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High levels of fluctuating asymmetry in populations of *Apodemus flavicollis* from the most contaminated areas in Chornobyl

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Abstract

Random deviations from the perfect symmetry of normally bilaterally symmetrical characters for an individual with a given genotype occur during individual development due to the influence of multiple environmental factors. Fluctuating asymmetry (FA) is often used as a measure of developmental instability, and can be estimated as the variance of the distribution of differences between the left and right sides. We addressed the question of whether levels of FA were elevated in radioactively contaminated populations living around Chornobyl compared to those in reference populations of the yellow-necked mouse (*Apodemus flavicollis*). In addition, we studied amounts of directional asymmetry (DA) when one side is larger than the other on average. There was a significant difference among populations, including reference populations, in the amount of both FA and DA. A higher level of FA was documented for the contaminated populations in close proximity to the failed Chornobyl reactor for both the asymmetry of size and shape. The FAs of size and shape were highest in populations from the most contaminated locations in the Chornobyl exclusion zone. Although the directional asymmetry of shape was also highest in the contaminated populations, it was not significantly different from those in most of the reference populations. Populations from less contaminated areas inside the Chornobyl exclusion zone did not express FA values different from those of the reference populations outside the affected area.

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FA of skulls of *A. flavicollis* may indicate the degree to which the level of radioactive contamination affects the development of animals at Chornobyl. However, the mechanisms leading to these effects are not clear and probably vary from population to population. There were significant correlations between the overall right to left differences for the Procrustes aligned shape configurations, centroid sizes, and intramuscular ^{137}Cs. Detectable effects of radiation on developmental stability probably start to occur between 0.132 and 0.297 μGy/h.

Keywords: Radiocesium; ^{137}Cs; *Apodemus flavicollis*; Chornobyl; Chernobyl; Size; Shape; Fluctuating asymmetry; Directional asymmetry; Geometric morphometrics

1. Introduction

Developmental instability (DI) is the tendency for the phenotypic value of a trait to deviate from the value expected for an individual of a given genotype in a given environment (Palmer, 1996). Random errors, which occur during development, can lead to small deviations from perfect symmetry between body sides for bilateral characters. DI has been argued to be controlled genetically through such mechanisms as levels of whole-genome heterozygosity or genomic coadaptation (Clarke, 1993). On the other hand, it is believed to be character-, taxon- and environment-specific (Batterham et al., 1996; Clarke, 1997). Moreover, much of the work recently described in the literature has failed to provide a general biological mechanism that would explain patterns and mechanisms of stability in natural populations (Clarke, 1997). DI is believed to increase as a response to the outside stress, when buffering mechanisms that are supposed to maintain symmetrical development fail to counteract an increased number of small random errors. These errors are difficult to observe directly on the trait, but can be estimated from the increased variance in the asymmetry of bilateral characters across a population (Klingenberg and McIntyre, 1998; Palmer, 1994).

Fluctuating asymmetry (FA) is an estimate of small, non-directional departures from the expected bilateral symmetry for certain traits (Palmer, 1996; Van Valen, 1962). Statistically, FA can be estimated as the variance of a distribution of the differences between the left and right sides among individuals. FA could provide an estimate of DI, because the two sides of a bilaterally symmetrical organism should share the same underlying genotype, and therefore should be identical in the same environment (Møller, 1997; Palmer, 1994). While FA remains a controversial measure of developmental instability (Van Dongen and Lens, 2002), it attracts increasing attention for its seemingly straightforward prediction of stress. A large number of studies show a positive relationship between FA and environmental stress at the population level (Møller and Swaddle, 1997), however, there are also studies that do not show these effects. Moreover, there is at least one study on rodents that demonstrates an opposite trend, where developmental stability actually increases in mice from the contaminated sites (Gileva and Kosareva, 1994).

Other common departures from the ideal asymmetry include directional asymmetry (DA) when one side is larger than the other on average, and antisymmetry when one of the sides is larger than the other consistently, but either side can be the one that is larger (Palmer, 1996). While most of the FA within populations is presumed to be environmental in origin, DA and antisymmetry are likely to be more dependent on changes in individual genotypes (Leamy et al., 2000). In contrast, genetic components of FA are generally low, whereas those of environmental components are usually high. Thus, FA has been used extensively as a measure of developmental stability, whereas DA with its genetic basis has not typically been recommended for this purpose (Leamy, 1999; Leamy et al., 2000). Unfortunately, the genetic-by-environmental interaction component is not typically measured.

FA may have many potential causes that are extremely difficult to isolate in the field, (Hershkovitz et al., 1993). Potential factors include loss of genetic variation (Leamy, 1985), mutations (Clarke and McKenzie, 1987), adverse temperatures (Clarke, 1992), nutritional stress (Swaddle and Witter, 1994), population density (Zakharov et al., 1985), habitat fragmentation (Anciaes and Marinio, 2000), and a variety of chemical factors (Ellegren et al., 1997; Graham et al., 1993; Møller, 1993; Pankakoski, 1985). Several hypotheses exist to explain the connection between environmental stress and FA (Klingenberg and Nijhout, 1999; Woods et al., 1999). Advances in molecular genetics have revealed some of the vast complexity of developmental processes and the way they may relate to asymmetry, and it is difficult to imagine that a single genetic mechanism could be responsible for regulating the developmental response to stress (Batterham et al., 1996).

