Heredity (2009) 102, 155–162

www.nature.com/hdy

ORIGINAL ARTICLE

Robertsonian polymorphism in the common shrew (*Sorex araneus* L.) and selective advantage of heterozygotes indicated by their higher maximum metabolic rates

A Banaszek, JRE Taylor, D Ochocińska and W Chętnicki
Institute of Biology, Department of Biology and Chemistry, University of Białystok, Białystok, Poland

Some cases of Robertsonian (Rb) polymorphism in the common shrew (*Sorex araneus* L.) are believed not to be associated with hybrid zones. One of the hypotheses explaining the persistence of such Rb polymorphism is that they are maintained by some form of selection for Rb heterozygotes. To test this hypothesis, we compared several parameters between homozygotes and Rb heterozygotes for the *mp* chromosome pair. We used shrews from Jurowce population in Poland, situated within the range of the Białowieża race, where Rb polymorphism persists far from any known hybrid zone. We found no differences between the two karyotypic classes in maximum metabolic rate during running (forced activity). However, the Rb heterozygotes showed significantly higher maximum metabolic rate during swimming (forced activity combined with thermal stress). The levels of fluctuating asymmetry (FA) of homozygous and Rb heterozygous shrews were indistinguishable, indicating no effect of chromosomal heterozygosity on developmental stability of shrews. We suggest that selective advantages, such as the higher metabolic performance in activity combined with cold stress, may outweigh the expected negative effects of Rb heterozygosity upon fertility, and help to maintain huge areas of the Rb polymorphism in this species.
Heredity (2009) **102,** 155–162; doi:10.1038/hdy.2008.102; published online 1 October 2008

Keywords: maximum metabolic rate; cold stress; developmental stability; fluctuating asymmetry; heterosis; Robertsonian fusion

Introduction

One of the best-described examples of exceptional chromosomal variability is the common shrew (*Sorex araneus* L., 1758) with chromosomal polymorphism generated by Robertsonian (Rb, centric) fusions, in which non-homologous acrocentric chromosomes join to form a metacentric element. In effect, three karyomorphs formed by one polymorphic pair of chromosomes may be present in one population: acrocentric homozygotes, simple Rb heterozygotes (with trivalent formed at meiosis I) and metacentric homozygotes. In the description of the karyotype of *S. araneus*, each chromosome arm is denoted by a letter of the alphabet with 'a' indicating the largest chromosome arm (Searle *et al*., 1991). Of the 20 chromosome arms comprising the common shrew's haploid karyotype, 12, denoted by 'g'–'r', have been found to show Rb variation. In addition to the Rb polymorphism occurring within populations, the common shrew is subdivided into numerous karyotypic races throughout its extensive Euro-Asiatic range (Wójcik *et al*., 2003). These races may differ in number or in arm composition of Rb metacentrics. When such karyotypic races meet, a hybrid zone arises containing hybrids, which are chromosomal heterozygotes. If the races differ in number of specific Rb metacentrics, the hybrids are just simple Rb heterozygotes. In those cases in which the races differ in the arm composition of metacentrics which share monobrachial homologies (that is, have one arm in common), the hybrids are complex heterozygotes and have complex meiotic configurations involving up to 11 chromosomes (Searle and Wójcik, 1998; Pavlova *et al*., 2007).

The maintenance of Rb polymorphism in the common shrew, as in any other species, requires explanation, as chromosomal heterozygosity is almost always connected with meiotic perturbations and lowered fertility, hence the heterozygotes would be selected against. Although it is not possible to show the reduced fertility in common shrew heterozygotes with the available sample sizes, the greater irregularity of meiosis in Rb heterozygotes leads one to infer that a small reduction in fertility does occur (Banaszek *et al*., 2000, 2002a). In such case, the forces opposing the negative pressure of natural selection must exist to maintain the chromosomal polymorphism. The hypotheses explaining the origin and maintenance of Rb polymorphism in the common shrew were described and thoroughly discussed by Searle and Wójcik (1998). Generally, most cases of polymorphism in the common

Correspondence: Dr A Banaszek, Institute of Biology, Department of Biology and Chemistry, University of Białystok, Świerkowa 20B, 15-950 Białystok, Poland.
E-mail: banaszek@uwb.edu.pl
Received 18 February 2008; revised 21 July 2008; accepted 31 August 2008; published online 1 October 2008

shrew can be explained by current hybrid zones (for review, see Searle and Wójcik, 1998). For example, the hybrid zone between the Drnholec (*jl*, *hi*, *gm*, *ko* and *nr*) and Ulm (*jl*, *hi*, *gm*) races, which differ in number of Rb metacentrics, generates the huge polymorphic area of *ko* and *nr* chromosome pairs covering most of former Czechoslovakia (Lukacova *et al*., 1994). The hybrid zones between the races, which differ in arm composition of metacentrics, may also maintain polymorphism in the surrounding area through the mechanism called acrocentric peak (Searle and Wójcik, 1998).

However, in the common shrew, some cases of polymorphism are not associated with any known hybrid zones (Searle and Wójcik, 1998). The first example is the chromosome pair, *jl*, which is characteristic of all known races of the common shrew and shows low-level polymorphism throughout almost all the species range. Moreover, Searle and Wójcik (1998) listed three examples of populations located far from the areas of contact (150–200 km), yet where polymorphism of one or two chromosome pairs was maintained and documented for more than 20 years.

