© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 4099-4107 doi:10.1242/jeb.111708

RESEARCH ARTICLE

Teaching an old jaw new tricks: diet-induced plasticity in a model organism from weaning to adulthood

Jeremiah E. Scott[1], Kevin R. McAbee[2], Meghan M. Eastman[2] and Matthew J. Ravosa[3,*]

ABSTRACT

Many organisms exhibit a decrease in the ability to modify their phenotypes in response to shifts in environmental conditions as they mature. Such age-dependent plasticity has important implications in a variety of evolutionary and ecological contexts, particularly with respect to understanding adaptive responses to heterogeneous environments. In this study, we used experimental diet manipulation to examine the life-history trajectory of plasticity in the feeding complex of a model organism, the white rabbit (*Oryctolagus cuniculus*). We demonstrate that, contrary to expectations derived from previous cross-sectional studies of skeletal plasticity, the jaws of weanlings and young adults exhibit similar increases in relative bone cross-sectional areas in response to the introduction of mechanically challenging foods into their diets. Furthermore, we present evidence that sensitivity to loading patterns persists well into adulthood in some regions of the masticatory apparatus in rabbits, indicating that there is an extended window of opportunity to respond to changes in dietary properties during an animal's life span. We conclude that certain aspects of the facial skeleton of rabbits, and perhaps mammals in general, are sensitive to environmental stimuli long after skeletal maturity is achieved, highlighting the importance of plasticity as a source of adaptive variation at later life-history stages.

KEY WORDS: Adaptive plasticity, Developmental window, Growth, Mammal, Masticatory apparatus, Reaction norm

INTRODUCTION

Numerous studies have demonstrated the importance of phenotypic plasticity as a source of morphological diversity in a wide range of organisms (e.g. Schlichting and Pigliucci, 1998; Agrawal, 2001; West-Eberhard, 2005; Gomez-Mestre and Buchholz, 2006; Suzuki and Nijhout, 2006; Pfennig et al., 2010; Scoville and Pfrender, 2010; Rajakumar et al., 2012). At the macroevolutionary level, some authors have argued that plasticity plays a major role in driving speciation and adaptive genetic evolution (Schlichting and Pigliucci, 1998; Palmer, 2004; West-Eberhard, 2005; Pigliucci et al., 2006; Schwander and Leimar, 2011; Standen et al., 2014). This 'phenotype precedes genotype' perspective (Palmer, 2004), which posits that phenotypic changes induced initially by environmental stimuli later become fixed through genetic assimilation, has been met with skepticism (Orr, 1999; de Jong and Crozier, 2003; Futuyma, 2011) but remains an active area of research (e.g. Gomez-Mestre and Buchholz, 2006; Suzuki and Nijhout, 2006; Pfennig et al., 2010; Rajakumar et al., 2012). By contrast, at the organismal level, it is widely recognized that plastic responses to environmental conditions are often adaptive (Gotthard and Nylin, 1995; Hoverman and Relyea, 2007; Svanbäck and Schluter, 2012). Plasticity may alter patterns of phenotypic covariance and genetic correlation structure, thus influencing how heritable variation and selection interact to produce evolutionary change (Via and Lande, 1985). Plasticity itself may also be a target of selection, with an organism's evolutionary history and ecological context influencing the magnitude of a plastic response and the length of the developmental window during which such a response can occur (Scheiner, 1993; Pigliucci, 1996; Pigliucci et al., 2006; Hoverman and Relyea, 2007).

The extent to which phenotypic sensitivity to environmental cues changes during development has been examined in a variety of species and traits (Hinton and McNamara, 1984; Meyer, 1987; Bouvier, 1988; Lieberman et al., 2001; Lieberman et al., 2003; Dufty et al., 2002; Marchinko, 2003; Taborsky, 2006; Hoverman and Relyea, 2007; Kotrschal and Taborsky, 2010; Serrat, 2013). Although these studies have documented substantial variation in how plasticity varies with age, there appears to be a general tendency for plasticity to decrease as organisms mature. The reasons for this age-dependency are not clear, but it presumably results from several interacting factors, including, among others, the costs of plasticity, an organism's ability to detect and reliably interpret information about the environment (especially fluctuations) and life-history strategy (DeWitt et al., 1998; Hoverman and Relyea, 2007; Auld et al., 2010; Fischer et al., 2014).

The biological system that has attracted the most attention in terms of age-dependent plasticity is the vertebrate skeleton, particularly with respect to how it responds to variation in loading patterns (i.e. bone functional adaptation). The literature devoted to this subject is vast (for reviews, see Lanyon and Rubin, 1985; Bertram and Swartz, 1991; Biewener, 1993; Pearson and Lieberman, 2004; Ruff et al., 2006; Ravosa et al., 2010b). This intensive focus is related to skeletal plasticity's relevance to biomedical applications (e.g. Bouvier and Hylander, 1981; Burr, 1997; Westerlind et al., 1997; Bass et al., 1998; Lanyon and Skerry, 2001; Engelke et al., 2006) and its potential to inform reconstructions of behavior in past populations and extinct species (e.g. Menegaz et al., 2009; Holmes and Ruff, 2011; Scott et al., 2014; Standen et al., 2014). The way in which bone responds to loading over the course of an organism's lifetime is complex and multileveled (Bertram and Swartz, 1991; Hsieh et al., 2001; Pearson and Lieberman, 2004; Hamrick et al., 2006; Ruff et al., 2006; Ravosa et al., 2008; Ravosa et al., 2010b). Several studies have observed an age-related decrease in the ability of an organism to modify a bone's cross-sectional geometry adaptively (i.e. bone modeling) in response to altered patterns of loading, such as increases in magnitude or frequency (Hinton and McNamara, 1984; Bouvier, 1988; Rubin et al., 1992; Kannus et al., 1995; Lieberman et al., 2001; Lieberman et al., 2003; Kontulainen

[1]Department of Anthropology, Southern Illinois University, Carbondale, IL 62901, USA. [2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA. [3]Departments of Biological Sciences, Aerospace and Mechanical Engineering, and Anthropology, University of Notre Dame, Notre Dame, IN 46556, USA.

*Author for correspondence (Matthew.J.Ravosa.1@nd.edu)

Received 25 July 2014; Accepted 3 October 2014

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111708

et al., 2003; Pearson and Lieberman, 2004; Ruff et al., 2006). However, the trajectory of this decrease in responsiveness and how it varies throughout the skeleton and among species is poorly documented, impeding our understanding of how organisms adjust to their environmental circumstances at different life-history stages and, therefore, our ability to interpret morphological variation and link it with ecological context.

Here, we report the results of a long-term (48 weeks), longitudinal dietary-plasticity experiment that are directly relevant to these issues. The experiment was designed to test the effects of seasonal variation in dietary mechanical properties on skull growth in our model organism, the New Zealand white rabbit (*Oryctolagus cuniculus* Linnaeus 1758), from weaning into adulthood. We examined three groups that differed in diet: control rabbits, 'early' rabbits and 'late' rabbits. All groups were fed rabbit pellets; the early and late rabbits also received hay cubes at different stages of the experimental period. The early rabbits first received hay just after weaning (weeks 1–6 of the experimental period) and then again during early adulthood (weeks 25–30), whereas the late rabbits first received hay around the time of skeletal maturity (weeks 19–24) and then again at the end of the experimental period (weeks 43–48). Given that seasonal dietary shifts can recur throughout an organism's lifetime in the wild, administering hay to the early and late groups twice during the experimental period simulates natural conditions and potentially allows us to make inferences regarding changes in phenotypic responsiveness within groups.

