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Original investigation

Developmental stability and protein heterozygosity in a local population of Iberian hares (*Lepus granatensis*)

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Abstract

Various studies have revealed a positive effect of heterozygosity on developmental stability in animals of diverse taxa. In homeothermic vertebrates, however, no clear picture has so far emerged in this context. Here, we test the influence of heterozygosity on the developmental stability of adult-sized skulls of 63 Iberian hares (*Lepus granatensis*) from a local population in Portugal. 44 allozyme and blood protein loci were screened by horizontal starch gel electrophoresis, agarose electrophoresis, and isoelectric focusing. This yielded eleven polymorphic loci, that were used to calculate individual heterozygosity. Levels of fluctuating asymmetry (FA) of three morphological character systems (15 epigenetic dental characters, ten non-metric skull traits, six metric skull variables) were determined and used as indicators of the levels of developmental homeostasis of single hares. Overall individual heterozygosity did not correlate with respective FA levels in any of the three morphological character systems. However, a trend towards a negative relationship between metric FA and heterozygosity suggested that there might be a slight positive influence of heterozygosity on developmental stability of the morphometric system, but it could be masked by various seasonal exogenic factors.

Key words: *Lepus granatensis*, heterozygosity, fluctuating asymmetry, developmental homeostasis

Introduction

Developmental homeostasis, i. e., the ability to buffer against minor random deviations from metabolic pathways during growth, can be reduced by environmental and genetic stress (e. g., Møller and Swaddle 1971). The level of fluctuating asymmetry (FA) is commonly used to evaluate the degree of developmental stability (e. g., Zakharov 1981; Palmer and Strobeck 1986; Novak et al. 1993). FA is defined as random departures from the ideal bilateral symmetry of morphological traits with a population mean around zero and a normal distribution (e. g., Palmer 1994). More symmetric animals apparently have higher fitness, as indicated by diverse fitness components, than asymmetric (Møller 1997). It has been proposed that heterozygosity stabilizes the ontogenetic development, so that genetically determined pathways are more

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precisely expressed in the phenotype of an organism (e. g., Handford 1980; Fleischer et al. 1983; Mitton and Grant 1984; Mitton 1993 a, b, 1995).

In local populations of adult brown hares (*Lepus europaeus*) from central Europe FA was negatively correlated with population-specific allozyme heterozygosity in non-metric but not in metric skull characters. This suggested a differential effect of heterozygosity on the developmental stability of non-metric and metric skull characters (Hartl et al. 1995). However, no such relationship was found in brown hares from Britain and New Zealand, that had lower levels of genetic variability than central European brown hares (Suchentrunk et al. 2000), and FA in both character systems was even lower than in brown hares from central Europe (Suchentrunk et al. 1998). Such inconsistencies of the relationship between heterozygosity and FA might result from different evolutionary histories of population sets. In addition, varying levels of environmental stress may conceal effects of heterozygosity on FA to different degrees (e. g., Pankakoski 1985; Pankakoski et al. 1992; Palmer and Strobeck 1986; Borisov et al. 1997; Møller and Swaddle 1997; Zakharov et al. 1997 a).

Here we, examine whether or not heterozygosity has a significant effect on developmental homeostasis of Iberian hares (*Lepus granatensis*) from one local population in a relatively homogeneous environment. In particular we examine whether overall individual heterozygosity is negatively correlated with FA in three character systems of the skull; and if so, whether such a relationship becomes generally apparent in all three character systems.

Material and methods

Collection of specimens and samples for genetic analysis

Sixty three adult-sized Iberian hares (*Lepus granatensis*) were collected at Pancas (38°48′ N/ 8°57′ W), approx. 15 km east of Lisbon, Portugal, during regular hunts between October 1997 and October 1999. Pancas is situated in a lowland region (29 m a. s. l.) that has a Mediterranean climate, with 500–600 mm average annual rainfall, mainly in October–April, and 16 °C mean annual temperature (C. N. A. 1983). The vegetation is characterized by tree stands of *Quercus suber*, *Pinus pinea*, as well as marshy areas, pastures, and arable land.

All hares were sexed by inspection of their primary reproductive organs. They were classified as "adults", based on body size and weight, absence of the epiphyseal protrusion of the ulna (cf. Suchentrunk et al. 1991 for European brown hares), the ossification pattern of skull sutures, and the shape and size of the processus supraorbitales (e. g. Palacios and Lopez 1980).