Here, we present another example of polymorphic population, named Jurowce, which is not directly influenced by current hybrid zones, where the polymorphism has been documented for 12 consecutive years: 1987–1999. The Jurowce population is situated in northeastern Poland within the range of the Białowieża race with specific metacentrics, such as *jl*, *hn*, *ki*, *gr* and *mn*. Four chromosome pairs are polymorphic in this population*: jl*, *ki*, *gr* and *mp*. The frequencies of chromosomes were stable over the 12 years of sampling and the population was in Hardy–Weinberg equilibrium (Szałaj *et al*., 1999). The distance to the closest hybrid zone is about 100 km and, with the extensive knowledge of the distribution of the races in Poland (Wójcik, 1993), we exclude the possibility of the existence of an undetected hybrid zone in the neighbouring area. Additionally, the analysis of the geographical position of chromosomally described sites in Belorussia and Ukraina (Mishta *et al*., 2000) also confirms that there is no hybrid zone in areas close to the eastern border of Poland.

Three chromosome pairs, namely *jl*, *ki* and *gr*, show a low level of polymorphism with most of individuals being metacentric homozygotes and frequency of acrocentrics not exceeding 5–10%. The *mp* chromosome pair is, on the other hand, highly polymorphic with the frequency of acrocentrics reaching about 30% (Szałaj *et al*., 1999). Although the low-level polymorphism of *ki* and *gr* pairs may be considered long 'tails' of the hybrid zones and *jl* polymorphism is a specific case of low-level polymorphism characterizing most of the races of the species (Searle and Wójcik, 1998), the *mp* polymorphism in this population is too high to be generated by a hybrid zone.

The aim of this study is to compare homozygous and Rb heterozygous shrews for *mp* chromosome pair in some parameters that describe fitness variability of individuals. The simplest way to explain the persistence of polymorphism in the common shrew populations would be to assume that Rb heterozygotes perform better, or in the case of developmental stability at least not worse, than homozygotes in parameters that could influence individual fitness. First, *Sorex* shrews are special in respect to very high metabolic rates that are much higher than would be expected for mammals of the same body mass (Taylor, 1998; Ochociñska and Taylor, 2005). Hence, the energetic budget and metabolic performance are very important fitness parameters in the common shrew. As the effect of genetic differences between individuals is most likely to be manifested during periods of stress, we decided to investigate the relationship between chromosomal heterozygosity and maximum metabolic rates measured during running (a forced activity) and during swimming (a forced activity combined with thermal stress). We assume that the ability to raise metabolism to a high level would be adaptive; it has been found that maximum rates of energy metabolism may determine the survival of small mammals in extreme conditions (Hayes and O'Connor, 1999). Second, we estimated the levels of fluctuating asymmetry (FA) in *mp* homozygous and Rb heterozygous shrews. FA, which is the variation in the small random differences occurring between the left and right side of normally bilaterally symmetrical traits, is commonly used as a fitness parameter describing developmental stability of an individual. Developmental stability may be understood as the ability of an organism to buffer genetically or environmentally induced perturbations during development (Zakharov, 1989).

Materials and methods

Trapping and housing conditions

The immature shrews were livetrapped in June and July of 1997 and 1998 in the Jurowce population near Bialystok, northeastern Poland. In shrews, the immatures are non-breeding animals in their first calendar year of life (Pucek, 1981). After capture, the animals were kept in controlled conditions of temperature of 18 °C (± 1 °C) and in long day conditions of light with natural hours of sunrise and sunset. Each shrew was housed individually in a glass terrarium measuring $32 \times 46 \times 30$ cm. A running wheel was provided for each shrew to prevent fattening and all shrews used the wheel very willingly. Shrews had constant access to tap water and food *ad libitum*. They were fed with a food mixture modified from the recipe of Searle (1984). The food mixture contained the following ingredients in proportion (by weight): 49 parts fresh ox heart, 32 parts fresh chicken, 16 parts fresh ox liver and three parts of vitamin and mineral supplement (NAFAG, Gossau, Switzerland). The meat was homogenized, thoroughly mixed and stored at −18 °C. The shrews were fed twice daily at 08.00 and 20.00 h. Additionally, once daily, the shrews were provided with live crickets (*Gryllus campestris*).

Measurements of maximum metabolic rates

Maximum metabolic rates were measured as maximum oxygen consumption during swimming ($VO_{2\,SWIM}$) and during running ($VO_{2\,RUN}$). Both metabolic rates were recorded after 2 weeks of acclimation to laboratory conditions. The acclimation to standardized laboratory conditions eliminates, at least partially, the environmental effects and leaves the genetic differences between the individuals easier to detect. The measurement of $VO_{2\ SWIM}$ on a given animal was performed 1 or 2 days after the measurement of $VO_{2\,RUN}$, and all metabolic trials were conducted between 15.00 and 19.00 hours.

Animals were weighed to the nearest 0.1 g before each measurement.

Oxygen consumption was measured in shrews with a positive-pressure open-flow respirometry system. A metabolic chamber with an animal inside received dry outside air from the upstream thermal mass-flow controller (β-ERG, Warsaw, Poland). Depending on the type of measurement, we interchangeably used different temperatures, two metabolic chambers and airflow rates (see below).

