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Repeatability of daily field metabolic rate in female Meadow Voles (*Microtus pennsylvanicus*)

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Summary

1. Repeated measurements of daily field metabolic rate (FMR) were made on 11 Meadow Voles (*Microtus pennsylvanicus* Ord.) by means of the doubly labelled water technique. The objective was to quantify the individual consistency of FMR by calculating the repeatability of successive measures on the same individuals. One more general goal was to test whether FMRs are sufficiently repeatable to be convenient for field studies of natural selection.

2. Voles were all non-reproductive females, 5–7-months old. They were maintained individually in outdoor enclosures 25 m^2 and were injected 1–3 times with doubly labelled water. Two to six measures of daily FMR were thus obtained per individual ($x = 4{\cdot}09$, SD = 1·51, $n = 11$) over a period of 42 days in July and August 1994.

3. Body mass and rate of change in body mass accounted for 31·6% of the observed variation in FMR; ground-temperature variations had no significant effect on FMR, probably owing to the small range of temperatures experienced by voles.

4. Repeatability of mass-corrected $\log_{10}$ FMR measurements was low ($r = 0{\cdot}261$). This low repeatability was very consistent with the results of a previous study on consistency of mass-specific daily energy expenditures of caged pouched mice.

5. A partition of the total variance observed in mass-corrected FMR showed that 63·0% of the variance originated from differences within individuals, 27·6% from differences among individuals, and 9·4% from measurement error. FMR was not consistently higher or lower on the first or second day of measurement, indicating no clear effect of handling stress.

6. A practical implication of these results is that single measurements of daily field metabolic rates are not necessarily a good predictor of the average 24 h energy budget for a given individual. On a more theoretical basis, these results indicate that, although the ability of an animal to manage its energy economics may largely determine its selective value, daily FMR seems to be a poor candidate variable to relate this ability to individual fitness in experimental studies.

7. We suggest that future studies of repeatability of FMR should concentrate on energetically constraining periods. This would allow further evaluation of the potential role of interindividual differences in FMR as a tool for understanding evolutionary pathways that shaped energy economics of animals.

Key-words: Daily energy expenditure, doubly labelled water, intraclass correlation, intraindividual variation

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Introduction

Biologists have long recognized the crucial role that energy plays in shaping the behaviour, ecology and physiology of animals. However, it was not until the advent of the doubly labelled water (DLW) technique (Lifson & McClintock 1966) that biologists could measure directly energy flux in free-ranging individuals. Now this non-invasive technique provides a powerful tool with which to examine energy expenditure or field metabolic rate (FMR) at the species level (Nagy & Montgomery 1980; Obst & Nagy 1992; Carlson, Moreno & Alatalo 1993), or between groups differing in time of year, behaviour, or reproductive stage (Karasov 1981, 1983; Vehrencamp, Bradbury & Gibson 1989; Mutze, Green & Newgrain 1991; Chappell *et al.* 1993; Weathers & Sullivan 1993;

Nagy & Gruchacz 1994). To date, most studies have focused on mean FMR, implicitly or explicitly portraying intra- or interindividual variation as noise (but see Tinbergen & Dietz 1994). Such noise, if truly it is, introduces variance that diminishes the capacity of statistical tests to detect between group differences (Berteaux *et al.* 1996).

Interindividual differences in metabolic rate, however, may represent biologically relevant variation rather than random noise. Because interindividual variability is the raw material upon which natural selection acts, a better understanding of the degree of individual variation in metabolic rate may help us understand the evolutionary pathways that shaped the metabolic capacities of organisms (Bennett 1987a). When viewed in this light, it becomes important to identify the sources of variation in FMR. Intra-individual variation may be of little evolutionary significance if it does not result in differential survival and hence it may truly be noise. Fundamental, repeatable differences between individuals may be the signal of interest because these can underlie differential survival and hence evolutionary change. Measures of both intra- and interindividual variation are clearly of key importance if we are to understand the nature of the variation upon which natural selection acts. Surprisingly, however, virtually no studies have attempted to quantify intra- and interindividual variation.