Blood samples were taken shortly after the death of the animals by cardiac puncture and collected in ETDA-coated tubes. Red blood cells were separated from plasma by centrifugation at 1 500 g for 5 min at 4 °C and stored at –20 °C. Liver, kidney, and spleen tissue samples were taken and frozen at –20 °C.

Protein heterozygosity

Fourty-four loci encoding for allozymes and blood proteins were initially screened for genetic variability. This set largely included loci that were already studied by Hartl et al. (1989, 1990, 1992, 1993, 1994, 1995) for brown hares (*L. europaeus*), Suchentrunk (1993, 2000), Suchentrunk et al. (1998, 1999, 2000 a, b, c, Suchentrunk, unpubl. data) for brown hares, mountain hares (*L. timidus*), Iberian hares, and several hare species from Mexico, and by Alves et al. (2000) for Iberian hares. Direct side-by-side comparisons of migrating allozymes on the same gels were made to infer alleles at polymorphic loci from zymograms (cf. Harris and Hopkinson 1976). Eleven loci revealed allelic polymorphism and were considered in the present analyses. They are listed in table 1 along with the respecitve allozyme/blood protein names, E. C. numbers, and methodological specifications.

Allele frequencies, locus-specific heterozygosities (h_o, h_e), and exact Fisher's tests for deviation of observed genotypes from Hardy-Weinberg expectations were calculated by using the BIOSYS-1 pc package, release 1.7 (Swofford and Selander 1989). For the combined analysis with the morphological data, genotypes at all polymorphic loci were categorized as "homozygous" or "heterozygous", irrespective of allele composition. The overall heterozygosity (H) of an individual was calculated as the percentage of heterozygous loci.

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Table 1. Enzyme systems/blood proteins and respective loci used in the combined analyses with fluctuating asymmetry of Iberian hares from Pancas. For allele frequencies, see Tab. 4. h_e – unbiased locus-specific heterozygosity, h_o – direct count (observed) locus-specific heterozygosity. Methodological specifications of protein screening (method. specific.) are in the footnote.

enzyme systems/blood proteins (name, code, E.C. number)	locus	method. specific.	h_e	h_o
NADH-diaphorase (DIA, 1.6.2.2)	Dia-2	SGE/G	0.258	0.143
Esterases (ES, 3.1.1.1)	Es-1	SGE/G	0.481	0.548
Acid phosphatase (ACP, 3.1.3.2)	Acp-2	SGE/G	0.016	0.016
Peptidase B (PEPB, 3.4.11)	Pep-B	SGE/A	0.338	0.320
Aminoacylase-1 (ACY-1,3.5.1.14)	Acy-1	SGE/G	0.182	0.127
Mannose phosphate isomerase (MPI, 5.3.1.8)	Mpi	SGE/G	0.284	0.176
Hemoglobin alpha chain (HBA)	Hba	SGE/A	0.501	0.366
Transferrin (TF)	Tf	AGE	0.154	0.083
Hemopexin (HPX)	Hpx	IEF	0.272	0.308
Vitamin D binding protein (GC)	Gc	IEF	0.146	0.154
Properdin factor B (BF)	Bf	IEF	0.370	0.333

SGE/A – horizontal starch gel electrophoresis and protein staining according to Alves et al. (2000), SGE/G – horizontal starch gel electrophoresis and protein staining according to Grillitsch et al. (1992), AGE – agarose gel electrophoresis (Teisberg 1970), IEF and HIEF – isoelectric focusing in carrier ampholytes and hybrid pH-gradients according to (Alves et al. 2000).

In a few individuals ambiguous allele interpretations at one or more loci resulted in a slightly reduced number of polymorphic loci for calculation of H. Hence, H-values were used in further analyses only if based on at least nine polymorphic loci. Sex-dependence of locus-specific heterozygosity (h) and H was tested by Mann-Whitney U-tests, respectively. Associations of homozygous or heterozygous genotypes among pairs of loci were checked by exact Fisher's or chi^2 tests, based on the Sequential Bonferroni procedure (alpha = 0.05) to account for multiple and partly dependent tests (Rice 1989). The Sequential Bonferroni procedure was also applied in all further test series involving non-metric characters, metric variables, as well as in test series of combined morphological and genetic data sets.