Stress resulting from excessive radiation may increase FA, because minor changes in environmental conditions would have more impact on the phenotype of individuals from exposed populations than from populations with no known exposure. Organisms under stress may also require more energy to perform the same functions as unstressed organisms, including energy spent for repair of the damage caused by stress on their bodies, as well as energy spent while functioning in stress-altered environments (Blum, 1988). On the other hand, genetic factors still influence the susceptibility of individuals and populations to the environmental factors creating a genotype-by-environment interaction (Møller and Swaddle, 1997). An increase in FA may reflect the expression of genetic variation at the phenotypic level due to the incorporation of mutant alleles in individual genomes (Møller and Swaddle, 1997). Finally, an increase of FA values would be expected in highly inbred populations (Zakharov and Sikorski, 1997). Thus, FA may only be used as a reliable indicator of environmental contamination when a substantial number of reference populations with no known contamination are sampled in order to account for the maximum number of relevant factors. In other words, it is essential to establish the ambient level of FA in a species and to know whether FA can vary significantly among reference populations even in the absence of assumed stressors.

Environmental radiation has imposed a significant amount of stress on populations in habitats contaminated during and since the 1986 Chornobyl meltdown. Several studies conducted in and around the Chornobyl nuclear power plant (ChNPP) have indicated that FA has significantly increased in plants and animals from the affected populations. FA was positively correlated with the level of contamination by ^{137}Cs in three different species of plants (Møller, 1998). In barn swallows, levels of FA, as well as the frequency of partial albinism, are increased near Chornobyl when compared to that of populations in reference areas (Ellegren et al., 1997; Møller, 1993). Some of these effects may have direct influences on individual fitness and could be expressed in lower competitive ability and survival (Møller, 1997). However, until recently mammalian populations from Chornobyl had not been tested for FA despite the fact that they live in the most contaminated areas around the failed reactor (Baker et al., 1996). In the absence of data on humans, small mammal populations may be one of the better models for evaluating risks of radioactive contamination for human populations.

Asymmetry has been estimated using many different indices (Palmer, 1996) that have varying degrees of reliability. Recently a new approach has been applied to the analysis of asymmetry using the linkage between geometric methods and conventional multivariate statistics and is called geometric morphometrics (Bookstein, 1996a,b; Klingenberg and McIntyre, 1998; Leamy, 1984; Palmer, 1996). A mixed model ANOVA is an essential part of this approach that allows reliable group-level estimation of measurement error (Palmer, 1996). In this method, asymmetry in overall size reflects positive correlations among differences between the inter-landmark distances on the left versus the right sides of skulls: 'the individual asymmetry parameter' (Leamy, 1997). Shape asymmetry is measured as the deviation between the pairs of the corresponding landmarks on the left versus those on the right side (Klingenberg and McIntyre, 1998). Using both of these approaches along with replicate measurements, allows the extraction and analysis of the patterns of covariation among landmarks and reliable estimates of FA, DA, and measurement error for each population. Our statistical approach followed closely that given by Klingenberg and McIntyre (1998).

Our overall objective was to determine whether asymmetry was elevated in radioactively contaminated populations compared to that in reference populations of the yellow-necked mouse (*Apodemus flavicollis*), a common species living in deciduous forests around the ChNPP and throughout Ukraine. Individual estimates for FA and DA were made for both size and shape of the skull from mice living in contaminated and reference areas. We wanted to also test for differences among reference populations for the amounts of FA and DA. Temporal heterogeneity in FA and DA were tested using samples from the same area, but from different years. Finally, we interpret our findings based on the radioactivity estimates for the same mice at each of the contaminated locations (Oleksyk et al., 2002).

2. Materials and methods

2.1. Populations

We collected 13 population samples totaling 843 individuals of *A. flavicollis* from 10 sampling locations (Fig. 1). Six sampled populations came from within a

