Developmental Stability in House Mice Heterozygous for Single Robertsonian Fusions

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Fluctuating asymmetry (FA) of tooth traits has been reported to be increased in Down syndrome patients as well as hybrids between chromosomal races of the house mouse differing in several Robertsonian (Rb) fusions. Developmental stability, assessed by FA, is thus thought to be impaired by spontaneous chromosomal abnormality or by chromosomal heterozygosity. Although the effect of a single fusion on developmental stability could theoretically be expected, it has never been documented. Crosses involving two chromosomal races of the house mouse diverging for one Rb fusion were performed to assess developmental stability in parental homozygous races as well as in their hybrids. Moreover, the occurrence of a spontaneous chromosomal mutation (WART type-b) allowed us to study the instantaneous effect of such a translocation on developmental stability. No difference in fluctuating asymmetry levels was detected among the groups considered in this study. This result suggested that a single stable or spontaneous balanced structural rearrangement did not inherently disturb developmental stability. In addition, the differential effect on developmental stability of one versus many heterozygous Rb fusions highlights the role of their quantitative accumulation in the disruption of coadaptation in chromosomal hybrids.

In hybrids between chromosomal groups, mispairing of structurally different chromosomes during meiosis is well known to decrease their fertility and to contribute to reproductive isolation between the parental groups (Nachman and Searle 1995; Saïd et al. 1993). In addition to this structural effect, Capanna and Redi (1994) hypothesized that chromosomal heterozygosity may also lead to perturbations of genome activity during early stages of gametogenesis. Chromosomal organization within the interphase nucleus has been shown to be spatially nonrandom and gene expression to depend on this organization (Heslop-Harrison and Bennet 1990; Marshall et al. 1997; Milot et al. 1996; Park and De Boni 1998). In chromosomal hybrids in which structural rearrangements modify the topology of chromosomes in interphase nuclei (Qumsiyeh 1995), transcriptional activity may thus be disturbed. Such a phenomenon may be related to the more general notion of genomic coadaptation, which refers to the selective processes favoring the accumulation in the gene pool of harmoniously collaborating genes. Coadapted gene complexes are thought to involve a relational balance of alleles within chromosomes between homologous chromosomes (Mather

1973 in Clarke 1993). Chromosomal rearrangements may thus be expected to disrupt genomic coadaptation (see Auffray et al. 1999 for a review).

Recently, Chatti et al. (1999) reported lower levels of developmental stability in wild hybrids between two Tunisian chromosomal races ($2n = 40$ and $2n = 22$) of the house mouse (*Mus musculus domesticus*) that differed by nine Robertsonian (Rb) fusions. In these samples of mice, developmental stability was appraised using fluctuating asymmetry (FA) of tooth characters, FA being an indicator of the bilateral variation of a given character over a sample of individuals. The lower developmental stability of the hybrids was interpreted as resulting from genomic incompatibilities between the two parental groups, that is, a breakdown of parental genomic coadaptation (Graham 1992). The low genic differentiation between the two races, as well as the trend for increasing levels of FA with the number of heterozygous fusions in hybrids, led Chatti et al. (1999) to suggest that the incompatibilities were more likely due to the structural reorganization of chromosomes than to genic divergence. The question addressed in the present study concerns the extent of disruption induced by a single balanced

translocation: FA was analyzed in two chromosomal races ($2n = 40$ and $2n = 38$) of house mice differing by one Rb fusion [Rb(4.12)], as well as in their F_1 and backcross progeny.

In addition, the spontaneous occurrence of a new balanced rearrangement due to a whole-arm reciprocal translocations (WART) between an Rb fusion and an acrocentric chromosome [WART type-b in Hauffe and Pialek (1997)] in the laboratory crosses allowed us to study two further aspects of the relation between chromosome change and genomic coadaptation. The new rearrangement [Rb(4.19)] was detected in the bone marrow of an F_1 ($2n = 39$) female that when backcrossed to an all-acrocentric male ($2n = 40$), produced three types of offspring: all-acrocentric mice and heterozygotes for one of the two Rb fusions [Rb(4.12) or Rb(4.19)], thereby indicating mosaicism of the germline (Catalan et al. 2000). Despite the small sample size of WART carriers, the comparative analysis of fluctuating asymmetry of mice in this lineage provided the opportunity to detect an effect of a spontaneous chromosomal rearrangement on developmental stability and to compare with the one of a stable rearrangement. Furthermore, as the extremely low frequency of Rb fusions involving chromosome 19 in wild mice is thought to be related to a high selective disadvantage (Nachman and Searle 1995), its specific effect on developmental stability was investigated.

