Relative warp analysis of skull shape across the hybrid zone of the house mouse (*Mus musculus*) in Denmark.

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(With 5 figures in the text)

The changes in skull shape that occur in the hybrid zone between the two European subspecies of the house mouse (*Mus musculus musculus* and *M. m. domesticus*) were studied by relative warp analyses. Landmarks observed on the ventral view of the skulls of 269 mice sampled in 18 localities in Denmark were analysed. Each population was also characterized by a hybrid index estimated from allozymic data. Although no clear pattern of within-population variability of shape could be established across the hybrid zone, the shape changes among the 18 populations were found to be correlated with the allozymic introgression but not with geographical location. Finally, the cline obtained for skull shape seems to be steeper than that corresponding to the average allozyme hybrid index which suggests that skull development could be slightly perturbed in hybrids.

Introduction

In a wide variety of organisms, secondary contact between two divergent taxa, which can still mate and produce offspring, has given rise to a hybrid zone. The geographical distribution of divergent morphological traits that distinguish between the two hybridizing taxa can be used to define the position of the contact zone. Morphological studies also reveal the effects of hybridization on developmental stability (see Graham, 1992, for review). Although, in a hybrid zone, one might predict that the stability of development will be favoured by the increased heterozygosity, selection against hybrids resulting from the disruption of co-adapted gene systems involved in development can also produce the opposite effect. The respective weights of these two forces will depend on the degree to which the co-adapted gene systems controlling development have diverged in the parental taxa. Although morphological developmental instability in hybrids is generally expressed as an increase in fluctuating asymmetry, it may also increase the phenotypic variability because of the appearance of developmental abnormalities. Szymura & Barton (1986) proposed that the greater morphological variation in genetically enriched hybrid populations is more likely to be due to an increase in abnormal phenotypes caused by a breakdown in developmental stability than to the higher genetic polymorphism expected in mixed genomes. Besides, the reduction of phenotypic variability potentially related to a higher level of heterozygosity (Eanes, 1978; Mitton, 1978; Leamy, 1982; Mitton & Grant, 1984;

Yezerinac, Loogheed & Handford, 1992) has never been observed in natural hybrid populations. Consequently, the phenotypic variability, estimated from the variance of one or several morphological traits across a hybrid zone, should provide an indication of the degree of the divergence and incompatibility between the hybridizing genomes. The width of the clines obtained for morphological characters across a hybrid zone may also reflect the level of selection on the hybrid phenotype (Barton & Gale, 1993). Finally, any increase in the size of the hybrids would suggest a heterotic effect.

It has been proposed that most natural hybrid zones resulting from secondary contact can be described by the tension zone model (Barton & Hewitt, 1985, 1989). This model supposes that the hybrid zone is maintained by two opposing processes: the dispersal of individuals leading to gene flow across the hybrid zone, and the selection against hybrids caused by a breakdown of the genomic co-adaptation.

The narrow hybrid zone between the two European subspecies of the house mouse, *Mus musculus musculus* and *M. m. domesticus* is generally considered to be a tension zone (see Boursot *et al.*, 1993; Sage, Atchley & Capanna, 1993 for reviews). Archaeozoological remains suggest that this zone which now crosses Europe from Bulgaria to Denmark results from a secondary contact which, in Northern Europe, occurred within the last 2500 years (Auffray, Vanlerberghe & Britton-Davidian, 1990; Auffray, 1993). The pattern of genetic variation that occurs across transects in Denmark, Germany, and Bulgaria has been investigated using allozymes (Hunt & Selander, 1973; Vanlerberghe *et al.*, 1986, 1988), mt-DNA (Ferris *et al.*, 1983; Vanlerberghe *et al.*, 1988; Prager *et al.*, 1993), and DNA markers on the X and Y chromosomes (Vanlerberghe *et al.*, 1986; Tucker *et al.*, 1992; Dod *et al.*, 1993). Although these transects occur in different geographical regions, the clines obtained for a given marker are all quite similar. The centres of the clines for these markers are approximately coincident but their widths vary from locus to locus. The X and Y chromosome clines are the steepest, which suggests that selection on certain gene combinations involved in sex chromosome function could play an important role in the dynamics of the hybrid zone. Similarly, the increase in the susceptibility of hybrid populations to infestation with intestinal worm (Sage *et al.*, 1986; Moulia *et al.*, 1991) appears to be due to the disruption of co-adapted gene systems involved in the immune response of the hybrid mice (Moulia *et al.*, 1993). However, a recent study on the fluctuating asymmetry of molars has shown that developmental stability increases towards the centre of the hybrid zone (Alibert *et al.*, 1994) which is evidence that there are also heterotic effects for certain characters in this zone.