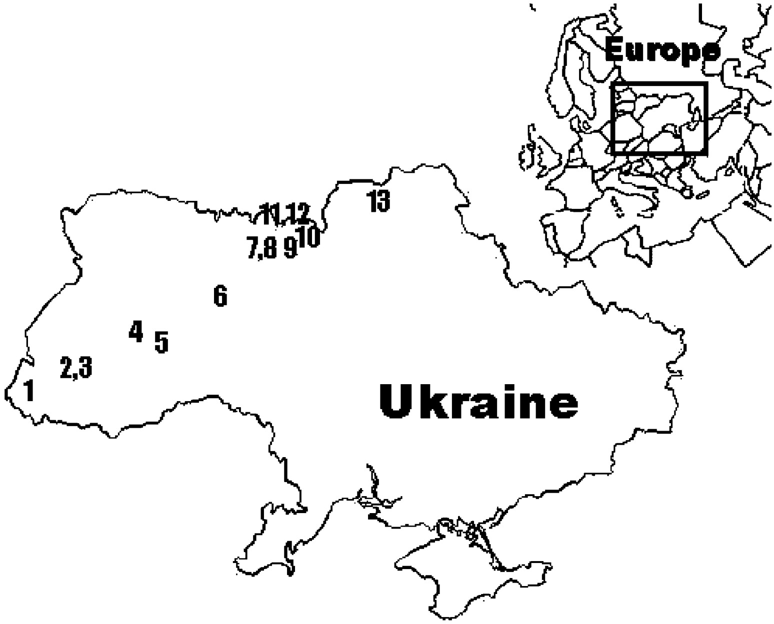


Fig. 1. Locations where populations of *Apodemus flavicollis* were collected: 1. Uzhgorod 1996 ($N = 33$; N 48° 44', E 22° 07'); 2. Kolochava 1996 ($N = 16$, N 48° 26', E 22° 37'); 3. Kolochava 1998 ($N = 17$; N 48° 26', E 22° 37'); 4. Stozhary 1998 ($N = 14$; N 49° 39', E 25° 43'); 5. Lysychyntsi ($N = 15$; N 49° 42', E 26° 11'); 6. Ruzhyn 1998 ($N = 20$; N 49° 42', E29° 14'); 7. Tovsty Forest 1997 ($N = 11$; N 51° 23', E 29° 42'); 8. Tovsty Forest 2000 ($N = 12$; N 51° 23', E 29° 42'); 9. Tovsty Forest (Forestry) 2000 ($N = 33$; N 51° 22', E 29° 43'); 10. Emerald Camp 1999 ($N = 15$; N 51° 20', E 30° 09'); 11. Gluboke Lake 1998 ($N = 10$; N 51° 27, E 30° 04'); 12. Gluboke Lake 2000 ($N = 13$; N 51° 27, E 30° 04'); 13. Vyshenky 2000 ($N = 17$; N 51° 40', 33° 05'). Populations 7, 8, and 9 are 10 to 30 km from the failed

roughly circular area of approximately 30 km from the failed reactor called the Chornobyl exclusion zone. Three of the six samples came from the most contaminated 10-km zone in close proximity to the failed reactor. The other three samples were obtained from the populations on the western edge of the 30-km exclusion zone. Latitude and longitude were recorded for each location using a geographical positioning system. The last group of sampled populations came from the uncontaminated locations along a southwest to northeast transect across Ukraine. None of the major plumes from the reactor went in the southwest direction. Two locations from the contaminated area and one reference location were sampled in 2 different years to check for the reproducibility of our results.

To estimate the degree of radioactive contamination affecting each of the contaminated populations, we used measures of ambient gamma activity collected with thermal luminescent dosimeters (TLDs), as well as measurements of concentrations of ^{137}Cs in dry muscle reported elsewhere (Oleksyk et al., 2002). Background values for the TLDs were estimated at the International Radioecology Laboratory in Slavutych, Ukraine. Some TLDs were also left at the Savannah River Ecology Laboratory, Aiken, SC.

2.2. Morphometrics

Our study concentrated on the fluctuating asymmetry (FA) of skulls. Each specimen was cleaned with dermestid beetles and dried. Skulls were leveled on a sand base with the ventral surface up. We took pictures of the ventral surface of each skull with a 35-mm camera using a close-up lens and a ring flash that went around the lens to provide even lighting. We used color film with an ASA of 100. Two pictures of each skull were taken to account for the effects of placement on the measurement error. Each skull was repositioned after the first picture was taken. Pictures were developed and scanned into individual bitmap files using a Nikon™ LS-2000 film scanner. Each picture was given a random name to prevent subjective bias during the subsequent measurement steps (Palmer, 1994). Evolutionarily homologous landmarks ($N = 24$) were chosen on each side of the skull similar to those used in a study with house mice (Auffray et al., 1996) (Fig. 2). Landmarks were distributed on the ventral surface of the skull to represent its entire surface (Fig. 2). Landmark positions were digitized using a standard software package TPSDIG (Rohlf, 2001). Landmarks were also independently placed on each of the pictures twice to assess the effects of digitizing on the measurement error. Statistical analyses were conducted using SAS 8.1 software (SAS, 1999).

2.3. Asymmetry of skull size

The FA of size and shape were estimated as described in Klingenberg and McIntyre (1998). Asymmetry in overall size was estimated using the unit centroid size (CS). The CS for each side of each skull was calculated as the square root of the sum of squared distances from all of the landmarks on each side to their centroid (Slice et al., 1996). Each skull was scaled to the unit CS in the analysis to eliminate the effect of individual size. To assess the FA of the total skull size, we used an ANOVA with CS as the dependent variable, side as a fixed effect and individuals as a random effect as recommended by Palmer (1994). The interaction term in this model represents the variation in left-right differences among individuals, which is a measure of FA. In addition, the main effect of sides accounts for the directional asymmetry and the main effect of individuals accounts for individual variation in size. The individual × side interaction was used as the mean square for error to test the significance of the main effects. The measurement error, which was the sum of the placement and digitizing errors, was used to test for the significance of the indi vidual × side interaction effect. The variance component of the interaction term provides an unbiased estimate of FA in each population.

Degrees of freedom were calculated using the Satterthwaite approximation (Palmer, 1994). FA of size was calculated in the same way separately for each sample in the study. The difference between the populations in the FA of size was assessed using Levene's test on the absolute differences between the right and left CSs