Excurrent air from the chamber was subsampled, redried (Drierite), scrubbed of CO_2 (Carbosorb AS, BDH Laboratory Supplies, Poole, England), and finally directed to the sensor of the two-channel oxygen analyzer (S-3A/II N 37 M, Ametek, USA). A computer with the A/D interface recorded the output of the O_2 analyzer (the difference in oxygen concentration between the ambient and the excurrent air), each 100 ms, averaged readings over 3 s intervals, and saved to a disc. We calculated metabolic rates using the equation 4 of Hill (1972), and corrected instantaneous values of maximum metabolic rates for the chamber washout time by applying a Z-transformation (Bartholomew *et al*., 1981, equation 1).

To measure maximum metabolic rate during swimming ($VO_{2\,SWIM}$), we used a vertically positioned cylindrical Plexiglas metabolic chamber (22.7-cm height, 11.5-cm diameter), vented with outdoor air at the rate of 750 ml min^{-1}. The chamber was partly filled with water, leaving the air volume of 560 ml above the water level. The temperature of water within the chamber was controlled at 25.0 ± 0.2 °C (this temperature was found earlier to produce maximum oxygen consumption during swimming). Each animal was placed just above the water level on a movable perforated platform and allowed 10 min for adaptation. The platform was then abruptly submerged to force the animal to swim. The duration of swimming was typically 5 min, and it was sometimes shortened up to 3.5 min if oxygen consumption started to decline, or prolonged to 6–9 min if oxygen consumption was rising. The behaviour of animals was recorded throughout the trial. After completing the swim trial, the platform was raised, and the shrew was removed from the metabolic chamber. Body temperature of the animal was measured to the nearest 0.1 °C, immediately after the removal with a thermocouple probe (BAT-12, Physitemp, Clifton, NJ, USA) inserted 1.5 cm into the rectum. All shrews were hypothermic, which indicated that they had achieved their maximal metabolic rates. The $VO_{2\,SWIM}$ was defined as the highest oxygen consumption averaged over 2 min.

For the measurement of maximum metabolic rate during running ($VO_{2\,RUN}$), the shrew was placed in the running wheel (12 cm diameter, 3.8 cm wide) enclosed in a circular Plexiglas metabolic chamber (13.3-cm diameter, 500 ml volume). The wheel was driven by an electric motor with a speed revolution regulator. All measurements of $VO_{2\,RUN}$ were performed at 20 °C. The airflow through the chamber was set at 1000 ml min^{-1}. After a 10-min adaptation period, the wheel was started, and then its revolution speed was increased until the maximum metabolic rate was achieved. Each trial lasted about 20 min and the $VO_{2\,RUN}$ was defined as the highest oxygen consumption averaged over 2 min.

Karyotypes

After measurements of metabolic rates, the shrews were killed and sexed by dissection. Chromosome preparations were carried out from the bone marrow by standard methods and stained for G-bands with Giemsa after treatment with trypsin (Seabright, 1971). Chromosome arms were identified following the rules of ISACC (Searle *et al*., 1991). A total of 56 individuals were karyotyped.

Fluctuating asymmetry

The skulls of 52 karyotyped shrews were cleaned manually. The right and left side of each mandible was photographed using a camera and a video capture board. For measurements, we used MultiScanBase software (CSS, Warszawa 2003). Eighteen measurements, designated A1–A10, B1–B4, C1, C3, L and H, from the left and right mandible were taken following the method of Pankakoski and Hanski (1989). All the measurements were taken three times by the same person (D Ochocińska). The series of measurements were compared by using Friedman's analysis of variance by ranks. This test compares the variables that were measured in dependent samples, for example, repeated measures. The first series of measurements was excluded as most different, and for further detailed analysis, two repeats of measurements were used.

The measurement error was evaluated by mixed model analysis of variance proposed by Palmer and Strobeck (1986). In general, this analysis allows partitioning of the between-side variance and the measurement error between series of measurements. The two-way analysis of variance with side as a fixed factor and individual as a random factor with repeated measurements of each side is used. The significant interaction variance means that the measurement error is smaller than the non-directional asymmetry variance. Unfortunately, six measurements (A2, A4, A5, A7, L and H) showed high measurement error and had to be excluded from further analysis.

The analysis of variance procedure, described above, tests simultaneously for the presence of directional asymmetry (DA) and for size or shape variation among the individuals, when considering the factors, such as side and individual, respectively (Palmer and Strobeck, 1986). Additionally, we used a two-sample *t*-test to compare the measurements on the right and left sides of the mandible and check for the direction of potential DA. The character size differences were also tested on the $(R+L)/2$ distributions for each variable. The size dependence of FA was evaluated by the non-parametric Spearman coefficient of rank correlation between $(R-L)$ and $(R+L)/2$ distributions. For normality testing, we used Shapiro–Wilks test. Skewness and kurtosis were also estimated. The presence of antisymmetry can be excluded if no distribution is platykurtic.

