942	–2.55*
S <sub>MUS</sub>	112	917	–1.46

\*Significant deviations from normality after sequential Bonferroni correction.

Sample size (N) is listed for each class.

M, mandible; S, skull.

similar to those seen before (Fig. 7G). Thus, it seems the asymmetry distributions within the hybrid and the *musculus*-like classes are not as similar as could be inferred from measures of multivariate skewness and kurtosis.

## DISCUSSION

Within the hybrid zone, the variation of right–left asymmetries, or fluctuating asymmetry, is expected to change as a result of shifts in DI. Disruption of genomic co-adaptation could increase random developmental noise or impair robustness to it, and hence

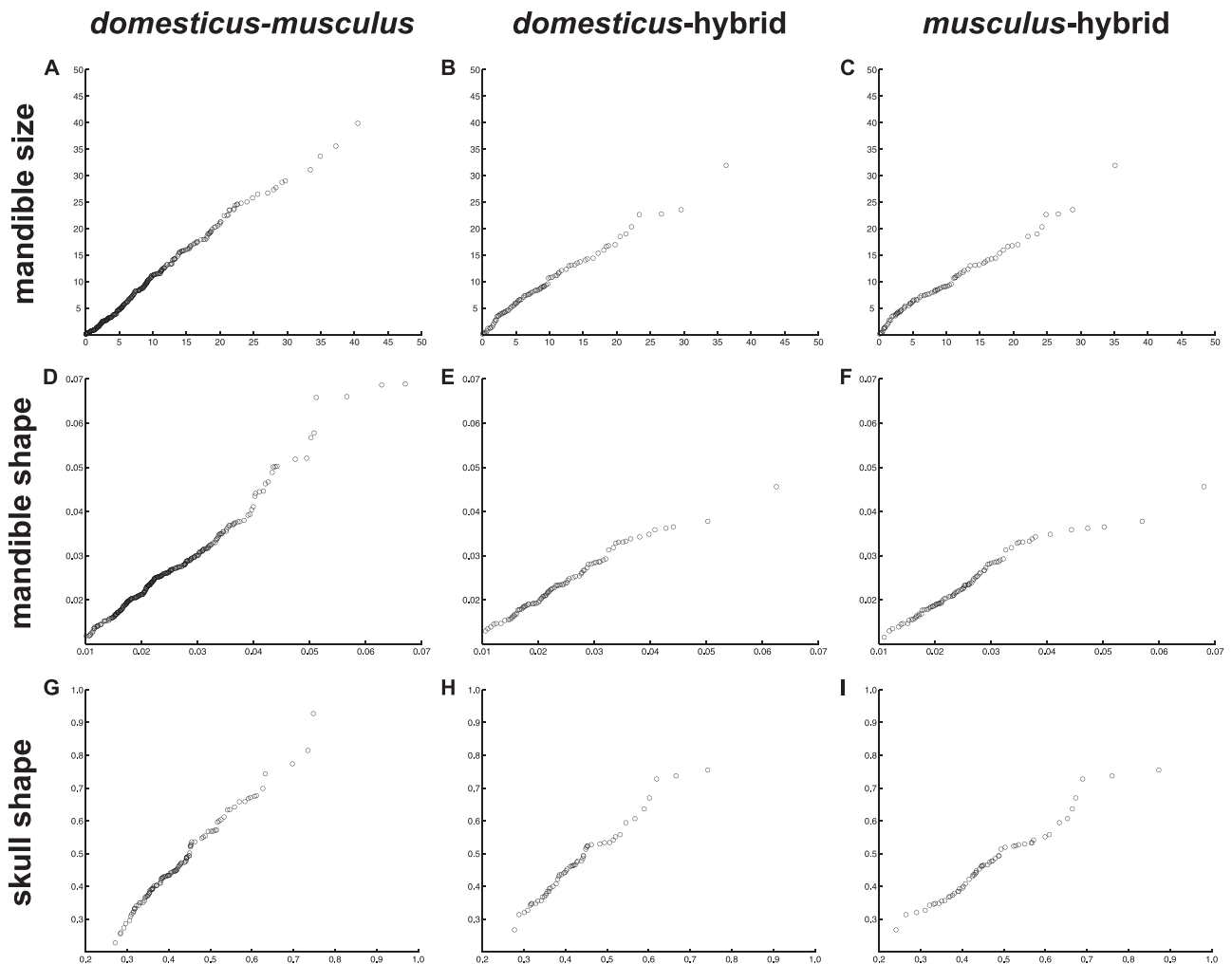
increase FA, whereas higher heterozygosity could have the opposite effect (Soulé, 1967; Graham, 1992; Clarke, 1993). At the same time, however, genetic variability for right–left patterning may be increased as a result of hybridization (Rego *et al.*, 2006). In such a case, FA results from the interplay between stochastic and deterministic factors.