In one of the few studies specifically examining variability in FMR, Speakman *et al.* (1994) found that intraindividual variability of metabolic rate of caged Pouched Mice (*Saccostomus campestris*) was as large as interindividual variability. They argued that the ecological relevance of measures of daily energy expenditure of free-living animals may be questionable if individuals do not balance their energy budgets over a 24-h time-scale, and concluded that studies addressing the intraindividual repeatability of field measures of energy expenditures were urgently required.

The aims of this study were two-fold. The first was to quantify the individual consistency of daily FMR in Meadow Voles (*Microtus pennsylvanicus* Ord.) to examine the relevance of 24-h periods of measurement. The DLW method was used to assess the repeatability of point measurements of daily energy expenditures on individuals of similar sex, age cohort and breeding condition. The second, more general objective was to quantify intra- and interindividual variation to assess whether variation in FMR can reasonably be used to study natural selection on metabolic rate.

Material and methods

APPROACH

Variability of FMR in a population can be separated statistically into a within-individual component, measuring the differences between successive daily expenditures of the same animals, and a between-individual component, measuring the long-term (physiological and/or behavioural) differences between individuals. It is the existence of long-term differences between individuals that was sought in this study. Ideally, the existence of such long-term differences (that can be considered permanent at the time-scale of the experiment) would be best demonstrated by repeating measurements of FMR on several individuals living in an environment uniform in both space and time. Field conditions, however, make this ideal situation difficult to obtain. This difficulty was circumvented by studying voles in enclosures; this enables researchers both to obtain realistic estimates of FMR and to control most of the environmental factors likely to influence daily energy expenditure.

The twenty Meadow Voles used in this experiment originated from a captive colony that was periodically outbred with wild voles (for details on rearing of voles, see Berteaux, Duhamel & Bergeron [1994]). All individuals were non-reproductive adult females that were 5–7-months old, had the same past life-history, and were not related. Voles were acclimatized to natural photoperiod and temperatures for at least 2 weeks before they were released into outdoor predator-free enclosures, 25 m^2, built in an old-field community on the campus grounds of Sherbrooke University, Québec, Canada (45 ° 15 'N, 27 ° 00 'W). Voles were allowed to habituate to the enclosures for 1 week before they were first trapped. They fed on natural vegetation (forbs and grasses); no supplementary food was added to the enclosures. Eight pens in total were used for the experiment. Each enclosure contained a single vole so that interactions between individuals were not possible. Measurements of daily energy expenditure were obtained during 12 observations days that were staggered over a 42-day period in July and August 1994.

MEASUREMENTS OF FIELD METABOLIC RATE

Voles were captured in Sherman traps baited with apple slices. Traps were opened from 1900 h to 2200 h and checked every hour. Immediately after capture, each individual was weighed to the nearest 0·1 g and given an intraperitoneal injection of 4 $\mu l\, g^{-1}$ body mass of water containing 77·9 atom % ^{18}O and 94 mCi $^{3}H\ ml^{-1}$. After 1 h, a 150-µl blood sample was taken under light anaesthesia (methoxyfluorane) from the suborbital sinus through heparinized glass capillary tubes. Animals were released at their point of capture; an attempt was made to recapture them at the same time of day 24 and 48 h later for collection of second and third blood samples. Blood samples were also taken before isotope injection in four individuals to determine natural ^{18}O abundance. Blood samples were stored under refrigeration before being vacuum distilled in Pasteur pipettes to recover the water

fraction (Nagy 1983). The ^3H was analysed by liquid scintillation in a Beckman LS 6000 counter and ^{18}O by the guanidine hydrochloride method to prepare CO_2 gas (Dugan *et al.* 1985; Wong, Lee & Klein 1987) which was measured with a VG-Isogas Sira 12 isotope ratio mass spectrometer (see Thomas, Martin & Lapierre [1995] for procedures). CO_2 production for voles was calculated by using equation 1 of Nagy (1983); energy expenditure was estimated by assuming an energy equivalence of $21{\cdot}7\,J\,ml^{-1}$ CO_2 (Nagy 1983). For each individual, the body-water volume at the time of initial capture was estimated from the ^{18}O dilution space (Nagy 1983). Body-water volumes at recaptures were estimated from body mass, assuming that the water fraction remained constant.