Even though the change from a short tail in *musculus* to a long one in *domesticus* was the first character used to describe the house mouse hybrid zone (Ursin, 1952 in Hunt & Selander, 1973), the morphological changes that characterize the transition are not obvious. This could be due to the close morphological proximity between the two subspecies as even a multivariate approach based on skull and mandible measurements does not allow a complete separation to be made (Gerasimov *et al.*, 1990). However, although Kraft (1985), using morphological traits, showed that *M. musculus* hybrids in Southern Germany were intermediate between the two parental subspecies, he found no clear pattern of within-population variability across the zone. Although, Hunt & Selander (1973) suggested that heterozygosity is higher in the hybrid zone than in the parental groups, Schnell & Selander (1981) found no change in the variability of the so-called diagnostic morphological characters in Danish hybrids. The morphological pattern of hybridization they found appears to be more complex. Several characters are intermediate in hybrids and others increase in size, suggesting heterotic effects. Finally, some rare non-metric morphological variants occurred, most of which were found in the centre of the hybrid zone. It is not clear

whether these morphological variants result from an increase in the mutation rate in the centre of the hybrid zone or result from the developmental instability of the hybrids. Both the increase in mutation rate and developmental instability are thought to be caused by genomic disruption (Barton & Hewitt, 1985; Fontdevilla, 1992; Sage et al., 1993). Recently, Prager et al. (1993) attempted to describe morphological patterns across the hybrid zone in northern Germany. Although most of the characters are intermediate in the hybrid populations, mandible shape in the most introgressed locality clearly diverges from an expected median shape between musculus and domesticus. The authors, therefore, suggest that this could be an example of a developmental abnormality caused by hybridization.

The results of these morphological studies are not unanimous and they cannot be combined to give a synthetic picture of the morphological modality of hybridization. The close morphological proximity between M. m. musculus and M. m. domesticus means that more powerful methods of
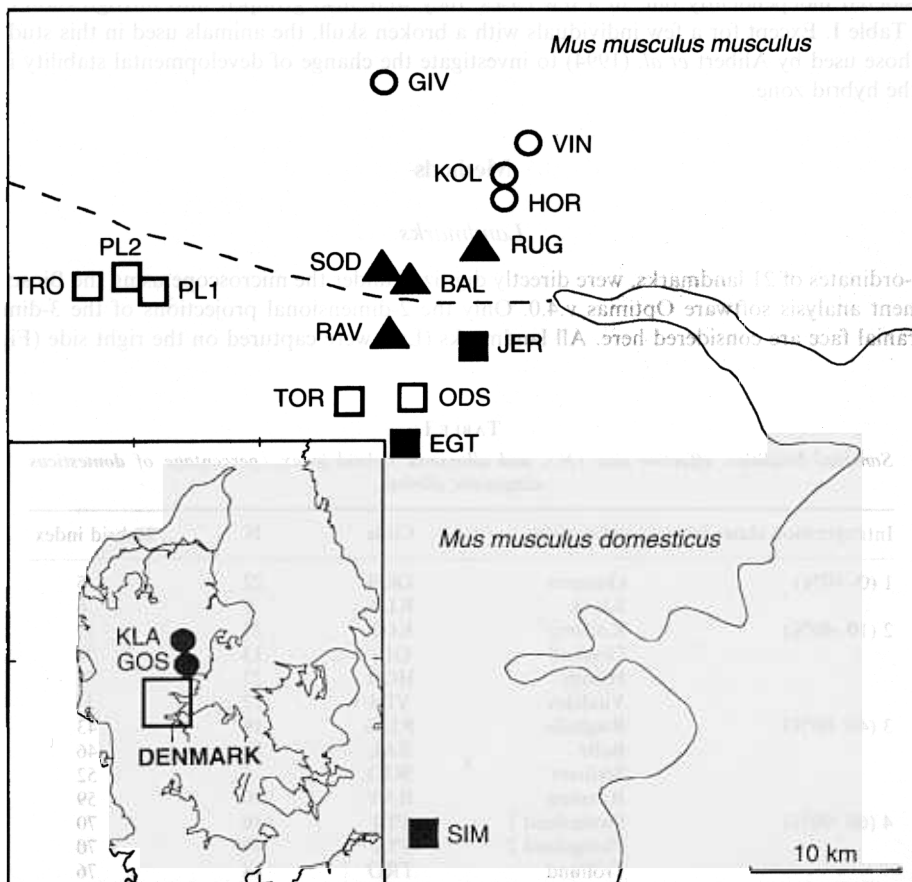

FIG. 1. Localities studied in the hybrid zone between the two European species of the house mouse in Jutland. The symbols correspond to the introgression classes which group localities on the basis of the percentage of Mus musculus domesticus diagnostic alleles present (●: introgression class 1 (0—10%); ○: class 2 (10—40%); ▲: class 3 (40—60%); □: class 4 (60—90%); ■: class 5 (90—100%)).

separation are required. Such methods have recently become available with the advent of image analysis and geometric morphometrics that has been established by Bookstein (see Bookstein, 1991 for a review). Several systematic studies have already demonstrated the power of the new techniques (Rohlf & Marcus, 1993); and this study is the first to apply relative warp analysis (Bookstein, 1991; Rohlf, 1993a) in a hybrid zone. It is used to obtain a synthetic pattern of within and between population changes in skull shape across the house mouse hybrid zone.

Material

A total of 269 individuals from 18 populations that were sampled between 1987 and 1992 along a transect of the hybrid zone between *M. m. musculus* and *M. m. domesticus* in Denmark (Fig. 1) were examined. These populations are characterized by their allozymic hybrid index, which corresponds to the percentage of *domesticus* alleles averaged over 7–10 diagnostic loci within each population (Table I) (Vanlerberghe *et al.*, 1986; Alibert *et al.*, 1994). All animals were adults. For most of the statistical analyses, these populations were considered independently but, in a few cases, they were also grouped into introgression classes as shown in Table I. Except for a few individuals with a broken skull, the animals used in this study are the same as those used by Alibert *et al.* (1994) to investigate the change of developmental stability in molars through the hybrid zone.