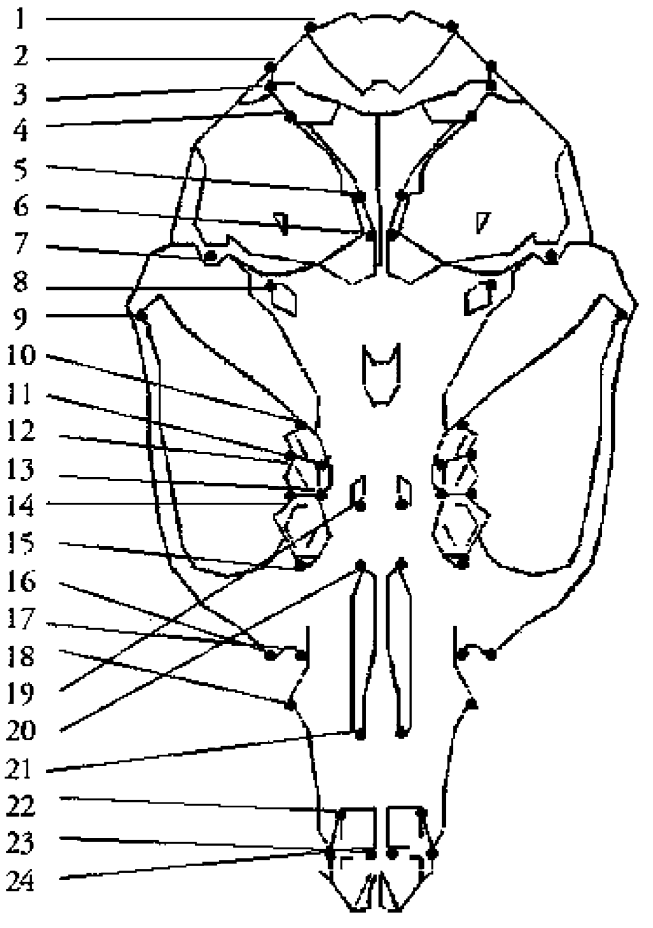


Fig. 2. Landmark positions on the ventral surface of the skull of *Apodemus flavicollis*. Landmarks on the left side are numbered 1 to 24. Identical landmarks were digitized on both left and right sides of the skull. This picture is not drawn to scale.

corrected for individual size (Palmer, 1994). Differences in individual population FAs were tested using pairwise *F*-tests with the approximate degrees of freedom calculated to the second decimal place. In determining significance, all probabilities generated from *F*-tests were tested using a sequential Bonferroni procedure to adjust for type I errors (Palmer, 1994). Differences between samples in DA were tested using a three-way ANOVA with sides (fixed), individuals (random) and populations (fixed) as main effects, and replicated measurements (Lamb et al., 1990). Differences among samples in the amount of DA were assessed as a sides $\times$ population interaction effect compared to the interaction individuals (sides $\times$ population) effect mean squares. Obtained probabilities were adjusted using a sequential Bonferroni procedure.

2.4. Asymmetry of skull shape

We analyzed the shape asymmetry of skulls by superimposing the configurations of landmarks from each side of the skull using a Procrustes superimposition (Klingenberg and McIntyre, 1998; Rohlf and Slice, 1990). First, landmark configurations of the left sides of the skulls were reflected to their mirror images by subtracting the *x*-values from a constant (e.g. 20) to align corresponding landmarks of right and left sides. After configurations were scaled to unit CS, a point with average coordinates (centroid) from the right side was given the same coordinates as the centroid from the corresponding left side of the skull. Then, configurations were rotated around their centroid to achieve the best fit. This procedure is included in the software TPSRELW (Rohlf, 2000). The output of the Procrustes procedure contains the coordinates of superimposed landmarks. Asymmetry can then be measured as the deviations between the pairs of the corresponding superimposed landmarks.

We used the same two-factor mixed-model ANOVA as previously to calculate sums of squares for each of the effects on each x and y coordinate. Then, we calculated the overall sums of squares for each of the main effects, interaction term and the error by adding the individual sums of squares for each of the effects across the x and y coordinates (Klingenberg and McIntyre, 1998). Degrees of freedom for the shape ANOVA were the degrees of freedom for each of the effects multiplied by the number of landmark coordinates minus four. The individual $\times$ side interaction was used to test the significance of the main effects. The measurement error was used to test for the significance of the interaction effect. The variance component for the individual $\times$ side interaction effect represented our best estimate of FA.

We used multiple F-tests to compare the values of FA and DA of shape between each pair of populations and to generate appropriate p-values. Then, once more, we applied the sequential Bonferroni procedure to ensure the appropriate table-wide probability of type I error as in Palmer (1994). The difference between populations was considered statistically significant only when the pairwise p-value was lower than the revised p-value.

2.5. Correlation of the overall asymmetry and ^{137}Cs in the dry muscle tissue

Two different estimates of FA were used to relate the amounts of the overall asymmetry in the exposed individuals to the amounts of ^{137}Cs in the dry muscle tissue. First, we used the absolute difference between the individual's CS of right and left sides as the approximation of the overall asymmetry of individual size. In addition, we used a measure of Procrustes distance between left and right sides (Bookstein, 1991). To calculate this measure, we subtracted the Procrustes aligned coordinates of the landmark configuration of right side from the corresponding coordinates of each individual landmark on the left side of the skull. Then we added the squared differences and calculated the square root of the resulting sum (Klingenberg and McIntyre, 1998). This distance measure is similar to the mean absolute difference between the left and right side (Palmer, 1994), but since it is initially standardized to unit CS during the Procrustes procedure, it is independent of overall size, except for allometry (Klingenberg and McIntyre, 1998). We used the nonparametric Spearman's correlation (r_S) to assess the degree of association between the individual CS differences, individual Procrustes distances, and the amounts of ^{137}Cs in the dry muscle of individual mice.

