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Profound reversible seasonal changes of individual skull size in a mammal

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Postnatal size changes in most vertebrates are unidirectional and finite once the individual reaches full size [1]. In rare cases, changes of body length may occur in response to harsh environmental conditions. Such reactionary changes are distinct from seasonal, often anticipatory morphological changes, such as the reversible size change of some adult bird brains [2]. A unique pattern of profound anatomical change known as Dehnel's phenomenon has been described for the body, skull and brain size of red-toothed shrews and some mustelids [3–5]. The seasonal 20% decrease and 15% re-growth of the most common proxy, braincase height, were documented at population level from extracted skulls post-mortem. Quantifying intra-individual change had so far been methodologically prohibitive. Here, we followed the intra-individual change in skull size and body mass throughout the full cycle in wild recaptured shrews (*Sorex araneus*). Using X-ray images we showed that individuals decreased the size of their braincases in anticipation of winter by an average of 15.3%. Braincases then partially regrew in spring by 9.3%. Body mass decreased by 17.6% and then dramatically increased by 83.4% in spring. Thus, we demonstrate that the dramatic changes incurred by Dehnel's phenomenon occur in the individual's bone and other tissues.

We trapped *S. araneus* in Möggingen, Germany (longitude 8.994, latitude 47.766) between June 2014 and October 2015 with wooden live-traps (PPUH A. Marcinkiewicz, Rajgród, Poland) baited with mealworms and checked at two-hour intervals. We anesthetized animals in an induction chamber (Surgivet; oxygen flow rate 1 l/min, 5% Isoflurane)

connected to a Titus System (Dräger, Lübeck). The anesthetized individual was placed into a form-fitting foam bed to ensure a standardized body position. We then took both ventral and lateral X-ray images of the skull in a Faxitron MX 20 cabinet (26 kV, 6 sec) using an OPG Imaging Plate (Gendex) and a scanner (DenOptix /Gendex) (Figure S1). While under anesthesia and before release at the place of capture, we individually marked shrews with subcutaneous passive integrated transponders (UNO PICO, 7 x 1.5 mm). We measured body mass to the nearest 0.01 g with an electronic scale. Animals were X-rayed once per month at most, except for individuals used for determination of technical measurement error (see Supplemental Experimental Procedures in Supplemental Information, published with this article online). All handling and sampling methods were approved by the Regierungspräsidium Freiburg, Baden-Württemberg (35-9185.81/G-11/21, 35-9185.81/G-14/28).

On the X-ray images we measured braincase height (BCH), skull length (SKL) and braincase width (BCW) (Figure S1). We size-corrected all skull measurements by dividing by the non-changing maxillary tooth row length (Figure S2): i.e., BCH_{cor} , SKL_{cor} , BCW_{cor} . We checked for non-linearity in the overall seasonal patterns for BCH_{cor} , SKL_{cor} , BCW_{cor} , tooth row and body mass using generalized additive mixed models (GAMM). In addition, we tested for significance of within-subject change of skull measures with paired t-tests (see Supplemental Experimental Procedures for details).

Our data show that all three skull measurements changed reversibly in individuals. BCH_{cor} decreased in shrews caught repeatedly in summer and then again in winter (paired t-test, d.f. = 28, $t = 10.39$, $P < 0.001$; Figure 1). BCH_{cor} of individuals we then recaptured after sexual maturation the following spring had partially regrown (paired t-test, d.f. = 13, $t = 2.60$, $P < 0.05$; Figure 1B). The few surviving post-reproductive individuals then began a second decline, but sample size for this second pre-mortal decline was not sufficient for analyses. Size changes were not sexually dimorphic (see Supplemental Experimental procedures).

All 12 individuals we caught during all three stages (summer juveniles, winter

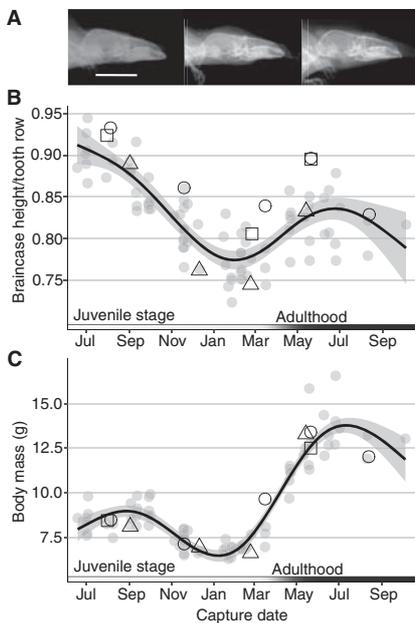


Figure 1. Individual changes in braincase height and body mass.