Results

Karyotype variation

Seven karyomorphs of three polymorphic arm combinations, *jl*, *gr* and *mp*, were present in samples from Jurowce population (Table 1). The individuals were divided into homozygotes and simple Rb heterozygotes

in respect of the *mp* chromosome pair only. In effect, the group of homozygotes comprises not only homozygotes for all chromosome pairs but also simple heterozygotes of *jl* and *gr* arm combinations (Table 1). Chromosome frequency analysis for the *mp* polymorphic arm combination showed a close fit to the Hardy–Weinberg expectations ($\chi^2 = 0.078$, df = 1, $0.8 > P > 0.7$), and frequencies of *mp* metacentric and *m*, *p* acrocentrics in this two-year sample were 0.715 and 0.285, respectively.

Maximum metabolic rates

The values of both maximum metabolic rates and body mass before the measurement were submitted to logarithmic (log 10) transformation. The maximum metabolic rates, $VO_{2\,SWIM}$ and $VO_{2\ RUN}$ were significantly correlated with body mass, hence they were tested by the analysis of covariance (ANCOVA) with body mass as a covariate. Before the analysis, we checked that body mass did not differ between karyotypic classes of individuals (body mass for $VO_{2\,SWIM}$ $F_{1,53} = 1.23$, $P = 0.27$, body mass for $VO_{2\,RUN}$ $F_{1,54} = 1.21$, $P = 0.28$).

The metabolic rates were not influenced by sex and year of capture of individual. No difference between *mp* homozygotes and simple Rb heterozygotes was found in maximum metabolic rate during forced running ($F_{1,53} = 1.12$, $P = 0.30$; Table 2), although body mass was highly significant in the ANCOVA model ($F_{1,53} = 79.46$, $P < 0.0001$). The interaction between the factor and body mass, when added to the model, was not significant ($F_{1,52} = 0.23$, $P = 0.63$). In respect to maximum metabolic rate during swimming, we found that individuals diving during the experiment had statistically lower oxygen consumption than those, which did not show such behaviour (one-way ANCOVA, $F_{1,50} = 15.77$, $P = 0.0002$). In consequence, maximum metabolic rate during swimming was analysed by two-way ANCOVA with diving (present or not) and karyotypic class as factors and body mass as the covariate. Both factors and the covariate were statistically significant ($F_{1,50} = 14.38$, $P = 0.0004$; $F_{1,50} = 5.59$, $P = 0.022$; $F_{1,50} = 7.83$, $P = 0.007$, respectively; Table 2, Figure 1), whereas the interaction between the factors was not ($F_{1,50} = 0.002$, $P = 0.96$). Interaction terms between factors and the covariate,

Table 1 Variable part of the karyotype of shrews used in this study

Karyotypic class	*Variable part of karyotype*	*2Na*	N
Hom	*jl jl, hn hn, ki ki, gr gr, mp mp, o o* and *q q*	20	22
	jl jl, hn hn, ki ki, gr/g, r, mp mp, o o and *q q*	21	4
	jl/j, l, hn hn, ki ki, gr gr, mp mp, o o and *q q*	21	3
	jl jl, hn hn, ki ki, gr gr, m m, p p, o o and *q q*	22	3
	jl/j, l, hn hn, ki ki, gr gr, m m, p p, o o and *q q*	23	2
	Total of *mp* homozygotes		34
Het	*jl jl, hn hn, ki ki, gr gr, mp/m, p, o o* and *q q*	21	16
	jl jl, hn hn, ki ki, gr/g, r, mp/m, p, o o and *q q*	22	6
	Total of *mp* heterozygotes		22
	Total		56

Abbreviations: 2Na, diploid number of autosomes; *N*, number of individuals.

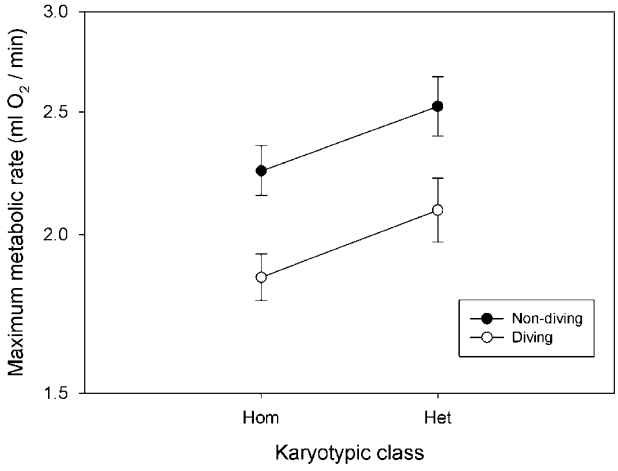

Figure 1 Maximum metabolic rates during swimming in Rb homozygotes and heterozygotes of the common shrew. Least square means from ANCOVA adjusted for body mass ± s.e. The data are shown separately for individuals which dived during measurements and those which did not. Logarithmic scale is on the Y-axis. ANCOVA, analysis of covariance; Rb, Robertsonian.

