Evolution, 51(4), 1997, pp. 1284–1295

DEVELOPMENTAL STABILITY, FITNESS, AND TRAIT SIZE IN LABORATORY HYBRIDS BETWEEN EUROPEAN SUBSPECIES OF THE HOUSE MOUSE

PAUL ALIBERT,[1] FABIENNE FEL-CLAIR,[1] KATERINA MANOLAKOU,[2] JANICE BRITTON-DAVIDIAN,[1] AND JEAN-CHRISTOPHE AUFFRAY[1,3]

[1]*Institut des Sciences de l'Evolution, UMR 5554 CNRS, cc 064, Université Montpellier II, France*
[2]*Laboratoire Génome et Populations, UPR 9060 CNRS, cc 063, Université Montpellier II, France*
[3]*E-mail: auffray@isem.univ-montp2.fr*

Abstract.—The effects of hybridization on developmental stability and size of tooth characters were investigated in intersubspecific crosses between random-bred wild strains of the house mouse (*Mus musculus domesticus* and *M. m. musculus*). Fluctuating asymmetry (FA) and trait size were compared within and between parental, F_1, backcross, and F_2 hybrid groups. The relationship between FA and reproductive fitness within the F_1 hybrids was also studied. The results indicated that both FA and character size levels differed significantly between the two subspecies. The F_1 hybrids and the recombined groups (backcrosses and F_2 hybrids) showed heterosis for both parameters. No significant differences in the FA of fertile and sterile F_1 hybrid individuals were found. Comparison of the FA levels obtained in this study with those found in wild populations from the hybrid zone in Denmark showed that the levels of FA were lower in laboratory-bred samples than in the wild populations. This study provides further evidence that, in hybrids, the developmental processes underlying most of the morphological traits we studied benefit from a heterotic effect, despite the genomic incompatibilities between the two European house mice revealed by previous genetical and parasitological studies.

Key words.—Developmental stability, experimental crosses, genomic coadaptation, heterosis, hybrid, *Mus musculus*, tooth characters, trait size.

Received February 26, 1996. Accepted March 25, 1997.

Coadaptation refers to the internal genomic balance between loci, at both the inter- and intrachromosomal levels, that was molded by selection during the evolutionary histories of populations (Dobzhansky 1937). The notion of coadaptation has played a prominent role in the study of the mechanisms of reproductive isolation and speciation because any breakdown of coadapted gene complexes would lead to a selective disadvantage. Two approaches have been used to estimate the degree of divergent coadaptation that occurs in differentiating populations. The first uses experimental crosses to assess the effects of hybridization on major fitness components such as viability and fertility. The second focuses on hybrid zones, i.e., sites where individuals from genetically distinct populations meet, mate and produce offspring (Barton 1979; Barton and Hewitt 1985; Hewitt 1988; Harrison 1990). In this case, the effects of different intergenomic combinations on hybrid fitness can be estimated indirectly from the introgression patterns of genetic markers.

The hybrid zone between the two European subspecies of the house mouse, *Mus musculus domesticus* and *M. m. musculus*, which crosses Europe from Denmark to Bulgaria, has been the focus of extensive studies (for review see Boursot et al. 1993; Sage et al. 1993). Autosomal, mitochondrial, and sex chromosome markers have been analyzed across several transects of the hybrid zone in Denmark, Germany, and Bulgaria. The similarity between the patterns of differential introgression that occur in these geographically different transects led to the conclusion that major incompatibilities exist between the two genomes (Boursot et al. 1984; Sage et al. 1986; Vanlerberghe et al. 1986, 1988a,b; Tucker et al. 1992; Dod et al. 1993; Prager et al. 1993). Moreover, the higher intestinal worm loads of hybrid populations compared to parental forms have been related to a disruption of the coadapted gene systems involved in the immune response to these parasites (Sage et al. 1986; Moulia et al. 1991, 1993, 1995). These results suggest that the house mouse hybrid zone is maintained by endogenous selective factors due to the disruption of coadapted gene systems in hybrids (Sage et al. 1986; Vanlerberghe et al. 1988a). However, the incompatibilities do not appear to affect all gene systems since a higher developmental stability has recently been reported in the Danish hybrids (Alibert et al. 1994). Although little is known about the processes underlying developmental stability, they are likely to involve numerous genes (Zakharov 1989) and may provide a valuable indicator of the extent of selection in the hybrid zone.

Developmental stability is one of the components of developmental homeostasis (for review see Zakharov 1989) through which organisms reduce phenotypic variation resulting from developmental accidents. It can be assessed by measuring fluctuating asymmetry (FA) which is the variation in the small random differences occurring between the left and right side of normally bilaterally symmetrical traits (Van Valen 1962). Levels of FA, which have been correlated with the intensity of both genomic and environmental stress, are thought to reflect the efficiency of the mechanisms controlling developmental stability of organisms (Zakharov 1989; Parsons 1990). It is generally assumed that genomic coadaptation and heterozygosity are the two genetic factors that increase developmental stability. In hybrid populations, it is therefore thought either to benefit from an increase in heterozygosity or to suffer from disruption of coadaptation, depending on the degree of divergence between hybridizing taxa (Vrijenhoek and Lerman 1982; Graham 1992). Until recently, it was also assumed that, at the level of genetic divergence observed between most naturally hybridizing taxa, breakdown in the coadaptation of gene complexes was more important than heterotic effects (Vrijenhoek and Lerman 1982; Graham

TABLE 1. Details of crosses, mating success (percentage of pairs having produced offspring after four months of mating), offspring samples, and sample sizes for each type of cross. Values in parentheses indicate sample sizes for measurements taken for M/3 (see text for further explanations). In offspring designation, M and D refer to the parental origin: M indicates a *musculus* mother in the initial inter-subspecific cross and D a *domesticus* one.

Crosses				Offspring			
	Number	Mating success		Offspring	Number	Number	
Type of crosses	of pairs	Number	%	designation	of males	of females	Total
Intra-subspecific							
♀ M × ♂ M	8	7	87.5	*musculus*	36 (27)	24 (22)	60 (49)
♀ D × ♂ D	8	6	75	*domesticus*	35 (32)	27 (20)	62 (52)
Inter-subspecific							
♀ M × ♂ D	8	8	100	fertile F_1M	12 (12)	14 (7)	26 (19)
				sterile F_1M	23 (20)	24 (18)	47 (38)
♀ D × ♂ M	11	11	100	fertile F_1D	8 (8)	7 (4)	15 (12)
				sterile F_1D	16 (16)	22 (21)	38 (37)
Backcrosses							
♀ F_1M × ♂ D_{father}	15	6	40	BC♀M	21 (21)	18 (18)	39 (39)
♀ F_1M × ♂ D_{strain}	38	10	26.3	BC′♀M	11 (10)	29 (29)	40 (39)
♂ F_1M × ♀ M_{mother}	—	—	—	BC♂M	—	—	—
♂ F_1M × ♀ M_{strain}	13	1	7.7	BC′♂M	9 (9)	14 (14)	23 (23)
♀ F_1D × ♂ M_{father}	13	2	15.4	BC♀D	—	—	—
♀ F_1D × ♂ M_{strain}	29	5	17.2	BC′♀D	4 (4)	14 (14)	18 (18)
♂ F_1M × ♀ D_{mother}	2	—	—	BC♂M	—	—	—
♂ F_1D × ♀ D_{strain}	9	2	22.2	BC′♂D	17 (17)	11 (11)	28 (28)
F_2							
♀ F_1M × ♂ F_1M	30	1	3.3	F_2M	3 (3)	6 (6)	9 (9)
♀ F_1D × ♂ F_1D	15	2	13.3	F_2D	39 (39)	37 (37)	76 (76)

1992; Clarke 1993). However, the presence of lower FA levels in natural hybrids between the two types of the European house mouse suggests that the relationship between developmental stability and genetic divergence may be more complex (Alibert et al. 1994).

The aim of this study was to assess the relative contribution of heterosis and breakdown in coadaptation to the developmental stability of successive hybrid generations of house mice. This was done by measuring and comparing FA levels of dental characters between F_1 hybrid and slightly recombined genomes (backcrosses and F_2 hybrids), as well as highly recombined (wild hybrids) ones. In order to see whether changes in developmental stability were accompanied by other changes in dental morphology, we also studied the size of the tooth characters in the crosses. The reduced introgression of the sex chromosome markers in the *Mus musculus* hybrid zone (Vanlerberghe et al. 1986, Tucker et al. 1992; Dod et al. 1993) suggested that hybrids were reproductively impaired despite their higher level of developmental stability. These results indicated that the positive correlation which is generally expected between developmental stability and fitness would not necessarily include reproductive fitness. We therefore investigated the relationship between FA and fertility of the male and female F_1 hybrids obtained in the laboratory.