Data on the mechanical properties of pellets and hay cubes demonstrate that hay cubes are more mechanically challenging than pellets in terms of the effort required to break them down using the postcanine dentition: hay has a greater elastic modulus (i.e. it is stiffer: pellets, E=29.2 MPa; wet hay, E=277.8 MPa; dry hay, E=3335.6 MPa) and it is probably tougher (Ravosa et al., 2007; Menegaz et al., 2009). Therefore, in comparison to the control rabbits, the early and late rabbits were expected to use higher-magnitude bite forces or greater repetitive loading, or both, in order to process the hay-cube component of their diets. Comparison of peak bone-strain levels along the working-side mandibular corpus (Weijs and de Jongh, 1977) suggest that rabbits do not tend to use higher-magnitude bite forces when processing hay in comparison to pellets. However, we lack such measurements in the context of our experimental design and therefore cannot conclusively rule out such differences in the present case. By contrast, preliminary observational data from a sample of 12 adults indicate that rabbits use approximately three times more chewing cycles per unit food mass when processing hay versus pellets (2.95 times more chews per g, 95% confidence interval: 2.58–3.35) (unpublished results), indicating that hay consumption does engender greater repetitive loading and correspondingly longer loading durations. Given the adaptive role of increased cyclical loading in bone formation (Bouvier and Hylander, 1981; Biewener et al., 1986), hay consumption should thus stimulate osteogenesis and result in larger jaw proportions in the two experimental groups in comparison with the control rabbits (Ravosa et al., 2007; Ravosa et al., 2008; Menegaz et al., 2009; Scott et al., 2014). With respect to differences between the experimental groups, we predicted an inverse relationship between age and the magnitude of diet-induced osteogenic responses; in other words: early rabbits at week 6>late rabbits at week 24>early rabbits at week 30>late rabbits at week 48.

RESULTS

Our analysis focuses on bone cross-sectional areas of the palate and mandibular symphysis, corpus and condyle, given their role in resisting bite forces generated during mastication. We used maximum cranial length to size-adjust the areas in order to control for the potentially confounding effects of subtle differences in organismal size (cf. Meyer, 1987; Lieberman et al., 2003; Gomez-Mestre and Buchholz, 2006; Hoverman and Relyea, 2007; Svanbäck and Schluter, 2012). For each individual at each time point, we divided the square root of a given bone cross-sectional area by maximum cranial length, creating a shape ratio that expressed the size of the cross-sectional area relative to cranial length. These ratios were logged (base e) for analysis.

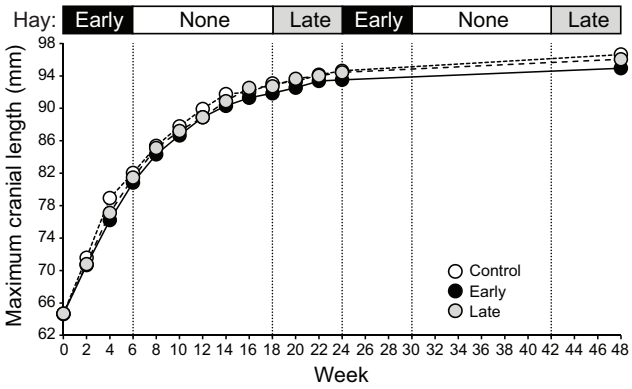

Fig. 1. Growth in maximum cranial length of the New Zealand white rabbit (*Oryctolagus cuniculus*). Mean maximum cranial lengths for each group are plotted against week. Note that there are slight differences in size among the groups, but such differences are not statistically significant, except in the comparison between the control and early groups in week 4. Weeks in which the experimental groups consumed hay are indicated; for example, only the early group received hay during weeks 1–6, and no group received hay during weeks 7–18.

To provide context for the analysis of shape ratios, Fig. 1 shows the growth curves for maximum cranial length in the three dietary cohorts. Note that the three groups were nearly indistinguishable at the start of the experiment (week 0; 5 weeks of age), with slight differences becoming apparent as development proceeded. In particular, the early rabbits tended to have shorter crania than the other two groups, especially the controls. However, such differences were only significant at week 4, and only in the comparison between the early and control groups (P<0.001). There is, therefore, no compelling evidence that differences in diet had a strong effect on the overall size of the skull. However, the fact that there were slight differences among the samples that persisted throughout the experiment highlights the importance of adjusting for skull size. Cranial growth began to level off around week 16 of the experiment (21 weeks of age). Cranial length increased after week 24, but only by 1–2% in each of the groups.

Relative growth curves (i.e. shape ratios plotted against time) for the cross-sectional areas up to week 24 are illustrated in Fig. 2. After week 24, the rabbits became too large to be imaged and were not scanned again until the end of the experiment, following sacrifice at week 48. This final time point is excluded from Fig. 2 in order to emphasize the changes that occurred during the first half of the experimental period. Fig. 3 presents box plots of the shape ratios from week 48, along with those from week 24 for comparison. Starting at the beginning of the experimental period, the relative areas of the mandibular symphysis, palate and mandibular condyle tended to decrease with age, indicating slower rates of growth relative to cranial length (Fig. 2). In contrast, the relative area of the mandibular corpus first decreased and then increased (Fig. 2).

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111708

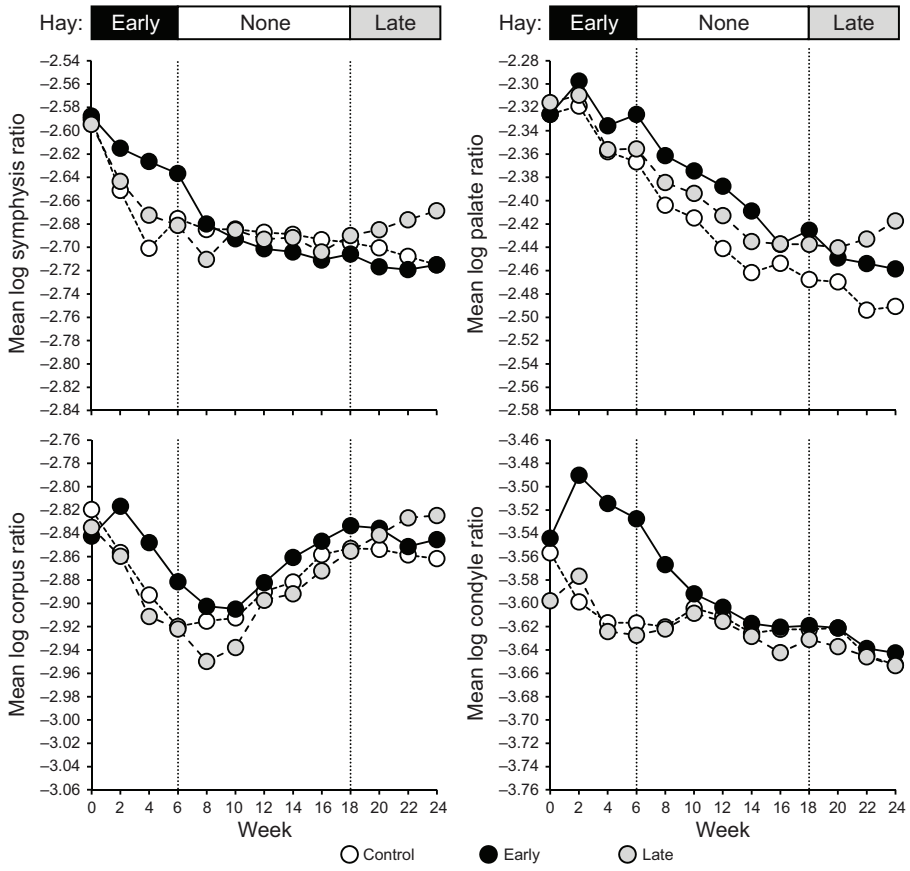

Fig. 2. The effects of dietary shifts on relative jaw cross-sectional areas in rabbits during the first half of the experimental period. Mean logged shape ratios for each group are plotted against week. Weeks in which the experimental groups consumed hay are indicated, as in Fig. 1. Note that the early rabbits diverged from the other two groups in all four shape ratios during weeks 1–6 after beginning their first hay regimen. Following their shift to an all-pellet diet after week 6, the early rabbits tended to converge on the other two groups. The late rabbits diverged from the early and control rabbits in three out of four shape ratios during weeks 19–24 after beginning their first hay regimen.