Table 2 Mean values ± s.e. of maximum metabolic rate during running ($VO_{2\,RUN}$) and maximum metabolic rate during swimming ($VO_{2\,SWIM}$) for *mp* homozygotes and Rb heterozygotes from Jurowce population

Metabolic parameters	*Karyotypic class*	N	*Mean VO_2 (ml min^{-1})*	*Mean body mass (g)*	F	P
$VO_{2\,RUN}$	Hom	34	2.33 ± 0.02	8.03 ± 0.14	1.115	0.296
	Het	22	2.31 ± 0.03	7.78 ± 0.17		
$VO_{2\,SWIM}$						
Non-diving	Hom	16	2.32 ± 0.13	7.79 ± 0.21	**5.591**[a]	**0.022**
	Het	11	2.57 ± 0.10	7.78 ± 0.23		
Diving	Hom	18	1.90 ± 0.09	7.79 ± 0.20		
	Het	10	2.04 ± 0.10	7.31 ± 0.15		

N, sample size, *F*- and *P*-value from the analysis of covariance (ANCOVA) with body mass as a covariate.
[a]*F*-value for the comparison of karyotypic classes.

when added to the model, were $P > 0.29$. The least square mean $VO_{2\,SWIM}$ from ANCOVA, adjusted for diving behaviour and body mass, was by 13% higher in the *mp* chromosomal heterozygotes than in homozygotes.

Fluctuating asymmetry

Twelve measurements were left for FA analysis after measurement error evaluation. Eight measurements (A6, A8, A9, A10, B2, B3, C1 and C3) out of twelve showed small DA (see Supplementary Table S1). The Shapiro–Wilk test showed a normal distribution of all 12 measurements left for analysis, thus excluding the presence of antisymmetry. In summary, four measurements A1, A3, B1 and B4 showed perfect FA and eight measurements showed small DA.

The presence of DA is a general problem in studies of FA as DA is the most common cause of departures of bilateral variation from the true FA. DA, in contrast to FA, has a genetic basis and the variation in (R−L) cannot be assumed to be 'developmental noise' and describe developmental stability (Palmer, 1994). Palmer and Strobeck (2003) proposed an arbitrary rule to estimate the significance of DA in any data set, that is, the DA does not influence the interpretation of FA variation, if it is no larger than the FA4a index for a particular variable (the way of computing FA4a is given in Palmer and Strobeck, 2003). In such cases, DA does not exceed the average deviation about the mean (R−L). In our study, the maximum value of DA for one trait was 0.06 and DA value for each trait was less than the FA4a value. We also noted that no side of mandible tended to be constantly larger, that is, five measurements A6, A10, B2, C1 and C3 were larger on the left side and three measurements A8, A9 and B3 on the right. In conclusion, we decided that the deviations from the perfect FA were very subtle and selected measurements could be used to reliably estimate FA of shrews' mandible.

Fluctuating asymmetry was calculated on the individual level as the absolute value of the difference between right and left $FA1 = |R-L|$, and the variance of the signed difference between right and left $FA4 = \mathrm{var}\,(R-L)$ (Palmer and Strobeck, 1986). As the measurements selected for FA analysis showed no scaling effects with character size (all Spearman corelations not significant), that is, FA was independent of size, we did not use any relative measures of FA that control for trait size. The FA4 index remains unaffected by DA in the absence of character size variation and with high character size variation, but FA independent of character size. Additionally, it does not require the correction for DA from a statistical point of view, as DA only shifts the mean of (R−L) distributions without modifying the variances, which are used to express FA (Palmer and Strobeck, 1986). In contrast to FA4, the FA1 index is influenced by DA, however, with low values of DA, as in this study, it still has high and sufficient discriminatory ability.

The comparisons of FA level between the groups of shrews were performed by Levene's test for heterogeneity of variance (Palmer and Strobeck, 2003). We found no difference between sexes and years of capture for both FA indices. Homozygotes and simple Rb heterozygotes showed no differentiation with respect to the level of FA (see Supplementary Table S2).

Discussion

Relationships between Rb heterozygosity and developmental stability

Robertsonian translocation is one of the most common chromosomal rearrangements in mammals. Even in humans, the occurrence of Rb fusions for some chromosomes is so high that it can be interpreted as natural polymorphism (Frydman *et al*., 2001). Hence, that could be important to establish a set of forces that would be reponsible for the persistence of some kinds of Rb polymorphism. Overdominance, frequency-dependent selection or variable selection in time or space can contribute to the adaptive character of chromosomal polymorphism. Additionally, in cases of chromosomal polymorphisms, the selective advantage of heterozygotes has to outweigh the disadvantage caused by lowered fertility, which is a common phenotype of Rb translocation heterozygotes on account of meiotic perturbations. In some species, there are specific adaptations in meiosis for proper segregation of heterozygous combinations of chromosomes that consist largely in the supression of recombination (Powell, 1997).

In the common shrew selection against simple Rb heterozygotes on account of meiotic and fertility problems is very small and often negligible (Searle and Wójcik, 1998). The trivalents segregate properly most probably because of proper metaphase orientation caused by terminal location of chiasmata (Giagia-Athanasopoulou and Searle, 2003). However, the analysis of many chromosomal hybrid zones indicates that chromosomal heterozygosity is disadvantageous, possibly because it generates changes modifying or disrupting the coadapted gene systems (Alibert and Auffray, 2003). The disruption in genomic coadaptation could result in phenotypic changes and affect the development, hence the disadvantage of chromosomal rearrangements may be caused not only through a fertility effect but also through the perturbations in developmental stability.