FA of epigenetic (non-metric) occlusal characters

Epigenetic occlusal characters in Leporids concern basically presence or absence of enamel folds, notches, grooves or islands, conformation patterns of enamel margins, and presence or absence of cement in folds (e. g., Forsyth Major 1898; Hibbard 1963; Angermann 1966; Palacios and Lopez 1980). Initially, 40 dichotomized (0/1) occlusal characters were scored for right/left differences in their respective character states (cf. Suchentrunk 1993; Suchentrunk et al. 1994, 1996, 2000 a, b). Bilateral asymmetry of a character was given, if different (0/1, 1/0) character states occurred on the right and left body sides. Only 15 characters were found with clear right/left-differences. They were used for the FA analysis; table 2 details the descriptions of the characters, character states, and character-specific bilateral asymmetry levels. The latter were calculated as percentages of individuals with asymmetric characters.

A Wilcoxon matched-pairs signed-rank test was run for each character to check for occurrence of FA or directional asymmetry (DA) (Palmer and Strobeck 1986). Since no character showed DA, all were considered indicators of developmental homeostasis (Palmer and Strobeck 1986; Palmer 1994; Møller and Swaddle 1997). Associations of FA between pairs of characters and sex-dependence of single characters were tested by exact Fisher's tests, respectively. Individual overall FA of occlusal characters (FA_{OC}) was calculated as the proportion of

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Table 2. Fluctuating asymmetry (FA) of epigenetic occlusal characters of Iberian hares from Pancas. Current character numbers (CN) and tooth allocation, character description, dichotomized character states (0/1), and level of FA in percent of unequal, i. e., (1)/(0) or (0)/(1) character states are given for each character.

CN	Tooth	Description of characters	dichotomized character states	FA
C-1	P_3	Mesial re-entrant fold (filled with cement):	present(1)/absent(0)	1.6
C-2	P_3	Additional mesial re-entrant fold (with cement):	present(1)/absent(0)	3.3
C-3	P_3	Anterior lingual re-entrant fold (with cement):	present(1)/absent(0)	13.1
C-4	P_3	Posterior external re-entrant fold breaking through the lingual enamel wall and separating trigonid and talonid completely:	yes(1)/no(0)	3.3
C-5	P_3	Margin of posterior external re-entrant fold forming one extra fold in its most lingual section, extending mesiad and/or distad:	yes(1)/no(0)	1.6
C-6	P_3	Mesial margin of posterior external re-entrant fold plicate (strong or slight plication)	yes(1)/no(0)	8.2
C-7	P_3	Distal margin of posterior external re-entrant fold plicate (strong or slight plication):	yes(1)/no(0)	8.2
C-8	P_3	Distal margin of posterior external re-entrant fold forming one distinct step or extra fold in its lateral part:	yes(1)/no(0)	11.5
C-9	P_3	Margin of anterior external angle with rather strong plication:	yes(1)/no(0)	1.6
C-10	P_3	Cement layer of mesial re-entrant fold stretching lingually (covering also anterior lingual re-entrant fold, if present):	yes(1)/no(0)	3.3
C-11	P_4	Distal margin of lateral fold with extra fold in the buccal section:	yes(1)/no(0)	1.7
C-12	M^2	Enamel island filled with cement lingually or buccally of lingual fold (hypostria):	present (1)/absent(0)	1.6
C-13	P^2	central fold with plicated margin:	yes(1)/no(0)	3.3
C-14	P^2	lingual fold with plicated margin:	yes(1)/no(0)	5.2
C-15	I^1	labial groove with cement:	yes(1)/no(0)	2.1

Table 3. Non-metric bilateral skull characters used for assessing fluctuating asymmetry (FA). Code, morphological designation, description of dichotomized (0/1) character states, and levels of FA (%) are given. f. – foramen (foramina).

code	description and character states (0/1)	FA
NM1	Foramen nervi hyperglossi internale: (0) two f. present, (1) > two f. present	6.6
NM2	Foramen nervi hypoglossi internale accessorium: (0) f. absent, (1) one or more f. present	27.9
NM3	Foramen condylare: number of f. on both sides: (0) equal, (1) unequal	51.6
NM4	Foramen alisphenoidale: number of f. on both sides: (0) equal, (1) unequal	29.5
NM5	Foramen ethmoidale accessorium: (0) absent, (1) present	30.0
NM6	Foramen palatinus: (0) one f. present, (1) two f. present	1.6
NM7	distinct foramen on os maxillare medial of P^2–M^1: (0) no, (1) yes	6.3
NM8	Foramen frontale mediale: (0) f. absent, (1) f. present	19.4
NM9	Foramen mandibulare: (0) one f. present, (1) more than one f. present	3.2
NM10	Foramina along the rostral sulcus of the mandibular ramus (0) equal, (1) unequal number	66.1

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asymmetric characters of the total set of occlusal characters studied per individual (Leary et al. 1985). Sex-specific variation of FA_{OC} was tested by a Mann-Whitney U-test.

