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Allozyme heterozygosity and fluctuating asymmetry in the brown hare (*Lepus europaeus*): a test of the developmental homeostasis hypothesis

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SUMMARY

The influence of allozyme heterozygosity on developmental homeostasis as indicated by fluctuating morphological asymmetry (FA) has been a controversial issue in evolutionary studies. In the present investigation, relationships between overall individual heterozygosity (H), calculated over 13 polymorphic enzyme loci, and fluctuating asymmetry (FA) in 27 non-metric and 9 metric bilateral skull and mandible traits were examined in a total of 417 brown hares. The respective tests were performed separately for juveniles and adults within and among five geographic arrays of samples, and among 17 single sampling localities. Within geographic units, neither in metric nor in non-metric characters could a clear relationship between FA and H be detected. Among geographic units and single sampling localities, for metric traits the result remained the same. In non-metric traits, however, a significant negative correlation between overall FA and $\bar{H}$ became apparent in adults. Thus, fluctuating asymmetry and heterozygosity are inversely related also in a mammalian species. The long lasting dispute as to the existence of a homeotherm–poikilotherm dichotomy may have been the result of a disregard of non-metric traits and of an interpopulation approach for assessing relationships between morphological FA and H in mammals.

1. INTRODUCTION

Based on a summary of earlier work on genetic variability and phenotypic variance, Lerner (1954) proposed that heterozygosity stabilizes development by buffering against environmental insult so that genetically determined pathways are more precisely expressed in the phenotype of an individual. Since that time the relationship between minor, nondirectional deviations from bilateral symmetry in morphological characters (fluctuating asymmetry: FA) and heterozygosity has been the subject of many studies. The results of these studies have been inconsistent, raising questions about the existence and potential causes of an association between heterozygosity and developmental stability (see Mitton & Grant 1984; Allendorf & Leary 1986; Palmer & Strobeck 1986; Mitton 1993, for reviews). Apart from inconsistencies due to differences in the various statistical approaches used (Palmer & Strobeck 1986), there are at least four possible biological reasons to account for the inconsistency of results.

First, heterozygosity has been assessed in different ways: by investigating inbred versus crossbred strains (see, for example, Mather 1953; Brückner 1976), interspecific hybridization (e.g. Lamb *et al.* 1990; Ross & Robertson 1990), variation for semi-lethal and subvital genes (e.g. Lewontin 1956), and by electrophoretic analysis of allozyme variation (see Mitton & Grant 1984; Mitton 1993, for reviews). Thus depending on the kind of analysis, an increased level of FA may have been found as a result of reduced heterozygosity *per se*, of homozygosity of deleterious genes, of the disruption of coadapted gene complexes, or a combination of these factors.

Secondly, the types of morphological characters used (metric/continuous or non-metric/meristic) vary widely among studies. This raises the possibility that the association between heterozygosity and developmental stability may differ among characters. For example, it may be stronger among those characters that have increased FA such as those with low heritability or little functional constraint (see Leary *et al.* 1985; Palmer & Strobeck 1986; Stearns 1992).

Thirdly, relationships between heterozygosity and developmental homeostasis have been examined both with respect to differences in heterozygosity and FA among individuals of the same population and to differences in mean heterozygosity and mean FA across populations. The expected negative association was more frequently detected in the latter than in the former case but the interpretation of these results is potentially confounded by environmental differences among populations (see Palmer & Strobeck 1986, for review).

Fourthly, besides FA, interindividual variation in

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morphological characters has also been used as an indicator of developmental homeostasis (e.g. Eanes 1978; Mitton 1978; Handford 1980; Fleischer *et al.* 1983; Yezerinac *et al.* 1992). This is justified only in traits with low heritability. Otherwise, morphological variation among individuals could be largely due to additive genetic variation, which is not indicative of developmental accidents (Soulé 1979; Allendorf & Leary 1986; Kieser & Groeneveld 1991).

Most of the negative associations between heterozygosity and FA reported so far have been detected in invertebrates and poikilothermic vertebrates (see Allendorf & Leary 1986; Palmer & Strobeck 1986; Beacham 1991, for reviews). In birds and mammals, findings are less consistent and it has been suggested that heterozygosity may be more closely related to developmental homeostasis in poikilothermic than in homeothermic species (Handford 1980; Wooten & Smith 1986). However, in birds interindividual morphological variation only has been used to estimate developmental stability (Handford 1980; Fleischer *et al.* 1983; Yezerinac *et al.* 1992), which may not be a valid approach (see above). In mammals, the few studies available have been limited as to the number and type (as yet only metric traits considered) of morphological characters (Smith *et al.* 1983; Wayne *et al.* 1986; Wooten & Smith 1986; Scribner & Smith 1990; Hartl *et al.* 1991; Kieser & Groeneveld 1991). Thus the inconsistent results obtained in mammals might reflect the choice of characters used. Similarly, an apparent difference in the significance of heterozygosity for developmental homeostasis among homeothermic and poikilothermic species may be due to a preponderance of non-metric traits examined in the latter.

In this study we asked whether there is a negative relationship between allozyme heterozygosity and FA in mammals and whether the result is different when comparing heterozygosity and FA among populations rather than among individuals from one population. We also examined the extent to which the relationship between heterozygosity and FA depends on the type of morphological characters investigated by scoring a large set of metric and non-metric traits; belonging to various morphological systems. As a model we chose a widespread, free-ranging mammal without known bottlenecks in population size, the European brown hare (*Lepus europaeus*).

2. MATERIALS AND METHODS

This study is based on 417 brown hares from 19 sampling localities in Austria (see figure 1). All animals were obtained during regular hunts in autumn 1988. Due to ontogenetic factors (body growth, differential mortality), relationships between FA and heterozygosity may differ between juveniles and adults, and so all analyses were carried out separately for both age classes. Dry eye lens mass (see Suchentrunk *et al.* 1991) was used to identify juvenile (not older than 6–8 months) and adult individuals.