However, we found that chromosomal heterozygosity had no effect on the level of FA of shrews from the Jurowce population. The single heterozygous chromosome pair *mp* does not influence the developmental stability of the individual. Some of the analysed shrews were double Rb heterozygotes for *mp* and additionally *gr* combination (Table 1), and the level of FA in these individuals was not higher. The chromosomal Rb heterozygotes are not at a disadvantage in relation to the developmental stability, although the results of the FA research in the house mice are equivocal. However, the comparison of FA levels of homozygotes and heterozygotes coming from the same polymorphic population has not been performed on any other species with Rb variability. In the case of house mice, chromosomal heterozygotes have been studied for developmental stability. However, they were collected from the hybrid zones, hence they arose as the effect of hybridization between chromosomal races that might harbour different levels of genic differentiation. Two geographical systems with a similar kind of chromosomal differentiation were studied in the house mice, and in one case, hybrids displayed higher levels of FA, whereas in the other, no difference from the parental groups was found (Chatti *et al*., 1999; Gazave *et al*., 2006). Gazave *et al*.

(2006) concluded that structural heterozygosity *per se* does not necessarily impair developmental stability. Another example of FA studies in the house mouse is the research performed on the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus* in Denmark, where the hybrids were triple chromosomal heterozygotes. Against expectations, the hybrids had lower levels of FA, showing better developmental stability than parental forms, hence the hypothesis was put forward that genic differentiation between mice subspecies resulted in the heterotic effect in hybrids (Alibert *et al*., 1997). These three examples show clearly that the differences between heterozygotes and their parental homozygous forms are not the result of chromosomal heterozygosity but rather genic differences and the extent of genetic differentiation between parental forms. In general, the chromosomal Rb heterozygotes are not at a disadvantage in relation to the developmental stability, and it is important that in the common shrew, they are also not strongly selected against because of meiotic and fertility problems (Searle and Wójcik, 1998).

Relationships between Rb heterozygosity and maximum metabolic rates

We found that Rb heterozygotes may be at a selective advantage under certain conditions, such as those imposing increased metabolic demands. We found that shrews heterozygous for the *mp* arm combination had a higher ability to increase metabolic rate, when they had to cope with forced activity and thermal stress (Table 2, Figure 1). This could be particularly advantageous for shrews during winter in temperate climates, when they must remain active. Field studies in harsh environments show that natural selection favours high metabolic capacity in thermogenesis in small mammals (Hayes, 1989; Hayes and O'Connor, 1999).

The relationship between chromosomal rearrangements and various metabolic or behavioural traits has also been reported for the house mouse. In Madeira, the mean daily energy intake has been compared between several chromosomal races. It was found that food energy intake was significantly correlated with the chromosomal race, once the effects of body mass were removed (Mathias *et al*., 2006). Another trait studied in two groups of mice, with standard and Rb karyotype, was daily motor activity pattern (Sans-Fuentes *et al*., 2005). The mice used for this research originated from the polymorphic area near Barcelona, hence they were heterozygotes for at least one pair of chromosomes. It was found that the presence of Rb fusion in the karyotype influenced the ultradian modulation of the circadian rhythm, through the effect on the motor activity effector system.

The effect of Rb heterozygosity on phenotypic traits

How does chromosomal heterozygosity affect metabolic rates or other metabolic or behavioural traits? One hypothesis suggests heterozygous advantage resulting from higher level of genic heterozygosity of Rb heterozygotes in comparison with chromosomal homozygotes (Searle and Wójcik, 1998). According to this hypothesis, acrocentrics and metacentrics of a polymorphic pair of chromosomes could accumulate different alleles. In effect, owing to the supression of crossing over in pericentric area in Rb heterozygotes, allelic differences between acrocentrics and metacentrics could be maintained in this part of chromosomes, resulting simultaneously in higher genic heterozygosity of Rb heterozygotes. In general, Rb polymorphism would be supported by natural selection, but operating through genic heterozygosity and non-directly through chromosomal heterozygosity (Searle and Wójcik, 1998). However, for this hypothesis, it has to be accepted first that there are allelic differences between the twin acrocentric and metacentric morphs of a particular chromosome and second that the crossing over is supressed in the centromeric region. If chiasma is always located distally in trivalents of the common shrew, then there would be no gene flow involving proximal loci on the metacentric, despite high or near normal fertility of the simple heterozygotes. This would allow acrocentrics and metacentrics to diverge genetically. There is genetic evidence of recombination supression in proximal regions of chromosomes for the house mouse (Davisson and Akeson, 1993; Pialek *et al*., 2001). In the common shrew, the available data are limited. However, chiasmata are significantly more frequently located in the distal half of the chromosome arm than in the proximal, which gives regular orientation of trivalent at anaphase I, and probably is responsible for low frequencies of nondisjunction. However, occasional proximal chiasmata do occur. Although the gene flow involving the proximal half of each metacentric arm will be reduced, compared to the rest of the genome, it is not absolutely supressed (Giagia-Athanasopoulou and Searle, 2003). Thus, it seems unlikely that metacentrics and acrocentrics will accumulate sufficient differences to give the effect of heterozygote advantage.