Superimposed on these general trends are clear signatures of plastic responses to dietary shifts. At week 0, none of the observed differences in shape ratios between the control and early groups were statistically significant ($P>0.25$). At week 6 – the end of the early rabbits' first hay-cube regimen – all four of the shape ratios for the early group were significantly larger than those for the control rabbits (symphysis: $P<0.001$; palate: $P=0.003$; corpus: $P=0.017$; condyle: $P=0.003$), indicating that the early group had developed relatively larger bone cross-sectional areas in response to feeding on hay in comparison to the control rabbits. The early rabbits also had larger shape ratios than the late rabbits at this time point (symphysis: $P=0.004$; palate: $P=0.003$; corpus: $P<0.001$; condyle: $P<0.001$). Notably, following week 6, the trajectories of the early rabbits began to converge on those for the control and late rabbits. By the end of the first half of the experiment (week 24), the early group did not differ from the control group at any of the sites ($P>0.06$) (Fig. 3).

The control and late groups were not statistically distinguishable at week 18 ($P>0.35$). Following week 18, with the onset of their hay-cube regimen, the late rabbits began to diverge from the control group, except at the condyle. By week 24, the late rabbits had significantly larger symphyseal ($P=0.005$), palatal ($P<0.001$) and corporal ($P=0.005$) shape ratios than the control group, and a significantly larger palate ratio than the early group ($P=0.015$) (Figs 2, 3). The *P*-value for the difference in mean symphysis ratios between the late and early groups at this time point was low ($P=0.026$) but not significant after adjusting for multiple comparisons using the false discovery rate (FDR) method (critical value: $P=0.021$) (Benjamini and Hochberg, 1995). A somewhat different pattern of relationships characterized the final time point (week 48; Fig. 3): the late rabbits were significantly larger than the other two groups at the palate (versus control: $P<0.001$; versus early: $P=0.002$) and larger than the control group at the symphysis ($P<0.001$); the early group was significantly larger than the controls only at the symphysis ($P=0.006$). There were no significant differences at the condyle or corpus, but the *P*-value for the difference in mean corpus ratios between the late and control groups was low ($P=0.033$; FDR-adjusted critical level: $P=0.023$).

Fig. 4 presents comparisons of the magnitudes of the plastic responses of each variable at different stages of the experiment. Given that neither of the experimental groups differed significantly from the control group prior to the onsets of their first hay regimen, we quantified the magnitude of the plastic responses in each of the experimental groups during the first half of the experiment using the difference in mean shape ratios between the control and experimental groups at the end of the hay regimen (early group at week 6; late group at week 24) and its 95% confidence interval, generated using the bootstrap (Efron and Tibshirani, 1993; Manly, 1997). We favored this approach over a repeated-measures ANOVA or a resampling-based equivalent because it allowed us to use the plastic responses in the first half of the experimental period (i.e. to week 24) to make inferences about the plastic response of the late group at the end of the experiment (i.e. week 48) using the same bootstrap procedure. In the case of late rabbits at week 48, we do not have a baseline comparison with the control group prior to the reintroduction of hay cubes beginning at week 43 because, as noted above, the rabbits were too large to be imaged during the second half of the experiment. Note also that we do not have data on the plastic response of the early rabbits during weeks 25–30 because of this constraint. The approach adopted here assumes that the shape ratios of the late and control group were not significantly different at week 42. Because our data do not allow us to directly examine this assumption, it follows that any conclusions about plasticity

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111708

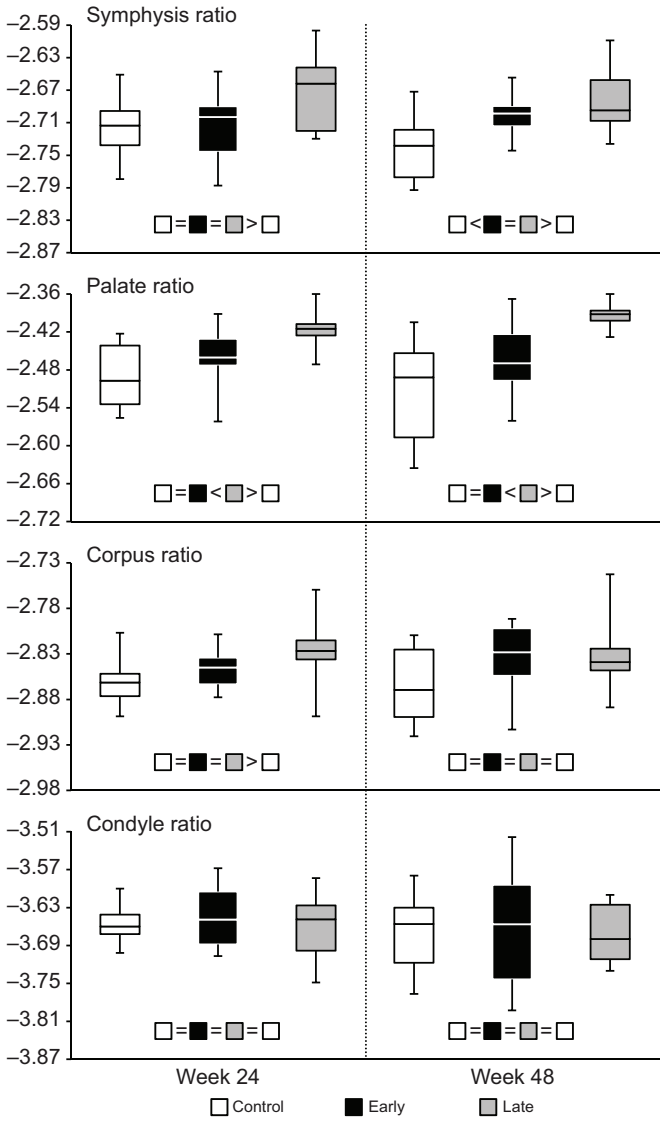

Fig. 3. Box plots of logged shape ratios for each group of rabbits at weeks 24 and 48. Significant differences between groups in mean logged shape ratios, and the directions of such differences, are indicated by greater-than and less-than symbols; equality signs indicate that the observed sample differences are not significant. For example, at top left (symphysis ratio, week 24), the control group is not statistically different from the early group (white versus black); the early group is not statistically distinguishable from the late group (black versus gray); and the late group is significantly larger than the control group (gray versus white).