MATERIAL AND METHODS

Samples

The animals used in this study came from two random-bred wild-derived strains, DDO (*M. m. domesticus*) and MDH (*M. m. musculus*), kept in the "Wild Mice Genetic Repository" in Montpellier, France. These strains originated from two Danish localities, Ödis (DDO) and Hov (MDH), which are located, respectively, at 34 km south and 40 km north of the center of the hybrid zone. These localities correspond to the extremes of the transect used in most previous studies conducted in Denmark (Vanlerberghe et al. 1986, 1988a; Nancé et al. 1990; Moulia et al. 1991; Dod et al. 1993; Alibert et al. 1994; Auffray et al. 1996a; Fel-Clair et al. 1996). At the onset of the experiment, the number of generations of random mating was 13 for DDO and 5 for MDH. These two strains were slightly introgressed as individuals of MDH carried 1.5% of *domesticus* alleles whereas 11% of *musculus* alleles were present in the genome of mice from the DDO strain (F. Bonhomme, pers. comm.). DDO is homozygous for three Robertsonian (Rb) fusions, Rb (3.8), Rb (2.5), and Rb (6.9), reducing the standard karyotype from 2n = 40 to 2n = 34 chromosomes (Nancé et al. 1990). Both strains were reared under identical conditions in the same animal room. Food and water were provided ad libitum.

To obtain F_1 hybrids, 11 pairs of *domesticus* (D) females and *musculus* (M) males and eight pairs of reciprocal crosses (female M × male D) were established. Sixteen intrasubspecific crosses (eight pairs for D × D and eight pairs for M × M) were used as reference groups. After four to six months, one group of F_1 hybrids was intercrossed to produce F_2 hybrids while another (both males and females) was backcrossed either to their parents, or to individuals belonging to the same parental strain in order to increase the size of the backcross samples (BC) (details of crosses are given in Table 1). All pairs were maintained for a minimum of four months, regardless of the reproductive outcome. All the animals used

in the morphometric analysis (see below) were adult (minimum 11 weeks old). A total of 481 individuals was analyzed.

Mating Success and Fertility Estimates

Reproduction within each type of cross was characterized by mating success, defined as the percentage of pairs which produced offspring after four months of mating. The fertility of F_1 hybrid individuals was estimated separately for each sex. In the backcrosses, F_1 hybrid females were considered to be fertile if they produced at least one offspring. The fertility of adult F_1 hybrid males was determined by the fresh weight of both testes. A reduction in testis weight has been shown to be correlated with sterility in the progeny of crosses between strains of these two subspecies (Forejt 1974; Forejt and Ivanyi 1975; Forejt et al. 1991). Forejt and Ivanyi (1975) have shown that hybrid males with a testis weight below 75 mg were sterile and fertile when above 120 mg. These values were used in the present experiment to distinguish between sterile and fertile F_1 hybrid males. When testis weight fell between 75 mg and 120 mg, the fertility/sterility status of these males was determined by germ cell analysis of histological sections of testes.

Tooth Characters

All skulls and mandibles were boiled and manually cleaned under running water. The bilateral characters measured were maximum length (L) and maximum width (W) of the three lower molars (M/1, M/2, and M/3). The age at which the animals were sacrificed was not standardized because of the technical constraints this would have imposed, but age is unlikely to be a source of bias since tooth size is definitive once it has erupted into the oral cavity i.e., 18 days and 28 days after birth for M/1-M/2 and M/3 respectively (Bader 1965a). The third molar (M/3) was absent in some individuals either due to the processing procedure or because of natural causes. The characters studied were the same as those used by Alibert et al. (1994) in their study of wild populations from the Danish hybrid zone. Measurements were taken with a Nikon measuroscope measuring to 0.001 mm accuracy.

Statistical Treatments

Statistical treatments of FA were essentially conducted according to Palmer (1994).

Preliminary Tests

To detect factors confounding analyses of FA, a series of preliminary tests was performed on the distributions of either signed asymmetries (right-minus-left values $[R_i - L_i]$) or absolute asymmetries ($|R_i - L_i|$) (Palmer and Strobeck 1986; Palmer 1994). As a first step, we looked for the presence of directional asymmetry (DA) and antisymmetry (AS), which are generally considered uninformative because they may possess a significant genetic basis (but see McKenzie and Clarke 1988; Graham et al. 1993). DA occurs when one side of a bilateral character is systematically larger than the other. In AS, a systematic deviation from symmetry also occurs but the side that is larger varies at random among individuals (Van Valen 1962; Palmer 1994). In DA the $(R_i - L_i)$ distribution is not centered on zero, while in AS the distribution appears bimodal. The presence of DA was assessed by testing for departures from zero of the means of signed differences $(R_i - L_i)$ for each trait within each sample using *t*-tests. Departures from normality were assessed using Kolmogorov-Smirnov tests and estimates of skewness and kurtosis. The relation between asymmetry and character size, defined as $([R_i + L_i]/2)$, was investigated by linear regression analyses of absolute asymmetry on character size both within each sample and in the whole dataset. Moreover, we tested for size dependence of FA among samples by linear regression of $\log(\text{var}[R_i - L_i])$ on $\text{mean}([R_i + L_i]/2)$ (Palmer 1994). Finally, differences in asymmetry levels between males and females were tested by ANOVAs of the absolute asymmetries both within and across samples.

Fluctuating Asymmetry

We used two indices to assess the differences in fluctuating asymmetry between samples: the means of the absolute right-minus-left differences and the variances of the signed differences between sides. These two indices correspond to FA1 and FA4 of Palmer (1994) who considered them as the most useful descriptors of FA. Using the absolute asymmetry values, we first performed a modified version of Levene's test for the heterogeneity of variances (two-way ANOVA: *sample* × *trait*) to test for differences between samples (Palmer 1994). If an overall significant difference was detected, an ANOVA with planned comparisons (Sokal and Rohlf 1995) was performed for each trait as follows. First, the fertility and sterility of the F_1 hybrids were compared. Contrast analyses allowed us then to test whether the sex and the origin of the partner (parent or individual of the same strain) of the F_1 hybrid were a source of variability in the backcrosses. Within each of the four groups (parental generation, F_1, BC, and F_2 hybrids), the samples of different parental origins were also compared, i.e., we distinguished between a *domesticus* and a *musculus* mother in the intra- and the intersubspecific crosses. Finally, to test the effect of hybridization and meiotic recombination on FA levels, we compared intrasubspecific samples versus pooled hybrid ones (F_1, BC, and F_2 hybrids), and F_1 hybrids versus recombined ones (BC and F_2 hybrids).

Using this design, it was also possible to partition out any effects due to litter size and to the litters themselves (Leamy and Touchberry 1974). Regressions of absolute asymmetry on litter size were done within all samples to test if litter size had to be considered as a covariate in our model. As only one regression (positive) out of the 78 performed was significant, it was not considered necessary to adjust asymmetry values for litter size. For the litter effect, the basic error variances of the ANOVAs were partitioned into between- and within-litter variances. If significant, the between-litter mean squares had to be used as the error terms for significance testing in the model (Leamy and Touchberry 1974).

Concordance between Characters

Kendall's coefficient of concordance (*W*) was used to test the concordance of FA indices (for both FA1 and FA4) for the six characters among the different samples (Siegel and

Castellan 1988). Testing concordance of FA levels among characters across populations was formerly introduced by Soulé (1967) and allowed him to define a 'population asymmetry parameter' (PAP). However, we chose not to use this term since the asymmetries estimated in this study only involved dental traits and not a set of uncorrelated morphological characters as originally proposed (Soulé 1967).

Measurement Error

Errors due to measurement were evaluated following the ANOVA procedure proposed by Palmer and Strobeck (1986). This procedure tests whether the between-side variance is significantly larger than the measurement error using a two-way ANOVA (*side* × *individual*) with repeated measurements of each side. If the interaction variance is significant, it means that the nondirectional asymmetry variance is significantly greater than the measurement error. When performed on the entire dataset, such an approach allows one to partition the measurement error out of the between-side variation (Palmer and Strobeck 1986; Palmer 1994) and gives an estimate of the true nondirectional asymmetry variance (FA10 in Palmer 1994). This analysis also tests for the presence of DA and for size or shape variation among individuals when considering the factors *side* and *individual*, respectively (Palmer and Strobeck 1986). However, we were only able to test the significance of the between-side variance relative to measurement error by the two-way ANOVA using a small subsample of 40 individuals chosen across all samples. The labor involved in taking duplicate measurements on the whole sample made it unreasonable to extend this approach to the estimation of FA10, DA, and size or shape variation.

