Supplementary information for

Evidence for inbreeding depression in captive Damaraland mole-rats

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Genetic analyses (taken from Leedale et al 2024 (1))

The genetic relatedness between pairs was estimated using Queller and Goodnight's (2) coefficient of relatedness, *r*, in SPAGeDi version 1.1.5 (3). This relatedness estimate has been found to be reliable when tested against known relationships (mother-offspring). DNA was extracted from tissue and amplified. Individuals were genotyped at 13 microsatellite loci (CH1; CH2; CH3; Cmech03; Cmech04; Cmech06; DMR2, DM4; DMR5; DMR7; LV25 and NCAM1). Population allele frequencies were generated using all genotyped individuals (*n* = 474) in CERVUS version 3.0.7 (4), to maximize accuracy in estimating rare allele frequency and ensure non-zero allele frequencies.

A wild population of Damaraland mole-rats at Tswalu in the Northern Cape, a study site around 80km from our field site, had mean intra-group coefficient of relatedness of r = 0.29 and mean inter-group coefficient of relatedness of r = -0.02 (5). An earlier study using animals from 3 different trapping sites (6) found a higher mean relatedness within groups (r = 0.46 ± 0.01) but that breeding pairs were generally unrelated (r = 0.02 ± 0.04).

Table S1. *Mean ± SD genetic relatedness of opposite-sex pairs taken from Queller & Goodnight's coefficient of relatedness. Note that one of the 8 inbred pairs couldn't be genotyped. Inbred parents were full siblings raised in different natal groups and outbred parents were unrelated individuals raised in different natal groups.*

Treatment	**Coefficient of relatedness (*r*)**
Inbred (*n* = 8)	0.435 ± 0.094
Outbred (*n* = 8)	-0.033 ± 0.118

Table S2. Categories of event that ended the period of natal philopatry for all 328 individuals in both treatments.

Category	Description	N	Inbred	%	MeanAge	Outbred	%	MeanAge
Alive	Individual was still alive at the end of the sampling period	144	74	43	730 days	70	45	596 days
Nat Death	Individual died of natural causes	102	71	42	44 days	31	20	66 days
Export	Individual was removed for the purpose of population control	45	11	6	945 days	34	22	465 days
Lab death	Individual died of lab related accident or killed by other mole-rats	26	14	8	251 days	12	8	451 days
Removal	Individual was removed from natal colony for experimental reasons or was evicted	11	1	1	740 days	10	6	388 days
Total		328	171			157		

Table S3. *Predictors of litter size from a generalised linear mixed model assuming a Gaussian error distribution. Models we fitted to 109 litters (59 inbred and 50 outbred) from 16 different breeding pairs (8 sibling and 8 unrelated pairs). Litter size is the number of pups processed following the end of a pregnancy but does not include bouts where no pups were found following the end of a pregnancy. Table provides estimates, standard errors, t-values and p-values.*

Model Term	**Mean Estimate**	**Std. Error.**	**Z-value**	**p-value**
Fixed Effects				
Intercept	3.08	0.38		
Treatment (Outbred)	0.18	0.34	0.52	0.60
Group Size	0.04	0.03	1.56	0.12
Density	-0.44	0.37	-1.21	0.23
Randon Effect	**Variance**	**Std Dev**		
Maternal ID	0.28	0.52		

Table S4. *Predictors of interbirth interval from a generalised linear mixed model assuming a Gamma error distribution. Models were fitted to 118 reproductive bouts (64 inbred and 54 outbred) from 16 different breeding pairs (8 siblings and 8 unrelated pairs) where the end date was known and represent the number of days between these bouts ending minus estimated gestation length (taken as 90 days). Table provides estimates, standard errors, t-values and p-values and estimates are provided on the link scale (log-link).*