Methods

Landmarks

The co-ordinates of 21 landmarks, were directly digitized under the microscope using the Bioscan image measurement analysis software Optimas v.4.0. Only the 2-dimensional projections of the 3-dimensional ventral cranial face are considered here. All landmarks (Lm) were captured on the right side (Fig. 2) and

TABLE I

*Sampled localities, effective size (N), and allozymic hybrid index (percentage of **domesticus** diagnostic alleles)*

| Introgression class	Locality	Code	N	Hybrid index
1 (0–10%)	Gossmer	GOS	22	5
	Klank	KLA	9	6
2 (10–40%)	Kollerup	KOL	25	19
	Givskud	GIV	13	28
	Hörup	HOR	23	31
	Vindelev	VIN	12	37
3 (40–60%)	Rugballe	RUG	16	43
	Balle	BAL	15	46
	Södover	SOD	5	52
	Ravning	RAV	10	59
4 (60–90%)	Plougslund 1	PL1	16	70
	Plougslund 2	PL2	10	70
	Tröllund	TRO	14	76
	Ödsted	ODS	14	82
	Törskind	TOR	12	88
5 (90–100%)	Egtved	EGT	21	96
	Jerlev	JER	23	97
	Simmersted	SIM	9	97
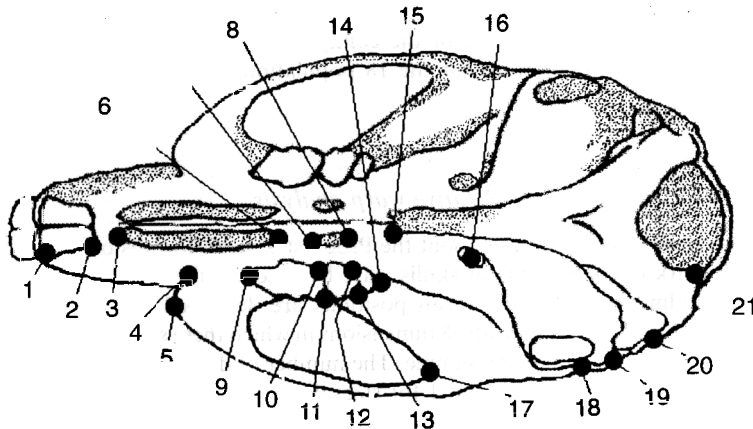
FIG 2. Location of landmarks on the ventral view of the skull of the house mouse

correspond to the external and anterior border of the incisor (Lm-1), the external extremity of the incisor (Lm-2), the anterior extremity of anterior palatine foramen (Lm-3), the juxtaposition of maxillar and malar process (Lm-4), the anterior extremity of the zygomatic plate (Lm-5), the posterior extremity of anterior palatine foramen (Lm-6), the anterior (Lm-7) and posterior (Lm-8) border of the posterior palatine foramen, the anterior extremity of the first upper molar (Lm-9), the lingual (Lm-10) and labial (Lm-11) juxtaposition of the first and second upper molars with the alveolar process, the lingual (Lm-12) and labial (Lm-13) juxtaposition of the second and third upper molars with the alveolar process, the posterior border of the third upper molar (Lm-14), the posterior border of the palatine (Lm-15), the centre of the ovale foramen (Lm-16), the posterior suture of the zygomatic (Lm-17), the posterior border of the external porus acousticus (Lm-18), the juxtaposition of the tympanic bulla with occipital (Lm-19), the juxtaposition of par mastoidea and jugularis process (Lm-20), the external extremity of the occipital foramen (Lm-21), respectively.

The raw data-set, therefore, corresponds to the individual configurations each consisting of 21 landmarks, i.e. 42 x- and y-co-ordinates.

Superimposition method

The variable volume of the mouse skull means that the image analyser has to be calibrated differently for each individual. Since an error attached to this recurrent operation has prevented us from making an accurate overall calibration, size has been excluded from this study. This exclusion was done by scaling all configurations to unit centroid size for each configuration. The centroid size is the square root of mean squared distance between the centroid and the landmarks within each configuration (Bookstein, 1991).

The 269 scaled configurations were then superimposed so as to be available for comparison for further analysis. This was done using the method called Procrustes generalized least square superimposition (GLS). Details of this method are given in Rohlf (1990), Rohlf & Slice (1990), and Bookstein (1991) and some applications are described in Auffray et al. (1996) and Corti et al. (1996). In the Procrustes GLS routine, the configurations were scaled to unit centroid size and then centred and rotated in order to minimize the scatter of landmarks, i.e. by minimizing the sum of squared distances between landmarks of each configuration to the corresponding landmarks of a reference configuration. Here the reference is the all-individual consensus configuration (all-individual mean configuration). The consensus configuration for each populations were also calculated for use in further analysis. The GLS superimpositions were

computed using the program Generalized Rotational Fitting of N-dimensional Data (GRF-ND) v.2.1 (Slice, 1993). Two data sets, one consisting of the configurations for the 269 individual and the other consisting of the consensus configurations for the 18 populations, were superimposed and subjected to relative warp analysis.