Character Size

The procedure used to detect differences between samples was similar to the one used in the FA analysis: planned comparisons were run for each trait on the $([R_i + L_i]/2)$ distributions. Character size values were not adjusted for litter size since, out of the 78 tested, only three regressions of character size versus litter size were significant (one positive and two negative). In a manner similar to the one used in the FA analysis, the basic error variances were partitioned into between-litter and within-litter components. Kendall's coefficient of concordance was used to test the correlation of the mean trait size for the six traits among samples. The effect of sex was assessed with a two-way ANOVA (*sex* × *sample*).

All the statistical tests used in this study, except the Levene, the Wilcoxon, and the Kendall tests, considered each of the six traits separately. Therefore, in order to limit the occurrence of the type-I error, the sequential Bonferroni correction was systematically applied to each of these collections of $k = 6$ tests according to Rice (1989). All the probability values provided correspond to the corrected probabilities.

RESULTS

Mating Success

Mating success is shown in Table 1. No fertility problems were apparent in the intra- or intersubspecific crosses. Intersubspecific matings were particularly successful as all pairs produced offspring, which was not the case for the intrastrain crosses. However, mating success clearly decreased in the F_1 intercrosses (3.3% and 13.3%) and in the backcrosses (7.7–40%). The F_1 hybrid females used in the latter crosses were classified as either fertile or sterile. In the case of F_1 males, testis weight varied between 42.5 mg and 250.9 mg. Comparison between reproductive success and testis weight confirmed that F_1 hybrid males with a testis weight below 75 mg were sterile and, except in one case, those with a testis weight above 120 mg were fertile. Germ cell analysis of testicular histological sections of males with intermediate testis weights (80–108 mg) allowed us to set the fertility/sterility threshold at 100 mg. Although heterozygosity for Rb fusions is known to reduce fertility through aneuploidy (Gropp and Winking 1981), the observed differences in testis weight cannot be related to the karyotype since all F_1 hybrids were heterozygous for three fusions. Fertility parameters will be reported in detail elsewhere (Fel-Clair et al., unpubl. data).

The origin and size of the 13 samples used are presented in Table 1. The data are absent for three types of backcrosses either because they were not tested (BC♂M), or because they produced few (BC♀D) or no litters (BC♂D).

Asymmetry

A detailed presentation of the results of the tests is provided in the Appendix.

Preliminary tests

Normality tests showed that all but one of the 78 signed asymmetry distributions were normally distributed. Nevertheless, kurtosis and skewness were significant for six and four distributions respectively (three of which were significant for both). Even though eight distributions out of the 78 tested exhibited a departure from normality, the presence of strong antisymmetry could be excluded as no distribution was platykurtic. Departures from normality did not affect a particular trait or sample and so were not considered to be a source of bias. Significant directional asymmetry (DA) was detected in three characters. We noted a significant right dominance for LM/3 in the *musculus* sample. WM/1 exhibited DA in *musculus* and sterile F_1D samples and, in both cases, the left side tended to be larger than the right one. To a larger extent, the means of $(R_i - L_i)$ distributions for WM/3 were significantly different from zero, the right side being larger than the left one, in seven of the 13 samples (samples: *musculus*, *domesticus*, sterile F_1M, BC′♀M, BC′♂M, BC′♀D, F_2D). This is the first report of DA for tooth characters in the house mouse although it has been shown to occur in a number of bone traits including mandibles where it represented less than 1% of the mean values of the characters (Leamy 1984, 1993). In our study, DA represents around 0.75% of the mean values obtained for the different characters. It does not seem to be related to the genetic status of the individuals since it occurred in the parental as well as the F_1, BC, and F_2 hybrid samples. From a statistical point of view, FA4 estimates do not require a correction for the significant DA, since the latter only shifts the mean of the $(R_i - L_i)$ distributions without modifying the variances which are used to express FA (Palmer and Strobeck 1986). The only

TABLE 2. Results of the analyses of variance of fluctuating asymmetry values for each of the six traits. Mean squares × 10^5 (MS), degrees of freedom (df) and probability values (asterisks) are presented. The value in parentheses for the residual df corresponds to the degrees of freedom for M/3.

		Trait					
Source of variation	df	LM/1 MS	LM/2 MS	LM/3 MS	WM/1 MS	WM/2 MS	WM/3 MS
Samples	12	9.59	7.74	27.38	38.91***	10.23	23.11
Intrasubspecific *musculus* vs. *domesticus*	1	23.04	1.93	0.99	44.02	10.14	18.02
F_1M vs. F_1D	1	5.27	0.09	96.45*	0.05	2.70	65.86
Sterile F_1 vs. fertile F_1	1	0.34	1.86	0.01	4.40	1.58	28.34
BC partner: parent vs. strain	1	11.38	1.82	17.42	31.14	11.04	13.02
BC partner: male vs. female	1	3.69	1.34	2.96	8.71	1.07	2.91
BCM vs. BCD	1	0.55	0.01	0.34	103.58**	0.65	0.80
F_2M vs. F_2D	1	1.11	0.10	3.50	0.35	0.00	5.78
Intrasubspecific vs. hybrids	1	57.58*	13.37	183.74**	220.44***	71.65**	36.43
Nonrecomb. vs. recomb. hybrids	1	4.79	6.06	4.89	12.56	20.70	0.00
Error	468 (426)	8.19	7.48	13.87	7.97	7.62	12.68

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ after correcting with the sequential Bonferroni technique per line.

problem could come from an allometry between tooth size and asymmetry, but this did not occur since there was no significant correlation between absolute asymmetry and character size, except in one case (sample BC′♀D for the trait LM/3) (see Appendix). Finally, ANOVAs and F-ratios between the FAs of males and females, did not detect any effect related to sex.

Measurement Error

In the subsample measured twice, the between-side variance was significantly larger than the measurement error for the six traits (all *P*-values of the interaction variance were less than 0.001). The variance due to measurement error varied between 10% and 14% of the nondirectional asymmetry variance, except for LM/3 for which the proportion was around 23%. These values agree with those reported in previous studies on the dentition of house mice (Bader 1965b). We considered measurement error values sufficiently low to assume that they did not contribute significantly to the asymmetry estimates. Imprecision due to measurement error also appeared to be negligible relative to mean character size.

Fluctuating Asymmetry

The modified Levene's test for heterogeneity of variances revealed that both *sample* and *trait* factors were significant sources of variability (*sample*: $F_{12,2724} = 6.27$, $P < 0.001$; *trait*: $F_{5,2724} = 16.6$, $P < 0.001$; *sample* × *trait*: $F_{60,2724} = 1.20$, ns). This meant that the levels of asymmetry differed between samples when information from all traits was pooled, and that the traits exhibited different levels of developmental stability (Palmer 1994). Indices of FA (FA1 and FA4) are given in the Appendix. Planned comparisons then allowed us to determine for each trait, which samples showed differences in FA (Table 2). The significance of the *sample* factor and of all contrasts was tested over the basic error variances since between-litter components were not significant for any of the characters. Offspring of the two intrasubspecific crosses did not significantly differ for any of the characters (Table 2), but the *musculus* samples were always more asymmetrical than the *domesticus* ones (Wilcoxon's signed ranks tests: $Ts = 0$; $P = 0.0156$). Within the F_1 hybrids, no significant differences were detected between fertile and sterile individuals, whereas FA for one character, LM/3, showed significant differences depending on the parental origin (F_1M was significantly more symmetrical than F_1D).

In the backcrosses, we found no significant relationships between the FA levels and the sex or the origin of the F_1 hybrid's partner (parent or individual from the same strain) (Table 2). To test the effect of the parental origin, BC♀M, BC′♀M and BC′♂M were then considered as a single sample, BCM, and tested against the sample BCD, established by pooling BC′♀D and BC′♂D. No global trend was found even though the difference in FA levels was significant for the trait WM/1 (BCM was significantly more symmetrical than BCD). Finally, there were no significant differences in asymmetry values between F_2D and F_2M. When the intrasubspecific samples were compared to the pooled hybrid samples, we found significant differences for four traits (LM/1, LM/3, WM/1, and WM/2) (Table 2), the hybrid samples being developmentally more stable than both subspecies (Appendix). Within hybrids, recombination between the *domesticus* and *musculus* genomes did not seem to affect the level of FA since we noted no significant differences between the first hybrid generation (F_1 hybrids) and the second one (backcrosses and F_2 hybrids).