FA of non-metric skull characters

Ten non-metric skull characters (foramina) were scored on both body sides. They could be easily scored and are largely a subsample of those characters that were used in FA analyses in brown hares (*Lepus europaeus*, see Hartl et al. 1995; Suchentrunk et al. 1998). Character descriptions, dichotomized character states (0/1), and respective asymmetry values appear in table 3. Statistical procedures were analogous to those for dental characters. Calculation of the individual-specific index of overall FA of non-metric skull characters (FA_{NM}) was based on all ten characters.

FA of metric skull variables

Six bilateral skull and mandible measurements (Fig. 1) were taken with digital calipers to the nearest 0.01 mm. Measurements were taken exclusively by one of the authors (PCA) to eliminate the possible inter-observer variability (Lee 1990), and were repeated once to obtain a data basis for evaluating the influence of measurement error on the FA estimation. The effect of measurement error on FA values of single variables was calculated by a two-way ANOVA for each variable, based on sides and repeated measurements in each individual. The influence of measurement error on the asymmetry measurement was considered insignificant, if the sum of mean variance of the side factor and the mean variance of the side/individual interaction factor was at least twice as high as the residual mean variance (Hartl et al. 1995; see also Palmer 1994).

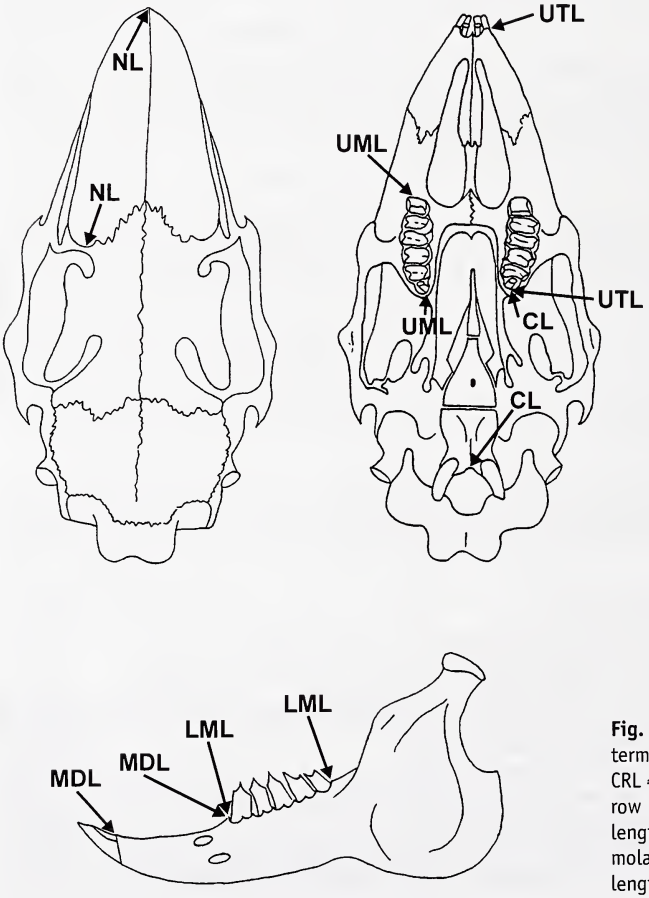

Fig. 1. Skull measurements used for determination of fluctuating asymmetry. CRL = cranium length, LML = lower molar row length, MDL = mandibular diastema length, NL = nasalia length, UML = upper molar row length, UTL = upper tooth row length.