Materials and Methods

Mating Design

All crosses involved laboratory offspring of wild-trapped house mice from different localities in Belgium. Three of these localities [Assesse (50.22N, 5.02E), Natoye (50.20N, 5.04E), and Space (near Gesves (50.24N, 5.04E)] yielded $2n = 40$ all-acrocentric individuals, and one [Corroy-le-Grand (50.40N, 4.40E)] yielded $2n = 38$ homozygotes for Rb(4.12) [Dallas et al. 1998; see Bauchau (1990) for information on Rb races in Belgium]. Six types of crosses were performed: two were intraracial ones ($2n = 40 \times 2n = 40$) and ($2n = 38 \times 2n = 38$), two were reciprocal interracial crosses ($2n = 40 \times 2n = 38$), and two were reciprocal backcrosses with all-acrocentric mice ($2n = 39 \times 2n = 40$). Each type of cross involved four pairs of mice. Mice of the different crosses were reared under identical conditions in the same animal room. Food and water were provided ad libitum.

The progeny were grouped into eight samples. Two of these corresponded to the parental types, the all-acrocentric mice with $2n = 40$ (coded ST40), and the Robertsonian ones homozygous for the Rb(4.12) fusion (RB38); two to the F_1 generation ($2n = 39$) separated according to the karyotype of the mother (^{40}F1) when the mother was all-acrocentric ($2n = 40$) and (^{38}F1) when the mother was Robertsonian ($2n = 38$). As the F_1 individuals were all backcrossed to all-acrocentric mice, the offspring were either all-acrocentric (BC40) or heterozygous for the Rb(4.12) fusion (BC39). Each of these groups was subdivided into two samples according to the sex of the F_1 parent: (^{39}BC40) and (^{39}BC39) when the mother was an F_1 individual and (^{40}BC40) and (^{40}BC39) when the mother was all-acrocentric. Only the progeny from the first two litters were examined and all the animals studied were at least 4 weeks old. A total of 257 individuals were analyzed and sample sizes are provided in Table 1.

WART Lineage

The mosaic female in which the WART mutation was detected was paired to an all-acrocentric male and gave birth to six litters, the second, fifth, and sixth of which contained four carriers of the WART mutation and 20 noncarriers, that is, all-acrocentric or $2n = 39$ individuals carrying Rb(4.12). These mice were grouped into two samples, the first one (WART) consisting in the five WART carriers including the mosaic female, and the second one (CONT), the control sample, grouping the 20 other mice from the same litters and six siblings of the mother.

Chromosomal Analysis

Mice were assigned to the different samples on the basis of karyotypes prepared from yeast-stimulated (Lee and Elder 1980) bone marrow cells using the air-drying technique. For each individual, diploid numbers were determined on conventionally stained slides by analyzing 3–30 metaphase plates under a Zeiss Axiophot microscope. Identification of chromosomes according to the nomenclature of Cowell (1984) was performed using the G-banding method (Seabright 1971) and the karyotypes were established with the Genevision software.

Tooth Characters

All mandibles were manually cleaned. Fluctuating asymmetry was estimated on the maximum length and width of the three lower molars (LM1, WM1, LM2, WM2, LM3, and WM3). These measurements are currently used in studies of FA in rodents (Alibert et al. 1994, 1997; Auffray et al. 1996, 1999; Bader 1965; Chatti et al. 1999), and levels of developmental stability in teeth and skull have recently been shown to be well related (Debat et al. 2000). The third lower molar was absent in some individuals either due to natural causes or to the cleaning procedure, leading to unequal sample size among traits (Table 1). Although the age at which the animal was sacrificed was not standardized, it was an unlikely source of bias since tooth size is definite once it has erupted into the oral cavity, that is, 18 and 28 days after birth for M1-M2 and M3, respectively (Bader 1965). To estimate the error of measurement, measurements were taken twice during two independent sessions with a Nikon measuroscope (1 μm accuracy).

Preliminary Treatments

Statistical treatments of FA were largely based on Palmer (1994). Preliminary tests involved Shapiro–Wilks (W) tests of normality for each trait of each of the 10 samples. Rejection of normality would potentially suggest the presence of antisymmetry, which corresponds to the bilateral variation in which one side is systematically larger than the other, but this side varies at random among individuals. These tests were performed on (R—L) distributions, R and L representing the mean right and the mean left values, respectively, of the trait over the two sessions of measurement. Skewness and kurtosis were also assessed. For each trait, size dependence of FA within samples was tested by linear regression of |R—L| on (R + L)/2 and, among samples, by linear regression of log(var(R—L)) on (R + L)/2. The significance of directional asymmetry—when one side is larger than the other one, resulting in the mean right measurement being statistically different from the left one—and the level of the error of measurement relative to FA were appraised for each trait within each sample, using the now classical two-way mixed model ANOVA (Palmer and Strobeck 1986). The importance of sex as a potential source of variation of |R—L| was appraised trait by trait using a two-way fixed model ANOVA involving sample, sex, and their interaction as effects. The relation between asymmetry and litter size was tested by a linear regression pooling all samples trait by