For further analyses, the samples which were not significantly different were pooled, reducing their number to seven (*musculus*, *domesticus*, F_1M, F_1D, BCM, BCD, and F_2). Kendall's coefficient of concordance clearly demonstrated that, whatever the FA index considered, the ranks of the FA levels of all six traits across the seven samples were not independent (FA1: $W = 0.607$, $P \ll 0.01$; FA4: $W = 0.692$, $P \ll 0.01$). In other words, a sample which tended to be more symmetrical for one character also tended to be more symmetrical for the others. As *W* was significant, the best estimate of the rank of the samples was obtained by ordering the values of Rj which are the sums of the FA indices after transformation to rankings (Soulé 1967; Siegel and Castellan 1988). Figure 1 depicts the overall ranking of the FA levels (with FA1) of the six traits across the seven samples. The two parental samples presented the highest levels of FA, whereas F_1M,

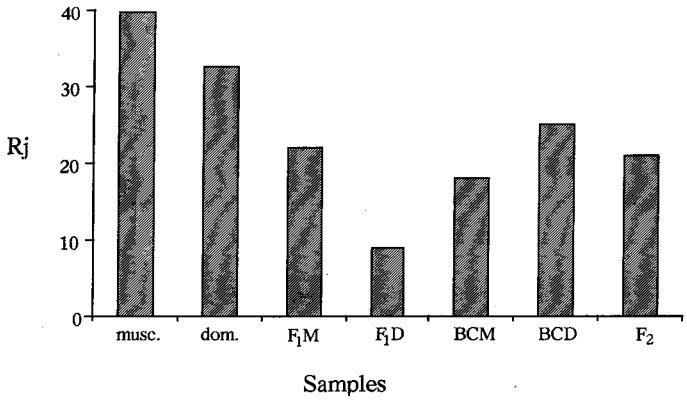

FIG. 1. Graphic representation of the sum, Rj, of the FA indices after transformation to rankings of the six characters within the seven samples (see text for further explanations); "musc." and "dom." refer, respectively, to *musculus* and *domesticus*.

F_2, and both BC samples showed intermediate rankings. F_1D yielded the lowest FA levels for all six characters.

Character Size

ANOVAs performed on the ($[R_i + L_i]/2$) values clearly showed that the *sample* factor was a highly significant source of variability of tooth character size among the 13 samples (Table 3). The *sex* factor was only significant for the trait LM/1 ($F_{1,455} = 8.30$, $P < 0.05$) which was larger in males. As the interaction between the *sex* and *sample* factors was not significant, and as the sex ratio of each sample did not appear unbalanced (sex ratios are shown in Table 1), we did not consider sex as a source of bias. Results of the planned comparisons run on the character size values are shown in Table 3. Partitioning of the basic error variances for the six traits revealed that the between-litter components were not significant. Thus, the significance of the *sample* factor and of all contrasts was tested over the basic error variances. Differences between the two intrasubspecific crosses were present. Character size values of the *musculus* sample were always higher than those of the *domesticus* one (Appendix), the differences being significant for four characters: LM/1, LM/2, WM/2, and WM/3 (Table 3). Within the F_1 hybrid group, fertile and sterile individuals did not display different levels of character size whereas the parental origin was found to be a source of variability since F_1 hybrids with a maternal *musculus* origin showed significantly larger character sizes than those with a *domesticus* one for all traits except LM/3.

In the backcross groups, character size did not depend on the origin of the F_1's partner (parental or strain), nor on its sex (Table 3). However, when the backcrosses were compared relative to their parental origin, BCM exhibited a significantly higher character size than BCD for WM/1 and WM/2. In the F_2 hybrid groups, the values of two of the six traits (LM/3 and WM/2) were significantly lower in F_2M than in F_2D. Moreover, F_2D values were always higher than those of F_2M (Wilcoxon's signed ranks test: $Ts = 0$; $P = 0.0156$).

All traits were significantly smaller in the intrasubspecific group than in the pooled hybrid one, i.e., including F_1 and F_2 hybrid groups and backcrosses (Table 3). Meiotic recombination may, however, affect trait size since the backcrosses and F_2 hybrids showed a significant size reduction for four of the six dental characters compared to the F_1 hybrids (Table 3, Appendix).

When we grouped the samples which were not significantly different, we obtained eight pools: *musculus*, *domesticus*, F_1M, F_1D, BCM, BCD, F_2M, and F_2D, among which Kendall's coefficient of concordance indicated a significant association between the character size levels ($W = 0.648$, $P \ll 0.01$). The sums of the character size estimates after transformation to rankings (Rj) are given in Figure 2. The hybrid samples F_1D, F_1M, BCM, and F_2D ranked higher than both parental ones. The *domesticus* parental sample showed the lowest overall ranking.

DISCUSSION

Developmental Stability and Heterozygosity

Hybrids between *M. m. domesticus* and *M. m. musculus* clearly benefit from a heterotic effect on the developmental stability and on the size of the tooth characters investigated. This effect is significant for four FA traits and the six size traits. Although the mechanisms underlying the general phe-

TABLE 3. Results of the analyses of variance of character size values for each of the six traits. Mean squares × 10^4 (MS), degrees of freedom (df) and probability values (asterisks) are presented. The value in parentheses for the residual df corresponds to the degrees of freedom for M/3.

		Trait					
Source of variation	df	LM/1 MS	LM/2 MS	LM/3 MS	WM/1 MS	WM/2 MS	WM/3 MS
Samples	12	449.73***	211.70***	241.83***	196.89***	153.74***	87.15***
Intrasubspecific *musculus* vs. *domesticus*	1	1,101.16***	769.10***	78.58	0.75	73.81*	120.87***
F1M vs. F_1D	1	667.96***	155.15**	23.66	727.85***	551.04***	80.56**
Sterile F_1 vs. fertile F_1	1	1.11	1.70	37.42	1.78	4.76	22.52
BC partner: parent vs. strain	1	79.07	8.09	16.40	24.08	1.66	11.25
BC partner: male vs. female	1	26.04	20.45	21.40	0.81	0.62	5.54
BCM vs. BCD	1	24.96	49.31	0.52	137.79***	130.63***	14.73
F_2M vs. F_2D	1	0.26	67.86	248.61**	33.61	111.97**	3.26
Intrasubspecific vs. hybrids	1	1,089.73***	844.32***	1,980.36***	405.34***	229.47***	196.19***
Nonrecomb. vs. recomb. hybrids	1	1,108.09***	40.54	9.36	500.88***	330.76***	293.13***
Error	468 (426)	14.68	12.20	23.07	8.49	8.75	7.56

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ after correcting with the sequential Bonferroni technique per line.

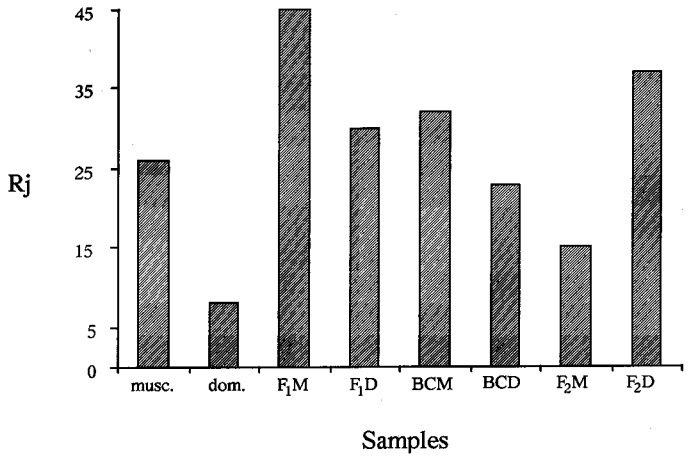

FIG. 2. Graphic representation of the sum, Rj, of the character size levels after transformation to rankings of the six characters within the eight samples (see text for further explanations); "musc." and "dom." refer, respectively, to *musculus* and *domesticus*.

nomenon of heterosis are still under debate, three hypotheses are generally advanced. These include dominance, overdominance, and the formation of fortuitous gene combinations. On the basis of the data available in the literature, Mitton and Grant (1984) have argued that the first two hypotheses, which imply heterozygosity per se, could explain 70–80% of the effect on growth and developmental stability. Higher developmental stability of heterozygotes has been reported by numerous authors (Mitton and Grant 1984; Palmer and Strobeck 1986) who have shown either a positive correlation between inbreeding and FA (Robertson and Reeve 1952; Bader 1965a; Leamy 1984, 1992) or a negative one between FA (or morphological variance) and allozyme heterozygosity in wild populations (for review see Mitton and Grant 1984; Clarke 1993; Markow 1995). However, it has been argued that these effects may not always be due to heterozygosity because both inbreeding in strains of and differences in evolutionary histories of wild populations could also lead to disruption of coadaptation and/or to fixation of deleterious recessive alleles due to a founder effect or drift (Patterson and Patton 1990; Clarke 1993; Markow 1995). The most cited examples that unambiguously support a relation between heterozygosity and developmental stability are the intrapopulation studies of the rainbow trout (Leary et al. 1983, 1984, 1992) and *Drosophila* (Biémont 1983) in which the most homozygous individuals displayed the highest FA levels. However, in a number of other studies no such correlation was found (Wooten and Smith 1986; Patterson and Patton 1990; Clarke et al. 1992; Yampolosky and Scheiner 1994).