Model Term	Mean Estimate	Std. Error.	Z-value	p-value
Fixed Effects				
Intercept	4.12	0.30		
Treatment (Outbred)	0.32	0.28	1.12	0.26
Group Size	-0.04	0.02	-1.96	**0.05**
Density	0.11	0.27	0.42	0.68
Random Effect	**Variance**	**Std Dev**		
Maternal ID	0.22	0.47		

Table S5. *Predictors of pup birthweights from a generalised linear mixed model assuming a Gaussian error distribution. Models were fitted to 292 pups (149 inbred and 143 outbred) from 16 different breeding pairs (8 siblings and 8 unrelated pairs) for which a weight was taken within 5 days of birth. Table provides estimates, standard errors, t-values and p-values.*

Model Term	Mean Estimate	Std. Error.	Z-value	p-value
Fixed Effects				
Intercept	11.49	0.45		
Treatment (Outbred)	1.13	0.32	3.58	**<0.001**
Group Size	-0.05	0.02	-2.24	**0.03**
Density	-0.19	0.31	-0.60	0.55
Litter Size	-0.72	0.09	-8.29	**<0.001**
Difference in days between birth and weighing	0.21	0.08	2.45	**0.01**
Random Effects	**Variance**	**Std Dev**		
Maternal ID	0.27	0.52		
Litter Reference	0.15	0.38		

Table S6. *Predictors of pup survival to 30 days from a generalised linear mixed model assuming a binomial error distribution with pups alive at 30 days assigned 1 and those that have died assigned 0. Models were fitted to 292 pups (149 inbred and 143 outbred) from 16 different breeding pairs (8 siblings and 8 unrelated pairs) for which a weight was taken within 5 days of birth. Table provides estimates, standard errors, t-values and p-values.*

Model Term	Mean Estimate	Std. Error.	Z-value	p-value
Fixed Effects				
Intercept	-1.22	1.71		
Treatment (Outbred)	1.09	0.65	1.68	0.09
Group Size	0.02	0.07	0.25	0.81
Density	-1.78	1.05	-1.69	0.09
Weight at Birth	0.55	0.16	3.42	**<0.001**
Random Effects	**Variance**	**Std Dev**		
Maternal ID	Negligible			
Litter Reference	3.47	1.86		

Table S7. *Predictors of pup survival to 30 days from a generalised linear mixed model assuming a binomial error distribution with pups alive at 30 days assigned 1 and those that have died assigned 0. Models were fitted to 328 pups (171 inbred and 157 outbred) from 16 different breeding pairs (8 siblings and 8 unrelated pairs). Table provides estimates, standard errors, t-values and p-values.*

Model Term	Mean Estimate	Std. Error.	Z-value	p-value
Fixed Effects				
Intercept	2.91	0.94		
Treatment (Outbred)	3.41	1.10	3.10	**0.002**
Group Size	0.18	0.09	2.07	**0.04**
Density	-2.72	1.02	-2.67	**0.007**
Treatment*Group Size	-0.22	0.10	-2.24	**0.03**
Random Effects				
Maternal ID	Negligible			
Litter Reference	3.36	1.83		

Table S8. *Predictors of individual survival across development from a cox proportional hazard model. The model was fitted to 328 individuals (171 inbred and 157 outbred) born to 16 different breeding pairs (8 siblings and 8 unrelated pairs). Table provides estimates, standard errors, hazard ratios, t-values and p-values.*

Model Term	Mean Estimate	Std. Error.	HR	Z-value	p-value
Fixed Effects					
Treatment (Outbred)	-0.98	0.42	0.36	-2.33	**0.02**
Group Size	-0.05	0.05	0.96	-0.88	0.38
Density	1.25	0.73	3.48	1.72	0.09
Random Effects	**Variance**	**Std Dev**			
Maternal ID	0.01	0.10			
Litter reference	2.69	1.64			