Because the washout rate of ^3H and ^{18}O is too high in small mammals to allow 5–6 repeated measurements of daily energy demand after a single injection, most individuals were injected several times. Animals were allowed a minimum of 1 week between two successive injections to avoid excessive stress. In the course of this study, CO_2 production was measured successfully 45 times from 11 voles.

DAILY WEATHER PATTERNS

In the field, thermoregulatory costs depend on the standard operative temperature, T_{es} (Bakken 1992), which combines shaded air temperature, radiation and wind speed. Ideally, T_{es} should be included as an independent variable in any attempt to explain variation in FMR, because it reflects most of the variation in daily weather patterns likely to influence energy demand. However, because T_{es} could not be measured in this study, the difference between the lower limit of thermoneutrality of *M. pennsylvanicus* (thermoneutrality zone: 25–29 °C) (Wiegert 1961) and the daily mean temperature at ground level (under the vegetation) was used as the independent variable most likely to reflect the thermoregulatory costs. We consider this environmental temperature index to be adequate in the context of this study: the influences of wind and solar radiation were minimal owing to the dense vegetation cover in which animals lived.

STATISTICAL ANALYSES

Body mass is the main determinant of FMR, so its influence must be factored out before any other effect can be detected. The $\log_{10}$ FMR was therefore regressed on $\log_{10}$ body mass by using standard least-squares techniques. All the measurements of FMR and body mass performed in the study were included in the least-squares analysis, although some measures were repeated on the same individuals. Mass-corrected FMRs (in units of $kJ\,g^{-x}day^{-1}$, where x is the slope of the log–log regression line) were then used in subsequent regression analyses to determine the extent to which variation in FMR was related to variation in (1) rate of change of body mass or (2) temperature index, two variables most likely to affect FMR.

The repeatability of FMR measurements was assessed by calculating the intraclass correlation coefficient (r) of metabolic rates. This measure of repeatability describes the proportion of variance in a character (FMR in this study) that occurs among rather than within individuals, and is based on variance components derived from Model II single-factor ANOVA (Lessells & Boag 1987; Sokal & Rohlf 1981; Zar 1984). Repeatability thus expresses the proportion of the variance of single measurements that is due to permanent differences between individuals, whereas the proportion of variance that originates from intra-individual differences is represented by $1-r$. Because there was concern that the estimates of repeatability in this study might be confounded by effects of body mass, the intraclass correlation coefficient was calculated on residuals previously derived from the log–log regression of FMR on body mass.

Variability in FMR has both biological and analytical error components. To quantify the proportion of variability that might originate from analytical error, the error introduced by the DLW technique in the final estimates of FMR was quantified. Estimates of FMR are most critically dependent on the accuracy of the initial and final isotope enrichments (Nagy 1975, 1980). Two replicate measurements were made on both initial and final samples for ^{18}O (I_O and F_O) and three replicates were run for both initial and final samples for ^3H (I_H and F_H). The potential error for each FMR was estimated based on the propagation of analytical errors in initial and final estimates of isotopes enrichments (Speakman & Racey 1987). For each FMR measurement, a range of estimates of FMR was generated from a random sample of combinations of estimates of I_O, F_O, I_H and F_H. Of a total of 36 combinations of enrichment estimates that were possible for each measure of FMR ($3\times3\times2\times2$), ten were chosen at random. The resultant distribution of FMR estimates was then used to define the error (SD/mean) associated with the calculation of each FMR measurement.

Finally, the variation in FMR was partitioned among the three possible components (interindividual, intraindividual and analytical error) by using a two-level nested ANOVA of residuals of estimates of FMR (body mass factored out after log–log regression of estimates of FMR on body mass). Variance components were expressed as percentages, following Sokal & Rohlf (1981).

Statistical analyses were carried out with STATVIEW for Macintosh (Abacus Concepts 1987). Values are the mean ± SD.

Results

Mean daily ground temperature always remained below the lower critical temperature of the

thermoneutral zone of *M. pennsylvanicus* (25 °C) during the 12 observation days (Fig. 1). The difference between the lower critical temperature and the mean daily temperature ranged from 1·4 to 8·6 °C and averaged 5·3 °C (SD = 2·3, $n = 12$).