In the present study, the heterotic effect on the FA found in the different generations of hybrid mice provides additional evidence for a relationship between the stability of development and genomic heterozygosity. We are not able, however, to determine the relative role of the different mechanisms responsible for the observed heterosis. As we used recently established random-bred strains it is unlikely that any major breakdown of coadaptation or expression of deleterious recessive alleles due to inbreeding occurred in the parental strains. This assumption is supported by the fact that, for the same characters, the original wild populations exhibited slightly lower levels of developmental stability (see below). Moreover, homogeneous conditions of rearing avoided environmentally-related differences in FA levels between samples.

Developmental Stability and Genomic Coadaptation

Genomic coadaptation is considered to be a necessary genetic condition for developmental stability. Most of the evidence for this relationship comes from studies on natural hybrid zones in which lower levels of developmental stability are reported for hybrid populations (for review see Graham 1992). This was interpreted as being the direct consequence of a disruption of the coadaptation of the gene systems controlling development due to the admixture of two different genomes. Hence, even though nearly half the studies reported no differences between hybrid and parental groups, outbreeding depression was thought to be the main effect at the specific or subspecific taxonomic level. However, recent studies have demonstrated that this is not always the case. Hybrids in the Danish hybrid zone between the two subspecies of the house mouse have lower levels of FA than the parental groups which suggests that developmental stability of the dental characters at least, is increased (Alibert et al. 1994). Similarly, hybrids between two subspecies of sagebrush were found to be developmentally more stable than the parental taxa for several characters (Freeman et al. 1995).

In the case of laboratory hybrids between *M. m. domesticus* and *M. m. musculus*, it is clear that recombination between the two genomes did not lead to major perturbations in development as both the backcrosses and F_2 hybrids still showed heterosis. The morphological characters analyzed showed no significant differences in FA levels before (F_1 hybrids) and after meiotic recombination (backcrosses and F_2 hybrids). However, the significant decrease in size of four molar characters out of six in recombined hybrids could be due to a slight breakdown in one or more coadapted gene systems or, more likely, to the decrease in heterozygosity that is expected in these groups. It is also of interest to note that there are some differences in levels of FA, and to a larger extent in character size, between the reciprocal crosses. F_1 hybrids obtained from crosses involving a *musculus* female (F_1M) showed a higher FA level than did the offspring of the reciprocal crosses (F_1D). This suggests the possibility of a maternal effect since the developmental stability of the *musculus* sample was significantly lower. However, the pattern was reversed in the backcrosses, and did not support the idea of a *musculus* maternal effect. As far as character size was concerned, offspring from crosses with a maternal *musculus* origin tended to be larger than those with a *domesticus* one, except in the F_2 samples where this trend was reversed. However, it is difficult to interpret this pattern in terms of a maternal effect or heritability because certain categories of crosses were missing and the size of the F_2M sample was small. It is clear that further investigations are needed before more definitive conclusions can be drawn.

Comparison with Wild Populations

Since the strains used in this study originated from Denmark and the dental characters studied were the same as those

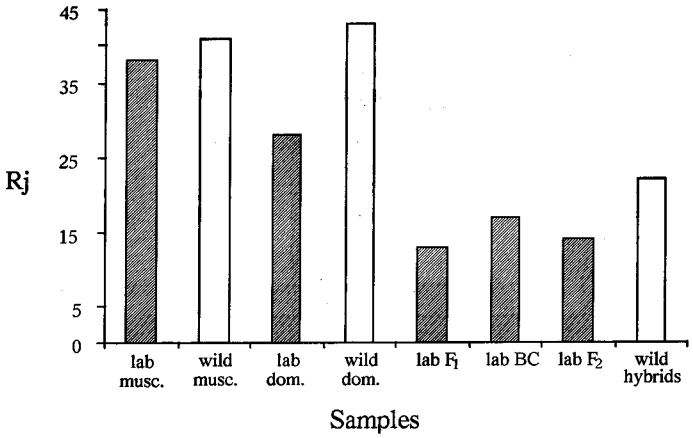

FIG. 3. Graphic representation of the sum, Rj, of the FA indices after transformation to rankings of the six characters within the eight samples (see text for further explanations), for the laboratory crosses and three wild-caught populations. Shaded bars represent laboratory populations (lab) and nonshaded bars represent wild populations (wild); "musc." and "dom." refer, respectively, to *musculus* and *domesticus*; "wild musc." and "wild dom." correspond, respectively, to classes 1 and 5 in the study of Alibert et al. (1994), whereas "wild hybrids" corresponds to class 3.

analyzed in wild house mice from the Danish hybrid zone (Alibert et al. 1994), direct comparisons between laboratory-bred and wild samples were possible. Figure 3 shows the sums of the FA indices after transformation to rankings, for the laboratory samples (the two strains and the three hybrid groups) and three wild-caught populations ("pure" *domesticus*, "pure" *musculus*, and a hybrid sample).

Here again, the correlation of FA levels for the six traits among samples was statistically significant ($W = 0.704$, $P \ll 0.01$). Interestingly, the ranking of the FA levels was lower in the laboratory *domesticus* and *musculus* samples than in the wild ones from the hybrid zone in Denmark. This result suggests that the founder effect, drift, and inbreeding that the random-bred strains have experienced were not very important since they did not lead to a decrease in developmental stability. On the contrary, wild populations appeared to be developmentally less stable. Since environmental stress is known to decrease developmental stability (Parsons 1990; Markow 1995), exogenous stress which is likely to be greater in the wild may be responsible for the observed differences (Auffray et al. 1996b).

The differences in FA between the wild and laboratory hybrid samples are only slight. This is interesting as it suggests that the breakdown in coadapted gene systems regulating development is not more pronounced in the wild hybrid individuals which have highly recombined genomes (up to now no F_1 hybrids have been found in the house mouse hybrid zone) than in the first two hybrid generations obtained in the laboratory. This is not expected if a large number of coadapted gene systems had been disrupted during hybridization.

Relationship with Sterility

We failed to find any relationship between F_1 hybrid sterility and the level of FA. This agrees with the previous study performed on wild populations (Alibert et al. 1994), since the lower level of FA found in the hybrid populations contrasted with the decrease in reproductive fitness suggested by the limited introgression of the sex chromosome markers across the hybrid zone (Vanlerberghe et al. 1986, 1988a; Tucker et al. 1992; Dod et al. 1993). Differences in developmental stability have been proposed to reflect differences in fitness (Soulé 1982; Leary et al. 1984; Møller and Pomiankowski 1993; Swaddle and Witter 1994). However, if the efficiency with which an organism produces an optimal phenotype under given conditions can be considered as one component of fitness, the use of developmental stability as a marker of overall fitness must be questioned. Studies reporting clear relationships between developmental stability and fitness are quite limited. Besides, most of them concern the direct consequences of asymmetry in terms of sexual selection, but very few demonstrate the outcome in terms of natural selection (Markow 1995). Our results clearly show that, at least in the case of hybrids, a correlation between developmental stability and overall fitness cannot be generalized.

Dynamics of the House Mouse Hybrid Zone

Even though the European house mouse hybrid zone has been the focus of numerous studies during the last two decades, the contrasting results obtained by different approaches demonstrate that the degree of hybrid dysgenesis is not yet well defined. The reduced introgression of the sex chromosome markers and the dysfunction of certain immune responses suggest that genomic incompatibilities do exist between *M. m. domesticus* and *M. m. musculus*. However, the greater developmental stability of tooth characters found in the natural hybrid populations provides no evidence for disruption of coadaptation in hybrids. An alternative hypothesis is that the pattern of FA observed in the hybrid zone reflects an overall hybrid dysgenesis. The decrease in FA levels could simply be due to the elimination by natural selection of the less fit individuals which are also the more asymmetric, thereby creating a heterotic-like effect. However, as we have shown that heterosis occurs before as well as after meiotic recombination, the first hypothesis appears to be the more plausible. The results reported here therefore provide additional evidence that selection acting on the hybrid genome between *M. m. domesticus* and *M. m. musculus* is likely limited to relatively few gene systems making a major contribution to the hybrid disadvantage.