3. Results

3.1. Measures of radioactivity

Populations at Gluboke Lake received the highest amounts of ionizing radiation from their environments: 4.146 μGy/h. The second highest doses were found at Emerald Camp averaging at 0.297 μGy/h. Populations at Tovsty Forest and Tovsty Forest (Forestry) received the lowest dose averaging at 0.132 and 0.107 μGy/h. These results, as well as the amounts of the intramuscular ^{137}Cs in these animals, were reported previously in our study of frequency distributions of radioactive contaminants at Chornobyl (Oleksyk et al., 2002).

3.2. Departures from normality and measurement error

Antisymmetry was examined using Kolmogorov–Smirnov tests of the frequency distribution of the CSs compared to an expected normal distribution. If present, antisymmetry would artificially inflate the levels of FA. The frequency distributions of data for each population were inspected for the presence of bimodality or unusual outliers. Outliers were traced back to the corresponding individuals whose landmarks were digitized again. The three types of outliers that existed were associated with handling (e.g. broken skull), severe trauma, and marks of disease. Individuals with these particular problems were removed from the analysis. Finally, after adjusting the overall error rate to the 0.05 level, there were no significant deviations from normality as indicated by the Kolmogorov–Smirnov test. Thus, we concluded that there was no evidence of antisymmetry in any of the studied population samples.

Measurement error was addressed during the *F*-tests. We tested whether our FA estimates were significantly larger than predicted due to error alone. There were three populations (Vyshenky, Tovsty Forest (Forestry), and Ruzhyn), where it was not possible to calculate a reliable estimate of the FA of shape because of the high measurement error. All of the other measurements of FA of size and shape were statistically significant with $p = 0.001$ in every case. Overall, our estimates of shape asymmetry appeared to yield results with higher significance and more degrees of freedom than those of size asymmetry.

3.3. Directional asymmetry

The presence of DA indicates that one side is consistently and significantly different than the other side. In the mixed model ANOVA procedure, DA is tested along with the FA for both the size and shape. The main effect of sides in the two-way ANOVA estimates directional asymmetry and sides $\times$ population interaction in the three-way ANOVA tests for the differences among populations in directional asymmetry. DA of size was significant only for two populations: Tovsty Forest (Forestry) ($DA = 8.76 \times 10^{-8}$, $p < 0.005$) and Tovsty Forest 1997 ($DA = 4.79 \times 10^{-7}$, $p < 0.0001$). There was an overall difference between populations in the amount of DA of size (mixed-model ANOVA, $F_{12,216} = 4.29$, $p < 0.0001$). DA of shape was significant for all populations except the one from Emerald Camp (Table 1). There was an overall difference between populations in the amount of DA of size (mixed-model ANOVA, $F_{528,5544} = 9.04$, $p < 0.0001$). Values of DA, degrees of freedom and the Bonferroni corrected significant differences in multiple pairwise comparisons are presented in Table 1. Most of the populations in the upper part of the table came from the northern contaminated localities at Chornobyl.

3.4. Fluctuating asymmetry

Asymmetry in overall size represents a positive correlation among left–right differences of the average distance between all of the landmarks on that side and their geometrical average (centroid size, CS). In our case, FA of size was significantly greater than the variance expected due to the measurement error for all of the samples except the three mentioned earlier (Vyshenky, Tovsty Forest (Forestry) and Ruzhyn). These samples were not used in the pairwise comparisons. The results of Levene's test indicate significant differences among samples in the differences between the left and right CS ($F_{12,831} = 8.36$, $p < 0.0001$). All of the samples from the 10-km exclusion zone had high values for FA, while those for the reference samples had low values of FA and samples from the 30-km exclusion zone had intermediate values (Fig. 3). On average, FA in samples from Gluboke Lake and Emerald Camp was 3.6 times as high as in the reference areas, and 2.3 times higher than in the less contaminated samples from Tovsty Forest and Tovsty Forest (Forestry) 30-km from the reactor (Fig. 5). However, none of the samples from the same location but different years was significantly different for FA.

Asymmetry of shape represents a positive correlation of the differences between the coordinates of the optimally aligned landmarks of the superimposed configurations of the left and right sides resulting from application of the Procrustes procedure. All FAs of shape were significantly larger than the variance expected due to measurement error ($p = 0.0001$; Table 2). We ranked our populations by their corresponding FA values and performed multiple *F*-tests with the subsequent Bonferroni correction. The samples from the 10-km exclusion zone have the highest ranks and FA values, while the reference samples and those from the less contaminated parts of the exclusion zone had lower ranks. The FAs of shape in both of the samples from Gluboke Lake and a sample from Emerald Camp were significantly greater than those in the rest of the samples, but were not significantly different from each other (Table 2, Fig. 4). The FAs of the samples from the Tovsty Forest

Table 1
Summary of analyses of directional asymmetry (DA) of shape within and among populations of *Apodemus flavicollis* from Ukraine