The mean number of daily field metabolic rates measured per individual was 4·1 (SD = 1·5). The range of values of FMR obtained for each individual was often large (Fig. 2); this range is reflected in the high standard deviations of FMR obtained for some of the voles (Table 1). Mean body mass of individuals ranged from 30·4 to 40·7 g and averaged 35·6 g (SD = 2·8 g, $n = 11$) (Table 1).

The average body-water content of individuals was 75·6% (SD = 2·9%, $n = 11$), indicating that voles in this study were lean. FMR was not consistently higher or lower on the first or second day of measurement (two-tailed paired t-test performed on FMR before data are mass-corrected: $t = 0{\cdot}455$, $P = 0{\cdot}65$, df = 20); this result indicates that there was no clear effect of handling stress. The measurement error (SD/mean) on FMR estimates originating from variation in measures of isotope enrichments ranged from 1·9 to 16·6% ($x = 7{\cdot}2 \pm 3{\cdot}2\%$, $n = 45$) (Fig. 3).

Across all daily measurements of energy expenditure, there was a significantly positive relation between $\log_{10}$ FMR and $\log_{10}$ body mass ($r^2 = 0{\cdot}21$, $f = 11{\cdot}37$, $P = 0{\cdot}002$, df = 1,43) (Fig. 4). The equation of the regression line was:

$$\log_{10} \text{FMR (kJ day}^{-1}) = 1{\cdot}249 \log_{10} \text{body mass (g)} + 0{\cdot}06. \quad \text{eqn 1}$$

Mass-corrected values of FMR (kJ $g^{-1{\cdot}249}$ day^{-1}) were significantly and positively related to body-mass change rates ($r^2 = 0{\cdot}13$, $f = 6{\cdot}68$, $P = 0{\cdot}013$, df = 1,43) (Fig. 5) but not to variations in the temperature index ($r^2 = 0{\cdot}01$, $f = 0{\cdot}27$, $P = 0{\cdot}61$, df = 1,43) (Fig. 6). Stepwise multiple-regression analysis was employed to determine the best predictive equation for FMR by

Table 1. Field metabolic rate (FMR), body mass and number of repeated measurements per individual (n) obtained on 11 enclosed female Meadow Voles in a study aimed at measuring individual consistency in daily energy expenditures; x represents the mean and SD the standard deviation of field metabolic rates and body masses

		FMR (kJ day^{-1})		Body mass (g)	
Individual	n	x	SD	x	SD
A	5	113·0	9·6	36·6	2·7
B	4	115·6	6·5	39·3	1·6
C	6	111·2	7·5	34·5	2·0
D	6	98·2	13·0	35·4	0·7
E	6	78·5	20·3	30·4	1·0
F	2	84·0	10·0	35·6	0·1
G	3	113·9	29·3	36·8	1·4
H	2	100·1	37·5	32·8	0·1
I	4	110·2	21·2	34·9	0·6
J	3	64·0	2·3	34·9	2·5
K	4	115·6	32·4	40·8	0·7

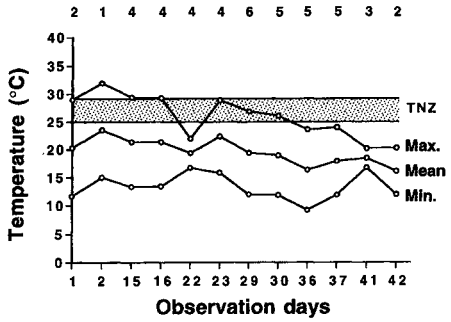

Fig. 1. Minimum (Min), mean and maximum (Max) daily temperatures at ground level experienced by adult female Meadow Voles during 12 observation days scattered over a 42-day study period aimed at measuring individual consistency of field metabolic rates. Day 1 is July 12 1994; Day 42 is August 22 1994. The thermoneutrality zone (TNZ) of *Microtus pennsylvanicus* is represented by the dotted area (25–29 °C). Numbers at the top of the graph give, for each observation day, the number of individuals for which field metabolic rate was measured.

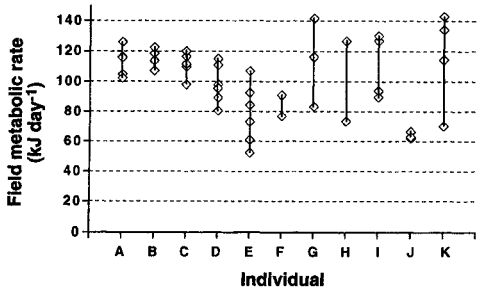

Fig. 2. Values of daily field metabolic rate obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2.