Modelling the effects of inbreeding on Damaraland mole-rat growth

Inbreeding effects on growth

To test whether inbreeding affected growth, we compared the skeletal and body mass growth of inbred versus outbred individuals. Individual body mass was measured regularly as individuals developed, whereas our two skeletal measures – total body length and upper incisor width – were measured under anaesthesia and were therefore collected less regularly. Incisor width is a repeatable measure of skull size and was measured at the widest point using digital callipers (± 0.1 mm). Body length was measured dorsally from the tip of the nose to the base of the tail using a tape measure (± 0.1 mm). Both skeletal traits were measured in duplicate by two observers, and we used the average of these two values for analysis. Because of large sexual size dimorphism in Damaraland mole-rats, we modelled the growth of males and females separately throughout. There were 4001 body mass measures taken from 40 females (median = 104 per female), and 5125 body mass measures taken from 52 males (median = 102 per male). For the skeletal traits, there were 174 measures taken from 39 females (median = 4 per female), and 217 measures taken from 52 males (median = 4 per male).

Previous work on captive Damaraland mole-rats has demonstrated that growth rates are fastest at birth and decelerate thereafter (7, 8). To capture this shape of growth, we modelled the growth of each skeletal trait as a monomolecular curve of the form:

$$S_t = A\left(1 - e^{-k_0(t-t_0)}\right),$$

where S_t is the size of the skeletal trait at time t, A is the asymptotic size, k_0 is a growth rate constant and t_0 is the age of onset of growth. We then extended the basic curve to allow the trajectories of individuals to vary according to whether they were inbred or outbred:

$$S_t = (A + A_{inb}.I).\left(1 - e^{-(k_0+k_{0inb}.I)(t_0-t)}\right).$$

Here, I is an indicator variable noting inbred (given as 1) vs outbred (given as 0) individuals, so that A_{inb} and k_{0inb} and k_{1inb} estimate the deviation of inbred individuals from the equivalent

population-level estimate for outbred individuals. We assumed that there should be no difference in t_0.

For body mass, the growth deceleration has been shown to be more pronounced around weaning (7), requiring a biphasic formulation of the monomolecular curve to model growth more accurately:

$$M_t = \begin{Bmatrix} A\left(1 - e^{-k_0(t-t_0)}\right); & \text{for t} < t_1 \\ A\left(1 - e^{-k_0(t-t_0)-k_1(t-t_1)}\right); & \text{for t} \geq t_1 \end{Bmatrix},$$

which includes two growth rate constraints, k_0 and k_1, either of the threshold age t_1, which was set at 50 days. As above, this curve was then extended to allow for the mass trajectories of individuals to vary according to whether they were inbred or outbred:

$$M_t = \begin{Bmatrix} (A + A_{inb}.I).\left(1 - e^{-(k_0+k_{0inb}.I)(t_0-t)}\right); & \text{for t} < t_1 \\ (A + A_{inb}.I).\left(1 - e^{-(k_0+k_{0inb}.I)(t-t_0)-(k_1+k_{1inb}.I)(t-t_1)}\right); & \text{for t} \geq t_1 \end{Bmatrix}$$

The growth curves were fitted as nonlinear mixed effects models (NLMM) to account for individual repeated measures. All 'population-level' parameters were included as fixed effects (A, A_{inb}, k_0, k_{0inb}, k_1, k_{1inb}, t_0), while A, k_0, k_1, and t_0 were allowed to vary as individual level random effects. The random effects were modelled as uncorrelated to aid convergence. We also included an autoregressive correlation of order 1 in all models to estimate the correlation between measurements taken from the same individual over successive time intervals (corAR1), which also acted to reduce serial autocorrelation in the residuals. To generate population-level confidence intervals for the predicted growth curves we generated 'population prediction intervals' according to Bolker (9). NLMMs were fit using the *nlme* R package (10).