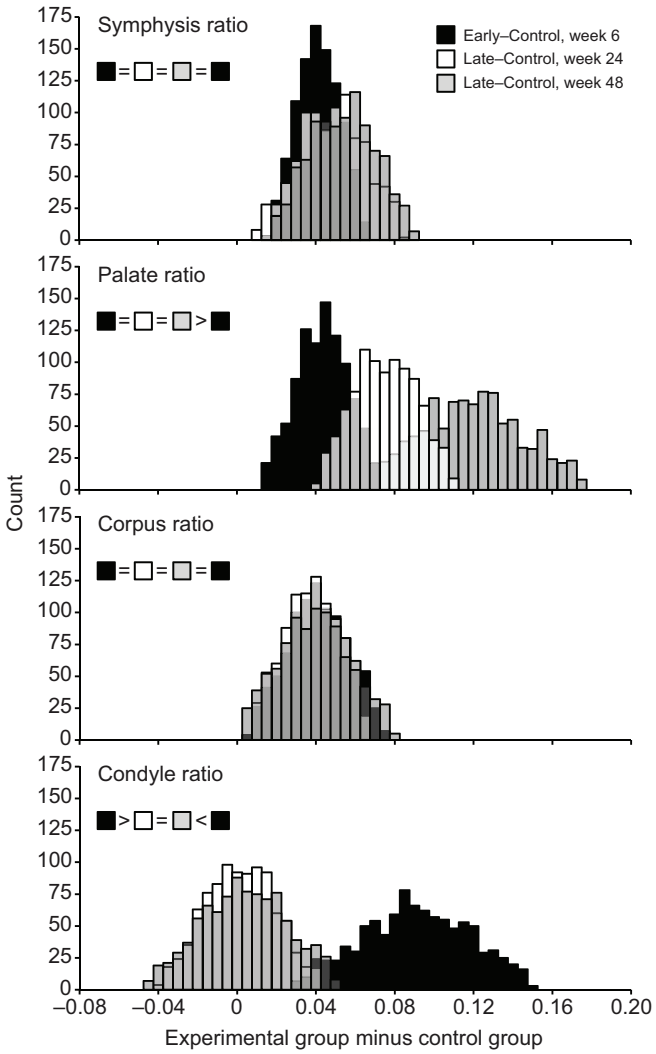

Fig. 4. Magnitudes of plastic responses in jaw shape ratios in rabbits. The distributions in each histogram represent the 95% bootstrap confidence intervals for the differences between the experimental and control groups at the end of each experimental group's hay regimen (i.e. the plastic responses). Distributions for week 48 should be interpreted with caution (see text for discussion). Significant differences, and the directions of such differences, are indicated by greater-than and less-than symbols; equality signs indicate that the observed sample differences are not significant. For example, at bottom (condyle shape ratio), the difference between the early and control groups at week 6 is significantly larger than the difference between the late and control groups at week 24 (black versus white); the difference between the late and control groups at week 24 is not statistically different from the difference between the late and control groups at week 48 (white versus gray); and the difference between the early and control groups at week 6 is significantly larger than the difference between the late and control groups at week 48 (black versus gray).

drawn from comparisons between week 48 and weeks 6 or 24 should be considered tentative and treated with due caution. However, the growth trajectory for the early group during the first half of the experiment indicates that diet-induced differences between this group and the control group faded over time once the early group switched to an all-pellet diet, with the shape ratios of the early group being statistically indistinguishable from those of the controls at week 24 (Figs 2, 3). To the extent that this pattern of change also characterized the late rabbits following week 24, the assumption of no differences (or only minor differences) between the late and control groups at week 42 may be reasonable, at least at some of the sites examined.

Each histogram in Fig. 4 contains three distributions: the first two represent the 95% bootstrap confidence intervals for the magnitudes of the plastic responses of the early rabbits at week 6 (early minus control) and the late rabbits at week 24 (late minus control). The third distribution represents the 95% bootstrap confidence interval for the magnitude of the difference between the late and control groups at week 48 – or the inferred plastic response of the late rabbits at week 48 assuming no difference between the late and control rabbits at week 42. The only variable that conformed to our expectation of decreasing plasticity with age was the mandibular condyle: the response of the early rabbits during weeks 1–6 was significantly larger than that for the late rabbits during weeks 19–24

(P=0.007) and the inferred response for the late rabbits at weeks 43–48 (P=0.01). In contrast to the condyle, the responses of the early and late groups at the symphysis and mandibular corpus were similar in magnitude and indistinguishable statistically, indicating similar levels of bone sensitivity to shifts in dietary mechanical properties at these sites just after weaning (weeks 1–6) and around the time of skeletal maturity (weeks 19–24), and perhaps well into adulthood (weeks 43–48). The results for relative palate cross-sectional area, taken at face value, suggest that plasticity increased with age (Fig. 4); however, only the comparison between weeks 6 and 48 was significant (P=0.006; two-tailed test), and it is important to keep in mind that the interpretation of plasticity magnitudes at the end of the experimental period is dependent on the assumption that the late and control groups had similar shape ratios at week 42. As we discuss below, we cannot rule out an alternative explanation.

DISCUSSION

The capacity to modify anatomy, physiology or behavior in response to changing environmental conditions is an important part of organismal adaptation and has been documented in a variety of biological systems and diverse organisms (e.g. Bernays, 1986; Meyer, 1987; Agrawal, 2001; Marchinko, 2003; Hoverman and Relyea, 2007; Muschick et al., 2011; Svanbäck and Schluter, 2012; Serrat, 2013; Standen et al., 2014). Our experiment was designed to examine how the mammalian masticatory apparatus – represented here by a model organism, the white rabbit – responds adaptively to temporal variation in food mechanical properties at different stages of the life span. Importantly, we were able to track changes in form longitudinally and the experimental treatment was neither invasive nor highly unusual in terms of the physical demands it placed on the subjects. Additionally, the experimental conditions mimicked the seasonal reliance of a species on foods that are usually avoided because they present more of a mechanical challenge to process than preferred foods, but which are critical with respect to survival during times of the year when preferred resources are scarce (Marshall and Wrangham, 2007).

In accord with our predictions derived from previous plasticity studies in rabbits (Ravosa et al., 2007; Ravosa et al., 2008; Ravosa et al., 2010a; Menegaz et al., 2009; Scott et al., 2014) and current understanding of bone functional adaptation (e.g. Bouvier and Hylander, 1981; Lanyon and Rubin, 1985; Biewener, 1993; Pearson and Lieberman, 2004; Ruff et al., 2006; Ravosa et al., 2010b), we found that our experimental groups responded to mechanically challenging foods – and the increase in loading such foods are expected to engender – by increasing the cross-sectional areas of various jaw structures, thereby presumably reducing bone strain. However, in contrast to our prediction that the magnitude of the plastic responses would decrease with age, we found remarkably similar effect sizes in juveniles (11 weeks of age) and young adults (29 weeks of age) at three out of the four sites we examined. Our data further indicate that the rabbits maintained the ability to respond to dietary shifts as older adults (53 weeks of age), and that the magnitude of these later responses may have been relatively high in some features, though we lack the data to establish this conclusively.

Before discussing the latter finding in greater detail and critically evaluating the case for relatively high levels of plasticity in older adults, it is worth noting that, in some respects, our results confirm previous views of bone functional adaptation. First, the clear contrast in plasticity between juvenile and adult rabbits at the mandibular condyle is consistent with the idea that the capacity of bone to respond to increased loading diminishes as organisms mature in some regions of the skeleton (Bertram and Swartz, 1991; Pearson and Lieberman, 2004; Ruff et al., 2006). Moreover, low plasticity in condylar cross-sectional area in our late rabbits mirrors the results of previous experimental studies of age-dependent plasticity in the temporomandibular joint (TMJ) conducted on rhesus macaques (*Macaca mulatta*) (Hinton and McNamara, 1984) and rats (*Rattus rattus*) (Bouvier, 1988) that also found decreased TMJ plasticity in older individuals. It is important to note, however, that, in our study, failure to detect a plastic response in condylar cross-sectional area does not mean that there was no response in the TMJ, given that there are a variety of other ways in which joints and skeletal elements can respond to increased loading – for example, by increasing bone mineralization and altering the trabecular architecture (e.g. Ruff and Runestad, 1992; Lieberman et al., 2001; Pearson and Lieberman, 2004; Ruff et al., 2006; Ravosa et al., 2007; Ravosa et al., 2008).

Also relevant in this context are the findings of Lieberman et al. (Lieberman et al., 2001) showing that, in sheep (*Ovis aries*), the joint surfaces of limb bones are potentially less plastic than the cross-sectional areas of the shafts. The authors interpreted their result as support for the hypothesis that joint geometry is more genetically canalized – and therefore has a relatively low level of plasticity – because of functional constraints imposed by the need to maintain joint mobility and congruence between the opposing articular surfaces (Ruff and Runestad, 1992). Our results for plasticity at the mandibular condyle in adults (i.e. no detectable plastic responses in the late rabbits at weeks 24 and 48) are consistent with this hypothesis, especially when considered in the context of the effects observed at the other three sites. Note, however, that whereas Lieberman et al. (Lieberman et al., 2001) found no plastic response in joint geometry in their sample of juvenile sheep, the response in our juvenile experimental group (early rabbits, week 6) was quite marked. Moreover, given that a prior study of rats observed that the TMJ condyle experienced the highest levels of plasticity in the growing skull (Bouvier and Hylander, 1984), it is also possible that variability in joint reaction norms is due to diet-related variation in joint-loading regimes, with some loading conditions being more stable and thus inducing relatively lower levels of plasticity.