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Occurrence of DA or antisymmetry (AS) was tested for each variable by a sign test (right minus left measurements), and by a Kolmogorov-Smirnov test of the frequency distribution of the right-left paired differences, respectively (Palmer and Strobeck 1986; Hartl et al. 1995). To check for size-dependence of asymmetry, a Spearman correlation between individual values of |right-left| differences and respective arithmetic means was performed in each variable. Despite absence of a correlation, we used an FA index for single metric variables that allowed the calculation of overall individual FA indices, even in cases of missing data for single values in partly damaged skulls. For single variables the following FA index was used:

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

where R and L are the measurements on the right and left sides, respectively (cf. Palmer and Strobeck 1986; Hartl et al. 1995; Palmer 1994). Sex-dependence of FA of each variable was checked by a one-way ANOVA. Pairwise correlations of FA of variables were tested by Spearman correlations. Overall FA of metric variables (FA_M) was calculated as the arithmetic mean of FA indices of the six (CRL, LML, MDL, NL, UML, UTL), and only in few partly damaged skulls based on five variables. Sex-dependence of FA_M was checked by a one-way ANOVA and size-dependence by a Pearson correlation between FA_M and individual condylobasal length (CBL) (e. g., Palmer 1994).

Relationships between non-metric and metric FA

Relationships between non-metric dental and skull characters were examined by Fisher's exact tests, and those between non-metric and metric variables by Mann-Whitney U-tests. Relationships between FA_{OC}, FA_{NM}, and F_{AM} were examined by Spearman correlations, respectively.

FA, skull length, and protein heterozygosity

Relationships between either FA_{OC}, FA_{NM}, F_{AM}, CBL, and H were tested by Spearman correlations, respectively. For the relationship between FA_M and heterozygosity, the following additional statistical approach was carried out: H-values were classified as low (< 20%) and high (20–45. 5%); and within each group FA values of single variables were calculated by:

$$\text{var}\,[(R - L)/(R + L)/2],$$

where R and L are the right and left side measurements per individual and var is the H-group variance. To maximize the information on FA of all variables and individuals in a comparison of metric FA between the two heterozygosity groups, we performed a two-way ANOVA with variable and group as factors (cf., Palmer 1994).

Results

The allele frequencies at polymorphic loci are presented in table 4. One significant deviation of genotype frequencies from Hardy-Weinberg expectations at the Dia-2 locus was found; it was due to a slight heterozygote deficiency. Locus-specific expected heterozygosities ranged between 0.016–0.501 and direct count heterozygosities between 0.016–0.548 (Tab. 1). There were no sex-specific differences of frequencies of homozygote and heterozygote genotypes at any locus. Also, no significant pairwise associations of homozygous or heterozygous genotypes were found among loci. H values ranged from 0.0% to 45.5% with a mean of 22.52% and a standard deviation of 12.68%. H values did not vary significantly between the sexes.

Levels of asymmetry of non-metric characters appear in tables 2 and 3 and those of metric variables in table 5. Only one metric variable (DIA) showed DA. No character showed AS. Apart from DIA, all metric characters were used as indicators of developmental homeostasis (Palmer and Strobeck 1986). In single non-metric characters and metric variables no sex-specific differences of FA were found. Also, no significant associations or correlations of symmetric or asymmetric expressions were detected between pairs of non-metric characters or metric variables, respectively. The two-way ANOVA of measurement repeats and side (Tab. 5) did not suggest that FA values of the six metric variables used for FA calculations were confounded by measurement errors.

FA of single metric variables and FA_M values were not significantly correlated with CBL. Also, in non-metric characters no significant differences of CBL values were

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Table 4. Allele frequencies (%) of polymorphic loci in hares from Pancas. Allele designations are not necessarily in alphabetical or numerical order because alleles were assigned in a combined analysis of *L. granatensis* and *L. europaeus* and some alleles were not found in the hares from Pancas. Allele designations of the loci Gc, Pep-B, HBA, Bf, and Hpx conform to those in ALVES et al. (2000)

locus	allele	frequency	locus	allele	frequency	locus	allele	frequency
Dia-2	A	0.857	Acy-1	A	0.100	Bf	1	0.786
	B	0.082		B	0.900		2	0.107
	C	0.061					3	0.060
							5	0.048
Mpi	A	0.157	Acp-2	A	0.992	Tf	C	0.083
	B	0.833		B	0.008		D	0.917
	C	0.010						
Es-1	B	0.643	Pep-B	1	0.800	Hpx	1	0.846
	C	0.333		3	0.150		2	0.115
	D	0.012		4	0.050		3	0.010
	F	0.012					4	0.029
Gc	1	0.923	Hba	3	0.451			
	3	0.019		4	0.549			
	4	0.058						