Another more plausible explanation is the so-called fusion effect of Capanna and Redi (1994), which is consistent with modern research on the interphase nucleus structure. The chromosomes tend to occupy exclusive territories in the interphase nuclei and there is a correlation between nuclear architecture and gene expression. The position of chromosomes changes during the cell cycle and cell differentiation, hence the architecture of the nucleus supports the global genomic coordination and regulation of cellular processes (Cremer and Cremer, 2001). The chromosomal rearrangenments may have an effect on the nuclear spatial organization and may affect some aspects of gene regulation related to metabolic pathways through the change of the position of the chromosome in the nucleus. It was shown in house mice, that the presence of Rb heterozygosity produces marked changes in the chromosome territories (Garagna *et al*., 2001). In the Rb heterozygotes, the spatial relationship of the chromosome arms of the metacentric and the acrocentric homologues differs from that of the homozygotes. The Rb metacentric occupies a different position than acrocentrics, hence one of the homologous arms is separated from the other. The spatial changes give, in effect, meiotic problems as the correct nuclear architecture is necessary for the synapsis, recombination and segregation of the chromosomes (Garagna *et al*., 2001).

Large-scale changes in chromosome territories change physical interactions between parts of the genome, and as a result act as the epigenetic factors in controlling gene expression. The changes in gene expression or activity

may by chance result in metabolic or behavioural traits with selective advantage. Under this hypothesis, the differences in various traits between homozygotes and heterozygotes are purely accidental; once they appear and show any adaptive value, they can be selectively supported in the populations and help to maintain polymorphic systems. The differences between homozygotes and heterozygotes would be specific for each chromosome arm combination and would depend solely on the genic composition of the metacentric. Hence, any rearrangement in any species could have a different effect. Similarities in the polymorphic systems of different chromosomes in various species would not be expected. This principle would explain why numerous traits have been described that differ between homozygotes and Rb heterozygotes (Gerard *et al*., 1994) and why they do not show a consistent pattern of variability. Moreover, it would also explain the results of our earlier analysis on this material where all the heterozygotes of different arm combinations were pooled together and compared with homozygotes, and no differences were found (Banaszek *et al*., 2002b). In conclusion, this study found no evidence that Rb heterozygosity impairs developmental stability. On the other hand, it may influence other phenotypic traits, such as maximum metabolic rate. The positive effects, such as higher metabolic performance in activity combined with cold stress, may outweigh the expected negative effects of Rb heterozygosity upon fertility and maintain Rb polymorphism in populations well outside the hybrid zones.

Acknowledgements

We express our gratitude to Bogdan Lewończuk and Małgorzata Lewoc, who took care for the animals. We are very grateful to Dr Sara Churchfield and Professor R Nichols who improved the English.

References

Alibert P, Auffray J-C (2003). Genomic coadaptation, outbreeding depression and developmental stability. In: Polak M (ed). *Developmental Instability: Causes and Consequences*. Oxford University Press: New York. pp 116–134.

Alibert P, Fel-Clair F, Monolakou K, Britton-Davidian J, Auffray JCh (1997). Developmental stability, fitness, and trait size in laboratory hybrids between European subspecies of the house mouse. *Evolution* **51**: 1284–1295.

Banaszek A, Fedyk S, Fiedorczuk U, Szałaj KA, Chętnicki W (2002a). Meiotic studies of male common shrews (*Sorex araneus* L.) from a hybrid zone between chromosome races. *Cytogenet Genome Res* **96**: 40–44.

Banaszek A, Fedyk S, Szałaj KA, Chętnicki W (2000). A comparison of spermatogenesis in homozygotes, simple Robertsonian heterozygotes and complex heterozygotes of the common shrew (*Sorex araneus* L.). *Heredity* **84**: 570–577.

Banaszek A, Taylor J, Ochocińska D, Chętnicki W, Ratkiewicz M (2002b). Robertsonian polymorphism in the common shrew (*Sorex araneus* L., 1758)—are there any differences between homozygotes and Rb heterozygotes? Abstract in: Volobouev VT (ed). *Evolution in the Sorex Araneus Group. Cytogenetic and Molecular Aspects*. ISACC Sixth International Meeting, Paris, France, p 13.

Bartholomew GA, Vleck D, Vleck CM (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J Exp Biol* **90**: 17–32.

Capanna E, Redi C (1994). Chromosomes and microevolutionary processes. *Boll Zool* **61**: 285–294.

Chatti N, Said K, Catalan J, Britton-Davidian B, Auffray JCh (1999). Developmental instability in wild chromosomal hybrids of the house mouse. *Evolution* **53**: 1268–1279.

Cremer T, Cremer C (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat Rev Genet* **2**: 292–301.

Davisson MT, Akeson EC (1993). Recombination supression by heterozygous Robertsonian chromosomes in the mouse. *Genetics* **133**: 649–667.

Frydman N, Romana S, Le Lorc'h M, Vekemans M, Frydman R, Tachdjian G (2001). Assisting reproduction of infertile men carrying a Robertsonian translocation. *Hum Reprod* **16**: 2274–2277.

Garagna S, Zuccotti M, Thornhill A, Fernandez-Donoso R, Berrios S, Capanna E *et al.* (2001). Alteration of nuclear architecture in male germ cells of chromosomally derived subfertile mice. *J Cell Sci* **114**: 4429–4434.

Gazave E, Catalan J, Ramalhinho M, Mathias M, Nunes AC, Davidian JB *et al.* (2006). Do chromosomal hybrids necessarily suffer from developmental instability? *Biol J Linn Soc* **88**: 33–43.

Gerard D, Bauchau V, Smets S (1994). Reduced trappability in wild mice, *Mus musculus domesticus,* heterozygous for Robertsonian translocations. *Anim Behav* **47**: 877–883.