To account for uneven age distributions within sampling localities and to obtain reasonable sample sizes for both intra- and interpopulational studies, we pooled the 19 sampling localities into larger units. For examining relationship between heterozygosity and fluctuating asymmetry, four groups of sampling localities (NWA, NEA, EA, SA; see figure 1) were formed, based on geomorphologic criteria (see figure 1) and ecogeographic differences across the study area (Margl 1982). In particular, the geographic units NEA and EA (separated by the river Danube) represent hares from favourable, NWA hares from less favourable, and SA hares from unfavourable habitats, as indicated by the key parameters for the quality of hare habitats: precipitation and temperature (Spittler 1976; Zörner 1981; Eiberle & Matter 1982). According to electrophoretic and mtDNA data (see Hartl *et al.* 1993) there is no population genetic objection against this arrangement. The three remaining sampling localities (VRT, OP, and GB, see figure 1) which could not be assigned to one of these natural geographic units because of ecogeographic or population genetic objections (cf. Hartl *et al.* 1993), were pooled to a fifth, intentionally artificial or mixed group (MG). It was used to test the effect of mixing specimens from very different geographic origins on the perceptibility of relationships between heterozygosity and FA. Altogether, two age classes in five geographic units resulted in a set of ten study groups. In all analyses concerning single skull and mandible traits, sequential Bonferoni procedures were used with a nominal α of 0.05 to correct the critical values for multiple tests (Rice 1989).

(*a*) *Allozyme heterozygosity*

Data on individual allozyme heterozygosity were taken from a previous investigation (see Hartl *et al.* 1993). The alleles found at 13 polymorphic loci and mean levels of heterozygosity across the 19 sampling localities examined are listed in table 1. Overall individual heterozygosity (H) was calculated by dividing the number of loci heterozygous in each individual by all loci found to be polymorphic in this study. H was not sex dependent in our material (Hartl *et al.* 1993). Relationships between H and age (dry eye lens masses) within study groups were examined using Spearman rank correlation analysis. For comparison among study groups, in each age class per geographic unit mean H ($\bar{H}$) was calculated as the arithmetic mean over all individuals. The influence of study group on levels of $\bar{H}$ was tested by means of a two-way ANOVA using arc-since transformed H-values. In each age class, $\bar{H}$ was tested for significant differences among single samples using the Kruskal–Wallis test.

(*b*) *FA in non-metric traits*

A total of 27 non-metric skull characters were used for assessing asymmetry in non-metric traits. For each trait only the presence or absence of symmetry (i.e. the occurrence of equal or different character states on both body sides) was scored (see Appendix). For differentiating between fluctuating and directional

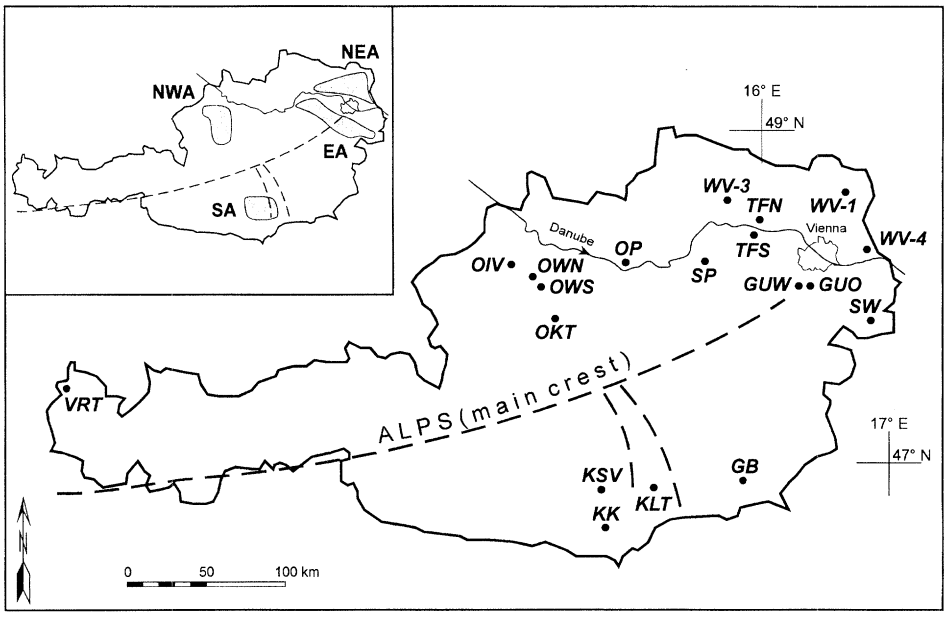

Figure 1. Sampling sites of the brown hare in Austria (districts and sample sizes in parentheses). VRT = Fussach, Höchst, Gaißau (Bregenz, $n = 12$), OIV = Wendling (Grieskirchen, $n = 25$), OWN = Edt/Lambach, Steinerkirchen (Wels-Land, $n = 26$). OWS = Ried i. Trkr. (Kirchdorf/Kr., $n = 23$), OKT = Nußbach (Kirchdorf/Kr., $n = 13$), OP = Naarn (Perg, $n = 16$), WV-3 = Hohenwarth (Hollabrunn, $n = 28$), TFN = Bierbaum, Frauendorf (Tulln, $n = 30$), WV-1 = Bullendorf (Mistelbach, $n = 30$), WV-4 = Zwerndorf (Gänserndorf, $n = 30$), SP = Haindorf, Markersdorf (St. Pölten-Land, $n = 23$), TFS = Rust (Tulln, $n = 20$), GUW and GUO = Guntramsdorf (Baden, $n = 20$ and 15, respectively), SW = Illmitz (Neusiedl/S., $n = 24$), GB = Gralla (Leibnitz, $n = 26$), KLT = St. Margarethen/L. (Wolfsberg, $n = 24$), KK = Grafenstein, Poggersdorf (Klagenfurt-Umg., $n = 15$), KSV = St. Georgen, Goggerwenig, Mente, Schratt (St. Veit/Gl., $n = 17$). The four natural geographic units used in the present analysis are depicted in the insert: NWA = northwestern Austria, NEA = northeastern Austria, EA = eastern Austria, SA = southern Austria. A fifth, mixed geographic unit (MG) was created by pooling the specimens from VRT, OP, and GB.