Table 1. Detailed presentation of trait size and fluctuating asymmetry results for tooth traits

Trait	Sample	Sample size	Character size (R + L)/2 Mean	Variance (× 1000)	Size dependence within sample Slope[a]	±SE	(a)	FA4 (variance) (× 10000)	Skew	±SE	(b)	Kurtosis	±SE	(c)	Shapiro-Wilk's W	(d)	|R—L| FA1 (mean) (x 100)	Mixed model ANOVA FA10 (× 10000)	df
WM1	ST40	38	0.897	0.808	0.036	0.052	ns	1.874	−0.645	0.383	ns	0.307	0.750	ns	0.94	ns	1.037	0.70	20.26
	RB38	39	0.885	0.553	0.019	0.046	ns	0.888	0.497	0.378	ns	−0.014	0.741	ns	0.96	ns	0.804	0.32	18.53
	40F1	40	0.900	1.048	0.016	0.062	ns	2.554	−1.416	0.374	ns	6.576	0.733	***	0.87	**	1.115	1.16	32.08
	38F1	34	0.912	1.078	0.013	0.033	ns	1.296	−0.665	0.403	ns	−0.380	0.788	ns	0.94	ns	1.128	0.48	17.39
	39BC40	29	0.917	0.527	0.083	0.057	ns	1.447	−0.262	0.434	ns	−0.334	0.845	ns	0.98	ns	1.029	0.48	11.80
	39BC39	25	0.914	0.505	0.027	0.082	ns	1.906	−0.568	0.464	ns	0.543	0.902	ns	0.97	ns	1.174	0.74	14.11
	40BC40	24	0.896	0.384	−0.042	0.095	ns	2.300	−0.246	0.472	ns	−0.914	0.918	ns	0.95	ns	1.225	1.01	17.63
	40BC39	28	0.893	0.355	0.081	0.092	ns	2.021	−0.122	0.441	ns	−0.402	0.858	ns	0.98	ns	1.173	0.92	22.20
	WART	5	0.926	0.235	−0.249	0.193	ns	1.806	0.557	0.913	ns	−2.067	2.000	ns	0.91	ns	1.080	0.51	1.18
	CONT	26	0.924	0.319	0.021	0.075	ns	1.022	−0.374	0.456	ns	−0.138	0.887	ns	0.98	ns	0.887	0.30	5.56
WM2	ST40	38	0.916	0.741	0.032	0.053	ns	1.572	−0.943	0.383	ns	4.077	0.750	***	0.93	ns	0.838	0.67	26.79
	RB38	39	0.923	0.525	0.065	0.053	ns	1.714	−0.366	0.378	ns	−0.577	0.741	ns	0.96	ns	1.092	0.79	31.84
	40F1	40	0.930	0.839	−0.005	0.026	ns	0.921	0.155	0.374	ns	−1.166	0.733	ns	0.94	ns	0.835	0.37	24.45
	38F1	34	0.940	1.276	−0.041	0.042	ns	1.763	−0.270	0.403	ns	−0.390	0.788	ns	0.94	ns	1.057	0.81	27.43
	39BC40	29	0.943	0.681	−0.031	0.045	ns	1.046	−0.039	0.434	ns	−0.121	0.845	ns	0.98	ns	0.905	0.44	19.20
	39BC39	25	0.940	0.544	0.015	0.058	ns	1.326	−0.253	0.464	ns	−0.885	0.902	ns	0.96	ns	0.986	0.59	19.00
	40BC40	24	0.911	0.511	0.109	0.104	ns	2.107	0.512	0.472	ns	1.510	0.918	ns	0.95	ns	1.138	0.94	18.32
	40BC39	28	0.911	0.414	−0.043	0.063	ns	1.395	0.676	0.441	ns	−0.265	0.858	ns	0.94	ns	0.964	0.58	18.37
	WART	5	0.963	0.472	0.087	0.105	ns	0.303	−1.359	0.913	ns	2.993	2.000	ns	0.84	ns	0.310	−0.17	1.78
	CONT	26	0.965	0.410	−0.030	0.102	ns	2.175	−1.287	0.456	ns	2.904	0.887	ns	0.92	ns	1.217	1.14	19.73
WM3	ST40	37	0.626	0.929	0.065	0.073	ns	2.895	0.695	0.388	ns	0.938	0.759	ns	0.96	ns	1.570	1.14	21.74
	RB38	36	0.666	0.333	0.119	0.102	ns	3.020	−0.063	0.393	ns	0.499	0.768	ns	0.99	ns	1.329	1.20	21.53
	40F1	31	0.675	0.569	0.014	0.088	ns	2.741	−0.903	0.421	ns	0.553	0.821	ns	0.93	ns	1.239	1.16	21.21
	38F1	30	0.668	1.059	0.065	0.060	ns	4.124	−0.331	0.427	ns	−1.066	0.833	ns	0.93	ns	1.703	1.94	25.59
	39BC40	29	0.676	0.325	0.054	0.097	ns	2.893	0.207	0.434	ns	−0.949	0.845	ns	0.95	ns	1.447	1.23	20.04
	39BC39	25	0.670	0.385	−0.028	0.076	ns	1.464	0.517	0.464	ns	−0.184	0.902	ns	0.97	ns	0.964	0.57	14.02
	40BC40	23	0.646	0.437	0.150	0.091	ns	2.048	−0.058	0.481	ns	0.833	0.935	ns	0.97	ns	1.076	0.86	15.35
	40BC39	27	0.648	0.467	0.125	0.080	ns	1.877	−0.457	0.448	ns	−0.359	0.872	ns	0.96	ns	1.087	0.69	13.75