In the house mouse hybrids, Alibert *et al.* (1994, 1997) found FA of the lower molars to be decreased relative to parental subspecies. A decrease of DI was suggested as the cause, because genetic variability would be hardly decreased by the mixing of different hereditary information. The same was concluded by Debat *et al.* (2000) for shape, although not for size, of the dorsal side of the skull. However, Mikula & Macholán (2008), analysing material from another portion of the hybrid zone, and using different traits and a different statistical approach, did not find any significant pattern suggesting heterotic effects upon developmental stability in hybrids.

Fluctuating asymmetry was repeatedly demonstrated to be trait dependent (Polak *et al.*, 2003), and thus any conclusion about the impact of hybridization on DI should be based on more traits. The mandible serves as a model object in mammalian developmental biology (Atchley & Hall, 1991; Chai *et al.*, 2000; Leamy, Routman & Cheverud, 2002; Ramaesh & Bard, 2003), which enhances the relevance of our results for researching a developmental basis of FA.

## DIRECTIONAL AND FLUCTUATING ASYMMETRY

Traditionally, only FA was supposed to be useful as an indicator of DI. It should be true, if the variation of



**Figure 7.** Quantile–quantile plots comparing unsigned asymmetry distributions among three broad introgression classes. Pairs of compared classes are indicated at the top, and the traits concerned are given at the left margin of the figure.

right–left asymmetries arises purely from stochastic deviations accumulated independently on both sides in the course of development. Genetic variability for right–left asymmetry may, however, be segregating in a population, and development of body sides may be interdependent, especially in well-integrated symmetrical structures.

Thus both the magnitude of mean asymmetry (DA) and variation of asymmetries (FA) may be influenced by stochastic as well as deterministic factors. Under such conditions, DA and FA may also be dynamically interrelated, as suggested by Graham *et al.* (1998), and occasionally demonstrated by some other authors (Lens & Van Dongen, 2000; Kark, 2001; Stige *et al.*, 2006). Thus, we inspected changes of both asymmetry components across the hybrid zone in our study.

Size DA as well as shape DA did not vary according to a quadratic polynomial model, but in particular the

shape DA appears to be larger in introgression classes with  $HI > 0.50$ . A difference between introgression classes with  $HI$  above and below 0.5 was also apparent in the principle components analysis of mean shape asymmetries, where they tended to be separated along the first principle component axis. It seems, therefore, that developmental backgrounds of shape symmetry determination are slightly different in the *domesticus* and *musculus* populations, depending on the proportion of genetic/epigenetic information that predominates.

Size FA did not show any clear trend. It varied widely between highly introgressed classes (cf. M-06, M-07, and M-08 on Fig. 3B). Shape FA appeared to be lower in the centre of the hybrid zone. The trend was, however, asymmetric, as on the *domesticus* side only the class M-01 had a relatively high shape FA. Overall, our results are in line with the results of

Debat *et al.* (2000), who found that the shape FA but not the size FA of the skull was lower in  $F_1$  laboratory hybrids. In the context of interspecific hybridization, it is not feasible to explain decreased FA as a consequence of lower genetic variability for right–left differences. Lower DI is a more likely explanation (Alibert *et al.*, 1994, 1997).

Traditionally, lower DI was predicted to be caused by the higher heterozygosity of hybrids (Lerner, 1954), but the generality of this relationship is doubtful (Clarke, 1993; Vøllestad, Hindar & Møller, 1999; Alibert & Auffray, 2003; Leamy & Klingenberg, 2005, but see Van Dongen, 2006). In any case, in mice, Leamy *et al.* (2001) did not find any difference in mandible-shape FA between inbred and outbred populations, and Leamy, Routman & Cheverud (cited by Leamy & Klingenberg, 2005) did not find significant correlation between heterozygosity and size or shape FA of molars.

Alternatively, and perhaps more likely, lower DI may be a by-product of over-dominant effects on developmental processes. In mice, a decrease of limb FA was observed in a certain period of prenatal development (Hallgrímsson *et al.*, 2003). Slight changes in the timing of developmental events resulting in a prolongation of this period could change FA levels. Similarly, interactions with surrounding tissues may be involved. It is known that the proper formation of bones depends on stimulation by contractions of embryonic muscles (Hall & Herring, 1990), and this fact was invoked to explain changes in FA (Hallgrímsson *et al.*, 2003) and shape variation (Zelditch, Lundrigan & Garland, 2004). In hybrids, these contractions might accidentally become less autocorrelated within body sides, making them more prone to symmetric development.

We observed an inverse relationship between average size and shape FA limited to classes with at least slightly introgressed gene pools (HI different from 0.000 or 1.000). Nevertheless, the traditional explanation of heterosis is not feasible here as the mean size dropped towards the *musculus* side (not shown) rather than towards the centre, where maximal heterozygosity is expected. Thus, the observation also argues for shape FA being dependent on the hybridization-induced changes in developmental dynamics, rather than on overall genomic parameters like heterozygosity.

This is in line with explanations discussed by Van Dongen *et al.* (2009) in their study of freshwater sticklebacks. Here, some directionally selected traits showed an increase of FA, but this was probably not caused by a concomitant loss of genetic variation. The authors preferred environmental and structural explanations: namely the limited availability of calcium and more scope for asymmetry in low-plated upland fish.