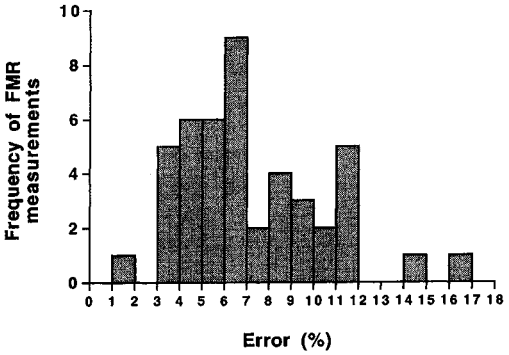

Fig. 3. Distribution of percentage measurement errors (SD/mean) of the DLW technique associated with estimates of FMR ($n = 45$) in a study aimed at measuring individual consistency of field metabolic rates in adult female Meadow Voles.

using body mass and rate of change of body mass as independent (predictor) variables. The best-fit equation obtained with $\log_{10}$ values of FMR and body mass was:

$$\log_{10} \text{FMR (kJ day}^{-1}) = 1{\cdot}148 \log_{10} \text{body mass (g)} + 0.02 \text{ mass change rate (g day}^{-1}) + 0{\cdot}218. \quad \text{eqn 2}$$

The equation explained 31·6% of the variance of $\log_{10}$ FMR. Inclusion of polynomial factors did not significantly improve the explained variation.

The importance of timing blood samples at exactly 24 h intervals was highlighted by Speakman & Racey (1988a). Because of field constraints, it was often not possible in the present study to time blood samples at exactly 24 h. The mean elapsed time between two successive blood samples was 23 h 18 min (SD = 32 min, n = 45); the maximum deviation from 24 h was 1 h 55 min (Fig. 7). To evalute the effect of these deviations on our FMR estimates, residuals of $\log_{10}$ FMR measurements obtained in the multiple-regression analysis (thus factoring out the confounding effects of body mass and mass change rate) were regressed against the deviation of intersample interval from 24 h. There was no relation between $\log_{10}$ FMR residuals

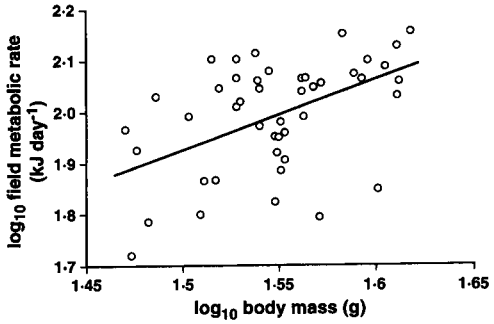

Fig. 4. Point measurements of $\log_{10}$ daily field metabolic rate as a function of $\log_{10}$ body mass. Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2; $y = 1{\cdot}249x + 0{\cdot}06$; $r^2 = 0{\cdot}21$; $P = 0{\cdot}002$.

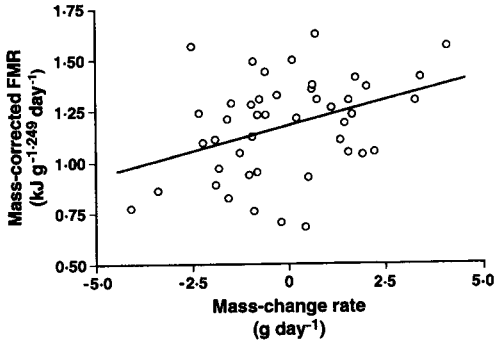

Fig. 5. Daily field metabolic rate (FMR) after the effect of body mass has been factored out (see Fig. 4), as a function of body-mass change rate. Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2; $y = 0{\cdot}049x + 1{\cdot}179$; $r^2 = 0{\cdot}13$; $P = 0{\cdot}013$.