ACKNOWLEDGMENTS

We would like to thank J. H. Graham for his advice and encouragement, B. Dod for her very accurate and relevant language revisions, F. Bonhomme for providing mice and breeding facilities, A. Orth and J. Catalan for their excellent technical assistance, A. Zaegel for maintenance of animals, and J. Lopez for her histological contribution. We are also grateful to L. Leamy and two anonymous reviewers for very helpful comments on the manuscript. This research was supported by a grant from the GREG: "Coadaptation génomique—Dysgénèse hybride et spéciation chez la souris: Une approche globale des interactions mutligéniques dans les génomes hybrides" (coordinator: P. Boursot), décision N.83/94.

KM, and part of this research, was supported by the European Community Biotech programme, contract BIO2-CT92-0476 to P. Boursot. This is publication ISEM N.97-040

Literature Cited

Alibert, P., S. Renaud, B. Dod, F. Bonhomme, and J.-C. Auffray. 1994. Fluctuating asymmetry in the *Mus musculus* hybrid zone: a heterotic effect in disrupted co-adapted genomes. Proc. R. Soc. Lond. B Biol. Sci. 258:53–59.

Auffray, J.-C., P. Alibert, C. Latieule, and B. Dod. 1996a. Relative warp analysis of skull shape across the hybrid zone of the house mouse (*Mus musculus*) in Denmark. J. Zool. Lond. 240:441–455.

Auffray, J.-C., P. Alibert, S. Renaud, A. Orth, and F. Bonhomme. 1996b. Fluctuating asymmetry in *Mus musculus* subspecific hybridization: traditional and Procrustes comparative approach. Pp. 275–283 *in* L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. Slice, eds. Advances in morphometrics. NATO. Adv. Study Inst. Ser. A Life Sci. Vol. 284. Plenum, New-York.

Bader, R. S. 1965a. Fluctuating asymmetry in the dentition of the house mouse. Growth 29:219–300.

———. 1965b. A partition of variance in dental traits of the house mouse. J. Mammal. 46:384–388.

Barton, N. H. 1979. The dynamics of hybrid zones. Heredity 43: 341–359.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

Biémont, C. 1983. Homeostasis, enzymatic heterozygosity and inbreeding depression in natural populations of *Drosophila melanogaster*. Genetica 61:179–189.

Boursot, P., F. Bonhomme, J. Britton-Davidian, J. Catalan, H. Yonekawa, P. Orsini, S. Gerasimov, and L. Thaler. 1984. Introgression différentielle des génomes nucléaires et mitochondriaux chez deux semi-espèces européennes de souris. Compt. Rend. Acad. Sci. Paris 229:691–693.

Boursot, P., J.-C. Auffray, J. Britton-Davidian, and F. Bonhomme. 1993. The evolution of house mice. Annu. Rev. Ecol. Syst. 24:119–152.

Clarke, G. M. 1993. The genetic basis of developmental stability. I Relationships between stability, heterozygosity and genomic coadaptation. Genetica 89:15–23.

Clarke, G. M., B. P. Oldroyd, and P. Hunt. 1992. The genetic basis of developmental stability in *Apis mellifera*: heterozygosity versus genic balance. Evolution 43:753–762.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia Univ. Press, New York.

Dod, B., L. S. Jermiin, P. Boursot, V. H. Chapman, J. T. Nielsen, and F. Bonhomme. 1993. Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. J. Evol. Biol. 6: 529–546.

Fel-Clair, F., T. Lenormand, J. Catalan, J. Grobert, A. Orth, P. Bourset, M.-C. Viroux, and J. Britton-Davidian. 1996. Genomic incompatibilities in the hybrid zone between house mice in Denmark: evidence from steep and noncoincident chromosomal clines for Robertsonian fusions. Genet. Res. 67:123–124.

Forejt, J. 1974. Nonrandom association between a specific autosome and the X chromosome in meiosis of the male mouse: possible consequences of the homologous centromeres separation. Cytogenet. Cell Genet. 13:369–383.

Forejt, J., and P. Ivanyi. 1975. Genetic studies on male sterility of hybrids between laboratory and wild mice *Mus musculus* L. Genet. Res. Camb. 24:189–206.

Forejt, J., V. Vincek, J. Klein, H. Lehrach, and M. Loudov-Mickov. 1991. Genetic mapping of the t-complex region on mouse chromosome 17 including the *hybrid sterility-1* gene. Mammal. Genome 1:84–91.

Freeman, D. C., J. H. Graham, D. W. Byrd, E. D. McArthur, and W. A. Turner. 1995. Narrow hybrid zone between two subspecies of big sagebrush, *Artemisia tridentata* (Asteraceae). III. Developmental instability. Am. J. Bot. 82:1144–1152.

Graham, J. H. 1992. Genomic coadaptation and developmental stability in hybrid zones. Acta Zool. Fenn. 191:121–131.

Graham, J. H., D. C. Freeman, and J. M. Emlen. 1993. Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica 89:121–137.

Gropp, A., and H. Winking. 1981. Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. Pp. 141–181 *in* R. J. Berry, ed. Biology of the house mouse. Academic Press, London.

Harrison, R. G. 1990. Hybrid zones: windows on evolutionary process. Oxf. Surv. Evol. Biol. 7:69–128.

Hewitt, G. M. 1988. Hybrid zones-Natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.

Leamy, L. 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. Am. Nat. 1935: 579–593.

———. 1992. Morphometric studies in inbred and hybrid house mice. VII. Heterosis in fluctuating asymmetry at different ages. Acta Zool. Fenn. 191:111–119.

———. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. Genetica 89:139–154.

Leamy, L., and R. W. Touchberry. 1974. Additive and nonadditive genetic variance in odontometric traits in crosses of seven inbred lines of house mice. Genet. Res. Camb. 23:207–217.

Leary, R. F., F. W. Allendorf, and L. K. Knudsen. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. Nature 301:71–72.

———. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat. 124:540–551.

———. 1992. Genetic, environmental, and developmental causes of meristic variation in rainbow trout. Acta Zool. Fenn. 191:79–98.

Markow, T. A. 1995. Evolutionary ecology and developmental stability. Annu. Rev. Entomol. 40:105–120.

McKenzie, J. A., and G. M. Clarke. 1988. Diazinon resistance, fluctuating asymmetry and fitness in the Australian sheep blowfly, *Lucilia cuprina*. Genetics 120:213–220.

Mitton, J. B., and M. C. Grant. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu. Rev. Ecol. Syst. 15:479–499.

Moulia, C., J. P. Aussel, F. Bonhomme, P. Boursot, J. T. Nielsen, and F. Renaud. 1991. Wormy mice in a hybrid zone: a genetic control of susceptibility to parasite infection. J. Evol. Biol. 4: 679–687.

Moulia, C., N. Le Brun, J. Dallas, A. Orth, and F. Renaud. 1993. Experimental evidence of genetic determinism in high susceptibility to intestinal pinworm infection in mice: a hybrid zone model. Parasitology 106:387–393.

Moulia, C., N. Le Brun, C. Loubes, R. Marin and F. Renaud. 1995. Hybrid vigour against parasites in interspecific crosses between two mice species. Heredity 74:48–52.

Møller, A. P., and A. Pomiankowski. 1993. Fluctuating asymmetry and sexual selection. Genetica 89:267–280.

Nancé, V., F. Vanlerberghe, J. T. Nielsen, F. Bonhomme, and J. Britton-Davidian 1990. Chromosomal introgression in house mice from the hybrid zone between *M. m. domesticus* and *M. m. musculus* in Denmark. Biol. J. Linn. Soc. 41:215–227.

Palmer, R. A. 1994. Fluctuating asymmetry analyses: a primer. Pp. 335–364 *in* T. A. Markow, ed. Developmental instability: its origins and evolutionary implications. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Palmer, R. A., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst. 17:391–421.

———. 1992. Fluctuating asymmetry as a measure of developmental stability: implication of nonnormal distributions and power of statistical tests. Acta Zool. Fenn. 191:57–72.

Parsons, P. A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biol. Rev. 65:131–145.

Patterson, B. D., and J. L. Patton. 1990. Fluctuating asymmetry and allozymic heterozygosity among natural populations of pocket gophers *Thomomys bottae*. Biol. J. Linn. Soc. 40:21–36.

Prager, E. M., R. D. Sage, U. Gyllensten, W. K. Thomas, R.

HÜBNER, C. S. JONES, L. NOBLE, J. B. SEARLE, AND A. C. WILSON. 1993. Mitochondrial DNA sequence diversity and the colonization of Scandinavia by house mice from east Holstein. Biol. J. Linn. Soc. 50:85–122.