Relative warp analysis

For statistical purposes, shape variation about the Procrustes mean form is obtained by superimposing each of the 269 landmark configurations of skulls, case by case, on the mean form (Rohlf, 1993*a*). The residuals of the 21 landmarks from their mean positions are described landmark by landmark by 42 Cartesian coordinates ($21x$, $21y$) that span all 38 dimensions in which these shapes can vary from their mean, either specimen by specimen, or, sample by sample. The number of dimensions is given by the 4 degrees of freedom (2 for position, 1 for orientation and 1 for size) that are removed by the GLS fit from the total number of dimensions, i.e. the 42 co-ordinates. Picture by picture, these residuals are not independent (Bookstein, 1991), so their diagrams are not a very useful way of describing large-scale shape differences. However, this is not important as they are used to compute the principal components of the dimensions giving the residual variability. These components are equivalent to a principal component analysis of the Procrustes distances among all pairs of forms in the original sample of 269 individuals and, therefore, do not depend significantly on the way the average shape is computed. This analysis corresponds to the first step of the standard routine for relative warp analysis described by Bookstein (1996*a*) and the principal components obtained correspond to the relative warps (RW).

The intra-population variability as a function of the level of introgression was computed from the scores obtained for all the individuals on the significant RW-axes. The centroid of each of the 18 populations within the RW space was computed in order to evaluate the distance between each individual and its own population centroid. These distances were compared among populations by multifactor ANOVAs. Two models were considered: the first (model 1) includes sex and locality and their interactions, the locality being nested within the introgression class. The second (model 2) excludes sex. The individual scores obtained for the significant RW were also used to estimate the variability among populations. Differences in their individual scores among populations and among introgression classes were estimated and tested by multifactor MANOVAs for model 1 (including the sex factor) and model 2 (excluding sex).

The 18 population consensus configurations were then subjected to relative warp analysis using the overall population consensus configuration as a reference.

This principal component analysis of shape variation can be computed in several modes (Rohlf, 1993*a*; Bookstein, 1996*a*) which allows one to concentrate on the subspace of a particular shape variability. Any shape change consists of 2 parts, the uniform or affine transformation and the non-uniform part of deformation. Both the uniform and non-uniform parts are complementary subspaces of the full vector space of shape change. The uniform shape change may be expressed using several methods. Here, the uniform transformation described by Bookstein (1996*b*) was used as it is especially suited to the procedure we have followed, i.e. the relative warp analysis of individuals superimposed by GLS after the reference configuration has been rotated with respect to its first principal component. The significance of a purely uniform transformation is easily understood if one considers all the landmarks of the reference configuration are fastened to a squared grid. The uniform transformation of the reference into another configuration will leave parallel lines. The uniform part is then described by 2 coefficients U1 and U2. The transformation corresponding to U1 converts the horizontal rectangles of the grids to parallelograms of the same height without changing the length along the *x*-axis. U2 corresponds to a pure dilatation—or compression—away from the *x*-axis (Bookstein, 1996*b*). Because our data set consists of only half of the skull, the 'uniform term' of the standard analysis would confound uniform and non-uniform shape changes of the skull (Bookstein, 1991). The uniform analysis corresponds to the second step of the relative warp analysis (Bookstein, 1996*a*).

The third step of the relative warps analysis concerns the non-uniform part of the shape-variation. This

corresponds to the subspace that describes the variation of the shape around the affine Procrustes fits relative to the Procrustes average shape. This analysis also leads to the emergence of principal components, the RWs, describing the non-uniform subspace. Only significant RWs were taken into account. The matrix of the generalized distance between populations in the RW space was compared to the distance matrix based on population differences for the introgression index and to the geographic distance matrix by Mantel *t*-tests using NTSYS-pc software.

For the relative warps analysis, the required principal components, and the corresponding case-scores specimen by specimen are computed by the programs Thin-Plate Spline Relative Warp (TPSRW-Rohlf, 1993*b*). This program supplies the convenient diagrams that display each component as deformation. The programmes TPS (Rohlf, 1994) or Morphometrika v.2. (Walker, 1993) were used to obtain the thin plate spline deformations between any pair of mean forms. The thin-plate spline deformation that is necessary for each landmark to be precisely superimposed on the landmarks of the reference configuration is represented as the deformation of a grid to which the reference configuration is attached. The thin-plate spline also corresponds to a fitting function used in shape analyses that is defined by Bookstein (1991). Here, the thin-plate spline is only a way of visualizing the non-uniform shape change as a whole, but the thin plate spline function is not used to define or compute the non-uniform or uniform shape changes. Theoretical details of this type of method are to be found in Bookstein (1991), Rohlf (1993*a*), Reyment & Jöreskog (1993), Bookstein (1996*a, b*) and some of their applications are given in Zelditch, Bookstein & Lundrigan (1992, 1993), Loy, Corti & Marcus (1993), Rohlf (1993*a*) and Swiderski (1993).

Results

Within-population variability

The variability within each of the 18 populations was estimated as the mean of the distance between individuals and the population centroid within the shape space defined by the 34 significant axes. The statistical routine used to test the significance of the axes is provided in TPSRW and details are given in Reyment & Jöreskog (1993). The model 1 ANOVA performed on these distances (Table II) shows that the sources of variability related to sex are not significant. The model 2 ANOVA (Table II) shows that within-population

TABLE II

ANOVAs on the morphological variability within and among populations expressed by the distance of each individual to the centroid of its own population (see text)

Source of variation	d.f.	Sum of square $(\times 10^{-8})$	Mean square $(\times 10^{-8})$	F	P
MODEL 1 (sex included)					
Sex		0.03	0.03	0.10	0.7401
Introgression class	4	0.92	0.23	0.85	0.4958
Locality (Introgression class)	13	13.96	1.07	3.93	< 0.0001
Sex*Introgression class	4	1.53	0.38	1.40	0.2332
Sex*Locality (Introgression class)	13	2.88	0.22	0.81	0.6483
Residual	232	63.40	0.27		
MODEL 2 (sex excluded)					
Introgression class	4	0.95	0.24	0.88	0.4765
Locality (Introgression class)	13	13.90	1.07	3.95	< 0.0001
Residual	251	67.96	0.27		

variability differs among populations but their plots against the allozymic hybrid index (not shown here) does not give a clear pattern across the hybrid zone. Moreover, the introgression class effect is shown not to be statistically significant.