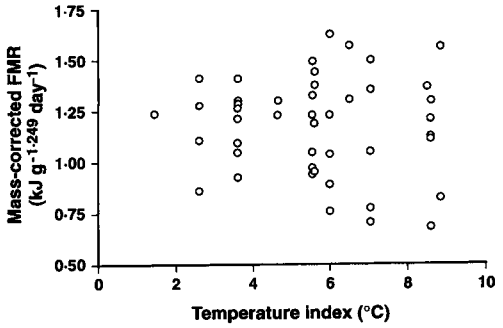

Fig. 6. Daily field metabolic rate (FMR) after the effect of body mass has been factored out (see Fig. 4), as a function of the difference between the lower limit of thermoneutrality of *M. pennsylvanicus* and the daily mean temperature at ground level (referred to as 'Temperature index' on the figure). Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2; $r^2 = 0{\cdot}01$; $P = 0{\cdot}61$.

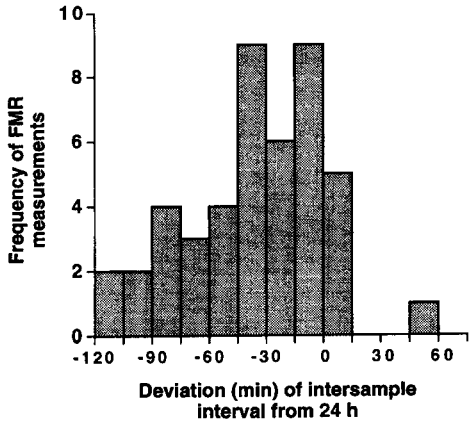

Fig. 7. Distribution of the deviations of intersample interval from 24 h in a study on adult female Meadow Voles aimed at measuring individual consistency of field metabolic rates by means of doubly labelled water.

and the deviations of intersample interval from 24 h ($r^2 = 0{\cdot}006$, $f = 0{\cdot}243$, $P = 0{\cdot}624$, df = 1,43) (Fig. 8); this result indicates that this potential bias did not affect estimates of FMR.

The individual consistency of FMR across days of measurement was low: the repeatability of residuals of $\log_{10}$ FMR was only 0·261 (based on the ANOVA results in Table 2). Thus, 73·9% (1 − 0·261) of the variance observed in residuals of individual measurements of FMR originated from intraindividual variation. The proportion of variance coming from interindividual variation was, however, significant ($P = 0{\cdot}023$) (Table 2). Another way to express the variability in individual FMR is to give the coefficient of variation (CV) of successive measures. The average CV was 16·9% (SD = 11·1%, n = 11) if the two individuals are included for which only two measurements of FMR were obtained, and 15·1% (SD = 9·7%, n = 9) if they are excluded. Because mass-change rate

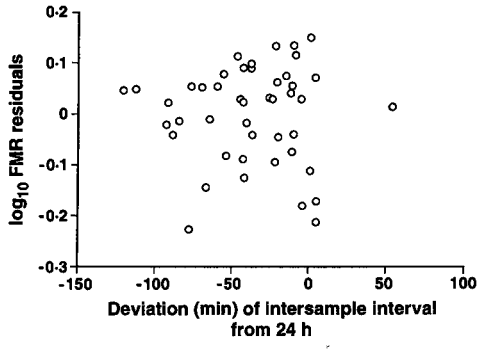

Fig. 8. Residual variation of $\log_{10}$ daily field metabolic rate (FMR) after the effects of body mass and body-mass change rate have been factored out (Figs 4 and 5), as a function of the deviation of the intersample interval from 24 h (intersample interval minus 24 h) (Fig. 7). Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2; $r^2 = 0{\cdot}006$; $P = 0{\cdot}624$.

was a predictor of FMR, it was also interesting to assess its repeatability. Rates of change of body mass were not consistent at all: the intraclass coefficient of correlation was only 0·004 ($f = 1{\cdot}01$, $P = 0{\cdot}452$, df = 10,34); this result indicates that virtually all the variation observed in body-mass change rates came from intraindividual differences. Body-mass change rates thus did not contribute to the between-individual component of variation of $\log_{10}$ FMR residuals.