Table 1. *Enzyme systems screened, and loci and alleles detected in the brown hare*

($\bar{h}$ = mean observed heterozygosity across 19 populations, s.d. = standard deviation.)

enzyme system (abbreviation, E.C. number)	locus (alleles)	$\bar{h}$ (s.d.)
Sorbitol dehydrogenase (SDH, 1.1.1.14)	*Sdh* (100, 300)	0.007 (0.03)
Lactate dehydrogenase (LDH, 1.1.1.27)	*Ldh-2* (100, 83)	0.005 (0.02)
Malate dehydrogenase (MDH, 1.1.1.37)	*Mdh-2* (100, 79)	0.042 (0.09)
Isocitrate dehydrogenase (IDH, 1.1.1.42)	*Idh-2* (100, 130, 83)	0.054 (0.08)
6-phosphogluconate dehydrogenase (PGD, 1.1.1.44)	*Pgd* (100, 170, 129, 117, 64)	0.108 (0.10)
Hexokinase (HK, 2.7.1.1)	*Hk-2* (100, 67)	0.016 (0.03)
Esterases (ES, 3.1.1.1)	*Es-I* (−100, −108, −75, −42)	0.482 (0.12)
	Es-d (100, 141)	0.294 (0.16)
Peptidases (PEP, 3.4.11)	*Pep-2* (100, 114, 104)	0.292 (0.14)
Aminoacylase-1 (ACY-1, 3.5.1.14)	*Acy-1* (100, 81, 66)	0.669 (0.10)
Adenosine deaminase (ADA, 3.5.4.4)	*Ada-2* (100, 121, 75)	0.238 (0.12)
	Ada-3 (100, 111)	0.196 (0.15)
Mannosephosphate isomerase (MPI, 5.3.1.8)	*Mpi* (100, 126, 77)	0.035 (0.06)

asymmetry we used Wilcoxon tests, and no cases of directional asymmetry were detected. In single non-metric traits, the occurrence of pairwise associations of FA among characters, sex and age dependence of FA within each study group, and the influence of age class and geographic unit on the distribution of FA were tested by chi-square and Kruskal-Wallis tests, respectively. We also examined whether the tendency of a non-metric trait to show FA generally depends on the number and the respective frequencies of character states found in this trait. For this purpose inter-individual variability of each trait (IV) was assessed in each of the ten study groups using the Shannon–Weaver information function:

$$IV = -\sum_{i=1}^{n} p_i \cdot \ln(p_i),$$

where n is the number of character states found on one body side (see Appendix), and p_i denotes the group-specific frequency of each character state. Variation of IV in single non-metric traits among sexes, study groups, and single sampling localities, respectively, was

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

examined by the Kruskal–Wallis test. In each of the ten study groups, the relationship between FA and IV of the 27 traits was tested using Spearman rank correlation analysis. Following the procedure outlined by Soulé & Baker (1968) we calculated the coefficient of concordance (Kendall's W) of FA in 27 non-metric traits separately for geographic units.

As an index for overall non-metric fluctuating asymmetry (FA_{NM}) we chose the proportion of traits asymmetric per individual (Leary *et al.* 1985). In 253 skulls at least one morphological trait was not scorable due to damage by shooting. To save large sample sizes of both individuals and characters we evaluated the influence of missing values on estimates of FA_{NM} in the following way. Separately for each age class we calculated FA_i, $i = 1(1)27$, the mean fluctuating asymmetry for each trait over all individuals where this trait was scorable. Then, in each individual with at least one unscorable trait, we computed the mean of the respective FA_i-values over all missing traits and defined the resulting value as the 'non-metric defect' (DF_{NM}). Calculated in this way, a high DF_{NM}-value found in an individual is indicative of one or more missing traits with a high tendency towards FA. Using Spearman rank correlation analysis, separately in each age class we tested for a negative relationship between DF_{NM} and FA_{NM} using all individuals with at least one unscorable trait, which would indicate that estimates of FA_{NM} are biased due to missing traits. In adults no such relationship was detected. In juveniles there was a weak, but statistically significant negative correlation ($r_s = -0.15$, $p = 0.05$, one-tailed probability). Thus DF_{NM} was considered in all analyses concerning the relationship between heterozygosity and FA_{NM} (see below). Relationships between FA_{NM} and age (dry eye lens masses) within groups were examined using Spearman rank correlation analysis. For comparisons among study groups, mean FA_{NM} ($\overline{FA}_{NM}$) was calculated as the arithmetic mean of FA_{NM} over all individuals in the respective group. The influence of age class and geographic unit on levels of $\overline{FA}_{NM}$ was checked by means of a two-way ANOVA using arc-sine transformed $\overline{FA}_{NM}$-values.

(*c*) *FA in metric traits*

For assessing FA in metric traits, twelve bilateral skull and mandible measurements were taken on the left and right side of each specimen using calipers (see figure 2). Measurements were taken exclusively by one of the authors (F.S.), eliminating the possible inter-observer variability (Lee 1990). The relative contribution of measurement error to asymmetry was determined as follows. Each measurement was repeated for three times on each side in 20 individuals. This produced a total data set of 120 measurements (2 sides × 3 repeated measurements × 20 individuals) in each metric trait. Based on this data set a two-way ANOVA (individual, side) was carried out for each metric trait. We then used the ratio of: (variance due to side + variance due to side × individual interaction)/residual variance as indication of the relative influence of measurement error on asymmetry. If the sum of the variance due to side and of the variance due to side × individual interaction was at least twice as high as the residual variance we considered the influence of the measurement error on the asymmetry assessment negligible. Using this criterion, fluctuating asymmetry estimates were not affected by measurement error (see §3).