A further counterexample to the idea that some aspects of joint morphology are, in general, less plastic than other components of the skeleton comes from our results for the mandibular symphysis. Although the rabbit symphysis is a joint, it maintained a juvenile level of plasticity at least into young adulthood. However, the surfaces of the symphyseal joint are characterized by numerous prominent rugosities that project and interlock with each other (Ravosa et al., 2007). This configuration indicates that the joint is functionally immobile, which has perhaps relaxed the constraints that may limit plasticity in other joints. Indeed, Ravosa et al. (Ravosa et al., 2008) found that symphyseal hard tissues in older rabbits maintain a level of plasticity unlike that of the TMJ, which exhibits decreased biomineralization and apparent increases in porosity. The notion that tissue plasticity might be site-specific is supported by comparisons of proximal limb joints in growing pigs subjected to exercise-induced dynamic loading, where differences in bone and cartilage responses were noted between the proximal femur and proximal humerus in the same experimental subjects (Congdon et al., 2012). Osteogenic responses of cortical bone to exercise may also vary along the length of a limb element, with distal regions exhibiting more pronounced morphological changes to altered mechanical stimuli (Hamrick et al., 2006; see also Hsieh et al., 2001).

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111708

Our results also confirm that adult behaviors can leave clear signals in bone morphology. A key question in the study of skeletal plasticity is the extent to which adult skeletal morphology reflects current or very recent events versus the loading regime experienced during the juvenile period, when bone is thought to be at its most responsive because of the interaction between postnatal growth and external loading (Bertram and Swartz, 1991; Pearson and Lieberman, 2004; Ruff et al., 2006). Our longitudinal data and experimental design provide an important perspective on this issue. First, the early rabbits revealed that the signals left by loads experienced early in ontogeny can be lost, or at least greatly diminished, over time if the loading regime in question is not sustained throughout the juvenile period (e.g. in behaviors that are highly seasonal). Second, the late rabbits provide compelling support for a juvenile level of responsiveness to increased loading in young adults. Thus, at week 24 (29 weeks of age), the late rabbits – in addition to being statistically distinguishable from the control group at three out of four sites – had significantly larger relative palate cross-sectional areas than the early rabbits (and the *P*-value for the symphysis comparison was low), whereas the early rabbits could not be statistically distinguished from the control rabbits at any of the four craniomandibular sites (Fig. 3). That the jaws of young adults were responsive to the shift in diet is not unexpected (Bouvier and Hylander, 1981; Ravosa et al., 2007); what is surprising, especially when considered in the context of overall skull growth (Fig. 1), is that the magnitude of the effect was indistinguishable from that observed in the juvenile rabbits at three out of four sites (Fig. 4).

With respect to the results for the late rabbits at week 48 (53 weeks of age), interpretation is complicated by our lack of longitudinal data following week 24. When comparing the late and control groups at this time point, we assumed (1) that the plastic response of the late rabbits at week 24 was transient, based on the relative growth curves of the early rabbits in the first half of the experimental period, and (2) that therefore, by week 43 (the onset of the late group's second round of hay cubes), the differences between the late and control groups were minimal, or at least not statistically significant. Under this set of assumptions, the results indicate three patterns of response in the final weeks of the experiment: first, there was no plastic response in relative condylar cross-sectional area, as in week 24. Second, the level of plasticity at the symphysis – and perhaps the corpus – was unchanged from earlier time points. In the case of the corpus, however, the results are somewhat ambiguous: comparison of the bootstrap confidence intervals for the magnitudes of the differences between the late and control groups at this time point (Fig. 4) suggests no change in sensitivity to loading at this site during the experimental period, but recall that although the *P*-value for the comparison between the late and control rabbits at week 48 was low (P=0.033), it was not significant (FDR-adjusted critical value: P=0.023). The third pattern was an apparent increase in plasticity at the palate in the later stages of the experimental period.

Given our lack of data between weeks 24 and 48, alternative explanations for the results from the final time point must be considered. In addition to the interpretation presented above, there are at least two other possibilities. The first of these is that there was a sharp decline in plasticity following week 24, such that the differences among groups at week 48 – particularly between the late and control groups – were holdovers from first half of the experimental period. We view this explanation as unlikely, however, given that we have clear evidence that a plastic response occurred in the early rabbits during their second round of hay cubes in weeks 25–30, at least in one feature. Specifically, note that the early rabbits did not differ from the control rabbits in any of the variables in week 24, but we detected a significant difference in their mean symphysis ratios at week 48 (Fig. 3), indicating that the rabbits were still capable of responding to changes in loading at the start of the second half of the experimental period. This observation is in line with current views of skeletal plasticity in mammals, which hold that the responsiveness of the skeleton to loading does not end abruptly at skeletal maturity or soon thereafter, but rather declines gradually throughout adulthood (Pearson and Lieberman, 2004; Ruff et al., 2006). Moreover, it is likely that plastic responses also occurred at the palate and corpus of the early group at the beginning of the second half of the experimental period, but that those responses did not result in differences between the early and control groups at week 48 that were large enough to be statistically detected (palate: P=0.089; corpus: P=0.06).

The second alternative explanation for the results from week 48 is that as the rabbits passed from early adulthood to later adulthood, they developed a lag in the response time to dietary shifts, particularly a shift from greater loading (hay) to reduced loading (pellets only). In other words, in contrast to the pattern exhibited by the early group in the first half of the experiment, which was characterized by relatively quick reversals in the plastic responses to hay consumption, the plastic responses of the late group during weeks 19–24 may have faded more slowly during the second half of the experiment. Time lags in plastic responses are predicted to evolve under certain conditions, and asymmetry in the lag for producing a response versus the lag in reversing it may be adaptive in some cases (e.g. Gabriel, 1999; Relyea, 2003; Hoverman and Relyea, 2007). Such phenomena may also be age-dependent (Fischer et al., 2014). The significant difference in relative symphyseal cross-sectional area between the early and control groups at week 48 supports the idea of a time lag in reversing diet-induced changes during the second half of the experimental period.

Notably, if the experiment had continued past week 48, the early rabbits would have received hay cubes during weeks 49–54, and any plastic response in the symphysis of this group during this time would have built on the existing difference between the early and control groups at week 48. In such a situation, if there is asymmetry in how the jaw structures of adults respond to different types of changes in loading (i.e. quicker response to increased loading, slower response to decreased loading), then the current plastic response combined with the residual from the earlier plastic response may interact to produce what appears to be – in the absence of longitudinal data – a degree of plasticity that is similar to, or perhaps greater than, that observed in juveniles and young adults. We cannot rule out such a scenario for our results from week 48 at any of the sites we analyzed in this study, meaning that we cannot rule out a slight reduction in the magnitude of the plastic responses in the late rabbits during weeks 43–48 relative to the juveniles and young adults, as predicted by previous studies of skeletal plasticity. Thus, with respect to the apparent increase in plasticity at the palate in the late rabbits at the end of the experimental period, a more likely explanation, in our view, is that this result reflects an interaction between the effects of past and current episodes of plasticity rather than greater plasticity. Regardless of how the results from week 48 are interpreted, it is clear that some aspects of jaw morphology remained sensitive to changes in diet well into adulthood.