Giagia-Athanasopoulou EB, Searle JB (2003). Chiasma localisation in male common shrews *Sorex araneus*, comparing Robertsonian trivalents and bivalents. *Mammalia* **67**: 295–300.

Hayes JP (1989). Field and maximal metabolic rates of deer mice (*Peromyscus maniculatus*) at low and high altitudes. *Physiol Zool* **62**: 732–744.

Hayes JP, O'Connor CS (1999). Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* **53**: 1280–1287.

Hill RW (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J Appl Physiol* **33**: 261–263.

Lukacova L, Pialek J, Zima J (1994). A hybrid zone between the Ulm and Drnholec karyotypic races of *Sorex araneus* in the Czech Republic. *Folia Zool* **43** (Suppl. 1): 37–42.

Mathias ML, Nunes AC, Marques CC, Auffray J-C, Britton-Davidian J, Ganem G (2006). Effects of climate on oxygen consumption and energy intake of chromosomally divergent populations of the House Mouse (*Mus musculus domesticus*) from the island of Madeira. *Functional Ecol* **20**: 330–339.

Mishta AV, Searle JB, Wójcik JM (2000). Karyotypic variation of the common shrew *Sorex araneus* in Belarus, Estonia, Latvia, Lithuania and Ukraine. *Acta Theriol* **54** (Suppl 1): 47–58.

Ochocińska D, Taylor JRE (2005). Living at the physiological limits: field and maximum metabolic rates of the common shrew (*Sorex araneus*). *Physiol Biochem Zool* **78**: 808–818.

Palmer AR (1994). Fluctuating asymmetry analyses: a primer. In: Markov TA (ed). *Development Instability: Its Origins and Evolutionary Implications*. Kluwer: Dordrecht, Netherlands. pp 335–364.

Palmer AR, Strobeck C (1986). Fluctuating asymmetry: measurement, analysis, patterns. *Ann Rev Ecol Syst* **17**: 391–421.

Palmer AR, Strobeck C (2003). Fluctuating asymmetry analyses revisited. In: Polak M (ed). *Developmental Instability: Causes and Consequences*. Oxford University Press: New York. pp 279–319.

Pankakoski E, Hanski I (1989). Metrical and non-metrical skull traits of the common shrew *Sorex araneus* and their use in population studies. *Ann Zool Fenn* **26**: 433–444.

Pavlova SV, Bulatova NSh, Shchipanov NA (2007). Cytogenetic control of a hybrid zone between two *Sorex araneus* chromosome races before breeding season. *Genetika* **43**: 1619–1626.

Pialek J, Hauffe HC, Rodriguez-Clarck KM, Searle JB (2001). Raciation and speciation in house mice from the Alps: the role of chromosomes. *Mol Ecol* **10**: 613–625.

Powell JR (1997). *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press: New York, pp 562.

Pucek Z (1981). *Keys to Vertebrates of Poland. Mammals*. Polish Scientific Publishers: Warsaw, pp 367.

Sans-Fuentes MA, López-Fuster MJ, Ventura J, Diez-Noguera A, Cambras T (2005). Effect of Robertsonian translocations on the motor activity rhythm in the house mouse. *Behav Genet* **35**: 603–613.

Seabright M (1971). A rapid banding technique for human chromosomes. *Lancet* **2**: 971–972.

Searle JB (1984). Breeding the common shrew (*Sorex araneus*) in captivity. *Lab Anim* **18**: 359–363.

Searle JB, Fedyk S, Fredga K, Hausser J, Volobouev VT (1991). Nomenclature for the chromosomes of the common shrew (*Sorex araneus*). *Mem Soc Vaud Sci Nat* **19**: 13–22.

Searle JB, Wójcik JM (1998). Chromosomal evolution: the case of *Sorex araneus*. In: Wójcik JM, Wolsan M (eds). *Evolution of Shrews*. Mammal Research Institute, Polish Academy of Sciences: Białowieża. pp 219–268.

Szałaj K, Banaszek A, Ratkiewicz M, Fedyk S, Chętnicki W (1999). Long-lasting chromosome and allozyme studies in the Białowieża race population of the common shrew. Abstract in: Wójcik JM, Wójcik AM (eds). *Evolution in the Sorex Araneus Group. Cytogenetic and Molecular Aspects*, ISACC Fifth International Meeting, Białowieża, Poland, p 39.

Taylor JRE (1998). Evolution of energetic strategies in shrews. In: Wójcik JM, Wolsan M (eds). *Evolution of Shrews*. Mammal Research Institute, Polish Academy of Sciences: Białowieża. pp 309–346.

Wójcik JM (1993). Chromosome races of the common shrew *Sorex araneus* in Poland: a model of karyotype evolution. *Acta Theriol* **38**: 315–338.

Wójcik JM, Borodin PM, Fedyk S, Fredga K, Hausser J, Mishta A *et al*. (2003). The list of chromosome races of the common shrew *Sorex araneus* (updated 2002). *Mammalia* **67**: 169–178.

Zakharov VM (1989). Future prospects for population phenogenetics. *Soviet Sci Rev/Sect F Physiol Gen Biol* **4**: 1–79.

Supplementary Information accompanies the paper on Heredity website (http://www.nature.com/hdy)