A two-level nested ANOVA made it possible to partition the total variance observed in residuals of FMR estimates (body mass factored out) according to each level of variation: among individuals, within individuals, and because of analytical error. Table 3 shows that, in spite of the measurement error associated with the use of DLW, there was a significant difference in mass-corrected residuals of FMR, both between and within individuals. This is reflected in the percentage of variation attributable to the several factors: 27·6% among individuals, 63·0% within individuals, and 9·4% among different estimates for single measurements of FMR. These results indicate that 30·5% of the biological variation (total variation minus measurement error) in FMR estimates originated from differences among individuals, whereas 69·5% of the biological variation originated from intraindividual differences. As one would have expected, this partition of variance is close to that obtained in the previous analysis of repeatability, where measurement error had not been taken into account.

Table 2. Results of a single classification ANOVA of the residuals of $\log_{10}$ transformed individual field metabolic rates (body mass factored out; see Fig. 4). Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2

Source of variation	Sum of squares	df	Mean square	*f*	*P*
Among individuals	0·168	10	0·017	2·501	0·023
Within individuals	0·229	34	0·007	—	—
Total	0·397	44	—	—	—

Table 3. Results of a two-level nested ANOVA of mass-corrected residuals of $\log_{10}$-transformed estimates of individual field metabolic rates. Ten estimates of FMR were performed for each measurement of daily energy demand by analysing the propagation of errors associated with initial and final estimates of isotope enrichments. Data obtained by means of the doubly labelled water technique on 11 adult female Meadow Voles maintained individually in outdoor enclosures 25 m^2

Source of variation	Sum of squares	df	Mean square	*f*	*P*
Among individuals	1·930	10	0·193	133·7	0·0001
Within individuals	2·280	34	0·067	46·4	0·0001
Measurement error	0·585	405	0·001	—	—
Total	4·795	449	—	—	—

Discussion

Two predictors of variations in daily field metabolic rate were identified (body mass and mass change rate) and then the variability of mass-corrected FMR was separated into its interindividual, intraindividual and analytical error components. The effect of body mass on energy metabolism has long been recognized (see, for example, Schmidt-Nielsen 1984; Nagy 1987, 1994). In this study mass had a relatively small effect, primarily because of the narrow range of body masses in animals selected for study.

The positive relation between mass-change rate and energy expenditure probably reflects the cost and payoff of foraging. Those animals that spent more time in locomotory and foraging activities, and hence had high energy expenditures, were those that also gained mass. The nature of the mass changes, however, was unknown. It is thus not possible to calculate the energy increment corresponding to a given mass gain, and this prevents any speculation about the benefit : cost ratio of foraging activities.

It is interesting to note that no significant effect of temperature on FMR was found. Bryant (1989) obtained similar results for birds. It is likely that the relatively high temperatures during the study, the low range of temperatures (33 out of 45 days had a temperature range of only 3·5 °C), and the heat production associated with digestion and locomotion combine to reduce the variability in thermoregulatory costs.

ANALYTICAL ERROR

Analytical error from estimates of initial and final isotope enrichments was calculated. This source of error involved variability at the machine level, within individual blood samples, and between replicate blood samples from the same animal. In this study, the mean error associated with FMR estimates was approximately 7%, but the range of errors was large. This degree of variation is consistent with previous

validation studies on mammals (Speakman & Racey 1988b; Nagy 1989).

The consequences of measurement error on our capacity to interpret results are directly related to the amount of biological variation in energy demand that exists. According to our partitioning of variance of mass-corrected estimates of FMR, biological variation was 9·6 times greater than the measurement error. We conclude that, althougy analytical error cannot be ignored, it alone does not account for the major part of the variation in FMR measurements. The DLW technique is thus sufficiently precise to allow the study of biological variability in FMR. It now becomes important to investigate whether the signal of interest in this study, namely interindividual variability, is large enough compared with noise (intraindividual variability) to be studied effectively.