Among-population variability

MANOVAs were performed on the scores of all individuals on the 34 significant RWs (Table III). Again, the effect of sex has been rejected in the model 1 MANOVA, suggesting the absence of sexual dimorphism in our samples. Model 2 MANOVA shows that both populations and introgression classes present different patterns within the RW space. The differences in the population variability between the introgression classes suggest that a pattern of morphological introgression might exist. The presence of 34 significant axes in the RW space indicates that the pattern of differentiation both within and among populations is very complex. In order to simplify the description of between population differentiation in further analyses, we only considered their consensus configuration, i.e. the 18 average configurations of populations were subjected to relative warp analysis.

TABLE III

MANOVAs on the relative warp scores obtained from the relative warp analysis on the all-individual data set (see text)

Source of variation	Value	F	Num. *d.f.*	Den. *d.f.*	P
MODEL 1 (sex included)					
Sex					
Wilks' Lambda	0.88	0.79	34	199	0.7923
Roy's Greatest Root	0.13	0.79	34	199	0.7923
Hotelling-Lawley Trace	0.13	0.79	34	199	0.7923
Pillai Trace	0.12	0.79	34	199	0.7923
Introgression class					
Wilks' Lambda	0.15	3.51	136	795	< 0.0001
Hotelling-Lawley Trace	2.54	3.68	136	790	< 0.0001
Pillai Trace	1.44	3.34	136	808	< 0.0001
Locality (Introgression class)					
Hotelling-Lawley Trace	6.02	2.68	442	2563	< 0.0001
Pillai Trace	3.62	2.39	442	2743	< 0.0001
Sex*Introgression class					
Wilks' Lambda	0.56	0.92	136	795	0.7326
Hotelling-Lawley Trace	0.63	0.91	136	790	0.7499
Pillai Trace	0.54	0.92	136	808	0.7150
Sex*Locality (Introgression class)					
Hotelling-Lawley Trace	2.24	0.99	442	2563	0.5022
Pillai Trace	1.81	1.00	442	2743	0.4788
MODEL 2 (sex excluded)					
Introgression class					
Wilks' Lambda	0.15	3.85	136	870	< 0.0001
Hotelling-Lawley Trace	2.55	4.05	136	866	< 0.0001
Pillai Trace	1.44	3.67	136	884	< 0.0001
Locality (Introgression class)					
Hotelling-Lawley Trace	5.86	2.86	442	2810	< 0.0001
Pillai Trace	3.51	2.51	442	2990	< 0.0001

TABLE IV
Significance of the first three relative warps obtained for the analysis based on the average consensus configurations of each population

Relative warp	Singular value	χ^2	d.f.	P	High (medium) x-displacement of landmarks	High (medium) y-displacement of landmarks
RW1	0.00732	217.8	152	0.0004	2,15 (4,5,6,7,16,20)	— (5)
RW2	0.00716	185.5	135	0.0026	3,15 (1,5,7,9,16,21,20)	
RW3	0.00515	140.1	119	0.0908	13,19,20 (18)	20 19

Morphological pattern of introgression

Relative warp analysis of the non-uniform components of the shape change mode on the consensus configurations of 18 populations gives two significant axes (Table IV).

The first RW representation (Fig. 3a) and the RW loadings, expressed as x- and y-displacements on each landmark, suggests that most of the landmark displacement occurs along the x-axis, which
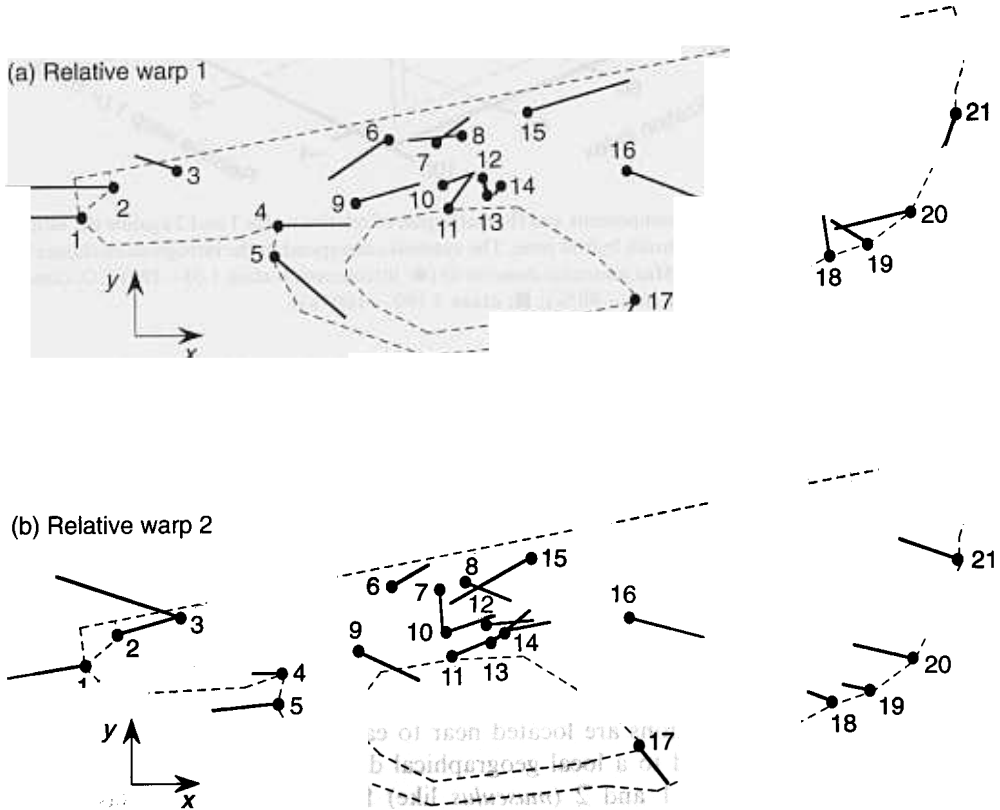