(A) X-ray images of one individual as a juvenile in July (left), size-decreased subadult in February (center), and re-grown adult in May (right). White scale bar is 10 mm. (B) Repeated BCH_{cor} from X-ray images of all recaptures (grey dots). Solid line and shaded area represent fitted values and standard error of the model (GAMM, $n = 95$ observations, $N = 37$ individuals, e.d.f. (smooth term)=3.906, $P < 0.001$). Symbols highlight three individuals with time between first and last capture >8 months. Individual in (A) corresponds to the open squares. The shaded bar along the x-axis indicates progression from juveniles (white portion of the bar) to adults (black). (C) Individual changes in body mass from recaptures. Solid line and shaded area represent fitted values and standard error of the model (GAMM, $n = 89$ observations, $N = 37$ individuals, e.d.f. (smooth term) = 3.974, $P < 0.001$).

subadults, and spring/summer adults) exhibited the full phenotypic cycle — first summer peak, winter decrease, and spring regrowth. Mean decrease and later increase in BCH_{cor} as assessed by X-rays were -15.3% and $+9.3\%$, respectively. The greatest changes within a single individual we recaptured during all stages were -16.3 and $+11.9\%$ (September to February, and February to May). The greatest changes in individuals caught only during two of the stages were -20.1% and $+13.2\%$. These are conservative assessments, because it is unlikely that any individual was captured at the exact time of size extremes. The other metrics followed the same pattern, but to a lesser extent

(Figure S2; SKL_{cor}: GAMM, $n = 87$, e.d.f. = 3.784, $P(\text{smooth t.}) < 0.001$; BCW_{cor}: GAMM, $n = 83$, e.d.f. = 3.598, $P(\text{smooth t.}) < 0.01$). We also found a reversible change in individual body mass (Figure 1C). On average, shrews decreased their body mass by 17.6% from July to February; but then they showed a dramatic increase during spring (83.4% from February to May). These results also match a previous study [6] in northeastern Poland which reported an average body mass decrease of 20.9% from summer to winter in recaptured animals.

We thus document the greatest postnatal reversible individual change in mammalian skull size along with mass known to date, matching previously published patterns from post-mortem skulls [5,7]. Relative or absolute BCH of recaptured and non-recaptured first-summer individuals was the same (t-test: d.f. = 59, $t = 0.226$, $P > 0.5$). Thus, a possible size-biased seasonal selection or a cohort turnover did not enhance the seasonal patterns we observed.

The morphological changes that we document are part of an extraordinary adaptive process that enables individuals to morph from a larger summer phenotype to winter phenotype by reducing the size of the body. The winter reduction in the body size of the shrew including the braincase and the correlated change in brain mass [4] are hypothesized to be an over-wintering strategy, an alternative to hibernation or migration [7]. An average decrease in body mass of 19% leads to a winter reduction of 18.2% in shrews' absolute resting metabolic rate [6], which means animals may partially compensate for a higher energy budget due to colder temperatures. This might also reduce the absolute food requirements. Thus, reducing size may improve the probability for survival of the high-metabolic shrews [8]. The partial regrowth into the adult phenotype in spring may then increase competitiveness during their only reproductive period when both sexes expand and aggressively defend their territories [8]. A recent study showing similar patterns in post-mortem skulls of two weasel species indicates that this pattern may be taxonomically more important than we currently know [5,9].

The changes in skeletal size in the common shrew we describe illustrate

how environmental forces shape the evolution of the mammalian body, skeleton and organs, including the skull and brain, and offer opportunities for further exploration and study. Our results show the extraordinary extent to which the postnatal mammalian skeleton can maintain flexibility if the proper genetic programs are activated. This opens new and important avenues in understanding how certain degenerative processes can potentially be reversed in the skeleton and other tissues.

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Supplemental Information: Profound reversible seasonal changes of individual skull size in a mammal