RICE, W. R. 1989. Analyzing tables statistical tests. Evolution 43: 223–225.

ROBERTSON, F. W., AND E. C. R. REEVE. 1952. Heterozygosity, environmental variation and heterosis. Nature 170:286.

SAGE, R. D., D. HEYNEMAN, K. C. LIM, AND A. WILSON. 1986. Wormy mice in a hybrid zone. Nature 3246:60–63.

SAGE, R. D., W. R. ATCHLEY, AND E. CAPANNA. 1993. House mice as models in systematic biology. Systematic Biology 42:523-561.

SIEGEL, S. AND J. N. CASTELLAN JR. 1988. Nonparametric statistics for the behavioral sciences. 2d ed. McGraw-Hill, New-York.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. 3d ed. Freeman, New York.

SOULÉ, M. E. 1967. Phenetics of natural populations. II Asymmetry and evolution in a lizard. Am. Nat. 101:141–160.

———.1982. Allometric variation. 1. The theory and some consequences. Am. Nat. 120:751–764.

SWADDLE, J. P., AND M. S. WITTER. 1994. Food, feathers, and fluctuating asymmetries. Proc. R. Soc. Lond. B Biol. Sci. 255: 147–152.

TUCKER, P. K., R. D. SAGE, J. WARNER, A. C. WILSON, AND E. M. EICHER. 1992. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution 46:1146–1163.

VAN VALEN, L. 1962. A study of fluctuating asymmetry. Evolution 16:125–142.

VANLERBERGHE, F., B. DOD, P. BOURSOT, M. BELLIS, AND F. BONHOMME. 1986. Absence of Y-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. Genet. Res. Camb. 48:191–197.

VANLERBERGHE, F., P. BOURSOT, J. T. NIELSEN, AND F. BONHOMME. 1988a. A steep cline for mitochondrial DNA in Danish mice. Genet. Res. Camb. 52:185–193.

VANLERBERGHE, F., P. BOURSOT, J. CATALAN, S. GERASIMOV, F. BONHOMME, B. ATANASSOV, AND L. THALER. 1988b. Analyse génétique de la zone d'hybridation entre les deux sous-espèces de souris *Mus musculus domesticus* et *Mus musculus musculus* en Bulgarie. Genome 30:427–437.

VRIJENHOEK, R. C., AND S. LERMAN. 1982. Heterozygosity and developmental stability under sexual and asexual breeding systems. Evolution 36:768–776.

WOOTEN, M. C., AND M. H. SMITH. 1986. Fluctuating asymmetry and genetic variability in a natural population. J. Mammal. 67: 725–732.

YAMPOLOSKY, L. Y., AND S. M. SCHEINER. 1994. Developmental noise, phenotypic plasticity, and allozyme heterozygosity in Daphnia. Evolution 48:1715–1522.

ZAKHAROV, V. M. 1989. Future prospects for population phenogenetics. Soviet Sci. Rev./Sect. F. Physiol. Gen. Biol. 4:1–79.

Corresponding Editor: L. Leamy

APPENDIX

Detailed presentation of the data for each of the six traits and the 13 samples.

Trait	Sample	N[a]	Character size (R + L)/2 Mean	± SE	Slope	± SE[b]	Asymmetry (R − L) Mean	± SE	Dmax[c]	Skew	Kurtosis	Variance (= FA4)[d]	|R − L| Mean (= FA1)[e]	± SE
LM/1	M	60	1.538	0.0034	−0.0378	0.0607	−0.0046	0.0024	0.0617	0.030	0.482	33.9312	14.8650	0.0015
	D	62	1.478	0.0056	−0.005	0.0268	−0.0017	0.0019	0.0758	−0.119	0.078	22.4958	12.1161	0.0011
	Fertile F1M	26	1.592	0.0053	0.0906	0.0689	0.0050	0.0029	0.1442	0.273	−0.783	21.8359	12.2692	0.0018
	Sterile F1M	47	1.599	0.0048	−0.0342	0.0428	−0.0021	0.0023	0.0776	0.109	1.197	23.8583	11.9787	0.0014
	Fertile F1D	15	1.546	0.0069	0.0401	0.0742	0.0018	0.0033	0.1002	0.067	−0.338	15.9457	10.2000	0.0019
	Sterile F1M	38	1.544	0.0052	0.0033	0.0353	0.0008	0.0021	0.1193	0.096	−0.987	17.3650	11.2105	0.0011
	BC♀M	39	1.512	0.0054	−0.0142	0.0435	−0.0007	0.0024	0.0878	−0.365	−0.349	22.9113	12.1795	0.0014
	BC′♀M	40	1.523	0.0052	0.0637	0.0526	−0.0046	0.0023	0.1216	−1.357**	2.843**	22.0554	11.1000	0.0017
	BC′♂M	23	1.552	0.0076	−0.0208	0.0331	−0.0003	0.0021	0.1554	0.824	0.552	10.2419	7.9130	0.0013
	BC′♀D	18	1.530	0.0115	−0.0124	0.0238	−0.0033	0.0027	0.1801	−0.322	−0.802	12.7154	9.7222	0.0015
	BC′♂D	28	1.510	0.0069	0.0899	0.0574	0.0014	0.0030	0.1476	0.768	0.404	25.4905	11.9286	0.0020
	F2M	9	1.536	0.0120	−0.068	0.0666	−0.0088	0.0027	0.2258	0.400	−0.875	6.4194	9.8889	0.0021
	F2D	76	1.537	0.0061	0.0097	0.0175	−0.002	0.0015	0.0662	0.268	−0.323	17.7400	11.0658	0.0009
LM/2	M	60	0.994	0.0033	0.0554	0.0551	0.0026	0.0021	0.0661	−0.037	0.528	26.4952	12.6833	0.0013
	D	62	0.944	0.0046	−0.0237	0.0286	0.0026	0.0018	0.0803	−0.211	−0.668	19.7529	11.8871	0.0010
	Fertile F1M	26	1.011	0.0048	−0.0648	0.0490	−0.0032	0.0021	0.0924	0.187	−0.546	11.1225	9.0769	0.0012
	Sterile F1M	47	1.004	0.0042	−0.0463	0.0524	0.0021	0.0024	0.0999	0.046	0.805	26.2793	11.8936	0.0016
	Fertile F1D	15	0.983	0.0077	0.1202	0.0722	−0.0005	0.0036	0.1919	1.084	0.626	19.8981	10.8667	0.0022
	Sterile F1M	38	0.984	0.0050	0.0539	0.0394	0.0007	0.002	0.0794	0.374	−0.308	14.7681	9.7368	0.0012
	BC♀M	39	0.998	0.0063	−0.0097	0.0340	0.0025	0.0021	0.0889	0.440	0.665	16.7202	10.2564	0.0013
	BC′♀M	40	1.000	0.0066	−0.0321	0.0324	−0.0022	0.0025	0.1146	0.545	−0.202	25.5908	13.4500	0.0014
	BC′♂M	23	1.024	0.0067	0.0667	0.0373	0.0016	0.0023	0.1203	0.494	−0.757	11.7431	8.9130	0.0013
	BC′♀D	18	0.999	0.0075	0.0395	0.0590	0.0029	0.0029	0.1075	0.376	−0.153	14.6298	9.7778	0.0017
	BC′♂D	28	0.990	0.0064	−0.0685	0.0439	0.0015	0.0027	0.0862	−0.041	−0.933	20.8184	12.1071	0.0014
	F2M	9	0.992	0.0083	0.0734	0.1735	−0.007	0.0053	0.2246	−0.605	−0.947	25.3068	12.3778	0.0039
	F2D	76	1.021	0.0051	−0.014	0.0223	−0.0002	0.0018	0.0734	0.237	−0.685	23.9779	12.7368	0.0010
LM/3	M	49	0.579	0.0066	0.0604	0.0426	0.0088	0.0032	0.0714	0.003	0.512	51.4723	18.8367	0.0022
	D	52	0.562	0.0077	0.0059	0.0321	0.0000	0.0031	0.1100	−0.306	−0.409	48.7078	18.2115	0.0017
	Fertile F1M	19	0.637	0.0094	0.0028	0.0752	0.0022	0.0052	0.0995	0.073	−0.597	52.2585	18.1579	0.0031
	Sterile F1M	38	0.625	0.0039	0.0743	0.0864	0.0044	0.003	0.1312	−0.724	1.396	33.7389	14.2895	0.0020
	Fertile F1D	12	0.628	0.0098	0.0354	0.0594	0.0003	0.003	0.1723	−0.354	0.472	10.4750	7.5833	0.0019
	Sterile F1M	37	0.613	0.0059	−0.0071	0.0340	0.0012	0.0022	0.1015	0.122	−0.638	18.0356	11.3514	0.0012
	BC♀M	39	0.626	0.0085	0.0157	0.0316	−0.0013	0.0032	0.1384	0.413	−0.48	38.8429	16.4359	0.0017
	BC′♀M	39	0.626	0.0070	0.0187	0.0460	0.0025	0.0028	0.0959	0.456	1.008	30.7466	13.0513	0.0019
	BC′♂M	23	0.643	0.0085	−0.1166	0.0570	−0.0007	0.0037	0.1504	−0.308	0.640	31.7874	13.4348	0.0024
	BC′♀D	18	0.634	0.0105	0.1926	0.0501*	0.0008	0.0046	0.1405	−0.227	0.923	38.4418	15.0000	0.0029
	BC′♂D	28	0.632	0.0098	0.0263	0.0360	−0.0029	0.0033	0.1413	0.505	0.548	30.4439	14.2857	0.0019
	F2M	9	0.568	0.0130	0.0637	0.0650	0.0002	0.0046	0.2297	0.556	−0.614	18.9944	11.1111	0.0024
	F2D	76	0.623	0.0069	0.0192	0.0234	0.0013	0.0021	0.0941	0.021	1.901**	32.5643	13.1974	0.0014