Table 5. FA of single metric skull variables selected for estimating overall metric skull FA of hares from Pancas. n = sample size, m. e. = measurement error (see material and methods), (R + L)/2 – mean of the variable size, s. e. = standard error of mean. Mean and standard errors of the differences between the sides (R–L) are also given to indicate the absence of DA (cf. PALMER 1994) in conjunction with the not significant sign-test results, based on the Sequential Bonferroni procedure. For variable acronyms and calculation of the FA index, see material and methods.

variable	n	m. e.	(R + L)/2 (+/–s. e.)	(R–L) mean (+/–s. e.)	FA index mean s. e.
CRL	59	3.3 : 1	29.70 (0.145)	0.046 (0.036)	0.007 (0.001)
LML	61	2.0 : 1	17.90 (0.08)	0.044 (0.043)	0.013 (0.002)
MDL	61	3.5 : 1	20.42 (0.13)	–0.083 (0.045)	0.013 (0.001)
NL	61	15.1 : 1	40.11 (0.2)	–0.097 (0.047)	0.007 (0.001)
UML	61	3.72 : 1	16.80 (0.07)	–0.06 (0.03)	0.011 (0.001)
UTL	61	2.92 : 1	43.46 (0.17)	0.048 (0.035)	0.005 (< 0.001)

found between symmetric and asymmetric character states. And there were neither significant correlations between CBL and FA_{OC} or FA_{NM} values nor significant correlations between FA_{OC}, FA_{NM}, FA_M values. No significant correlations between FA_{OC}, FA_{NM}, FA_M, CBL, and H were found. However, there was a slight tendency towards lower FA_M values in hares with greater H values. The respective correlation coefficients and associated significance levels are listed in table 6. The two-way ANOVA of the variance-based metric FA in-

Table 6. Relationships between overall individual heterozygosity (H) and overall fluctuating asymmetry of non-metric occlusal characters (FA_{OC}), non-metric skull characters (FA_{NM}), metric skull variables (FA_M), condylobasal length (CBL). One-tailed Spearman rank correlation coefficients (r_s), individual numbers (n), and significance levels (p) are given; n. s. = not significant.

		FA_{OC}	FA_{NM}	FA_M	CBL
H	r_s	0.0485	–0.0017	–0.2164	0.0824
	n	48	49	49	48
	P	0.372	0.495	0.068	0.289
		n. s.	n. s.	n. s.	n. s.

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Table 7. Fluctuating asymmetry (FA) values of single metric skull variables in hares with low (< 20%) and high (> 19%) heterozygosity. Means (M), standard errors (SE), minimum (MIN), maximum (MAX), and sample sizes (n) are given for each variable and heterozygosity group. M, SE, MIN, and MAX values are multiplied by 10^4. For acronyms of variables, see Fig. 1.

		FA index $\lvert R - L \rvert / [(R + L)/2]$			
Variable	N	M	SE	MIN	MAX
group: heterozygosity < 20%					
CRL	21	90.79	17.32	6.82	307.27
LML	20	145.66	25.58	5.3	373.68
MDL	20	153.31	27.24	13.54	444.44
NL	21	77.49	12.94	2.65	191.87
UML	21	115.73	20.88	0.0	402.68
UTL	20	44.38	6.83	2.22	126.26
group: heterozygosity 20–45.5%					
CRL	26	47.00	10.51	0.00	183.36
LML	26	101.05	18.96	0.00	341.83
MDL	25	109.17	21.43	0.00	387.81
NL	26	68.09	10.97	2.51	235.12
UML	28	103.3	17.8	6.13	341.3
UTL	27	56.22	8.24	0.00	222.09

dices revealed a significant effect by variables ($p < 0.0001$) but only a tendency ($p = 0.019$; Bonferroni criterion for multiple testing: $p = 0.01$) towards an H group effect. The H group/variable interaction factor was not significant ($p = 0.457$). The means, standard errors, and extreme values of FA in single metric variables are listed in table 7, separately for each H group.