	WART	4	0.680	0.222	0.557	0.385	ns	5.526	0.719	1.014	ns	1.788	2.619	ns	0.93	ns	2.313	2.39	2.24
	CONT	21	0.686	0.299	0.176	0.136	ns	2.891	−0.005	0.501	ns	−0.182	0.972	ns	0.99	ns	1.390	1.18	10.77
LM1	ST40	38	1.490	2.265	0.016	0.036	ns	2.503	0.389	0.383	ns	0.697	0.750	ns	0.97	ns	1.221	1.00	23.36
	RB38	39	1.522	0.974	0.008	0.051	ns	3.131	−0.161	0.378	ns	−0.920	0.741	ns	0.96	ns	1.479	1.31	26.16
	40F1	40	1.527	1.012	−0.016	0.044	ns	2.666	−0.296	0.374	ns	−0.836	0.733	ns	0.95	ns	1.375	1.19	30.64
	38F1	34	1.518	1.325	0.043	0.047	ns	3.287	0.037	0.403	ns	−0.869	0.788	ns	0.97	ns	1.506	1.49	26.97
	39BC40	29	1.517	1.167	−0.083	0.037	ns	2.223	0.299	0.434	ns	−1.324	0.845	ns	0.91	ns	1.288	0.96	20.47
	39BC39	25	1.515	1.462	0.010	0.058	ns	3.426	−0.024	0.464	ns	−0.412	0.902	ns	0.97	ns	1.490	1.42	16.21
	40BC40	24	1.493	0.996	0.070	0.062	ns	2.303	0.093	0.472	ns	0.078	0.918	ns	0.97	ns	1.171	0.94	15.15
	40BC39	28	1.487	1.331	0.014	0.047	ns	2.134	0.218	0.441	ns	−0.780	0.858	ns	0.97	ns	1.170	0.86	17.01
	WART	5	1.549	0.492	−0.110	0.106	ns	0.986	−0.763	0.913	ns	0.304	2.000	ns	0.95	ns	0.800	0.09	0.12
	CONT	26	1.552	0.803	0.016	0.048	ns	2.473	0.484	0.456	ns	−1.272	0.887	ns	0.88	ns	1.546	1.05	15.35
LM2	ST40	38	1.010	0.906	−0.048	0.046	ns	1.848	−0.555	0.383	ns	0.595	0.750	ns	0.97	ns	1.182	0.80	27.41
	RB38	39	1.016	1.364	0.021	0.048	ns	3.611	0.047	0.378	ns	−0.789	0.741	ns	0.97	ns	1.558	1.64	31.15
	40F1	40	1.024	2.015	0.053	0.036	ns	2.629	0.354	0.374	ns	0.158	0.733	ns	0.97	ns	1.299	1.13	28.37
	38F1	34	1.013	1.006	0.007	0.046	ns	1.606	0.137	0.403	ns	−0.473	0.788	ns	0.97	ns	1.207	0.55	14.97
	39BC40	29	1.040	1.085	0.037	0.053	ns	2.532	−0.065	0.434	ns	−0.733	0.845	ns	0.97	ns	1.336	0.98	16.30
	39BC39	25	1.043	1.193	−0.103	0.081	ns	3.741	0.220	0.464	ns	1.450	0.902	ns	0.95	ns	1.428	1.66	18.78
	40BC40	24	1.020	0.470	0.086	0.099	ns	2.156	0.370	0.472	ns	−0.590	0.918	ns	0.96	ns	1.292	0.82	13.12
	40BC39	28	1.031	1.615	0.030	0.040	ns	1.848	0.504	0.441	ns	0.649	0.858	ns	0.96	ns	1.102	0.69	14.57
	WART	5	1.069	1.499	0.013	0.104	ns	0.489	−1.786	0.913	ns	3.611	2.000	ns	0.81	ns	1.600	−0.15	0.72
	CONT	26	1.061	1.106	0.110	0.058	ns	2.261	−0.017	0.456	ns	0.049	0.887	ns	0.98	ns	1.277	0.91	11.12
LM3	ST40	37	0.574	0.931	0.065	0.066	ns	3.465	−0.029	0.388	ns	0.235	0.759	ns	0.99	ns	1.488	1.27	18.65
	RB38	36	0.563	1.049	0.005	0.055	ns	3.109	0.025	0.393	ns	−0.219	0.768	ns	0.99	ns	1.432	1.14	18.36
	40F1	31	0.588	1.870	−0.020	0.060	ns	4.044	0.582	0.421	ns	1.996	0.821	*	0.94	ns	1.474	1.74	22.09
	38F1	30	0.582	2.246	0.076	0.039	ns	3.465	0.364	0.427	ns	−0.916	0.833	ns	0.94	ns	1.513	1.54	22.66
	39BC40	29	0.564	1.518	0.023	0.056	ns	3.135	−0.276	0.434	ns	0.589	0.845	ns	0.96	ns	1.374	1.29	18.64
	39BC39	25	0.564	1.603	0.029	0.046	ns	2.535	0.015	0.464	ns	−0.733	0.902	ns	0.99	ns	1.324	1.07	16.84
	40BC40	23	0.567	1.594	0.006	0.056	ns	2.338	−0.466	0.481	ns	1.130	0.935	ns	0.94	ns	1.187	0.70	7.38
	40BC39	28	0.571	0.913	−0.004	0.070	ns	2.996	1.092	0.441	ns	1.967	0.858	*	0.93	ns	1.248	1.00	11.42
	WART	4	0.548	0.553	−0.311	0.441	ns	2.687	−1.361	1.014	ns	1.697	2.619	ns	0.89	ns	1.388	0.55	0.44
	CONT	21	0.546	0.249	0.015	0.105	ns	2.401	0.077	0.501	ns	−1.112	0.972	ns	0.96	ns	1.386	0.64	4.21