FIG. 3. Vectors of the landmark changes for (a) the first and (b) the second relative warps of the 18 populations across the hybrid zone. The reference was the consensus configuration of all population means superimposed by the generalized least square method (GLS).
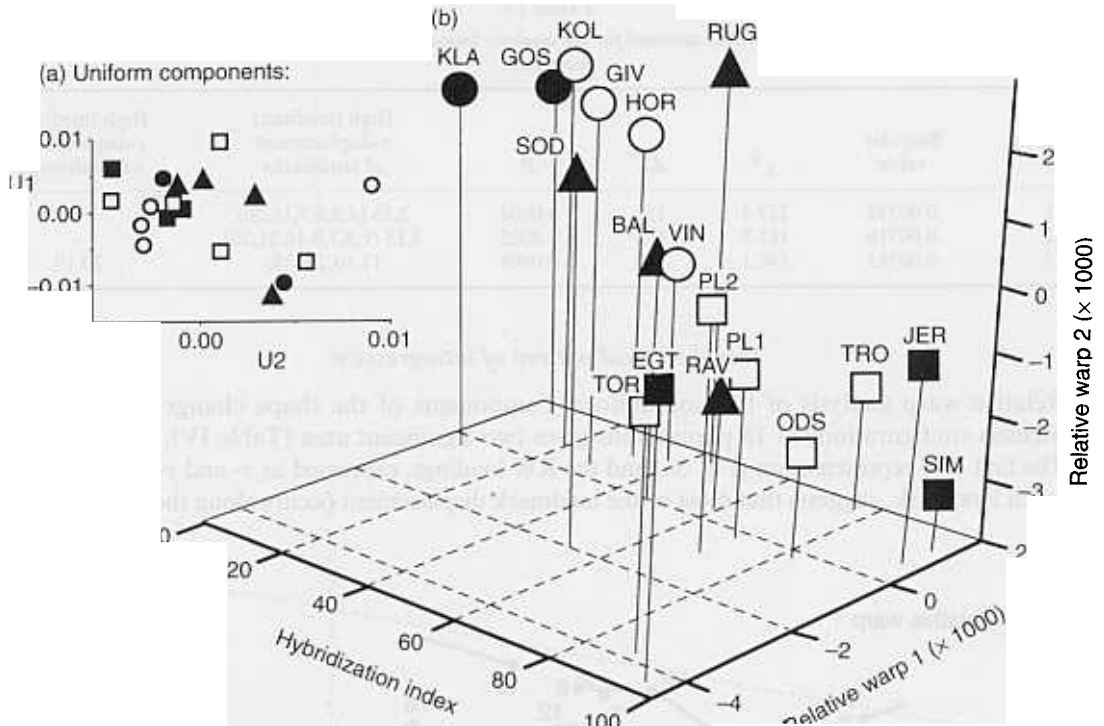
FIG. 4. (a) Scatterplot of the uniform components and (b) scatterplot of relative warps 1 and 2 against the introgression index for all populations studied in the Danish hybrid zone. The symbols correspond to the introgression classes based on the percentage of diagnostic alleles for *Mus musculus domesticus* (●: introgression class 1 (0—10%); ○: class 2 (10—40%); ▲: class 3 (40—60%); □ class 4 (60—90%); ■: class 5 (90—100%)).

is the first principal component of the reference configuration. The RW1 expresses a complex pattern of morphological change. Opposite displacements of Lm-2 and 6 versus Lm-15, 5, 7, and 4 suggest an elongation of the palatine, while Lm-20 and closer ones are slightly displaced towards the centre of the configuration, indicating a compression of the posterior part of the skull.

The second RW is more clearly defined (Fig. 3b). Lm-1 and 3 suggest an elongation of the rostrum. The displacement of Lm-16 opposed to the one of Lm-21 and closely located ones, again suggests a posterior compression of the cranial cavity. Finally, the common displacement of all landmarks related to teeth in the opposite direction to most of the other landmarks, especially 15, shows a backward movement of the teeth row.