To test for the occurrence of directional asymmetry we used sign-tests (right versus left measure). Antisymmetry was examined using Kolmogorov–Smirnov tests of the frequency distributions of right–left paired differences compared to an expected normal distribution (Palmer & Strobeck 1986). Size dependence of asymmetry in each metric trait was examined using Spearman rank correlations (difference between right–left against their mean). Although no significant size dependencies occurred in any trait (no growth factor), we chose an asymmetry-index, that accounted for potential size differences. This allowed us to calculate an overall asymmetry-index for each individual, even so if not all of the traits could be scored (see below). For single traits the following index was used:

$$|R - L|/[(R + L)/2],$$

where R and L are the measurement on the right and the left body side, respectively. This formula corresponds to the *FA*-index 2 of table 1 in Palmer & Strobeck (1986), who compared several indices for evaluation of FA. In each trait, sex dependence of asymmetry was examined using the Kruskal–Wallis test, and age dependence of asymmetry using Spearman rank correlations between individual dry eye lens masses and *FA* values. Pairwise intercorrelations of asymmetry among the various metric characters were examined by Spearman rank correlations. Influences of age class and geographic unit on the distribution of asymmetry in single metric characters were checked by two-way ANOVAs (see Willig & Owen 1987). After rejecting those traits which showed directional asymmetry, antisymmetry, and age or sex dependence among individuals (see §3), the remaining 9 (CL, UTL, DIA, UML, NL, ZAL, MDL, LML, DH, see figure 2) were considered in all further analyses. The coefficient of concordance (Kendall's W) of *FA* in these nine metric traits was calculated as described for non-metric traits.

An overall *FA*-index that combines all metric traits within one individual (FA_M) was calculated as the arithmetic mean of all scorable *FA*-values. We evaluated the relationship between the 'metric defect' (DF_M) and FA_M in the same way as described for non-metric traits. Neither in juveniles nor in adults was a significant negative relationship detected. Nevertheless, DF_M was considered in all analyses concerning the relationship between heterozygosity and FA_M (see below). Relations between FA_M and age (dry eye lens masses) within geographic units and age classes were examined using Spearman rank correlation analysis. For comparison among study groups, mean FA_M ($\overline{FA}_M$) was calculated as the arithmetic mean over all individuals in the respective group. The influence of age class and geographic unit on levels of $\overline{FA}_M$ was checked by means of a two-way ANOVA.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

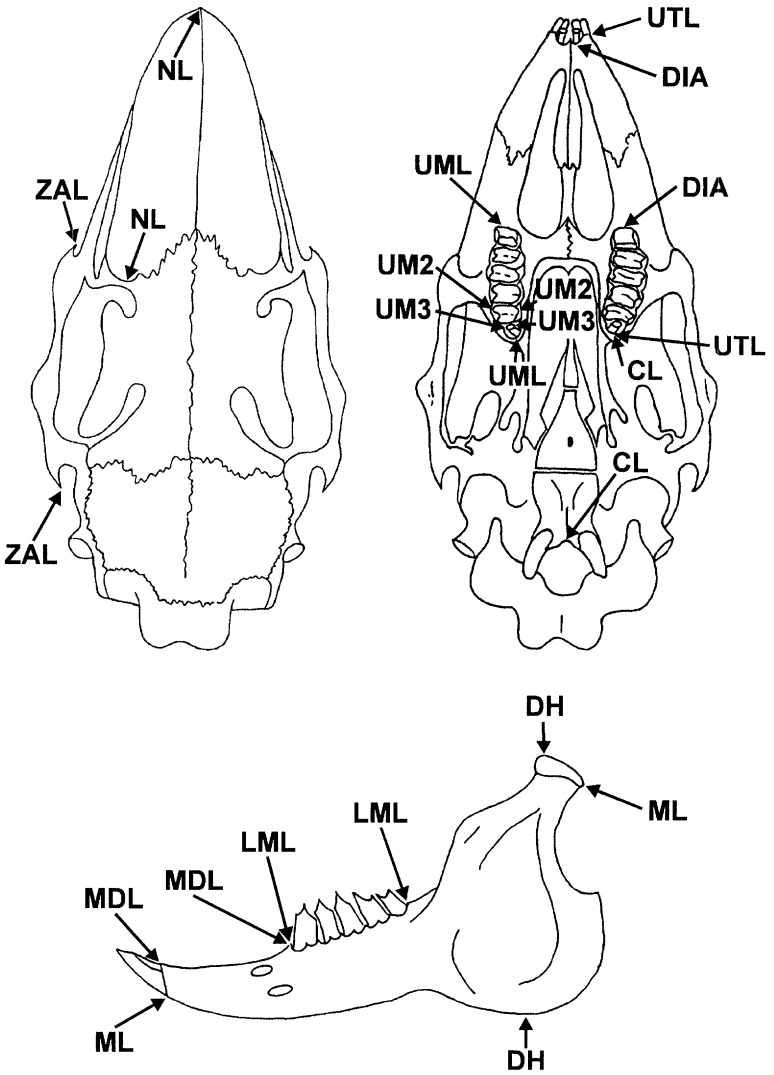

Figure 2. Bilateral and mandible skull measurements (metric traits) examined in the brown hare. CL = cranium length, DH = dentale height, DIA = diastema length, LML = lower molar row length, MDL = mandibular diastema length, ML = mandible length, NL = nasal length, UML = upper molar row length, UM2 = bucco-lingual diameter of second upper molar, UM3 = bucco-lingual diameter of third upper molar, UTL = upper teeth row length, ZAL = zygomatic arch length. The two points used for taking each measurement are indicated by arrows.