***Table S9**. Summary tables for non-linear mixed effects models of Damaraland mole-rat body mass growth. Females (n = 40, 26 inbred and 14 outbred) and males (n = 52, 27 inbred and 25 outbred were modelled separately. Growth was modelled as a biphasic monomolecular curve as indicated in the main text.*

Females

Fixed effect	Estimate (SE)	t-value	p-value
A	151.11 (9.43)	16.01	<0.001
A_{inb}	-36.06 (11.28)	-3.20	0.001
k_0	0.00351 (0.0004)	9.38	<0.001
k_{0inb}	0.00063 (0.0004)	1.41	0.16
k_1	0.00215 (0.0002)	10.00	<0.001
k_{1inb}	0.00095 (0.003)	3.43	<0.001
t_0	-15.14 (1.78)	-8.51	<0.001

Individual random effect	Standard deviation
A	30.67
k_0	0.0012
k_1	0.0006
t_0	0.004
Residual	0.61

AR(1) corr: phi = 0.84

Males

Fixed effect	Estimate (SE)	t-value	p-value
A	220.17 (7.77)	28.34	<0.001
A_{inb}	-50.56 (9.98)	-5.06	<0.001
k_0	0.00220 (0.0002)	10.26	<0.001
k_{0inb}	0.00049 (0.0002)	2.39	0.017
k_1	0.00182 (0.0002)	10.92	<0.001
k_{1inb}	0.00053 (0.0002)	2.17	0.030
t_0	-11.13 (4.87)	-2.29	0.022

Individual random effect	Standard deviation
A	28.03
k_0	1.55e-06
k_1	7.06e-04
t_0	0.00
Residual	9.17

AR(1) corr: phi = 0.86

Table S10. *Summary tables for non-linear mixed effects models of Damaraland mole-rat body length growth. Females (n = 39, 25 inbred and 14 outbred) and males (n = 52, 27 inbred and 25 outbred) were modelled separately. Growth was modelled as a monomolecular curve.*

Females

Fixed effect	Estimate (SE)	t-value	p-value
A	17.32 (0.31)	55.06	<0.001
A_{inb}	-0.52 (0.37)	-1.40	0.16
k	0.00309 (0.0002)	12.44	<0.001
k_{inb}	0.00063 (0.0002)	0.66	0.51
t_0	-278.08 (25.69)	-10.83	<0.001

Individual random effect	Standard deviation
A	0.89
k_0	1.75e-07
t_0	0.002
Residual	0.57

AR(1) corr: phi = 0.60

Males

Fixed effect	Estimate (SE)	t-value	p-value
A	19.22 (0.25)	76.43	<0.001
A_{inb}	-0.92 (0.31)	-2.96	0.0036
k	0.0029 (0.0002)	17.81	<0.001
k_{inb}	0.00025 (0.0002)	1.97	0.052
t_0	-227.16 (17.20)	13.21	<0.001

Individual random effect	Standard deviation
A	0.92
k_0	0.0002
t_0	0.003
Residual	0.447

AR(1) corr: phi = 0.10

Table S11. *Summary tables for non-linear mixed effects models of Damaraland mole-rat incisor width growth. Females (n = 39, 25 inbred and 14 outbred) and males (n = 52, 27 inbred and 25 outbred were modelled separately. Growth was modelled as a monomolecular curve.*

Females

Fixed effect	Estimate (SE)	t-value	p-value
A	6.28 (0.15)	40.72	<0.001
A_{inb}	-0.36 (0.18)	-1.97	0.051
k	0.00350 (0.0003)	11.05	<0.001
k_{inb}	0.00012 (0.0003)	0.46	0.64
t_0	-99.12 (17.13)	-5.79	<0.001

Individual random effect	Standard deviation
A	0.42
k_0	3.05-06
t_0	0.009
Residual	0.32

AR(1) corr: phi = 0.36

Males

Fixed effect	Estimate (SE)	t-value	p-value
A	7.38 (0.15)	50.31	<0.001
A_{inb}	-0.55 (0.17)	-3.21	0.0016
k	0.00285 (0.0002)	14.81	<0.001
k_{inb}	0.00040 (0.0002)	1.91	0.058
t_0	-88.69 (11.89)	-7.46	<0.001