In summary, this study represents the first examination of plasticity throughout postnatal development in multiple regions of the mammalian masticatory apparatus. Given that most previous

work on skeletal plasticity in mammals has focused on the postcranium, particularly single-site analyses of limb bones (Bertram and Swartz, 1991; Pearson and Lieberman, 2004; Ruff et al., 2006), our results provide a novel perspective, revealing a protracted period of phenotypic responsiveness to dietary shifts that is characterized by a surprisingly uniform level of plasticity in some regions (symphysis, palate and corpus) throughout the juvenile stage and extending into early adulthood, and perhaps beyond. We also found evidence for the age-dependent changes in plasticity recorded in previous studies, most clearly in the cross-sectional area of the mandibular condyle, which was much less responsive to changes in diet in adults compared with juveniles. Finally, we inferred the presence of further age-dependent changes in plasticity in the later stages of the experiment, including a possible lag in response time to dietary shifts, though we were unable to fully document these because of methodological constraints. Our results thus highlight the mammalian skeleton's remarkable capacity for adaptive plasticity in the face of changing environmental conditions and the complexity of such responses, both in terms of variation among different components of the system and with respect to changes in a single site during the lifetime of an organism.

MATERIALS AND METHODS

Animal model and experimental design

All procedures used in this study were approved by the University of Notre Dame Institutional Animal Care and Use Committee (IACUC). Our sample consisted of *N*=30 male rabbits obtained at weaning (5 weeks of age) from Harlan Laboratories (www.harlan.com) and housed at the University of Notre Dame's animal care facility, Friemann Life Science Center. Day-to-day care of the animals, including periodic health evaluations, was handled by trained veterinary staff. The animals were raised for 48 weeks, making them 53 weeks old at the conclusion of the experimental period. In white rabbits, weaning typically occurs at 4–5 weeks of age, and skeletal maturity and sexual maturity are attained at ~26 weeks of age (Masoud et al., 1986; Isaksson et al., 2010).

Previous experimental work has established that the masticatory apparatus of growing rabbits is sensitive to variation in dietary mechanical properties (Ravosa et al., 2007; Ravosa et al., 2008; Ravosa et al., 2010a; Menegaz et al., 2009; Scott et al., 2014). Importantly, *Oryctolagus cuniculus* resembles other mammalian herbivores in key features of the masticatory apparatus and bone biology that make it a suitable model organism. These features include: (1) the configuration of the skull, which is characterized by a vertically deep facial skeleton, tall mandibular ramus, and a TMJ situated high above the occlusal plane; (2) mandibular kinematics, with a TMJ capable of rotational and translational movements, and transverse jaw movements during mastication; (3) intracortical bone remodeling; and (4) patterns of covariation among dietary mechanical properties, jaw-muscle activity, and jaw-loading regimes, which are well-characterized (Weijs and de Jongh, 1977; Weijs et al., 1989; Hirano et al., 2000; Langenback and van Eijden, 2001).

Beginning the experiment at weaning was important, not only because we were interested in testing the hypothesis that phenotypic sensitivity to environmental stimuli decreases across the life span, but for two additional reasons. First, it mitigates the effects of other dietary influences that might confound comparisons among groups subjected to different dietary protocols. Second, because mammals begin to adopt adult diets and chewing behaviors around the time of weaning (Herring, 1985; Weijs et al., 1989), commencement of diet manipulation at this early developmental stage facilitates a more naturalistic experiment.

Upon arrival, the rabbits were divided equally into three cohorts (*N*=10 each). Animals in the first group, the control rabbits, were fed a diet consisting solely of Purina rabbit pellets throughout the experiment. Animals in the second group, the 'early' rabbits, were each given three hay cubes (~3.2×1.9×1.9 cm) per day in addition to pellets for the first 6 weeks of the experimental period and were then switched to an all-pellet diet for the next 18 weeks (weeks 7–24). Animals in the third group, the 'late' rabbits, were put on the opposite feeding schedule – i.e. pellets-only for the first 18 weeks, then three hay cubes daily for the next 6 weeks. Thus, the early rabbits consumed hay directly after weaning, whereas the late rabbits were not exposed to hay until around the time they achieved skeletal maturity. These schedules were repeated in the second half of the experimental period, giving each of the experimental groups two periods of exposure to hay and mimicking seasonal variation in dietary composition. The amount of pellets received by each rabbit was determined by veterinary staff based on established standards. Because the rabbits initially exhibited a preference for pellets, animals receiving hay cubes were given a reduced amount of pellets to ensure that they consumed all of their hay cubes while receiving adequate nutrition.

Data acquisition

Longitudinal skull growth was tracked *in vivo* using micro-computed tomography (Bioscan/Mediso X-CT, Budapest, Hungary; settings: 70 kVp and 100 μA, with a 71 μm reconstructed isotropic voxel size). Prior to scanning, each rabbit was anesthetized using a cocktail of ketamine (25 mg kg^{-1}), xylazine (5 mg kg^{-1}) and acepromazine (2.5 mg kg^{-1}) administered by intramuscular injection to the quadriceps femoris muscle. Rabbits were scanned at the beginning of the experiment upon arrival (week 0; 5 weeks of age) and then every 2 weeks thereafter until week 24, the end of the first half of the experimental period. Week 24 corresponds with a chronological age of 29 weeks, or 6.67 months (i.e. young adulthood). At this point in the experiment, the rabbits were too large to be scanned. We therefore lack longitudinal data for the second half of the experiment. The subjects were scanned one last time at the end of the experimental period (week 48, or 53 weeks of age) following death. Animals were killed by veterinary staff following established standards and IACUC guidelines using the following procedures: subjects were first anesthetized using the ketamine–xylazine–acepromazine cocktail and then given a pentobarbital overdose (100 mg kg^{-1}) via cardiac puncture, with bilateral thoracotomy used as a secondary means of assuring death.

Reconstructed scans were opened in the program PMOD version 3.3 (PMOD Technologies Ltd, Zurich, Switzerland) and oriented so that the sagittal plane was parallel to the computer monitor and the occlusal plane was horizontal. Following orientation, we measured maximum cranial length and bone cross-sectional areas at three sites on the mandible (symphysis, corpus, condyle) and one site on the cranium (palate) using the measurement and segmenting tools available in PMOD. Longitudinal data are complete for 27 of the 30 rabbits.

Measurements used in this study

Cranial length is the distance from the most posterior point on the neurocranium to the most anterior point on the premaxilla between the maxillary central incisors, taken in the sagittal plane. Symphyseal area is the bone cross-sectional area in the coronal plane at 25% of the distance from the posterior border to anterior border of the main body of the joint (i.e. not including the long inferior shelf of bone that extends posteriorly under the diastemata and mesial portion of the postcanine tooth rows). Palatal area is the bone cross-sectional area between the maxillary tooth rows and inferior to the paranasal sinuses and the nasal cavity, taken in the coronal plane at the level of the second maxillary postcanine tooth (P^3). Condylar area is the bone cross-sectional area in the coronal plane, taken at the mediolaterally widest point of the condyle. Corporal area is the bone cross-sectional area at the second mandibular postcanine tooth (P_4), taken with the specimen oriented so that the section is perpendicular to the long axis of the corpus.

Data analysis

We evaluated the statistical significance of differences among groups at selected time points using the following bootstrap procedure (Efron and Tibshirani, 1993; Manly, 1997): (1) At week *K*, bootstrap (i.e. resample with replacement) from the sample of shape ratio *Q* for group *X* 1000 times, with each bootstrap sample being identical in size to the original sample. (2) Compute the mean shape ratio *Q* for each of the 1000 bootstrap samples. (3). Perform steps 1 and 2 on group *Y*. (4) Randomly pair the 1000 mean shape ratios for group *X* with those for group *Y*. (5) For each pairing, subtract the mean shape ratio for group *X* from the mean shape ratio for

group *Y*. This step produces a distribution of pairwise differences between groups *X* and *Y* for shape ratio *Q*. (6) Center the distribution of pairwise differences on zero by subtracting the mean of the 1000 pairwise differences generated in step 5 from each pairwise difference. This step is necessary because the distribution of pairwise differences will be centered on the observed difference between groups *X* and *Y*. In order to derive a *P*-value for the observed difference between the two cohorts, the distribution must be centered on – i.e. the mean of the distribution must equal – zero, which approximates the distribution of the test statistic when the null hypothesis of no difference between samples is true (Manly, 1997). (7) Using the zero-centered distribution, count the number of values that are as extreme as or more extreme than the observed difference between groups *X* and *Y*. The resulting value is *M*. (8) Use the following formula to obtain the *P*-value for the comparison: $P=(M+1)/(N+1)$, where *M* is as above, *N* is the total number of bootstrap differences (i.e. 1000) and 1 is added to *M* and *N* to include the observed difference.