When populations are plotted on the plane defined by the the first two RWs (not shown here, but visible on Fig. 4(b)), it can be seen that RW1 separates EGT and TOR from all the other localities. As these two populations are located near to each other, their divergence from the other localities might be related to a local geographical differentiation (see **Discussion**). RW2 separates introgression classes 1 and 2 (*musculus* like) from introgression classes 4 and 5 (*domesticus* like) more clearly. In introgression class 3, i.e. centre of the hybrid zone, BAL, and SOD clearly occupy an intermediate position between the *musculus* and *domesticus* groups, whereas RUG and RAV are close to the *musculus* and *domesticus* groups, respectively. A linear

combination between RW1 and RW2 would probably be the best discriminating axis between the two parental groups.

The correlation between hybrid index and RW1 is just above the level of significance ($r = 0.42$; $0.05 < P < 0.1$), as is the linear regression between both ($F = 3.4$; $P = 0.087$). However, RW2 and the hybrid index are significantly correlated ($r = 0.74$; $P < 0.001$), as well as the linear regression ($F = 1.9$; $P = 4.54\ 10^{-4}$). This result suggests that RW2 represents the morphological pattern of hybridization, whereas RW1 seems to be mainly due to a local geographical divergence involving EGT and TOR.

A 3-D representation of RW1 and RW2 against introgression level is given in Fig. 4b. When one plots the RW scores of the populations against introgression index, one obtains a logistic-like pattern rather than a straight line, which shows that the morphological cline is steeper than the average allozyme cline. Unfortunately, it is not possible to estimate the width of the morphological cline by fitting a logit transformation (Barton & Gale, 1993) as no logical minimum and maximum are available for RW scores. Plots of the uniform components, as well as the low values of U1 and U2, suggest that the uniform transformation does not represent any systematic pattern of change through the hybrid zone, and it could well be a negligible component in the global shape change observed here (Fig. 4a).

In order to test the relative importance of geographical and genetic distances in the morphological divergence between populations, three distance matrices were constructed and compared. The first matrix is the generalized distances between groups in the plane defined by RW1 and RW2. It was then compared to the between-population geographical distances by a Mantel test (Mantel-$t = 0.805$; $P = 0.21$), which suggests there is no correlation between morphological divergence and geographical distance. The morphological distance matrix is, however, strongly correlated with a genetic matrix based on inter-population distances on the univariate axis of the introgression index (Mantel-$t = 4.267$; $P < 10^{-4}$). Thus, the morphological differentiation observed here is clearly related to introgression and is independent of the geographical distances between the localities under consideration.

Comparison of average morphs by the deformation grids

In order to visualize the morphological pattern across the hybrid zone, we used the all-individual consensus configuration of introgression class 5 (*domesticus*) as a reference and represented the non-uniform deformation as a thin-plate spline after superimposing on the consensus configuration of all the other introgression classes (Fig. 5). Such a representation is another way of expressing the overall pattern of shape change suggested by the RWs. From the *domesticus*-like class to the *musculus*-like one, it is possible to describe an elongation of the rostrum, the compression of the tympanic bulla, and the occipital region and the compression of the palatine with a slight posterior displacement of the teeth row. There is a certain continuity in the accentuation of the features described above through the introgression classes, i.e. across the hybrid zone. However, the pattern of introgression class 4 shows a slightly different pattern. This may be due to the differentiation of the EGT and TOR populations that is shown by RW1.

Discussion

The relative warp analyses performed here provide a synthetic image of morphological modalities of hybridization, that are affected but not masked by local geographical effects.
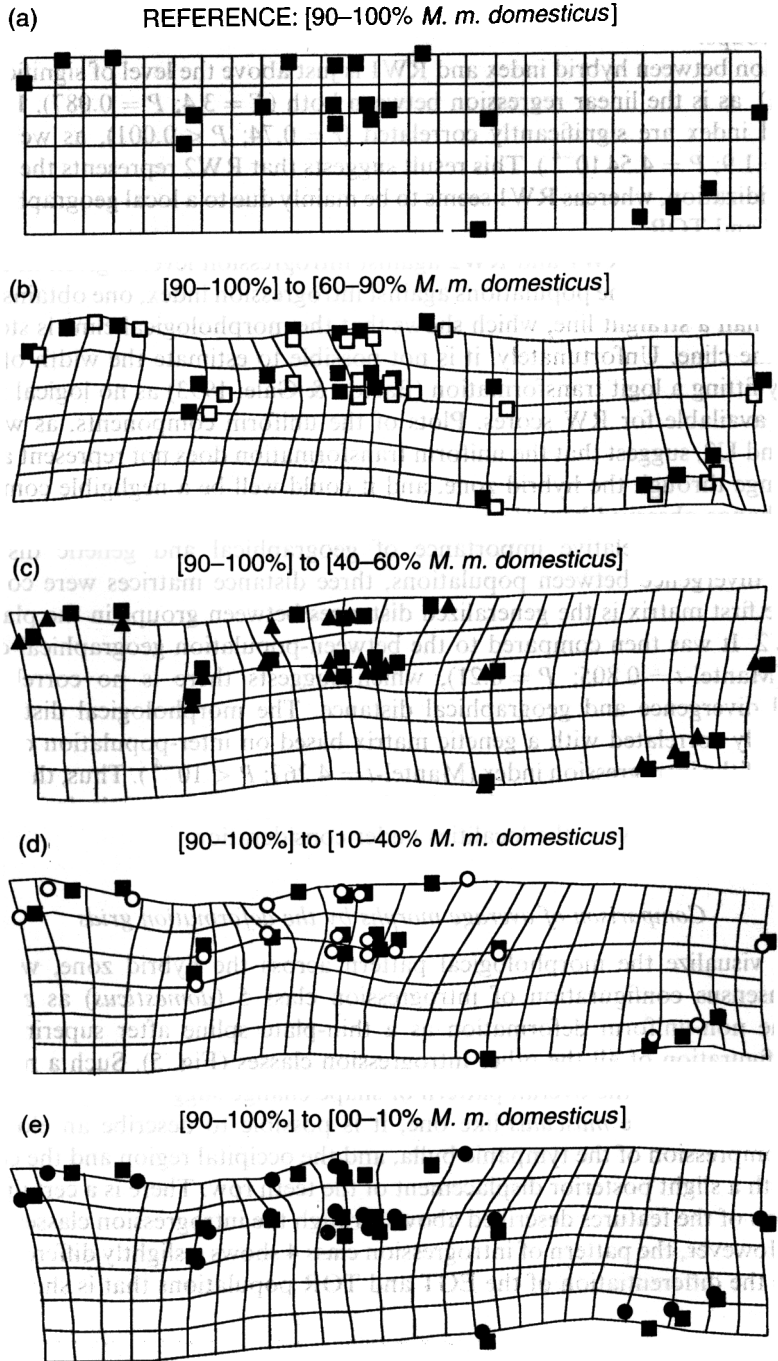
(a)   REFERENCE: [90–100% *M. m. domesticus*]