Discussion

Heterozygosity is commonly considered to indicate levels of genetic variability within individuals and populations (e. g., Mitton and Pierce 1980; Nei 1987). Hares with high heterozygosity may harbour less homozygous genotypes with rare recessive alleles that are detrimental to certain metabolic processes than hares with low heterozygosity. Hence, low heterozygosity could lead to higher developmental instability ("dominance hypothesis"). According to the "overdominance hypothesis" individuals with heterozygote genotypes at many unlinked polymorphic loci should have a better capability of buffering biochemical processes against various adverse environmental effects during ontogenesis (e. g., Turelli and Ginzburg 1983; Mitton 1993 b). In Oldfield mice (*Peromyscus polionotus*) the ability of an individual to maintain stable developmental trajectories under fluctuating environmental conditions is related to its genetic variability (Teska et al. 1991). Higher genetic variability may lead to the production of a higher variability of biochemical products to buffer diverse environmental influences. This in turn should lead to a more regular expression of bilateral symmetric morphological traits of an organism (e. g., Ginzburg 1979; Mitton and Grant 1984; Mitton 1995; Møller and Swaddle 1997).

Bilateral asymmetry of morphological characters is only indicative of developmental homeostasis if they show fluctuating asymmetry (FA) (Palmer and Strobeck 1986; Palmer 1994; Møller and Swaddle 1997). Among all traits presently studied, the only case of directional asymmetry (DA) was found in one metric variable (DIA). We do not have a convincing biological explana-

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tion for this significant deviation from FA. It might result from a systematic measurement bias due to different positions of the skulls when holding them for taking right and left measurements. Whatever reason, we excluded this variable from all combined analyses with heterozygosity. In three other metric variables (MDL, NL, UML) the standard errors of right-left differences (R–L) were quite low compared to the respective means (table 5). This might suggest DA in these characters. Nevertheless, we included these variables in the calculations of metric FA because of nonsignificant sign-tests when based on the Sequential Bonferroni procedure.

Our results demonstrate that there is FA in all three morphological character systems studied, but no concordance of FA levels among the three morphological systems. And we did not find a significant relationship between FA and heterozygosity. This corresponds to the "poikilotherm-homeotherm hypothesis" (Handford 1980; Wooten and Smith 1986; but see Hartl et al. 1995), according to which a negative relationship between FA and heterozygosity should be more likely in poikilothermic animals because of their supposedly greater sensitivity to environmental conditions, whereas homeotherms experience a more stable development during their ontogeny (see also Møller and Swaddle 1997). In some poikilothermic vertebrates, single-locus heterozygosity was found to be negatively correlated with FA (Mitton 1993 a, b, 1995 for overview). However, here we did not check for relationships with single-locus heterozygosity, to avoid too stringent significance levels by the Sequential Bonferroni procedure (see Palmer 1994).

Nevertheless, in the metric skull characters a tendency towards increased FA in hares with low heterozygosity became apparent. A weak inverse relationship might indeed exist, but could be largely masked by effects of diverse exogenic factors, despite the quite small and homogeneous study area. Mulvey et al. (1994) found a negative relationship between genic variability and FA in the fish *Gambusia holbrooki* only in certain environmental contexts. Exogenic factors might include diverse weather components or food stress in different seasons. At Pancas hares are born all year round (leverets can be observed in any season). Seasonal changes of ground temperature and moisture, wind and rainfall, together with varying food availability for leverets and lactating does could modify levels of FA of hares born in different seasons. Increased dental FA was observed in mice that were born and raised in cold environments (Siegel and Doyle 1975 a). Rats (*Rattus norvegicus*) exposed to cold and heat stress increased FA of long bones (Gest et al. 1986). FA of humeri of cotton mice (*Peromyscus gossypinus*) and Florida mice (*P. floridanus*) was raised by cold stress (Siegel and Doyle 1975 b). Levels of parasitic infections may also have varied within the sampling period. FA of antlers of a Norwegian reindeer (*Rangifer tarandus*) herd was enhanced by abomasal nematode infections, and there was a negative relationship between certain immune parameters and FA (Lagesen and Folstad 1998). In addition, seasonal changes of levels of psychogenic stress due to variable predation pressure by foxes, raptors etc. with possible effects on various hormone-based regulation systems may influence developmental stability. Social stress had a negative impact on FA of non-metric skull traits in laboratory rats (*Rattus norvegicus*) (Valetsky et al. 1997).