(*d*) *Relationships between non-metric and metric FA*

Within each geographic unit and age class, pairwise associations between FA in single non-metric and in single metric traits were examined using the Kruskal–Wallis test. Relationships between FA_{NM} and FA_M within and among study groups were examined by Spearman rank correlations.

(*e*) *FA and heterozygosity*

Allozyme heterozygosity and morphological asymmetry were examined. Within each study group, individual average heterozygosity over 13 polymorphic loci (H) was examined for correlations with FA in single metric traits using Spearman rank correlations. In single non-metric traits, associations among H and FA were examined using the Kruskal–Wallis test. Across geographic units, FA in single non-metric and metric traits (averaged over individuals per age class and geographic unit) was examined for correlations with $\bar{H}$ by means of the same method. All analyses concerning the respective relationships between H and FA_{NM} or FA_M within geographic units, and between $\bar{H}$ and $\overline{FA}_{NM}$ or $\overline{FA}_M$ across geographic units and single sampling localities were conducted by means of Spearman rank correlations.

Because Palmer & Strobeck (1986) stated that *FA* indices based on the absolute values |R–L| (right–left) are less useful for detecting true differences in FA among populations than those indices based on the variance (R–L) among individuals within each group, we recalculated mean metric *FA* in single traits for each age class, geographic unit, and single sampling locality using the following index (see index 6, table 1 in Palmer & Strobeck 1986):

$$\mathrm{var}\{(R-L)/[(R+L)/2]\}.$$

For estimating individual FA_M, we calculated the arithmetic mean over the scorable metric traits. Both mean metric *FA* in single traits and $\overline{FA}_M$ were examined

for a relationship with $\bar{H}$ across geographic units and single sampling localities, respectively, as described above.

3. RESULTS

(a) *Allozyme heterozygosity*

Allozyme heterozygosity (H) was not age dependent in any of the ten study groups examined. $\bar{H}$ was different among geographic units ($p = 0.001$) but not among age classes, both when the four natural and when all five geographic units were considered. Also at the level of single sampling localities, $\bar{H}$ was significantly different among sites ($p < 0.05$).

(b) *FA in non-metric traits*

No cases of directional asymmetry were found. There was no sex and age dependence of FA in any trait, and no associations of FA among characters within the ten study groups. Regarding differences in FA among age classes and geographic units, NM13 was significantly more asymmetric in adults of NWA ($p = 0.001$). When the tendency towards asymmetry of a character (see table 2) was examined for a relationship with the diversity of character states (which was not dependent on sex, study group or sampling locality) a high correlation became apparent in each study group ($r_s = 0.64$–0.94, $p < 0.001$ in all cases). Kendall's W over the 27 traits, examined separately for each age class, was constantly low (0.002–0.079) and statistically not significant among the four natural or all five geographic units. FA_{NM} was not age dependent within study groups, and there was no significant influence of age class and geographic unit on $\overline{FA}_{NM}$.

Table 2. *Fluctuating asymmetry (FA) in non-metric characters scored in this analysis*

(*FA* for each trait is given as the weighted mean of the percentage of individuals asymmetric over five geographic units and its standard deviation (s.d.) in juveniles and adults. n = total sample size of individuals for each trait. For description of character states see Appendix.)

	juveniles			adults		
code	mean *FA*	s.d. *FA*	*n*	mean *FA*	s.d. *FA*	*n*
NM1	0.155	0.359	212	0.297	0.456	165
NM2	0.393	0.491	219	0.341	0.478	170
NM3	0.144	0.353	222	0.161	0.367	174
NM4	0.489	0.501	229	0.548	0.500	179
NM5	0.294	0.457	214	0.272	0.445	162
NM6	0.209	0.410	201	0.168	0.373	149
NM7	0.293	0.458	232	0.243	0.428	181
NM8	0.286	0.452	238	0.239	0.430	184
NM9	0.225	0.412	235	0.161	0.345	180
NM10	0.296	0.469	220	0.306	0.213	183
NM11	0.219	0.403	233	0.265	0.444	181
NM12	0.060	0.218	230	0.022	0.128	179
NM13	0.175	0.369	234	0.309	0.445	168
NM14	0.008	0.051	240	0.0	0.0	189
NM15	0.292	0.456	243	0.278	0.450	187
NM16	0.246	0.428	240	0.231	0.423	186
NM17	0.022	0.134	234	0.027	0.138	186
NM18	0.215	0.407	228	0.173	0.369	179
NM19	0.112	0.312	231	0.126	0.331	174
NM20	0.004	0.024	237	0.006	0.022	184
NM21	0.0	0.0	233	0.006	0.022	182
NM22	0.0	0.0	237	0.032	0.137	185
NM23	0.0	0.0	229	0.006	0.038	173
NM24	0.126	0.324	223	0.234	0.416	175
NM25	0.0	0.0	243	0.005	0.026	186
NM26	0.054	0.196	205	0.050	0.169	141
NM27	0.017	0.096	233	0.055	0.213	183

Table 3. *Bilateral metric characters scored in the present analysis*

(FA in each trait is given as the weighted mean of mean *FA*-values over five geographic units and its standard deviation (s.d.) for juveniles and adults. n = sample size, ME = influence of measurement error on *FA* (ratio of the sum of mean squares of side and of the side–individual–interaction component of variance to residual as found by respective two-way ANOVAs involving repeated measurements of the two body sides in 20 individuals. For description of skull and mandible measurements see figure 2.)

	juveniles			adults			
code	mean *FA*	s.d. *FA*	*n*	mean *FA*	s.d. *FA*	*n*	ME
CL	0.0057	0.0060	212	0.0068	0.0071	164	18.9:1
UTL	0.0050	0.0045	225	0.0058	0.0056	177	6.4:1
DIA	0.0090	0.0083	230	0.0092	0.0099	178	9.1:1
UML	0.0087	0.0084	233	0.0126	0.0160	184	3.0:1
NL	0.0072	0.0060	234	0.0071	0.0062	188	5.1:1
ZAL	0.0082	0.0063	198	0.0088	0.0083	147	4.2:1
MDL	0.0122	0.0104	225	0.0130	0.0123	171	23.2:1
LML	0.0093	0.0096	223	0.0115	0.0122	171	3.2:1
DH	0.0064	0.0065	209	0.0073	0.0053	149	2.5:1