APPENDIX. CONTINUED.

			Character size (R + L)/2				Asymmetry (R − L)						Asymmetry |R − L|	
Trait	Sample	N[a]	Mean	± SE	Slope	± SE[b]	Mean	± SE	Dmax[c]	Skew	Kurtosis	Variance (= FA4)[d]	Mean (= FA1)[e]	± SE
WM/1	M	60	0.910	0.0036	−0.0546	0.0534	−0.01	0.0024	0.082	0.084	−0.335	34.3743	17.7833	0.0014
	D	62	0.908	0.0042	−0.0066	0.0378	−0.0013	0.0022	0.0837	0.205	−0.495	28.7842	13.9839	0.0012
	Fertile F1M	26	0.972	0.0048	0.1426	0.0491	−0.0029	0.0025	0.1296	−0.097	−1.08	16.0234	10.8462	0.0013
	Sterile F1M	47	0.978	0.0035	0.0331	0.0438	−0.0048	0.0015	0.0741	−0.23	−0.435	10.0927	8.7021	0.0010
	Fertile F1D	15	0.923	0.0065	0.0502	0.0773	−0.0027	0.0032	0.1482	0.183	−0.723	15.3667	10.1333	0.0018
	Sterile F1M	38	0.922	0.0039	−0.0618	0.0576	−0.0043	0.0020	0.1251	0.395	0.524	14.5713	9.6842	0.0013
	BC♀M	39	0.929	0.0038	0.0217	0.0530	0.0014	0.0019	0.0823	0.147	0.036	14.4884	9.1282	0.0013
	BC′♀M	40	0.933	0.0035	0.0273	0.0550	−0.0003	0.0020	0.1350	0.524**	−0.345	16.1076	10.075	0.0012
	BC′♂M	23	0.929	0.0055	0.0059	0.0797	−0.0019	0.0028	0.2095	−1.579	3.426**	17.6356	9.1304	0.0020
	BC′♀D	18	0.905	0.0065	0.01	0.1035	−0.0086	0.0047	0.1058	0.135	−1.013	40.3075	18.1667	0.0027
	BC′♂D	28	0.913	0.0051	0.0171	0.0664	−0.0015	0.0029	0.1566	−0.655	−0.559	24.2480	12.4643	0.0017
	F2M	9	0.907	0.0153	0.0153	0.0354	0.0011	0.0037	0.2276	−0.173	−1.371	12.0361	9.5556	0.0015
	F2D	76	0.928	0.0043	−0.0311	0.0295	−0.0023	0.0016	0.0904	0.321	2.040**	18.9077	10.2105	0.0011
WM/2	M	60	0.915	0.0032	−0.0305	0.0665	0.0013	0.0023	0.1080	1.109**	2.529***	32.3298	13.0333	0.0016
	D	62	0.900	0.0044	0.0141	0.0422	0.0025	0.0021	0.1595**	1.174**	2.812***	26.8122	11.2097	0.0015
	Fertile F1M	26	0.958	0.0037	0.0789	0.0660	0.0001	0.0021	0.1077	−0.526	0.138	11.1306	8.4231	0.0012
	Sterile F1M	47	0.965	0.0038	0.0248	0.0474	0.0005	0.0018	0.0906	0.357	1.178	14.4560	8.6596	0.0012
	Fertile F1D	15	0.914	0.0077	−0.0362	0.0300	−0.0021	0.0021	0.1258	−0.262	−0.352	6.8352	6.8667	0.0012
	Sterile F1M	38	0.917	0.0042	0.0269	0.0500	0.0011	0.0018	0.1074	0.565	0.912	12.0388	8.1842	0.0012
	BC♀M	39	0.919	0.0047	0.0285	0.0453	0.0045	0.0022	0.0919	0.224	−0.132	18.3730	11.6410	0.0013
	BC′♀M	40	0.930	0.0047	0.0101	0.0387	0.0029	0.0018	0.1181	0.285	−0.816	13.5497	9.7750	0.0011
	BC′♂M	23	0.927	0.0041	0.0787	0.0461	0.0016	0.0022	0.1871	−0.402	−1.134	10.7802	9.3043	0.0009
	BC′♀D	18	0.904	0.0065	0.0116	0.0846	0.0058	0.0028	0.2020	0.886	0.336	14.6183	9.4444	0.0022
	BC′♂D	28	0.905	0.0066	−0.0156	0.0367	−0.0015	0.0023	0.1108	−0.166	−0.989	14.5517	10.1071	0.0012
	F2M	9	0.893	0.0118	−0.0984	0.0599	0.0064	0.0032	0.1236	−0.225	−0.615	9.4778	9.3333	0.0022
	F2D	76	0.930	0.0040	0.0123	0.0241	0.0012	0.0013	0.0734	0.081	−0.159	13.6028	9.3158	0.0008
WM/3	M	49	0.627	0.0040	−0.0478	0.0762	0.0094	0.003	0.0927	0.413	0.058	42.9288	17.3265	0.0021
	D	52	0.605	0.0043	0.0004	0.0549	0.0074	0.0024	0.0536	0.013	0.290	30.5857	14.6538	0.0017
	Fertile F1M	19	0.649	0.0062	−0.1485	0.0872	0.0065	0.0036	0.0935	0.206	0.070	24.4374	12.9474	0.0024
	Sterile F1M	38	0.665	0.0043	0.0874	0.0944	0.014	0.0034	0.0774	0.146	−0.162	43.0837	20.0789	0.0024
	Fertile F1D	12	0.636	0.0076	0.1581	0.1029	0.0068	0.0037	0.1397	0.819	0.382	16.7242	10.8333	0.0027
	Sterile F1M	37	0.639	0.0050	0.0392	0.0387	0.003	0.0021	0.0867	−0.09	−0.722	16.4083	11.0270	0.0011
	BC♀M	39	0.617	0.0037	0.0747	0.0620	0.0035	0.0025	0.0731	−0.222	0.134	24.8834	12.7436	0.0016
	BC′♀M	39	0.626	0.0040	0.1596	0.0718	0.0112	0.0025	0.1552	0.364	1.959	23.5694	15.2564	0.0018
	BC′♂M	23	0.632	0.0035	0.2564	0.1113	0.0095	0.0031	0.1262	−0.344	−0.224	22.6534	14.7826	0.0020
	BC′♀D	18	0.619	0.0058	−0.1014	0.1065	0.0118	0.0038	0.2360	−0.221	−1.608	26.2301	16.5556	0.0026
	BC′♂D	28	0.618	0.0057	−0.0909	0.0551	0.0075	0.0027	0.1009	0.121	−0.908	20.2926	13.0000	0.0018
	F2M	9	0.626	0.0047	−0.1762	0.2350	−0.0046	0.0045	0.1590	−0.545	0.212	18.3778	10.5556	0.0030
	F2D	76	0.632	0.0035	0.0439	0.0407	0.0087	0.0017	0.0699	0.371	−0.196	21.0795	13.2368	0.0012

[a] Sample size.
[b] Slope ± SE from regression of |R − L| versus (R + L)/2.
[c] Kolmogorov-Smirnov test statistic.
[d] Values × 10^5.
[e] Values × 10^3.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ after correcting with the sequential Bonferroni method applied within each series of tests, per sample across the six traits.