BIOLOGICAL VARIATION WITHIN AND BETWEEN INDIVIDUALS

To our knowledge, there are no other studies on repeatability of FMR with which we can compare our results. However, Speakman *et al.* (1994) provided data on individual consistency of daily energy expenditure of caged Pouched Mice (*Saccostomus campestris*); this constitutes a valuable point of reference. Because they did not calculate the intraclass coefficient of correlation of daily energy expenditure in their study, we derived this coefficient from data provided in their Table 1 to permit a comparison with our results. We derived a value of r of 0·269 ($f = 2·03$, df = 10,20, $P = 0·08$). This intraclass coefficient of correlation was calculated on mass-specific energy expenditure (FMR divided by body mass) whereas repeatability in our study was calculated on mass-corrected residuals of $\log_{10}$ FMR. We tested for the effect of this methodological difference by recalculating the repeatability of our data with mass-specific FMR. We obtained a value of r of 0·236, very similar to the value obtained on residuals (0·261). We can thus conclude that the repeatability of individual energy expenditure was remarkably consistent between our study and that of Speakman *et al.* (1994), suggesting that the low repeatability of daily energy expenditures that we found was not unique to our study conditions.

For a better appreciation of the significance of these values of repeatability, it is worthwhile here to compare them with values from the literature obtained on other physiological parameters. In a study of lactation in British Friesian cattle, Barker & Robertson (in Falconer 1981) found r values of 0·40 and 0·67 for milk yield in first and second lactations and percentage fat in first and second lactations, respectively. Huey & Dunham (1987) found that the between-year repeatability of maximum sprint speeds of individual lizards varied from 0·56 to 0·63 depending on the population studied. Mass of fleece in different years was repeatable ($r = 0·74$) in a study of domestic sheep (Morley in Falconer 1981). Recently, Chappell, Bachman & Odell (1995) analysed repeatability of maximum oxygen consumption (both during exercise and thermogenesis) in a wild population of Belding's Ground Squirrels, *Spermophilus beldingi*. Repeatabilities of mass-corrected $V_{O_{2max}}$ ranged from 0·73 to 0·88 on a short-term basis (over 2 h interval), but declined in between-year measurements. These few examples demonstrate that the repeatability of FMR obtained in our study was low compared with that of some other physiological and performance measures.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

Our main objective was to determine whether interindividual variation in FMR is large enough to allow us to study and better understand the evolutionary pathways that shaped energy metabolism in free-living animals. Presumably the differences between species result from natural selection acting on interindividual variation within populations. Therefore, measuring the fitness consequences of differences among individuals may offer valuable insights as to how the evolution of FMR has proceeded. Surprisingly, individual consistency of FMR measurements was low. Differences in total energy demand among individuals thus appear so small compared with intraindividual variation as to preclude the possibility of statistically detecting any significant relations between FMR and other variables indicative of fitness.

The low repeatability of FMR found in this study may appear paradoxical given that energy availability and use are generally considered to be important constraints on animal function (Bennett 1987b). How can animals both be constrained by energy and have the freedom to vary their energy budget considerably (sometimes by a factor of more than 2)? Two explanations can be offered. First, animals may not balance their energy budget over 24 h periods. The time-frame used by convenience to evaluate the energy demands of free-living animals using DLW may thus not be ecologically realistic (Speakman *et al.* 1994). This point is critical: 24 h measurements of daily energy demand are often the rule in field bioenergetics of small endotherms. Second, energy may not always be constraining. In our study, mean FMR was on average three times BMR (Bradley 1976; Thomas, Samson & Bergeron 1988); this result is consistent with values found in many other species (see references in Kojeta 1991; Karasov 1992; Thomas, Brigham & Lapierre 1996). However, mean ground temperature was close to the lower critical temperature and voles were in a non-reproductive state. High primary productivity coupled with the fact that animals were working below their physiological maximum means that energy may not be a true constraint for voles in this

study. The situation may change when animals work near their maximum physiological capacity (for example, during reproduction or in periods of cold-stress) and/or when energy availability in the environment is reduced. In such conditions, animals may have less freedom to vary their energy budget from day to day; this constraint should translate to a lower intraindividual variability of FMR. Interindividual differences may be far more apparent in such conditions; as a consequence, repeatability of FMR may become much higher. Some support for this hypothesis comes from a recent experiment by Chappell *et al.* (1995) showing that maximal aerobic performance is repeatable (at least on a short-term basis) in Belding's Ground Squirrels. Future studies of repeatability of FMR should concentrate on energetically demanding periods, such as those under cold-stress; this may allow for the detection of individual differences, which we presume are the basis for natural selection. Until we can adequately document and quantify interindividual variation, the route by which differences in metabolic rate have evolved will remain obscure.

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