For comparisons between the control group and each of the experimental groups prior to the onset of an experimental group's first hay-cube regimen (week 0 for early rabbits; week 18 for late rabbits), the signs of the differences from the zero-centered distribution obtained in step 6 were disregarded in step 7, making the test two-tailed, because there is no *a priori* reason to expect a difference in a particular direction prior to hay cubes being introduced into the diets of the experimental rabbits. In subsequent weeks, however, we made directional predictions; in such cases, *M* represents counts of only the positive differences (i.e. experimental group minus control group), making the test one-tailed. We used the false discovery rate (FDR) procedure (Benjamini and Hochberg, 1995) to account for the issues associated with multiple testing. Two families of tests were recognized: comparisons of means within time points (40 tests) and comparison of plasticity magnitudes between time points (12 tests), discussed below.

We tested for significant differences between the magnitudes of the plastic responses at week 6 and week 24 using the bootstrap procedure outlined above, with the following additional step: (5b) perform steps 1–5 for control rabbits versus early rabbits (week 6), and for control rabbits versus late rabbits (week 24). Randomly pair values from the distribution of differences for control versus early with those from that for control versus late and subtract the week 24 differences from the week 6 differences. Continue on to step 6.

This step creates a distribution of differences between the plastic responses in the early and late groups (i.e. a distribution of differences between differences). Because we expected the early group to exhibit a greater plastic response in this comparison, the test is one-tailed. We also used this test to compare the inferred plastic responses for the late rabbits at week 48 to those for the early and late rabbits at weeks 6 and 24, respectively.

Acknowledgements
For assistance with various aspects of this research, we thank Matt Leevy and the Notre Dame Integrated Imaging Facility and the staff at Freimann Life Science Center, especially Mark Suckow, Kay Stewart, Vicki Mack, Valerie Schroeder, Brittany Pogotis and Ashley Sipocz. Erin Franks, Amy Remer and Khari Thompson provided helpful comments.

Competing interests
The authors declare no competing financial interests.

Author contributions
M.J.R. conceived and designed the experiment. J.E.S., K.R.M. and M.M.E. performed the experiment and acquired the data. J.E.S. analyzed the data. J.E.S. and M.J.R. interpreted the results and wrote the paper, with input from K.R.M. and M.M.E.

Funding
This work was supported by funding from the National Science Foundation [grant number BCS-1029149/1214767] to M.J.R.

References
Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science* **294**, 321-326.
Auld, J. R., Agrawal, A. A. and Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. Biol. Sci.* **277**, 503-511.
Bass, S., Pearce, G., Bradney, M., Hendrich, E., Delmas, P. D., Harding, A. and Seeman, E. (1998). Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. *J. Bone Miner. Res.* **13**, 500-507.
Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* **57**, 289-300.
Bernays, E. A. (1986). Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* **231**, 495-497.
Bertram, J. E. A. and Swartz, S. M. (1991). The 'law of bone transformation': a case of crying Wolff? *Biol. Rev. Camb. Philos. Soc.* **66**, 245-273.
Biewener, A. A. (1993). Safety factors in bone strength. *Calcif. Tissue Int.* **53 Suppl. 1**, S68-S74.
Biewener, A. A., Swartz, S. M. and Bertram, J. E. A. (1986). Bone modeling during growth: dynamic strain equilibrium in the chick tibiotarsus. *Calcif. Tissue Int.* **39**, 390-395.
Bouvier, M. (1988). Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. *J. Dent. Res.* **67**, 1206-1212.
Bouvier, M. and Hylander, W. L. (1981). Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). *J. Morphol.* **167**, 1-12.
Bouvier, M. and Hylander, W. L. (1984). The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. *Am. J. Anat.* **170**, 117-126.
Burr, D. B. (1997). Muscle strength, bone mass, and age-related bone loss. *J. Bone Miner. Res.* **12**, 1547-1551.
Congdon, K. A., Hammond, A. S. and Ravosa, M. J. (2012). Differential limb loading in miniature pigs (*Sus scrofa domesticus*): a test of chondral modeling theory. *J. Exp. Biol.* **215**, 1472-1483.
de Jong, G. and Crozier, R. H. (2003). A flexible theory of evolution. *Nature* **424**, 16-17.
DeWitt, T. J., Sih, A. and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77-81.
Dufty, A. M., Clobert, J. and Møller, A. P. (2002). Hormones, developmental plasticity and adaptation. *Trends Ecol. Evol.* **17**, 190-196.
Efron, B. and Tibshirani, R. J. (1993). *An Introduction to the Bootstrap*. New York, NY: Chapman & Hall.
Engelke, K., Kemmler, W., Lauber, D., Beeskow, C., Pintag, R. and Kalender, W. A. (2006). Exercise maintains bone density at spine and hip EFOPS: a 3-year longitudinal study in early postmenopausal women. *Osteoporos. Int.* **17**, 133-142.
Fischer, B., van Doorn, G. S., Dieckmann, U. and Taborsky, B. (2014). The evolution of age-dependent plasticity. *Am. Nat.* **183**, 108-125.
Futuyma, D. J. (2011). Expand or revise? The evolutionary synthesis today. *Q. Rev. Biol.* **86**, 203-208.
Gabriel, W. (1999). Evolution of reversible plastic responses: inducible defenses and environmental tolerance. In *The Ecology and Evolution of Inducible Defenses* (ed. R. Tollrian and D. Harvell), pp. 286-305. Princeton, NJ: Princeton University Press.
Gomez-Mestre, I. and Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proc. Natl. Acad. Sci. USA* **103**, 19021-19026.
Gotthard, K. and Nylin, S. (1995). Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**, 3-17.
Hamrick, M. W., Skedros, J. G., Pennington, C. and McNeil, P. L. (2006). Increased osteogenic response to exercise in metaphyseal versus diaphyseal cortical bone. *J. Musculoskelet. Neuronal Interact.* **6**, 258-263.
Herring, S. W. (1985). The ontogeny of mammalian mastication. *Am. Zool.* **25**, 339-349.
Hinton, R. J. and McNamara, J. A., Jr (1984). Effect of age on the adaptive response of the adult temporomandibular joint. A study of induced protrusion in *Macaca mulatta*. *Angle Orthod.* **54**, 154-162.
Hirano, T., Burr, D. B., Cain, R. L. and Hock, J. M. (2000). Changes in geometry and cortical porosity in adult, ovary-intact rabbits after 5 months treatment with LY333334 (hPTH 1-34). *Calcif. Tissue Int.* **66**, 456-460.
Holmes, M. A. and Ruff, C. B. (2011). Dietary effects on development of the human mandibular corpus. *Am. J. Phys. Anthropol.* **145**, 615-628.
Hoverman, J. T. and Relyea, R. A. (2007). How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology* **88**, 693-705.
Hsieh, Y. F., Robling, A. G., Ambrosius, W. T., Burr, D. B. and Turner, C. H. (2001). Mechanical loading of diaphyseal bone in vivo: the strain threshold for an osteogenic response varies with location. *J. Bone Miner. Res.* **16**, 2291-2297.
Isaksson, H., Harjula, T., Koistinen, A., Iivarinen, J., Seppänen, K., Arokoski, J. P. A., Brama, P. A., Jurvelin, J. S. and Helminen, H. J. (2010). Collagen and mineral deposition in rabbit cortical bone during maturation and growth: effects on tissue properties. *J. Orthop. Res.* **28**, 1626-1633.
Kannus, P., Haapasalo, H., Sankelo, M., Sievänen, H., Pasanen, M., Heinonen, A., Oja, P. and Vuori, I. (1995). Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. *Ann. Intern. Med.* **123**, 27-31.
Kontulainen, S., Sievänen, H., Kannus, P., Pasanen, M. and Vuori, I. (2003). Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female racquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. *J. Bone Miner. Res.* **18**, 352-359.
Kotrschal, A. and Taborsky, B. (2010). Environmental change enhances cognitive abilities in fish. *PLoS Biol.* **8**, e1000351.