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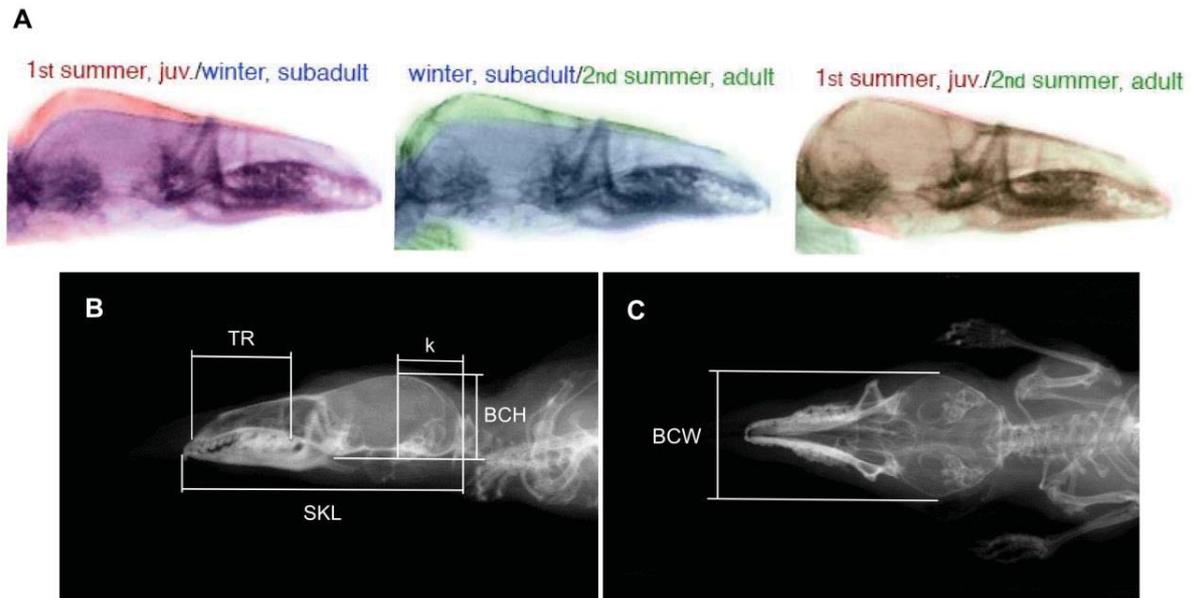


Figure S1. Overlapping X-ray images and linear measurements taken from X-ray images. **A**, Overlapping X-ray images from the skull of the same individual as summer juvenile in July and winter subadult in February (left), winter subadult and adult in May (center) and summer juvenile and adult (right). **B**, linear measurements we recorded from x-rays: skull length (SKL), braincase height (BCH), tooth row length (TR) and **C**, braincase width (BCW). See text for details.

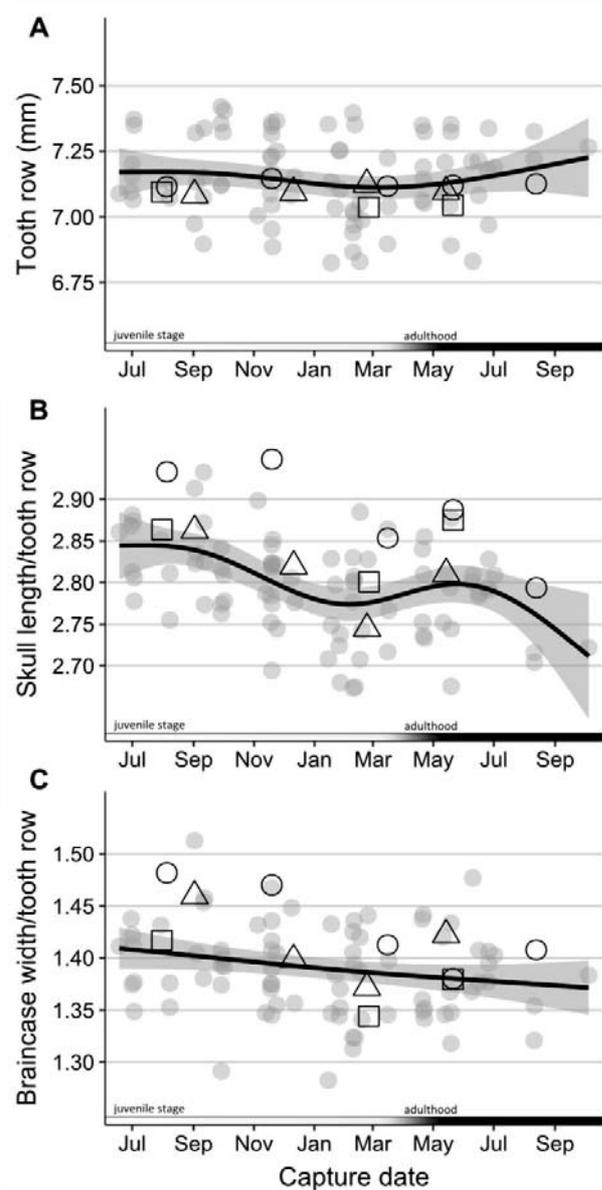


Figure S2. Seasonal changes in tooth row length, SKL_{cor} , and BCW_{cor} . A) Measurements of tooth row length from recaptured individuals show no seasonal (GAMM, e.d.f = 1, $n = 95$, $P > 0.1$) or sex-specific (GAMM, e.d.f = 1, male estimate = $+0.0776$ mm, $P > 0.05$) patterns. B) Seasonal pattern in SKL_{cor} and C) BCW_{cor} ; see model details in text. Solid line and shaded area represent fitted values and standard error of the model. Symbols highlight the same individuals as in Fig 1. The shaded bar along the x-axis indicates major age stages of individuals: juvenile and subadult (white portion of the bar) and adult (black).