[a] Regression slope of |R—L| on (R + L)/2.
Statistical significance of (a) regression slope, (b) skewness, (c) kurtosis, and (d) departure from normality.
* $P < .05$; ** $P < .01$; *** $P < .001$; ns, nonsignificant.

trait. At each step of these preliminary treatments involving the 10 samples, sequential Bonferroni tests (Rice 1989) were conducted on sets of $k = 10$ tests corresponding to the 10 samples. Finally, the effect of parental pairs of a potential source of variation within samples was appraised using a nested ANOVA, corrected for unequal sample size (Sokal and Rohlf 1995), considering pair effect nested into sample one. However, within each of the WART and CONT samples, individuals originated from a single parental pair. These two samples were thus not involved in the later analysis.

Fluctuating Asymmetry Estimates and Testing

Three indices of FA were considered for each trait and each sample, FA1 which corresponded to the mean of |R—L|, FA4 which was the variance of (R—L), and FA10 which resulted from the two-way mixed model ANOVA, and partitioned out the error of measurement as assessed from the two replicates from the FA estimate.

The differences in FA levels were tested using the modified Levene's test (Palmer 1994) on the |R—L| dataset. It corresponds to a two-way fixed model ANOVA involving sample, trait, and their interaction as effects. The concordance of FA estimates for all traits across samples was tested using a Kendall test of concordance on the three types of FA indices separately.

Results

Preliminary Results

In this section the P values provided were corrected by the Bonferroni procedure and are coded as follows: $P > .05$, ns; $P < .05$, *; $P < .01$,**; $P < .001$, ***. Normality was not rejected except for one distribution out of the 60 considered here (Table 1). Skewness was not detected in these distributions, but kurtosis was in two of them. The two-way mixed model ANOVAs showed that the side effect was significant in four of the 60 cases (appendix), suggesting a low occurrence of directional asymmetry. As the very few cases of departure from perfect normality centered on zero did not affect a particular trait or sample, neither directional asymmetry nor antisymmetry could be considered as affecting the estimates of FA in this study and all data were retained in further analyses. Individual asymmetry was not dependent on character size, either within samples (Table 1) or among them ($0.19 < F_{[1, 9]} < 1.84$, all P values ns).

For all samples except WART, the interaction term of the two-way mixed model ANOVA was significant for all traits (appendix). The nonsignificance of the interaction term for five of the six traits in the WART group (appendix) was likely due to several causes: a more important error of measurement for this sample (although all measurements were taken by the same operator) and a lesser sensitivity of F tests, both causes being potentially related to the extremely low sample size. However, the WART mutation was detected once all animals had been sacrificed. It was thus impossible to increase the sample size. Re-

sults concerning this sample should then be considered with caution as representing an indication rather than an evidence. This artifact led, however, to few negative values of FA10 in this sample.

Two-way fixed model ANOVAs demonstrated that sex had no effect on the level of FA when considered alone (sex effect, $0.08 < F_{[1, 243–268]} < 1.52$, all P values ns) or in interaction with the sample (interaction sex sample, $0.32 < F_{[1, 243–268]} < 1.39$, all P values ns). For no trait was linear regression of |R—L| on litter size significant ($0.05 < F_{[1, 261–286]} < 1.27$, all P values ns). Nested ANOVAs indicated that the differences among the progenies of the different parental pairs were not significant (parental pair effect nested in sample one, $0.50 < F_{[24, 206–225]} < 1.59$, all P values ns).

Fluctuating Asymmetry

Levene's test showed that (1) some traits were repeatedly less developmentally stable than others (trait effect, $F_{[5, 1619]} = 7.95$***), (2) the extent of FA variation among samples did not depend on the trait (interaction sample trait, $F_{[45, 1619]} = 0.93$ns), and (3) samples did not exhibit statistically different levels of developmental stability (sample effect, $F_{[9, 1619]} = 0.75$ ns). Even if FA levels were not significantly different, the ranking of samples for FA levels could have been concordant across traits. Such a pattern would have suggested a trend for differences in developmental stability among the samples. However, Kendall tests of concordance were not significant whatever the FA index considered (FA1, $W = 0.119$ ns; FA4, $W = 0.125$ ns; FA10, $W = 0.174$ ns).

These results suggested that the presence of the stable chromosomal rearrangement [Rb(4.12)], whether heterozygous (in [40]F1, [38]F1, [39]BC39, and [40]BC39 samples) or homozygous (in RB38), did not impair the developmental stability of carriers as compared to the all acrocentric group (ST40 sample). In addition, although based on a small sample size, the spontaneous WART mutation did not lead to a detectable increase in FA of carriers (in the WART sample) as compared to the control group (CONT) and to all other samples.

Discussion

No differences in FA were found between hybrid and parental groups of mice from Belgium differing by Rb(4.12). This indicated that this structural rearrangement did not impair developmental stability in

the chromosomal hybrids. These data contrast with those of a previous study showing a decrease in developmental stability of teeth in hybrids between Tunisian chromosomal races of the house mouse diverging for nine Rb fusions (Chatti et al. 1999). Thus the genomic incompatibility evidenced between races diverging with a large difference in the number of fusions is not detected between those differing for a single fusion. This differential effect on developmental stability of hybrids between chromosomal races could arise from several causes that are not mutually exclusive: the number of Rb fusions differentiating the two races, the level of genic divergence between them, and/or the type of Rb fusion involved.

The contrasting results of heterozygosity for one versus many Rb fusions suggest that the interphase chromosomal reorganization induced by only one of these rearrangements has no deleterious effect on genome expression. Alternately, it may indicate that although heterozygosity for one Rb fusion may in fact result in an effect, the latter is not sufficient to perturb developmental stability or for it to be detected. In this case, an additive and/or threshold relationship would be expected between the number of heterozygous Rb fusions and disruption of genomic coadaptation. A similar gradient in hybrid unfitness is observed in fertility assays of chromosome heterozygosity in house mice. Whereas the presence of one Rb fusion in a heterozygous state has only a small effect on the fertility of mice, the latter decreases drastically when many Rb fusions are involved (Nachman and Searle 1995).

The difference in hybrid developmental stability between the Belgian and Tunisian chromosomal races, if not due to chromosome structure, may be related to their level of genic differentiation. This level is a function of the extent of reproductive isolation and the maximum time since divergence, which is appraised by the date of colonization by house mice of regions in which chromosomal divergence is observed. Colonization dates, c.5000 years ago for Tunisia, and c.2000 years for Belgium (Auffray 1993; Auffray et al. 1990) and hybrid infertility estimates (Tunisia, Saïd et al. 1993; Belgium, Viroux and Bauchau 1992) suggest that the level of genic differentiation is expected to be higher between the Tunisian races than the Belgian ones. This is supported by estimates from allozyme studies indicating a higher level of differentiation in the Tunisian races

(Saïd and Britton-Davidian 1991) than in European ones (Saïd et al. 1999). Thus the contrasting results in levels of developmental stability between the chromosomal hybrids of these two regions could be due to different levels of genic differentiation, resulting in a higher degree of genomic incompatibility in the Tunisian than in Belgian hybrids.

Furthermore, it has been suggested that the absence of differences in fluctuating asymmetry levels between homozygous chromosomal races may be due to selective processes that restore developmental stability in the initially impaired derived race (Auffray et al. 1999). Such a selective process has received support from studies showing that an impaired developmental stability was rapidly restored following disruption of coadaptation due to the appearance of a mutant allele (Clarke and McKenzie 1987). If a perturbation of developmental stability had occurred in the recently formed Rb race from Belgium, the similar FA levels between the Rb and all-acrocentric races suggests that the restoration would have already taken place. That such a process (perturbation followed by restoration) is unlikely is suggested by results on mice carrying the spontaneous WART mutation, which showed no detectable increase in FA levels compared to those heterozygous for Rb(4.12). Although developmental instability of teeth has been shown to be impaired by a spontaneous chromosomal abnormality in Down syndrome patients (Barden 1980; Shapiro 1983), our study is the first one to report the neutral effect of a spontaneous balanced chromosomal rearrangement on developmental stability. Thus neither spontaneous nor stable single structural rearrangements seem to perturb developmental stability in chromosomal hybrid mice.

Even if based on a small sample size, the absence of a detectable effect related to the Rb(4.19) fusion is remarkable in itself in that it involves chromosome 19, which is only very rarely present in fusions in wild house mice (Britton-Davidian et al. 2000; Nachman and Searle 1995). This rare occurrence, compared to all other autosomes, was thought to be associated with the high selective disadvantage of Rb fusions involving this chromosome. The present study has demonstrated that such a counterselection, if it exists, does not involve impairment of developmental processes. Further studies should address the question of the nature and extent of this selective disadvantage.

The absence of an effect on developmental stability in hybrids carrying Rb(4.12) and Rb (4.19) may be related to the set of chromosomes present in these Rb fusions. Although the genetic basis of developmental stability is for the most part unknown, recent studies in house mice have detected a few quantitative trait loci (QTLs) for FA of mandibular traits, notwithstanding an overall low genetic variability and heritability for FA (Leamy et al. 1997, 1998). These QTLs were localized on many different autosomes including chromosomes 4, 12, and 19. Of interest, QTLs for a given trait FA were not located close to those for size or directional asymmetry of the trait but were adjacent, in some cases, to growth hormone and receptor loci. Even though chromosomes 4, 12, and 19 carry several loci related to growth factors (Mouse Genome Database 1999), our data show that a change in their structure did not lead to a detectable developmental instability of tooth traits. Similarly, Chatti et al. (1999) reported that none of the nine heterozygous Rb fusions they studied appeared to contribute preferentially to the high level of FA in the chromosomal hybrids. In this case, however, since all autosomes were involved except one, the specific effect of one or several Rb fusions could not be accurately tested as they were all partly associated. Thus the influence of individual Rb fusions and/or of specific chromosomal arm combinations on developmental stability has yet to be addressed.

In conclusion, we have shown that, contrary to chromosomal abnormalities, a single balanced rearrangement does not disturb genome activity at any stage of its fixation in populations. Further crossing experiments are now required to determine if the developmental instability observed in hybrids between highly divergent chromosomal groups is due to genic differentiation between them, to one or several specific heterozygous rearrangements and/or to an additive effect of the accumulation of Rb fusions.

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Appendix. Results of the two-way mixed model ANOVAs

Trait	Sample	Individual			Side (df = 1)		Interaction		Error	
		df	MS (× 10000)	F	MS (× 10000)	F	MS (× 10000)	F	df	MS (× 10000)
WM1	ST40	37	32.33	17.25***	0.25	0.13ns	1.87	4.02***	76	0.47
	RB38	38	22.12	24.90***	8.92	10.04*	0.89	3.47***	78	0.26
	[40]F1	39	41.93	16.42***	2.50	0.98ns	2.55	10.96***	80	0.23
	[38]F1	33	43.13	33.27***	12.91	9.96*	1.30	3.81***	68	0.34
	[39]BC40	28	21.08	14.57***	4.36	3.02ns	1.45	3.00***	58	0.48
	[39]BC39	24	20.21	10.61***	7.67	4.03ns	1.91	4.46***	50	0.43
	[40]BC40	23	15.35	6.67***	0.00	0.00ns	2.30	8.25***	48	0.28
	[40]BC39	27	14.22	7.04***	7.56	3.74ns	2.02	10.95***	56	0.18
	WART	4	9.39	5.20ns	0.24	0.13ns	1.81	2.29ns	10	0.79
	CONT	25	12.75	10.59***	1.06	0.88ns	1.20	2.00*	52	0.60
WM2	ST40	37	29.63	18.86***	0.28	0.18ns	1.57	6.91***	76	0.23
	RB38	38	21.00	12.25***	0.86	0.50ns	1.71	12.03***	78	0.14
	[40]F1	39	33.57	36.45***	0.48	0.53ns	0.92	4.99***	80	0.18
	[38]F1	33	51.04	28.95***	4.49	2.54ns	1.76	11.53***	68	0.15
	[39]BC40	28	27.26	26.07***	4.68	4.48ns	1.05	6.01***	58	0.17
	[39]BC39	24	21.77	16.42***	2.53	1.91ns	1.33	9.28***	50	0.14
	[40]BC40	23	20.46	9.71***	10.80	5.13ns	2.11	9.50***	48	0.22
	[40]BC39	27	16.57	11.87***	0.01	0.00ns	1.40	5.90***	56	0.24
	WART	4	18.90	62.38**	0.04	0.13ns	0.30	0.47ns	10	0.64
	CONT	25	16.39	6.42***	0.48	0.19ns	2.55	9.16***	52	0.28
WM3	ST40	36	37.18	12.84***	51.14	17.67**	2.89	4.67***	74	0.62
	RB38	35	13.31	4.41***	3.09	1.02ns	3.02	4.82***	72	0.63
	[40]F1	30	22.75	8.30***	5.54	2.02ns	2.74	6.48***	62	0.42
	[38]F1	29	42.37	10.27***	0.10	0.02ns	4.12	16.71***	60	0.25
	[39]BC40	28	12.99	4.49***	3.01	1.04ns	2.89	6.69***	58	0.43
	[39]BC39	24	15.40	10.51***	0.04	0.03ns	1.46	4.42***	50	0.33
	[40]BC40	22	17.46	8.53***	0.00	0.00ns	2.05	6.27***	46	0.33
	[40]BC39	26	18.67	9.95***	3.31	1.76ns	1.88	3.83***	54	0.49
	WART	3	8.86	1.60ns	8.85	1.60ns	5.53	7.50**	8	0.74
	CONT	20	11.96	3.77**	0.01	0.00ns	3.17	3.92***	42	0.81

* $P < 05$; ** $P < .01$; *** $P < .001$; ns, nonsignificant.

Trait	Sample	Individual df	MS (× 10000)	F	Side (df = 1) MS (× 10000)	F	Interaction MS (× 10000)	F	Error df	MS (× 10000)
LM1	ST40	37	90.60	36.19***	2.53	1.01ns	2.50	5.05***	76	0.50
	RB38	38	38.98	12.45***	0.78	0.25ns	3.13	6.07***	78	0.52
	^{40}F1	39	40.47	15.18***	0.02	0.01ns	2.67	9.01***	80	0.30
	^{38}F1	33	52.98	16.12***	0.96	0.29ns	3.29	10.64***	68	0.31
	^{39}BC40	28	46.70	21.01***	0.00	0.00ns	2.22	7.10***	58	0.31
	^{39}BC39	24	58.48	17.07***	0.83	0.24ns	3.43	5.81***	50	0.59
	^{40}BC40	23	39.83	17.29***	0.17	0.07ns	2.30	5.49***	48	0.42
	^{40}BC39	27	53.24	24.95***	1.40	0.65ns	2.13	5.03***	56	0.42
	WART	4	19.68	19.97*	0.16	0.16ns	0.99	1.24ns	10	0.80
	CONT	25	32.13	12.16***	7.11	2.69ns	2.64	4.80***	52	0.55
LM2	ST40	37	36.23	19.60***	10.42	5.64ns	1.85	7.38***	76	0.25
	RB38	38	54.54	15.11***	5.96	1.65ns	3.61	10.79***	78	0.33
	^{40}F1	39	80.59	30.65***	4.39	1.67ns	2.63	7.00***	80	0.38
	^{38}F1	33	40.22	25.04***	19.05	11.86*	1.61	3.22***	68	0.50
	^{39}BC40	28	43.39	17.14***	4.36	1.72ns	2.53	4.40***	58	0.58
	^{39}BC39	24	47.73	12.76***	2.31	0.62ns	3.74	8.87***	50	0.42
	^{40}BC40	23	18.79	8.71***	13.65	6.33ns	2.16	4.26***	48	0.51
	^{40}BC39	27	64.61	34.96***	3.40	1.84ns	1.85	3.93***	56	0.47
	WART	4	59.95	122.67**	12.80	26.19ns	0.49	0.62ns	10	0.78
	CONT	25	44.24	16.63***	1.21	0.45ns	2.66	3.15***	52	0.84
LM3	ST40	36	37.23	10.74***	10.22	2.95ns	3.47	3.73***	74	0.93
	RB38	35	41.97	13.50***	3.33	1.07ns	3.11	3.79***	72	0.82
	^{40}F1	30	74.82	18.50***	0.16	0.04ns	4.04	7.24***	62	0.56
	^{38}F1	29	89.86	25.93***	0.00	0.00ns	3.47	8.82***	60	0.39
	^{39}BC40	28	60.71	19.37***	0.78	0.25ns	3.13	5.62***	58	0.56
	^{39}BC39	24	64.13	25.30***	1.85	0.73ns	2.53	6.35***	50	0.40
	^{40}BC40	22	63.77	27.27***	3.68	1.57ns	2.34	2.50**	46	0.93
	^{40}BC39	27	36.51	12.18***	0.05	0.02ns	3.00	3.01***	56	1.00
	WART	3	22.14	8.24ns	7.70	2.87ns	2.69	1.69ns	8	1.59
	CONT	20	9.97	3.76**	0.84	0.32ns	2.65	1.95*	42	1.36