Langenbach, G. E. J. and van Eijden, T. M. G. J. (2001). Mammalian feeding motor patterns. *Am. Zool.* **41**, 1338-1351.
Lanyon, L. E. and Rubin, C. T. (1985). Functional adaptation in skeletal structures. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 1-25. Cambridge: Harvard University Press.
Lanyon, L. and Skerry, T. (2001). Postmenopausal osteoporosis as a failure of bone's adaptation to functional loading: a hypothesis. *J. Bone Miner. Res.* **16**, 1937-1947.
Lieberman, D. E., Devlin, M. J. and Pearson, O. M. (2001). Articular area responses to mechanical loading: effects of exercise, age, and skeletal location. *Am. J. Phys. Anthropol.* **116**, 266-277.
Lieberman, D. E., Pearson, O. M., Polk, J. D., Demes, B. and Crompton, A. W. (2003). Optimization of bone growth and remodeling in response to loading in tapered mammalian limbs. *J. Exp. Biol.* **206**, 3125-3138.
Manly, B. F. J. (1997). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Boca Raton, FL: Chapman & Hall/CRC.
Marchinko, K. B. (2003). Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): magnitude, age dependence, and speed of response. *Evolution* **57**, 1281-1290.
Marshall, J. and Wrangham, R. W. (2007). Evolutionary consequences of fallback foods. *Int. J. Primatol.* **28**, 1219-1235.
Masoud, I., Shapiro, F., Kent, R. and Moses, A. (1986). A longitudinal study of the growth of the New Zealand white rabbit: cumulative and biweekly incremental growth rates for body length, body weight, femoral length, and tibial length. *J. Orthop. Res.* **4**, 221-231.
Menegaz, R. A., Sublett, S. V., Figueroa, S. D., Hoffman, T. J. and Ravosa, M. J. (2009). Phenotypic plasticity and function of the hard palate in growing rabbits. *Anat. Rec.* **292**, 277-284.
Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* **41**, 1357-1369.
Muschick, M., Barluenga, M., Salzburger, W. and Meyer, A. (2011). Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol. Biol.* **11**, 116.
Orr, H. A. (1999). An evolutionary dead end? *Science* **285**, 343-344.
Palmer, A. R. (2004). Symmetry breaking and the evolution of development. *Science* **306**, 828-833.
Pearson, O. M. and Lieberman, D. E. (2004). The aging of Wolff's "law": ontogeny and responses to mechanical loading in cortical bone. *Am. J. Phys. Anthropol.* **125 Suppl. 39**, 63-99.
Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D. and Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* **25**, 459-467.
Pigliucci, M., Murren, C. J. and Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* **209**, 2362-2367.
Pigliucci, M. (1996). How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). *Trends Ecol. Evol.* **11**, 168-173.
Rajakumar, R., San Mauro, D., Dijkstra, M. B., Huang, M. H., Wheeler, D. E., Hiou-Tim, F., Khila, A., Cournoyea, M. and Abouheif, E. (2012). Ancestral developmental potential facilitates parallel evolution in ants. *Science* **335**, 79-82.
Ravosa, M. J., Kunwar, R., Stock, S. R. and Stack, M. S. (2007). Pushing the limit: masticatory stress and adaptive plasticity in mammalian craniomandibular joints. *J. Exp. Biol.* **210**, 628-641.
Ravosa, M. J., López, E. K., Menegaz, R. A., Stock, S. R., Stack, M. S. and Hamrick, M. W. (2008). Adaptive plasticity in the mammalian masticatory complex: you are what, and how, you eat. In *Primate Craniofacial Function and Biology* (ed. C. J. Vinyard, M. J. Ravosa and C. E. Wall), pp. 293-328. New York, NY: Springer.
Ravosa, M. J., Ning, J., Costley, D. B., Daniel, A. N., Stock, S. R. and Stack, M. S. (2010a). Masticatory biomechanics and masseter fiber-type plasticity. *J. Musculoskelet. Neuronal Interact.* **10**, 46-55.
Ravosa, M. J., Ross, C. F., Williams, S. H. and Costley, D. B. (2010b). Allometry of masticatory loading parameters in mammals. *Anat. Rec.* **293**, 557-571.
Relyea, R. A. (2003). Predators come and predators go: the reversibility of predator-induced traits. *Ecology* **84**, 1840-1848.
Rubin, C. T., Bain, S. D. and McLeod, K. J. (1992). Suppression of the osteogenic response in the aging skeleton. *Calcif. Tissue Int.* **50**, 306-313.
Ruff, C. B. and Runestad, J. A. (1992). Primate limb bone structural adaptations. *Annu. Rev. Anthropol.* **21**, 407-433.
Ruff, C., Holt, B. and Trinkaus, E. (2006). Who's afraid of the big bad Wolff?: "Wolff's law" and bone functional adaptation. *Am. J. Phys. Anthropol.* **129**, 484-498.
Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35-68.
Schlichting, C. D. and Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, MA: Sinauer Associates.
Schwander, T. and Leimar, O. (2011). Genes as leaders and followers in evolution. *Trends Ecol. Evol.* **26**, 143-151.
Scott, J. E., McAbee, K. R., Eastman, M. M. and Ravosa, M. J. (2014). Experimental perspective on fallback foods and dietary adaptations in early hominins. *Biol. Lett.* **10**, 20130789.
Scoville, A. G. and Pfrender, M. E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proc. Natl. Acad. Sci. USA* **107**, 4260-4263.
Serrat, M. A. (2013). Allen's rule revisited: temperature influences bone elongation during a critical period of postnatal development. *Anat. Rec.* **296**, 1534-1545.
Standen, E. M., Du, T. Y. and Larsson, H. C. E. (2014). Developmental plasticity and the origin of tetrapods. *Nature* **513**, 54-58.
Suzuki, Y. and Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science* **311**, 650-652.
Svanbäck, R. and Schluter, D. (2012). Niche specialization influences adaptive phenotypic plasticity in the threespine stickleback. *Am. Nat.* **180**, 50-59.
Taborsky, B. (2006). The influence of juvenile and adult environments on life-history trajectories. *Proc. Biol. Sci.* **273**, 741-750.
Via, S. and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505-522.
Weijs, W. A. and de Jongh, H. J. (1977). Strain in mandibular alveolar bone during mastication in the rabbit. *Arch. Oral Biol.* **22**, 667-675.
Weijs, W. A., Brugman, P. and Grimbergen, C. A. (1989). Jaw movements and muscle activity during mastication in growing rabbits. *Anat. Rec.* **224**, 407-416.
West-Eberhard, M. J. (2005). Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. USA* **102 Suppl. 1**, 6543-6549.
Westerlind, K. C., Wronski, T. J., Ritman, E. L., Luo, Z.-P., An, K.-N., Bell, N. H. and Turner, R. T. (1997). Estrogen regulates the rate of bone turnover but bone balance in ovariectomized rats is modulated by prevailing mechanical strain. *Proc. Natl. Acad. Sci. USA* **94**, 4199-4204.