Palmer and Strobeck (1986), and Palmer (1994), among others, recommended variance-based FA indices and comparisons of FA at the population/group level. In a meta-analysis, Vøllestad et al. (cf. Møller and Swaddle 1997) found a tendency towards an inverse relationship between FA and heterozygosity at the population level in poikilotherms but no clear pattern in homeotherms. In fact, in central European brown hares (*Lepus europaeus*) a significant negative relationship between non-metric FA and heterozygosity was only apparent at the population level Hartl et al. 1995). This might result from a better estimate of genic variability by groupspecific

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heterozygosity than by individual heterozygosity (Palmer and Strobeck 1986). To increase the power of the comparison of FA between groups of individuals (populations etc.), Palmer (1994) recommended combining the FA information of several traits in a two-way ANOVA with group and trait factors. By this variance-based approach, the initially found (not significant) tendency towards increased FA of metric skull variables in hares with low heterozygosity was also evident. However, we emphasize that in this second variance-based test of FA between the two heterozygosity groups, sample sizes per group were naturally lower compared to the full set of individuals in the correlation analysis between H and F_{AM}. Moreover, this second approach resulted in a more stringent significance level to account for multiple (and partly dependent) testing (Rice 1989). This might have reduced the chance of detecting a significant difference.

It has been questioned whether measuring heterozygosity at a comparatively small number of loci provides a good estimate of overall genome heterozygosity. Heterozygosity estimates might be valid particularly if: a) a large amount of the genome is structured in blocks, i. e. when there is a large amount of linkeage disequilibrium, b) there is a high degree of inbreeding, and c) there is non-random mating due to small population size and isolation (Møller and Swaddle 1997). The largely absence of concordant locus-specific deficiency of heterozygous genotypes does not particularly indicate a severe level of inbreeding in the hares from Pancas. We also did not find any significant linkage disequilibrium in our sample and we do not have any information on the mating structure of hares at Pancas. However, there is a substantial level of gene flow between the Pancas population and other populations in Portugal (Alves and Ferrand 1999). To increase the predictor quality of heterozygosity, we analysed most of the protein loci that have been found to be polymorphic in the genus *Lepus* (Hartl et al. 1990, 1992, 1993, 1995 for brown hares; Grillitsch et al. 1992; Alves et al. 2000; Suchentrunk et al. 1998, 1999, 2000, and unpublished data for diverse hare species).

Based on eleven polymorphic loci, we did not find a significant positive effect of overall individual heterozygosity on developmental stability. This, however, does not necessarily mean that there is no such effect. We found a tendency for a negative correlation between metric skull FA and heterozygosity; and such a relationship might indeed exist, but it could be masked by the combined influence of various unperceived seasonal environmental stressors. Weather parameters or food availability, among other stressors, might exert seasonal influences on growing hares. If so, such exogenic stress components would have a clearly higher overall effect on developmental stability than cross genic variability of the hares. This, however, should be tested by a further study based on hares from different birth seasons.

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Zusammenfassung

Heterozygotie und Entwicklungshomöostase bei Iberischen Hasen (*Lepus granatensis*) aus einer lokalen Population in Portugal

Bisherige Untersuchungen haben vielfach einen positiven Einfluß des Heterozygotiegrades auf die Entwicklungsstabilität von Tieren ergeben. Homeotherme Vertebraten zeigen diesbezüglich allerdings kein einheitliches Bild. In dieser Arbeit wird der Einfluß des individuellen Heterozygotiegrades von Iberischen Hasen aus einer lokalen Population in Portugal auf ihre Entwicklungshomöostase untersucht. Die Analyse von 44 proteinkodierenden Loci mittels Stärke- und Agarosegelelektrophorese sowie isoelektrischer Fokusierung ergab bei 63 Hasen elf polymorphe Loci, die zur Berechnung des individuellen Heterozygotiegrades herangezogen wurden. Das Niveau der Entwicklungshomöostase einzelner Hasen wurde anhand der fluktuierenden Asymmetrie (FA) in nicht-metrischen und metrischen Merkmalssystemen (Zahnmerkmale, Schädelmeßstrecken und Foramina) ermittelt. Das Ausmaß der individuellen FA war in keinem der drei Merkmalssysteme mit dem Heterozygotiegrad korreliert. Die FA der Schädelmeßstrecken zeigte aber tendenziell einen negativen Zusammenhang mit der Proteinheterozygotie. Solch ein (schwacher) Zusammenhang könnte tatsächlich bestehen, aber durch nicht erfaßte (kaum erfaßbare) exogene Streßfaktoren weitestgehend verdeckt sein.

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