Group	N	Populations	DA df	Mean DA values	Significant pairwise F-tests
30 km	8	Tovsty Forest 2000	40.81	2.77×10^{-06}	7, 8, 9, 10, 11, 12
10 km	12	Gluboke Lake 2000	37.03	2.11×10^{-06}	9, 10, 11, 12
10 km	11	Gluboke Lake 1998	31.26	1.99×10^{-06}	9, 10, 11, 12
Ref	13	Vyshenky	38.72	1.76×10^{-06}	9, 10, 11, 12
Ref	5	Zbarazh Lysychyntsi	39.11	1.68×10^{-06}	9, 10, 11, 12
30 km	9	Tovsty Forest Forestry	40.46	1.18×10^{-06}	12
Ref	1	Uzhhorod	39.80	1.02×10^{-06}	12
Ref	4	Zbarazh Stozhary	33.03	8.45×10^{-07}	7
Ref	3	Kolochava 1998	27.76	4.15×10^{-07}	1, 2, 3, 4, 5
Ref	6	Ruzhyn	26.23	3.73×10^{-07}	1, 2, 3, 4, 5
30 km	7	Tovsty Forest 1997	21.58	3.21×10^{-07}	1, 2, 3, 4, 5
Ref	2	Kolochava 1996	21.36	2.53×10^{-07}	1, 2, 3, 4, 5, 6, 7
10 km	10	Emerald Camp	3.54	7.41×10^{-08}	n/a

Groups include samples from the 10 km zone around the failed reactor (10 km), 30 km zone (30 km), and reference samples outside of the Chornobyl 30 km zone (Ref). Samples are listed in the table as ranked from the highest to the lowest for the amount of directional asymmetry so that populations with the highest DA are at the top of the table. DA is tested against the amount of experimental error. Results of significant F-tests after sequential Bonferroni correction at $p = 0.05$ for pairwise comparisons for all samples are listed, where N is the number that corresponds to the name of each population ranked from south to north as in figures.

n/a, not tested since the DA value was not significant when tested against the error.

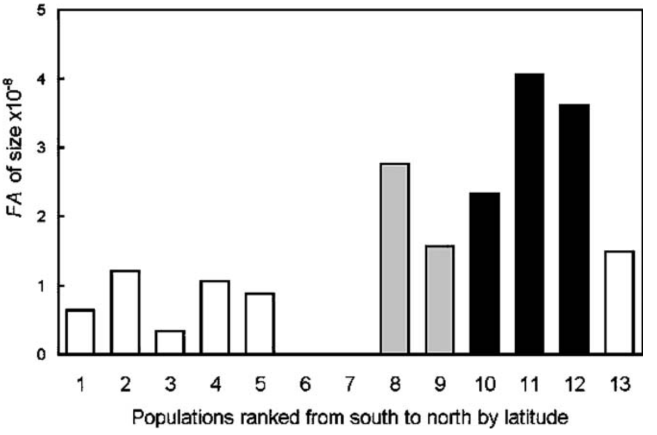


Fig. 3. Mean values of fluctuating asymmetry for size with populations arranged by their latitude from the southern most (1) to northern most location (13) as in Fig. 1. Values for reference populations are indicated by white bars, while populations from the 30 km contaminated zone are given in gray, and those from within the 10 km area from the Chornobyl reactor are given in black. Negative mean values for populations 6 and 7 are not shown.

population also did not differ from each other. Finally, the average FA in the 10-km exclusion zone is 1.8 times higher than the average FA in the reference populations, and 3.7 times higher than the average FA in the less contaminated populations within the 30-km distance from the reactor (Fig. 5).

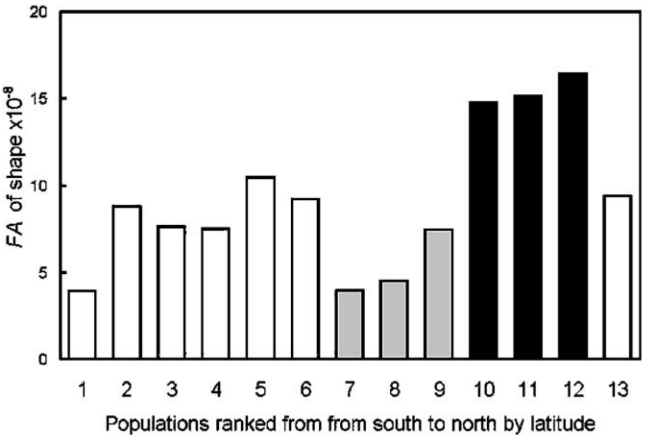
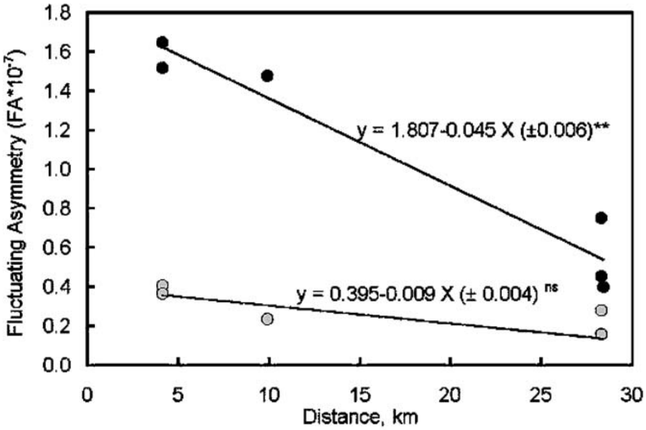


Fig. 4. Values of the fluctuating asymmetry (FA) of shape ranked by the latitude of the location from the southern most (1) to northern most location (13) as in Fig. 1. Control populations are indicated in white, populations from the 30-km zone are given in gray, and populations from within the 10-km area from the Chornobyl reactor are represented in black. Significance for each of the values and pairwise differences are presented in Table 2.