Individual random effect	Standard deviation
A	0.0015
k_0	1.29e-07
t_0	0.007
Residual	0.501

AR(1) corr: phi = 0.82

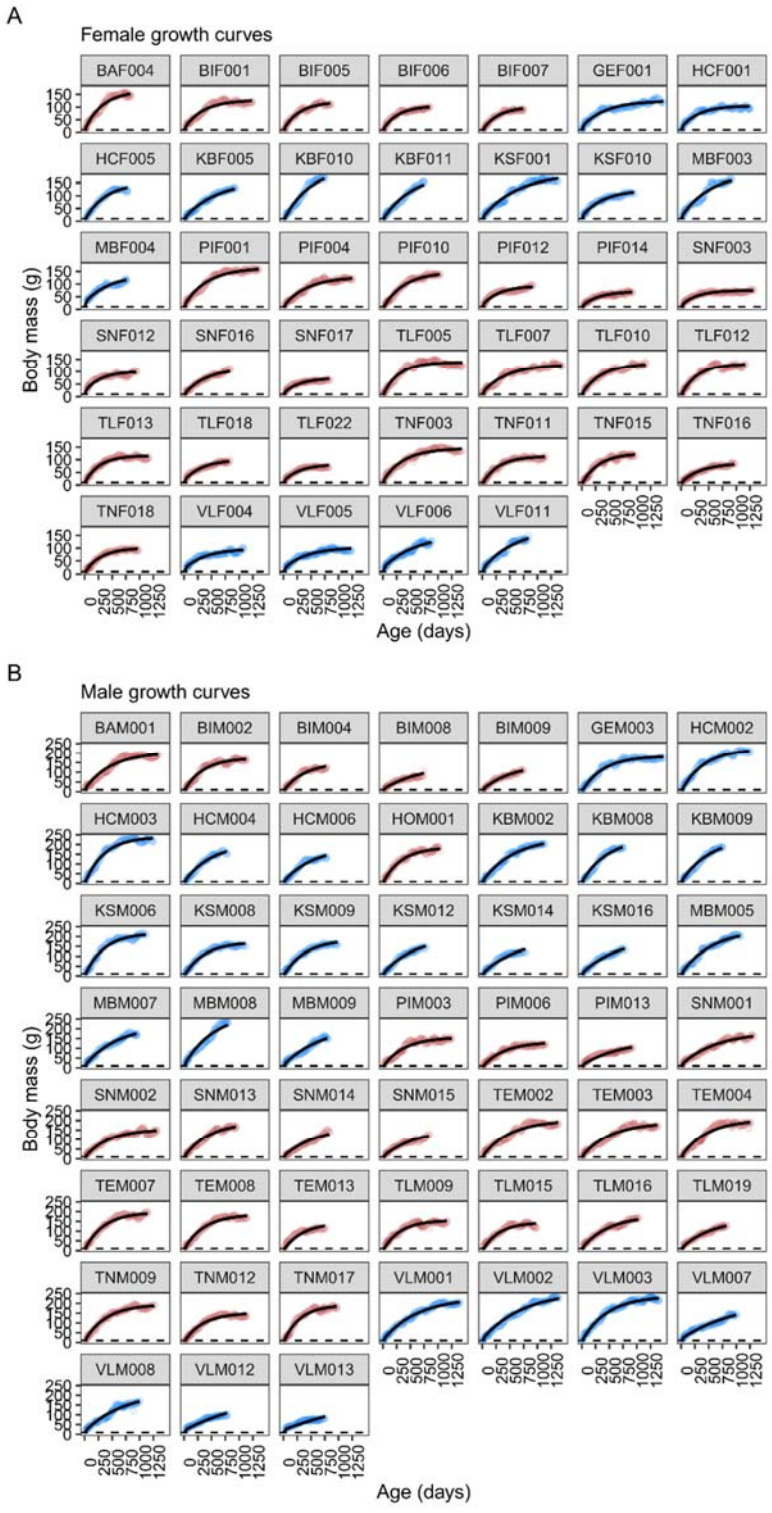

Figure S1. *Individual body mass curves, as predicted by sex-specific growth models (A- females, B- males). Each curve was predicted from a non-linear mixed effects model that specified body mass growth as a biphasic monomolecular curve, with the second phase of growth occurring once individuals were 50 days of age. The model allowed for individual varying growth parameters specified as random effects. Raw data is plotted as points (inbred individuals in red, outbred in blue), with the black line showing the predicted curve in each case.*