#### KURTOSIS OF ASYMMETRY DISTRIBUTIONS

The most general pattern involved kurtosis of individual asymmetry distributions. Towards the centre of the hybrid zone, distributions became more platykurtic, with the trend being evident for size FA (although one outlier occurred) as well as shape FA. We interpret shifts in kurtosis to be a consequence of a mixture of different underlying distributions. The mixtures are thought to be continuous, composed from  $N$  distributions, the means of which correspond to deterministic components, and variances to stochastic components, of individual asymmetries.

Platykurtosis arises when distributions with different means are mixed (Palmer & Strobeck, 2003), and hence the shift towards it should indicate increased genetic variability for right–left differences. The trend could reflect newly created genetic variability as well as a greater heterogeneity of gene pools in the centre of the hybrid zone. Platykurtosis also arises where developmental regulation amplifies already existing asymmetries, which was observed in lobster claws (Govind & Pearce, 1986), but seems unlikely in mouse mandibles.

From the range of possible phenotypic impacts of new genetic variation, changes in covariance structure of asymmetries deserve special attention. The mammalian skull, but also the mandible, is composed from multiple partly independent developmental modules (Klingenberg, Mebus & Auffray, 2003; Mitteroecker & Bookstein, 2008; Zelditch *et al.*, 2008; Porto *et al.*, 2009). In poorly integrated structures, module-specific asymmetries are likely to be less correlated, and in the computation of the overall asymmetry they can easily balance themselves. Consequently, the overall asymmetries should be less variable in a population. We invoked higher genetic variability for asymmetries as an explanation for their relatively platykurtic distributions. Therefore, we could speculate that in hybrids genetically determined asymmetries are more correlated across developmental modules.

Leptokurtosis is expected when the growth of a structure is multiplicative, but in such a case, size dependence of asymmetries also arises (Graham *et al.*, 2003), and in our study there is a poor correspondence between the classes with the most leptokurtic distributions and those with the most significant size dependence. Thus, leptokurtosis in *domesticus*-like and *musculus*-like classes is more likely to reflect a mixture of distributions with similar means but different variances (Palmer & Strobeck, 2003).

#### TRAIT DEPENDENCE OF FA VARIATION

The results of a reanalysis of skull asymmetry data are consistent with our earlier conclusions (Mikula &

Macholán, 2008). No decrease of shape FA in the centre of the hybrid zone was apparent. Conversely, the most notable trend was an increase of FA from the *domesticus*-like class S-01 to the hybrid class S-05 (Fig. 6B), although hybrid and *musculus*-like classes (from S-04 to S-08) had similar levels of FA.

The inspection of quantile–quantile plots (Fig. 7G, H, I), however, revealed asymmetry magnitudes to be similarly distributed within two outer classes. It suggests different factors being responsible for elevated FA in the centre and on the *musculus* side of the hybrid zone. Given the shift towards platykurtosis, the increased FA in the centre may be caused by an increase of genetic variability, whereas on the *musculus* side an inherently higher DI might be more important. The decrease of skull-shape FA observed by Debat *et al.* (2000) in F<sub>1</sub> hybrids between Danish mice might result from reduced genetic variability in experimental populations and a concurrent decrease of DI in hybrids. Alternatively, the difference in genetic variability might be limited to Central European populations.

In any case, changes in shape FA seem to be trait specific, at least in the Central European portion of the hybrid zone. Between-trait differences in FA are quite common (Polak *et al.*, 2003), and they were also reported in studies of hybrids (Alibert & Auffray, 2003). For example, lab-bred hybrids between two chromosome races of *Sceloporus grammicus* lizards showed higher FA in one trait, but lower FA in most of the other traits (Dosselman, Schaalje & Sites, 1998). Nevertheless, generally speaking, the causes of these differences remain obscure. In the case reported here, the difference might relate to the efficiency of bone remodelling. According to experience from human surgery, the efficiency of bone remodelling is appreciably higher in the mandible than in the skull base (Kleinheinz, Meyer & Joos, 1997). To our knowledge, the difference was never studied in the house mouse, but its mandible was shown to be very plastic (Renaud, Auffray & de la Porte, 2010).

## CONCLUSIONS

The most general result of our study was the change in shape of individual asymmetry distributions towards platykurtosis, observed in the mandible-size asymmetry, as well as in the mandible-shape asymmetry and the skull-shape asymmetry. It was interpreted as a result of increased genetic variability for right–left asymmetries.

Secondly, we documented the trait dependence of changes in shape FA, although it may be restricted to the Central European portion of the house mouse hybrid zone. A U-shaped trend (minimum at HI = 0.41) was predicted for the mandible-shape

FA, whereas a marginally significant non-monotonic increase towards the *musculus* side (maximum at HI = 0.86) was noted for the skull-shape FA. An evidence for a hybridization-induced change of mandible-size FA is lacking.

The decrease of mandible-shape FA was hypothesized to result from decreased developmental instability caused by subtle over-dominant effects on developmental dynamics, rather than from an increase of heterozygosity. The development of the skull seemed to be inherently less stable in the *musculus* mice, although the causes are hard to guess.

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