** $p = 0.001$
ns $p = 0.05$

Fig. 5. Correlation between the values of fluctuating asymmetry of shape (black circles) and the distance to the Chornobyl reactor ($r^2 = 0.94$), and lack of correlation between fluctuating asymmetry of size (gray circles) and distance to the reactor ($r^2 = 0.54$).

3.5. Correlation of asymmetry in the Chornobyl exclusion zone

There was an overall significant correlation between the differences of the landmark coordinates of the Procrustes aligned configurations of the left and right sides and the amounts of intramuscular ^{137}Cs in the individual mice from the Chornobyl area ($r_S = 0.28$, $p < 0.001$). There were also significant correlations between the radiocesium concentrations and CS ($r_S = 0.09$, $p = 0.03$) and between the two estimates of the overall asymmetry ($r_S = 0.41$, $p < 0.0001$). There were no significant correlations between the Procrustes differences and the concentrations of intramuscular ^{137}Cs or between the amounts of radiocesium and CS differences within any of the samples. We were unable to separate different types of asymmetry and the measurement error associated with the procedures used to collect the data in this analysis. These results represent an overall measure of asymmetry assuming consistent levels of measurement error across populations. There was a significant correlation between the FA of shape and the distance to the failed reactor for the locations in the Chornobyl exclusion zone (both the 10- and 30-km zones; $r^2 = 0.94$, $p = 0.001$: Fig. 5). However, FA of size did not correlate with distance from the reactor ($r^2 = 0.53$, $p = 0.083$: Fig. 5).

4. Discussion

Ionizing radiation should impose significant stress on individual animals in landscapes contaminated during the 1986 Chornobyl meltdown. We predicted that higher values of FA should be observed in animals from the contaminated sites

Table 2
Summary of analyses of non-directional asymmetry of shape within and among populations of *Apodemus flavicollis* from Ukraine

Group	N	Populations	FA *df*	FA values	Significant pairwise F-tests
10 km	12	Gluboke Lake 2000	366.00	1.643×10^{-07}	4, 5, 6, 7, 8, 9, 10, 11, 12, 13
10 km	11	Gluboke Lake 1998	69.92	1.513×10^{-07}	7, 8, 9, 10, 11, 12, 13
10 km	10	Emerald Camp	399.90	1.475×10^{-07}	4, 6, 7, 8, 9, 10, 11, 12, 13
Ref	4	Zbarazh Stozhary	368.50	1.043×10^{-07}	1, 3, 11, 12, 13
Ref	13	Vyshenky	457.10	9.363×10^{-08}	1, 12, 13
Ref	6	Ruzhyn	374.90	9.200×10^{-08}	1, 3, 12, 13
Ref	2	Kolochava 1996	427.10	8.786×10^{-08}	1, 3, 12, 13
Ref	3	Kolochava 1998	396.70	7.616×10^{-08}	1, 2, 3, 12, 13
Ref	5	Zbarazh Lysychyntsi	329.90	7.492×10^{-08}	1, 2, 3, 12, 13
30 km	8	Tovsty Forest 2000	259.10	7.483×10^{-08}	1, 2, 3, 12, 13
30 km	7	Tovsty Forest 1998	49.83	4.509×10^{-08}	1, 2, 3, 4, 12, 13
30 km	9	Tovsty Forest Forestry	859.60	3.964×10^{-08}	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Ref	1	Uzhhorod	735.00	3.920×10^{-08}	1, 2, 3, 4, 5, 6, 7, 8, 9, 10

Groups include populations from the 10 km zone around the failed reactor (10 km), 30 km zone (30 km), and reference populations (Ref). Populations are listed as ranked from the highest to the lowest for the amount of fluctuating asymmetry (FA) in non-directional asymmetry so the populations with the highest FA are at the top of the table. FA significance is tested against the amount of experimental error. Results of significant F-test after sequential Bonferroni correction at $p < 0.05$ for pairwise comparisons of all populations are listed where N is the number that corresponds to the name of the population as in Fig. 4. Populations in the 10-km zone around the Chornobyl Plant have the highest amounts of FA.

closest to the failed reactor as opposed to the uncontaminated reference sites elsewhere in the Ukraine. Indeed, higher FAs were documented for samples taken from populations of rodents living in areas closest to the failed Chornobyl reactor for asymmetry of both types of analysis measurements (size and shape). Fluctuating asymmetry of shape was highest in the three samples from the most contaminated locations in the Chornobyl exclusion zone (Fig. 4). Although directional asymmetry of shape was also highest in the contaminated samples, it was not significantly different from that of most of the reference samples because of the high variance among the samples (Table 2). Finally, values of shape FA were highly correlated with distance from the reactor. However, samples from the less contaminated areas around the failed reactor did not exhibit FAs significantly different from those of reference samples. Populations that were sampled more than once did not show a significant difference between years. Thus, our results were robust and replicable over time. These data support our hypothesis about expected increases of FA in rodents from the contaminated areas at Chornobyl (Oleksyk et al., 2001).

Differences between the landmark coordinates of the Procrustes aligned configurations of the left and right sides of the individual mice correlated with the concentration of intramuscular ^{137}Cs, but not within any of the individual samples. The lack of correlation within the samples is likely due to the limited range of the x and y variables for each sample relative to that across (Chesser et al., 2000; Oleksyk et al., 2002). There might also be other contaminants that cause elevated levels of asymmetry, such as radioactive ^{90}Sr that were not accounted for in this analysis. In addition, intramuscular contamination is not the only source of exposure for the affected populations. Animals could also receive a substantial dose of external radiation from their environment that may not correlate with the ^{137}Cs concentration in muscle. Thus radiocesium concentrations represent only a rough approximation of the total exposure. Finally, we may lack statistical power to detect this relationship in each population.