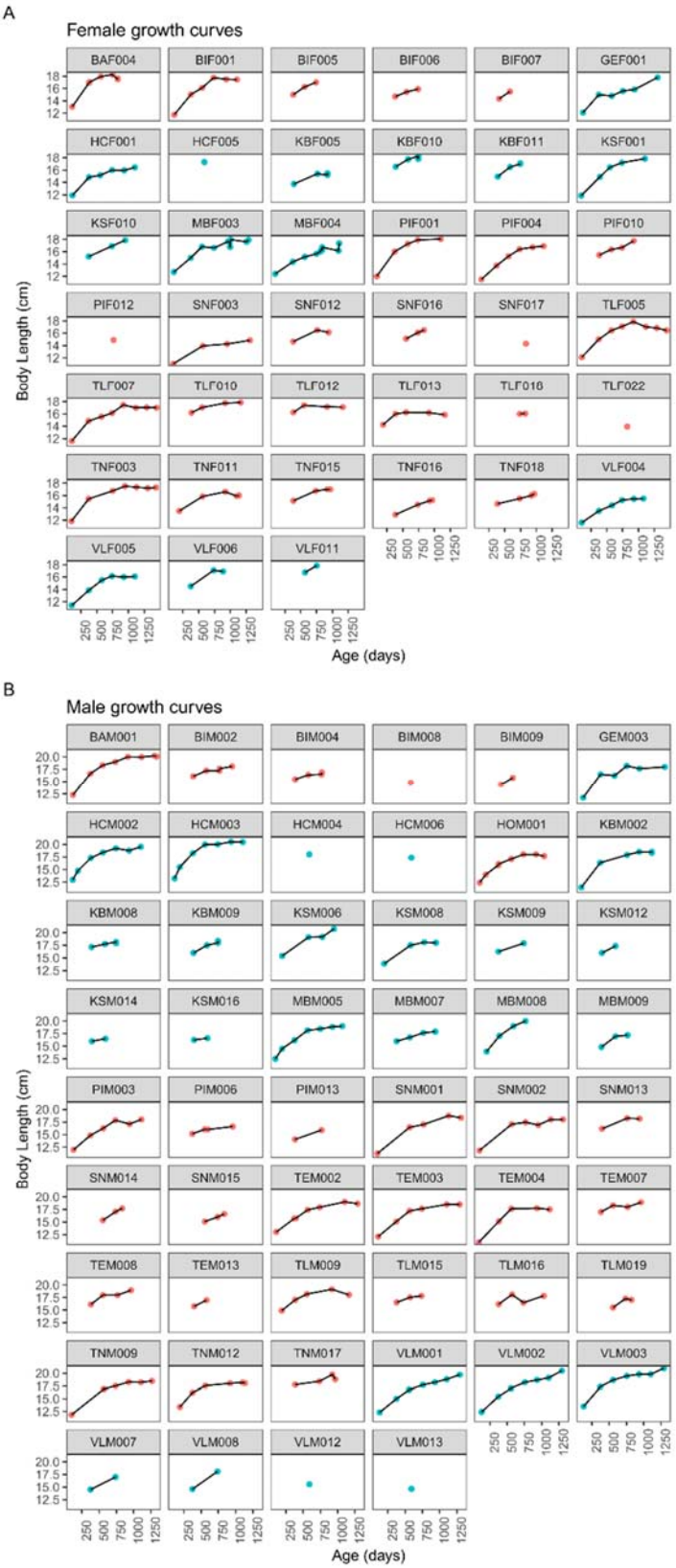

Figure S2. *Longitudinal body length measures across individuals. We do not predict individual curves as for body mass due to the lower sampling resolution.*