(c) *FA in metric traits*

There was no antisymmetry, but directional asymmetry was detected in the following characters (character in age class of geographic unit): ML in juveniles of NEA; UM2 in juveniles of EA; UM3 in adults of NWA; and ML in adults of EA. Asymmetry was not age or sex dependent in any trait, and not intercorrelated among characters. For the following analyses, ML, UM2, and UM3 were rejected because of the occurrence of directional asymmetry. For each of the nine remaining tracts, mean *FA* over five geographic units for juveniles and adults, and estimates of the associated measurement error are given in table 3. The two-way ANOVA (age class and geographic unit) revealed an influence of the age class on FA in UML ($p < 0.001$). Kendall's W across the four natural geographic units was higher in adults ($W = 0.161$) than in juveniles ($W = 0.042$), but nevertheless low and statistically insignificant. Across all five units, W was similarly low and did not differ between age classes. FA_M was not age dependent within groups. When levels of $\overline{FA}_M$ were tested for the simultaneous influence of age and geographic unit, in all three sample sets they turned out to be higher in adults (see table 3, $p < 0.05$) but independent of geographic units.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 4. *Relationships between heterozygosity and fluctuating asymmetry at the individual level*

(For grouping of samples into geographic units see figure 1. In each of these groups, juveniles and adults were treated separately, resulting in a total of ten groups consistently used in the present analysis. n = sample size in each group, H = overall heterozygosity (over 13 polymorphic loci), FA_{NM} = overall non-metric fluctuating asymmetry (over 27 non-metric traits), FA_M = overall metric fluctuating asymmetry (over nine metric traits), r_s = Spearman correlation coefficient.[a])

geographic unit:	NWA	NEA	EA	SA	MG
juveniles (n)	43	62	62	31	37
mean H	0.227	0.172	0.178	0.185	0.196
mean FA_{NM}	0.171	0.152	0.156	0.143	0.174
r_s H/FA_{NM}	0.18	0.25[a]	0.15	0.10	0.14
mean FA_M	0.009	0.007	0.008	0.008	0.009
r_s H/FA_M	−0.17	0.10	0.05	−0.35[a]	0.03
adults (n)	44	56	40	25	17
mean H	0.194	0.146	0.196	0.160	0.224
mean FA_{NM}	0.166	0.177	0.161	0.168	0.161
r_s H/FA_{NM}	0.08[b]	−0.05	0.16	0.13[b]	−0.17
mean FA_M	0.009	0.009	0.009	0.010	0.009
r_s H/FA_M	0.10	0.11	0.13	−0.16	0.51[a]

[a] Statistically significant at $p < 0.05$.
[b] FA_{NM} positively correlated with FA_M at $p < 0.05$.

(*d*) *Relationships between metric and non-metric FA*

No significant relationship between FA in single non-metric traits and FA in single metric traits was found. Significant relationships between FA_M and FA_{NM} within each of the ten study groups are indicated in table 4. Significant relationships between $\overline{FA}_{NM}$ and $\overline{FA}_M$ across geographic units are indicated in table 5.

(*e*) *FA and heterozygosity*

Within the ten study groups examined, overall individual heterozygosity (H) was not significantly associated with FA in single non-metric or metric traits. The relationship between H and FA_{NM} was generally more positive in juveniles than in adults. This pattern was confirmed by the few statistically significant correlation coefficients (see table 4). Regarding the relationship between H and FA_M the general pattern of correlation coefficients did not reveal any trend for a difference between juveniles and adults. With the exception of NWA, correlation coefficients were either positive or negative in both age classes of a geographic unit (see table 4).

When examined separately in each age class for relationships with $\bar{H}$ across geographic units and single sampling locations, neither mean FA in single non-metric nor in single metric traits was significantly correlated with $\bar{H}$, irrespective of the type of index used in metric traits. Nevertheless, in all cases correlation coefficients showed a tendency to be more negative (or less positive) in adults than in juveniles. Across geographic units, $\overline{FA}_M$ was consistently positively related to H in juveniles. In adults the relationship between mean $\overline{FA}_M$ and $\bar{H}$ was less positive or even negative, but not statistically significant (see table 5). Also in non-metric traits, $\overline{FA}_{NM}$ was positively related to $\bar{H}$ in juveniles, but FA_{NM} in adults was consistently negatively correlated with $\bar{H}$ (see table 5). DF_{NM} (the mean non-metric defect in a group, calculated over individuals with missing values) was significantly negatively correlated with $\bar{H}$ in juveniles (see table 5).

Table 5. *Relationships (r_s values) between heterozygosity and fluctuating asymmetry at the level of the population (geographic units, single sampling locations)*

($\bar{H}$ = mean overall heterozygosity (calculated over 13 polymorphic loci); $\overline{FA}_{NM}$ = mean overall non-metric fluctuating asymmetry (calculated over 27 non-metric traits); $\overline{FA}_M$ = mean overall metric fluctuating asymmetry (calculated over nine metric traits); r_s = Spearman correlation coefficient; 4GU = 4 geographic units; 5GU = 5 geographic units; SS = single sampling locations with specimens available in each age class. $\overline{FA}_M$ was calculated using two different indices, one based on the mean of the absolute values of |R–L| ($\overline{FA}_M - 1$), the other based on the variance of (R–L) ($\overline{FA}_M - 2$).)