Supplemental Experimental Procedures

Skull measurements and technical measurement error. We recorded the following measurements: skull length (SKL), from the anterior most projection of I1 to the occipital condyle; braincase width (BCW), greatest lateral diameter of the braincase; and braincase height (BCH), from the dorsal outline of the braincase to the orthogonal line defined by the ventral outlines of the pterygoid process and occipital condyle, passing over a constant proportional distance (k) on the line defined by SKL (Fig. S1), gauged on the digital X-ray files using Image-J [S1]. All measurements on the X-ray images were taken blind by a single observer (JL) regarding capture date and sex to avoid biases. We size corrected all skull measurements by dividing by the non-changing maxillary tooth row length (Fig. S1A, S2A).

We estimated the technical error of measurement [S2] based on repeated measurements of ten individuals. The same observer re-measured each individual in five independent sessions. Each session included the entire X-raying process, from the placement of the shrew under the X-ray source to the picture measurement using Image-J [S1]. We quantified the technical error of measurement as the within-subject standard deviation based on these repeated measurements. This gives 95% confidence intervals for repeatability error in normally distributed data [S2]. The technical measurement error as % of the mean common SD is 2.04% for BCH_{cor}, 1.35% for SKL_{cor}, 1.89% for BCW_{cor} and 1.64% for tooth row.

Age and sex determination. We classified individuals as summer juvenile, winter subadult or adult based on the annual life cycle of the shrews and external morphological characteristics. In the wild *S. araneus* has a maximum life span of ~18 months [S3]. Young shrews reach their first summer peak in body size after three weeks, pass the winter in an immature, subadult stage and reach sexual maturity in spring [S3]. Shrews mate only as adults during their second summer (June – September) and die shortly thereafter. There is only a brief period, June – September, when two generations overlap, only one of them sexually mature. During this period of overlap, we classified “summer juveniles” as follows: no developed testes or mammary glands present, little tooth wear usually with pigmented tooth tips [S4], furry tails, and body mass typically < 10 g. We classified individuals as “adults” if their teeth were blunt, tooth pigmentation was reduced or absent, tails were hairless and often scarred, and body mass was > 10 g.

To determine the sex of immature individuals (all individuals until the second summer) we used a PCR-based gonosomal sexing method (C. Roos; DPZ Göttingen unpublished). DNA was extracted from tail tip samples using Qiagen’s DNAeasy kit (Qiagen, GmbH, Hilden). Adults were sexed by the presence of testes or mammary glands.

Quantifying seasonal patterns in skull dimensions. We used the seasonal nonlinear predictions of the change for our variables of interest (i.e., BCH_{cor}, SKL_{cor}, and BCW_{cor}) to formulate our a priori expectations. We used generalized additive mixed models (GAMM) [S5] with sex as a parametric term, ‘individual’ as a random effect and age-corrected capture Julian day of the year (jday) as a non-parametric term to quantify potential nonlinear patterns. We used a Gaussian distribution and added a smoothing function to jday that included 5 knots in a thin plate regression spline. We restricted the number of knots to identify the a priori pattern while reducing the risk of model overfitting. To calculate jday, we first shifted June 1st of year one to jday = 1 until May 31st of the following year = jday 365, for the juveniles. For adults, the jday is calculated as 365 + jday of year one. For example, a juvenile captured on October 10th, jday = 132 and for an adult captured on July 18th, jday = 412. As shrews do not live beyond 18 months, there is no possibility of further jday cycles (i.e., our maximum jday = 515). This first model suggested no differences between the sexes at the factor level (BCH_{cor}: GAMM, n = 89, males estimate = 0.0007581, P > 0.1), nor did the model with an added interaction between sex and jday (BCH_{cor}: GAMM, n = 89, e.d.f.(females) = 3.812, e.d.f.(males) = 3.689, P(smooth t.) < 0.001 for both sexes). We then combined sexes and re-ran the model. Final models for SKL_{cor} and BCW_{cor} are depicted in Fig. S2. All analyses were performed in R (ver. 3.2.3) [S6], using the R package nlme [S7].

In addition, we tested for significance of within-subject change of skull measures with paired t-tests. For these tests, the closest value to each cycle peak (summer juvenile/winter subadult/spring-summer adult) was chosen for each recaptured individual. One test was performed for the shrinking phase (summer to winter) and a second one for the regrowth (winter to spring-summer).

Data availability. Data used for this paper can be found in Dryad: <http://dx.doi.org/10.5061/dryad.15r51> [S8].

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Author Contributions.

J.L. performed all the data collection and most of the analyses. J.L., D.K.N.D. and M.H. designed the study and wrote the paper. S.L. supervised and performed statistical analyses. D.K.N.D., M.W. and M.H. conceived the project. All authors contributed to the discussion and final draft of the paper.