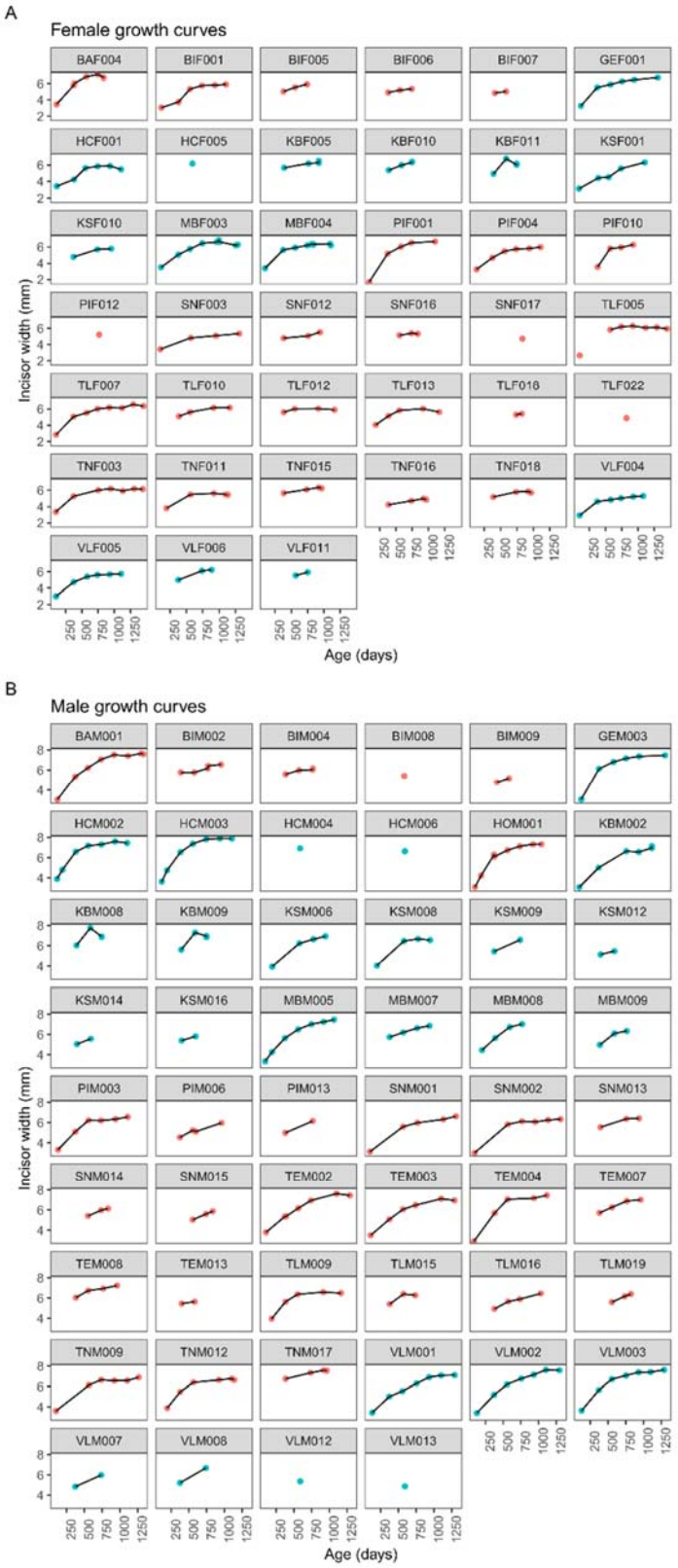

Figure S3. *Longitudinal incisor width measures across individuals. We do not predict individual curves as for body mass due to the lower sampling resolution.*

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