	$\overline{FA}_{NM}$	$\overline{FA}_M - 1$	$\overline{FA}_M - 2$
juveniles			
$\bar{H}$ (4GU)	0.40	0.80	1.00[g]
$\bar{H}$ (5GU)	0.60[a]	0.80[b]	0.90[c]
$\bar{H}$ (17SS)	0.66[a,f]	0.47[d]	0.71[h]
adults			
$\bar{H}$ (4GU)	−1.00[g]	0.40	0.20
$\bar{H}$ (5GU)	−0.90[e]	−0.30	−0.40
$\bar{H}$ (17SS)	−0.44[c]	0.0	0.11

[a] $\bar{H}$ negatively correlated with DF_{NM} at $p < 0.5$.
[b] $\overline{FA}_M$ positively correlated with $\overline{FA}_{NM}$ at $p < 0.05$.
[c] $p = 0.040$.
[d] $p = 0.028$.
[e] $p = 0.019$.
[f] $p = 0.002$.
[g] $p < 0.001$.
[h] $p = 0.001$.

4. DISCUSSION

(*a*) *FA in skull and mandible characters*

Albeit FA in our data set is generally low, we observed considerable differences among various non-metric traits (see table 2). Especially tooth characters are obviously only slightly affected by FA (cf. Wayne *et al.* 1986; Suchentrunk 1993; Suchentrunk *et al.* 1994). It has been proposed by various authors (e.g. Palmer & Strobeck 1986; Stearns 1992) that functionally important traits are subjected to stronger stabilizing selection which should result in increased bilateral symmetry. Our results show that levels of FA in non-metric traits are highly positively correlated with character state diversity in the respective traits. Thus if there are differences in development constraints among non-metric traits they are not only reflected by varying tendencies towards FA, but also by the general incidence of non-metric variants.

Within our study groups, there was practically no association of FA among single non-metric and metric

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

traits, respectively. Across geographic units or age classes, Kendall's coefficients of concordance among the single non-metric traits were generally low and statistically not significant. Essentially the same was found for metric traits. These results demonstrate that FA in a given character is hardly predictive for FA in other characters, both within and among geographic units and age classes. Heterogeneity of FA across populations due to a significant concordance of FA among characters has been defined to be the 'population asymmetry parameter' (PAP) by Soulé (1967). In contrast to a significant concordance of FA among characters across populations of a satyrine butterfly (Soulé & Baker 1968; $W = 0.36$, $p < 0.05$) and of the house mouse (Leamy 1984; $W = 0.35$, $p < 0.01$), our data do not support the existence of a PAP among free-ranging populations of the brown hare. As an important methodological consequence for relating FA in morphological characters to overall heterozygosity, the use of FA_{NM} and FA_{M} may yield results quite different from those obtained by considering FA separately for single non-metric or metric traits.

(*b*) *FA and heterozygosity within geographic units*

Our results show that there is no clear relationship between overall individual heterozygosity (H) and overall individual non-metric (FA_{NM}) and metric FA (FA_{M}) within age classes and geographic units. There is also practically no relationship between FA in single metric traits and H. Because even in the mixed group, consisting of specimens of very distinct geographic origin, the findings are in accordance with the general trends observed, we consider pooling of samples from slightly different environments not responsible for these results.

Our data are in accordance with previous studies, which failed to detect a relationship between metric FA and H within populations of mammals and supported the hypothesis of heterozygosity influencing developmental homeostasis only in poikilothermic animals (Wooten & Smith 1986). When emphasising differences in the influence of allozyme heterozygosity on developmental homeostasis among homeothermic and poikilothermic taxa it has been commonly overlooked that most of the negative associations among H and FA observed in the latter were based on non-metric (meristic) traits (see Beacham 1991, for review), which have not been considered in mammals so far. Consequently, we decided to test whether the 'poikilotherm–homeotherm dichotomy' is a result of the type of morphological characters investigated and re-examined relationships between H and FA within geographic units and age classes using a large set of non-metric traits. However, as in the metric traits, also overall individual non-metric FA (FA_{NM}) and FA in single non-metric traits did not show a clear relationship to H. These results remained the same, when the whole was repeated only using the individuals without missing values ($n = 164$).

Apart from the type of morphological characters examined, a further possible influence on the perceptibility of a relationship between H and FA within populations had to be considered. According to theoretical considerations and mathematical models, allozyme heterozygosity as calculated over one or two dozen of polymorphic loci should be in fact representative only for a very limited part of the genome (Mitton & Pierce 1980; Chakraborty 1981; Chakraborty & Ryman 1983; Mitton & Grant 1984). Thus as demonstrated by Palmer & Strobeck (1986), H is more likely to be indicative for differences in general heterozygosity among individuals from different populations rather than among individuals from the same population. In fact, all studies which failed to detect a negative relationship between H and FA in mammals have been conducted only in individuals from one geographic population so far (Smith *et al.* 1983; Wooten & Smith 1986). Therefore our next step of analysis was to investigate relationships between H and FA across geographic units.

(*c*) *FA and heterozygosity among geographic units*

When examined for correlations with $\bar{H}$ across geographic units separately for each age class, $\overline{FA}_{M}$ was consistently positively related to $\bar{H}$ in juveniles. In adults, relationships between $\overline{FA}_{M}$ and $\bar{H}$ were remarkably less positive or even negative, and essentially the same result was obtained when a *FA*-index based on the variance (R–L) rather than on the absolute values |R–L| was used (see table 5). Obviously the two different modes of calculating an overall asymmetry index in metric traits had no substantial influence on the perceptibility of a relationship between $\overline{FA}_{M}$ and $\bar{H}$ across geographic units. In non-metric traits, $\overline{FA}_{NM}$ was consistently positively related to $\bar{H}$ across geographic units and single sampling localities in the juvenile class. In contrast, there was a consistently significant negative correlation of $\overline{FA}_{NM}$ with $\bar{H}$ in adults (see table 5). These results remained the same when the whole analysis was repeated using only a reduced set of individuals without missing values ($n = 164$).

The significant negative correlation between $\overline{FA}_{NM}$ and $\bar{H}$ in adults suggests that in the brown hare developmental homeostasis is higher in the more heterozygous allozyme gene pools of geographic units and sampling sites. However, non-metric and metric traits appear to be different as to the perceptibility or the presence of homeostatic effects.