Although FA tends to increase in populations exposed to pollution (Møller and Swaddle, 1997), it should be considered significant only when the level of FA of stressed populations is above the background level of FA in unstressed populations. In our study multiple populations of the same species were sampled in different environments across a large geographical area south and north of Chornobyl. There were significant differences between the reference populations in the amounts of FA and DA (Tables 1 and 2). Therefore, conclusions about radiation effects at Chornobyl should always be questioned when there are no or very few reference populations. In these types of studies and probably others, it seems prudent to always expect that reference populations will differ significantly from each other, and there is no way to know whether a particular reference population has a high or low value for the response variable of interest without replicate sampling of reference populations. The majority of the samples should always come from the reference sites. However, despite the differences in geographical locations of the sampled populations or their environmental conditions, FA values were greatest in populations that should have been maximally affected by the radioactivity and were located closest to the reactor (Fig. 5).

Our results are consistent with the predicted increase in the levels of FA in populations exposed to anthropogenic contamination. However, the mechanism(s) by which this occurs is not clear. Some of the populations may experience high levels of inbreeding and associated higher mortality rates. If the differential mortality hypothesis were true for these populations, we would expect FAs in the most contaminated ones to be similar or lower than those of 'moderately' contaminated populations. However, this was not the case (Figs. 3 and 4). On the other hand, the highly skewed microspatial distributions of contaminants in the populations from Chornobyl (Oleksyk et al., 2002) could result in only a few individuals dying because they lived in close proximity to highly contaminated areas. Migrants from relatively uncontaminated sites in the area could constantly replace them. With high migration rates, even if selective mortality occurs because of life-threatening doses (Chesser et al., 2000), it is unlikely to result in a significant decrease in overall levels of FA in a population.

Other field studies have linked FA to toxic agents in contaminated areas. Radiation from Chornobyl affected levels of FA in three species of plants in areas near the Chornobyl exclusion zone (Møller, 1998). Several studies show that plants increase their FAs close to sources of aerial contamination (Graham et al., 1993; Kozlov et al., 1996). Higher levels of FA have been found in fish from the waters around sources of industrial pollution and in ponds with high concentrations of mercury and low pH (Zakharov, 1981). High heavy metal concentrations are correlated with high FA values in common shrews (Pankakoski et al., 1992). Gray seals have higher levels of FA in highly polluted areas than in relatively pristine areas (Zakharov et al., 1989). Finally, FA was associated with heavy metal pollutions in rodents (Cavedon et al., 1990; Nunes et al., 2001). On the other hand, some studies have failed to demonstrate increases in FA with pollution (Gileva and Kosareva, 1994; Rabitsch, 1997). However, overall FA seems to be associated with environmental contamination of various sorts, including radiation at least under some conditions.

Asymmetry represents a measure of the developmental instability of a phenotype and may be associated with important characteristics affecting the individual fitness. Some studies argue that asymmetric individuals generally have lower fecundity and poorer survival than more symmetrical individuals in populations (Møller, 1997). These differences arise from individuals with lower competitive ability, and higher risks of predation and parasitism compared to that of their more symmetrical counterparts. It is likely that individuals in highly contaminated areas would have overall lower average fitness values which creates population sinks (Pulliam, 1988). There is also a contrary point of view that the evidence for connection between developmental stability and fitness is not clear (Clarke, 1988). Finally, some other authors argue that FA is not always a good measure of environmental quality because of the selective process of 'differential mortality' among the animals exposed to toxic agents (e.g. Floate and Fox (2000)). A result of this type of selection would be that at locations with higher levels of exposure a robust subset of individuals would survive and express lower levels of FA. This in fact may be what is observed in some instances (Gileva and Kosareva, 1994). Thus, individuals in the contaminated areas around the Chornobyl plant may suffer changes that result in long-term evolutionary consequences. However, it is unlikely that these changes would persist in the local populations because of the substantial gene flow between among those populations and the populations in the uncontaminated areas nearby.

Failure to detect higher FAs in populations with lower levels of contamination may indicate a threshold of exposure somewhere between 0.132 and 0.297 μGy/h (i.e. the difference between the 10 km zone and 30 km zone exposure rates reported here) over which FA significantly increases above its ambient level (Figs. 3, 4 and 5). However, since most contaminated environments in the United States and Europe are expected to be decontaminated to the levels much lower than those seen in this study, FA may not be useful in validating the effectiveness of clean-up efforts at these lower levels.

In conclusion, highly contaminated populations of *A. flavicollis* expressed significantly higher levels of FA calculated both as asymmetry of size and shape. However, FA values of the less contaminated populations in the outer exclusion zone were not different from the FA values expressed in most of the reference populations from the relatively uncontaminated areas in Ukraine. In addition, we found large differences in the amounts of FA and DA among reference populations from the uncontaminated regions. Higher FA values probably indicate that populations from the localities close to the failed Chornobyl reactor are experiencing significant levels of stress during their development. These highly contaminated populations may also be accumulating mutations that could disrupt normal development in the affected individuals. Finally, future studies of FA in contaminated areas must include sufficient reference populations to establish the expected background level of FA in an area before conclusions can be reached about the effects of contaminants.

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