(b)   [90–100%] to [60–90% *M. m. domesticus*]

(c)   [90–100%] to [40–60% *M. m. domesticus*]

(d)   [90–100%] to [10–40% *M. m. domesticus*]

(e)   [90–100%] to [00–10% *M. m. domesticus*]

FIG. 5. Deformation grids expressing (a) the non-uniform part of transformation of the reference (class 5 all-individual GLS consensus) to (b, c, d, e) all the other classes averaged as for the reference (magnification ×8).

Although this morphometric analysis of the skull completely distinguishes *musculus* from *domesticus* (introgression class 1 and introgression class 5, respectively), the result is not shown here as it is only relevant to the populations analysed and may not be of any global systematic value for the two subspecies over the whole of their respective ranges. Besides, this separation is based on landmark locations within scaled configurations among both subspecies and may not be easily converted into one or several discriminatory measurements.

Whereas the among-population distances based on the combination of RW1 and RW2 scores are related to genetic distances in terms of introgression differences, and are not dependent on geographic ones, RW1 considered alone clearly underlines a local morphological divergence for the EGT and TOR samples. This divergence is important enough to emerge on the first RW, and obviously accounts for more variation than the second one, which is more clearly tied to the introgression index. It is difficult to determine what is responsible for the morphological divergence of EGT and TOR localities. It is unlikely to be related to the occurrence of several Robertsonian (Rb) fusions in the populations with more than 50% of *domesticus* alleles (Nancé *et al.*, 1990; Fel-Clair *et al.*, 1996) as these two populations are not chromosomally isolated from the others. Nor is it due to the heterozygous occurrence of Rb6–9 in EGT and TOR, as this chromosomal rearrangement also occurs in SIM without inducing any morphological divergence that might have been revealed on RW1. The close proximity of EGT to TOR, which is in an area that is geographically rather isolated from the other localities, suggests that it could be a local morphological divergence. The fact that the morphological difference that separates these two localities from the other populations is more important than the morphological changes related to hybridization, means that differences observed within a small number of populations should be interpreted with caution. For instance, it is possible that the divergence of the hybrid mandible shape that Prager *et al.* (1993) attributed to developmental abnormalities in the hybrid zone in Schleswig Holstein might not be related to introgression and could reflect local morphological divergence.

The heterogeneous intra-population variabilities do not show any clear pattern across the hybrid zone. Moreover, the rejection of the introgression class effect on intra-population variability suggests that morphological variability across the hybrid zone does not depend on the level of hybridization. The results of this analysis suggest that neither developmental abnormalities resulting from the breakdown of genomic co-adaptation (Barton & Hewitt, 1985, 1989; Szymura & Barton, 1986; Graham, 1992), nor changes in the morphological variability related to allelic diversity, occur across the contact zone. As size was not considered in this study, an eventual heterotic effect on skull size cannot be excluded. It is, nevertheless, interesting to note that the analysis of the molars belonging to the same individuals shows a decrease in fluctuating asymmetry and heterosis in hybrids (Alibert *et al.*, 1994), but does not reveal any pattern in the variation of tooth size across the hybrid zone (Alibert, unpubl. data).

This study shows that the morphological change across the hybrid zone is continuous, and allows us to describe expected and ordered intermediate phenotypes between the two subspecies. However, the fact that the morphological cline might be steeper than the change in the allozymic hybrid index across the zone, suggests that gene-systems involved in the developmental processes involved in skull formation could be slightly perturbed in hybrids, even though both the lack of morphological abnormalities and the general pattern of variability across the zone, suggest that the disruption would not be very strong. We cannot, therefore, exclude the possibility that the steeper morphological cline reflects selection on another gene system that is linked to gene(s) involved in skull formation. This would explain the apparent contradiction between the observed

increase in developmental stability of the teeth in hybrids, and the narrow morphological cline that suggests that the processes underlying skull development are only slightly disturbed in the hybrid genome.

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