The positive relationship between $\bar{H}$ and $\overline{FA}_{NM}$ and $\overline{FA}_{M}$, respectively, in juveniles is not easy to interpret. Possibly FA in juveniles is to a certain extent due to rapid alterations of the bilateral expression of traits in the course of regular ontogenesis. If such temporal FA is more pronounced in populations with a higher growth rate, and growth rate is positively correlated with H as observed in many cases (for a review see Mitton & Grant 1984), then also overall FA should be positively correlated with H. Unfortunately a relationship between H and growth rate could not be examined in this study due to an insufficient number of very young specimens available. The switch in the relationship between $\overline{FA}_{NM}$ and $\bar{H}$ from significantly positive in juveniles to significantly negative in adults

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

could also be interpreted as a result of differential mortality with respect to both parameters. However, in free-ranging hares this could be examined only if a multiple sampling of large numbers of individuals over the whole year were possible. Altogether our results show that a negative relationship between heterozygosity and developmental homeostasis may be detectable only within a particular age group of individuals. As demonstrated by Mulvey *et al.* (1994) in the fish *Gambusia holbrooki*, in some cases such a relationship may also be detectable only under particular environmental conditions.

As a possible alternative to our genetic interpretation of differences in FA_{NM} across geographic units it may be argued, that the latter are mainly due to differences in environmental conditions (see Pankakoski 1985), which cannot be fully ruled out in an interpopulation approach (Palmer & Strobeck 1986). However, in our data FA_{NM} is not consistently higher in geographic units where environmental conditions are considered less favourable for the brown hare (area of NWA and SA, see Margl 1982).

5. CONCLUSIONS

The objective of this study was to re-examine relationships between allozyme heterozygosity and fluctuating asymmetry in morphological traits in a representative of mammals. In this group the results available so far gave rise to very contradictory conclusions, most of them strengthening the argument that developmental homeostasis may be related to heterozygosity only in poikilothermic animals (Wooten & Smith 1986). The data obtained in this study demonstrate a significant negative correlation between heterozygosity and FA in a mammal. In contrast to the cheetah (see O'Brien *et al.* 1985; Wayne *et al.* 1986) this result cannot be explained by inbreeding depression due to extensive genetic depletion. It is most probably due to differences in developmental homeostasis as a result of moderate (albeit statistically significant) differences in heterozygosity. As stated previously by Palmer & Strobeck (1986), the negative relationship between genetic variability and fluctuating asymmetry became explicite only in a comparison of H and FA across rather than within geographic units and sampling sites, respectively. Furthermore, the postulated difference in the relationship between heterozygosity and developmental homeostasis between poikilothermic and homeothermic animals may indeed have been due to a disregard of non-metric traits in the latter. Similar to what has been stated for the assessment of heterozygosity (Mitton & Grant 1984; Palmer & Strobeck 1986), the low concordance of FA among morphological characters both within and among geographic units of the brown hare suggests that estimates of overall FA should be based on a large number of traits to reveal differences in developmental homeostasis.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

APPENDIX

Non-metric bilateral skull and mandible characters and character states scored in the brown hare

code and anatomical designation of character	code and description of character states
NM1 Foramen nervi hypoglossi internale	0 = two f. present, 1 = three or more f. present
NM2 Foramen nervi hypoglossi internale accessorium	0 = f. absent, 1 = one f. present, 2 = two or more f. present
NM3 Foramen condylare	0 = f. absent, 1 = one or more f. present
NM4 Foramen basioccipitale laterale	0 = f. absent, 1 = one f. present, 2 = two or more f. present
NM5 Foramen alisphenoidale	0 = f. absent, 1 = one f. present, 2 = two or more f. present
NM6 Fenestra orbitalis	0 = f. absent, 1 = one or more f. present
NM7 Foramina circumoptica	0 = 1–6 f. present, 1 = > 7 f. present
NM8 Foramen ethmoidale accessorium	0 = f. absent, 1 = one or more f. present
NM9 Foramen palatinum majus	0 = f. absent, 1 = one f. present, 2 = two or more f. present
NM10 Foramen intermaxillare	0 = f. absent, 1 = one or two f. present
NM11 Foramen processus intermaxillaris	0 = f. absent, 1 = one or two f. present
NM12 Foramen zygomaticum anterius	0 = f. absent, 1 = one of more f. present
NM13 Foramen zygomaticum	0 = f. absent, 1 = one f. present, 2 = two or more f. present
NM14 Processus nasalis ossis intermaxillaris	0 = p. shorter or reaching the nasofrontal suture, 1 = p. exceeding the nasofrontal suture
NM15 Foramen frontale mediale	0 = f. absent, 1 = one or more f. present
NM16 Foramen frontale postorbitale	0 = f. absent, 1 = one or more f. present
NM17 Foramen fossae articularis mandibularis ossis temporalis	0 = f. absent, 1 = one f. present, 2 = more f. present
NM18 Foramen mentale accessorium	0 = f. absent, 1 = one f. present, 2 = more f. present
NM19 Foramen mandibulare	0 = f. absent, 1 = one f. present, 2 = two f. present
NM20 Expression of I^2	0 = I^2 present, 1 = additional I^2 present, 2 = I^2 absent
NM21 Additional upper molar	0 = aum absent, 1 = aum present
NM22 Expression of M^3	0 = M^3 absent, 1 = M^3 present
NM23 Additional lower molar	0 = alm absent, 1 = alm present
NM24 Fenestra temporalis	0 = f. absent, 1 = f. present
NM25 Processus palatomaxillaris accessorius	0 = p. absent, 1 = p. present
NM26 Foramen basisphenoideo-pterygoideum	0 = f. absent, 1 = f. present
NM27 Foramen temporale	0 = f. absent, 1 = one or more f. present

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES