

Smaller, Weaker and Less Stiff Bones Evolve from Changes in Subsistence Strategy

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Mini Abstract

We propose a computational model with which to examine the evolution of bone. Our results indicate that changes in subsistence strategy have influenced the evolution of bone growth and mechanoregulation, and predict that bone size, stiffness and structural strength may decrease in future generations, bringing increased risk of fracture and prevalence of osteoporosis.

Conflict of Interest

All authors have no conflicts of interest and no disclosures.

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Abstract

Purpose

Archaeological data suggest that bone size and strength have decreased over evolution. We hypothesize that changing evolutionary pressures and levels of physical activity, both arising from changes in subsistence strategy, have affected the evolution of bone. We propose a computational model with which to examine the evolution of bone growth and mechanoregulation due to the transitions from hunter-gatherer to agricultural to modern lifestyles.

Methods

The evolution of genes governing growth and mechano-regulation in a population of bones is simulated, where each individual is represented by a 2-D bone cross-section. Genetic variability is assumed to modulate growth through mechanoregulatory factors that direct periosteal expansion, endosteal expansion/infilling, and ash content accretion in response to strains incurred during walking.

Results

The model predicts decreases in cortical area and section modulus (a measure of structural strength) and increases in maximum compressive strain over the course of the simulation, meaning evolution of smaller, less strong, and less stiff bones is predicted for the population average. The model predicts small but continued decreases in size, strength, and stiffness in modern populations, despite the absence of a strong evolutionary advantage to efficient bones during this phase.

Conclusion

In conclusion, our results show that changing loading regimes and evolutionary pressures may have influenced the evolution of bone growth and mechanoregulation, and predict that bone size and strength may continue to decrease in future generations, bringing increased risk of fracture and prevalence of osteoporosis.

Keywords: Bone adaptation, Evolution simulation, Hunter-gatherer, Physical activity, Bone fragility

Introduction

During the evolution of the *Homo* genus, there have been dramatic changes in the habitual loading patterns applied to bones; compare the physical lifestyle of the hunter-gatherer populations to the sedentary lifestyle many people lead today. Archaeological data suggest that bone size and strength may have decreased over evolution (1), and it is possible that changes in lifestyle, such as the switch from hunter-gatherer to agricultural lifestyles, are responsible (2). It has been proposed that evolutionary processes towards reduced bone weight have led to a system operating near the limits of safety, leading to skeletal fragility in some individuals (3), and a continued trend towards bone slenderness will lead to an increased risk of fracture (4, 5). Increasing levels of physical activity through exercise can reduce fracture risk in males (6), but decreasing levels of physical activity in today's developed countries may affect the genes governing the maintenance of healthy bone (7). Despite the physical evidence of bone evolution, we do not know how natural selection has acted upon the genes affecting our bones. Moreover, it remains to be seen how our bones will evolve in today's environment in which the size or strength of one's bones are not often critical to survival.

A number of studies have shown how changes in habitual loading regimes have affected bone size and shape. Stock and Pfeiffer (8) compared the bones of highly mobile foragers from the African Late Stone Age (ca 10,000 to 2,000 BP) with 19th century Andaman Islanders, a population accustomed to swimming and canoeing, and found that the latter population had greater size and section moduli in the upper limb, while the Later Stone Age had more robust bones of the lower limb. Ruff and colleagues (2) compared the structural characteristics of femora of pre-agricultural and agricultural groups, and demonstrated significant temporal decreases in almost every geometrical property such as cortical, marrow and total areas, and moments of inertia. Aside from the ontogenetic effects of mechanical loading, it is likely that changes in the mechanical environment have also affected the phylogeny of the genes influencing the growth and adaptation of bone. Ruff (1) showed how femoral mid-shaft cortical area and section modulus have decreased exponentially between the past 5,000 to 1.9 million years. Data from modern humans (within the past century) indicate that the relative cortical area and section moduli may have decreased even faster in the past 5,000 years than over the

previous 2 million years (9). However, with such archaeological studies, it is difficult, if not impossible, to examine many of the properties of the living bone, such as the adaptive response of the bone to low or high mechanical loads. Due to the small specimen numbers usually involved in archaeological studies, conclusions can be made only on the group or population average, and it is not possible to quantify the amount of variation in bone size present in a particular group, or to examine how the amount of variation in a population may evolve over time. These issues can be investigated using computational models. For example, Nowlan and Prendergast (10) simulated the evolution of mechanoregulation of bone by combining equations describing long bone growth with a genetic algorithm. The authors found that no population evolved a mechanoregulatory response that would lead to the ‘optimum’ bone. Their results also indicated that it is possible that a range of parameters governing mechanoregulation will persist in a population over many generations. Currey et al. (11) used a mathematical model to demonstrate how the level of uncertainty in the variables contributing to the tissue properties of bone may affect the evolution of an optimum balance between stiffness and toughness, and proposed that stochastic rather than deterministic values for stress and growth should be incorporated into mathematical models of bone evolution.

In this paper, we propose a computational model with which to examine the evolution of genes governing bone growth and mechanoregulation. A population of femoral bones is grown, where each bone is grown from a post-natal diaphyseal collar to maturity and growth is directed by heritable gene sets which determine baseline growth rates and the mechanobiological response of the periosteal and endosteal surfaces and of ash content accretion. Through changes in selection criteria, which convey an evolutionary advantage on the most fit individuals, and changes in loading regimes, we examine the effect of different subsistence strategies, from hunter-gatherer to agricultural to sedentary lifestyles, on the evolution of the size, structure and stiffness of bones. We hypothesize that decreasing levels of physical activity and changing selection pressures over evolution have affected the genes regulating bone. We also use the model to predict if and how the geometry and composition of bone may evolve in the future if a sedentary lifestyle continues to be the norm, and examine how increasing or decreasing modern levels of physical activity could affect bone evolution in the future.

Methods

The evolution of a population of 1,000 bones over two thousand generations, or 40,000 years, is simulated. For each generation, a population of femoral bones is grown, where growth of each individual bone is simulated from a post-natal diaphyseal collar to maturity at 20 years of age. Each individual is represented by a 2-D circular cross-section which approximates a section at the mid-diaphysis of the femur. Growth is directed by four diploid gene sets, each of which determine a genetic variable; the baseline growth rate (g), and the mechanobiological responses of the periosteum (p), the endosteum (e), and of ash content accretion (ac). At maturity, bones are assessed for fitness (viability), and from the set of functional (viable) individuals, a new generation is formed using genes selected from the functional set. For each 'mating', four monoploid germs (one for each variable, g , p , e and ac) are obtained from each parent, where a gene is randomly selected from each locus. The monoploid germs are combined to create four diploid chromosomes for the child. Mutations occur at a frequency of 1 in 100,000 for each locus in the chromosome (12), where the magnitude of the gene to be mutated is randomly doubled or halved, with equal probability assigned to either type of mutation as described by Nowlan and Prendergast, 2005 (10). When a new individual is created from the genes of two parents, one or more of their genes may be mutated before growth begins. A

flowchart of the steps involved in the simulation is given in

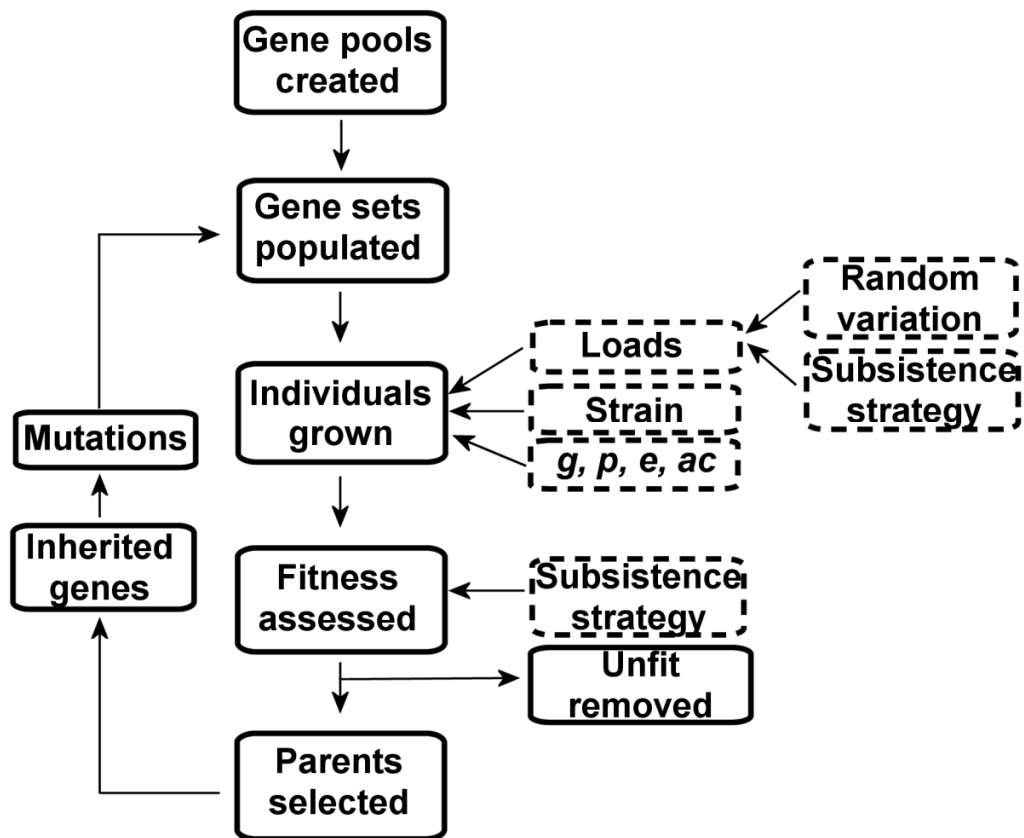


Figure 1.

Gene Pool Creation and Population Initialization

The minimum and maximum values for each genetic variable (g , p , e and ac) are specified so that a gene pool can be created for that variable (Table 1). Each gene pool contains 1000 numbers (genes), where the gene pool is populated by random numbers between the minimum and maximum bounds specified for the relevant variable, as specified in Table 1. Each gene set contains five loci, with two genes at each locus, as used previously by Nowlan and Prendergast (10) and as illustrated in Figure 2. At the start of the simulation, the population is initialized when for every individual, four diploid gene sets, one for each variable, g , p , e , and ac , are populated with genes from the relevant gene pool (10). Once the simulation is underway, each new generation is initialized with genes inherited from the parent generation rather than from the initial gene pool.

Subsistence Strategy

Three subsistence strategies (lifestyles) are considered in the simulation, which affect both the loading regime and the assessment of fitness, both of which are described in more detail below. With a hunter-gatherer lifestyle, it was critical to minimize bone mass (for faster movement and more efficient ambulation) while minimizing the risk of bone failure (13). Therefore, individuals with lighter bones and lower functional strains were more likely to survive and pass on their genes. During this phase in the model, which lasts for 1,000 generations, applied loads are considered to be higher than agricultural and modern loads, and the heaviest and least stiff bones in each generation are eliminated. Agriculture, a subsistence strategy still widely in use today, is thought to have become widespread about 10,000 years ago (14). With this lifestyle, levels of physical activity are likely to be lower (2), and resistance to failure not as critical to survival in comparison to a hunter-gatherer lifestyle. However, minimizing mass is still likely to be advantageous with an agricultural lifestyle, due to the intermittent scarcity of nutritional resources (15). During the agricultural phase (500 generations), applied loads are higher than modern walking loads, and the individuals with the heaviest bones are the most likely to be eliminated through natural selection. Many people today are engaged in little or no daily physical activity, which has negative effects on many aspects of health (7). In developed countries, as long as a bone is functional, it is unlikely that the fitness of one's bones will convey any evolutionary advantage. During this 'modern phase' of the model (500 generations), walking loads (16) are applied, and light or stiff bones convey no evolutionary advantage. The emergence of the modern lifestyle is very recent in evolutionary terms, and we consider that the current state of a developed country in the model is roughly 10 generations into the modern phase. This means that the final stage of the model is predicting what may happen to bones in the future in an environment with continuing low levels of physical activity and minimal selection on bones.

Loading Regime

Loads active at the mid-shaft of an adult femur were taken from measurements of the axial force, torque and anterior-posterior bending moment in a femoral prosthesis

shaft during walking activity, one year after implantation of the prosthesis by Taylor and Walker (16), as detailed in Table 2 and as illustrated in Figure 3. During each timestep during growth, loads are scaled to loads at maturity according to the body mass at that timepoint. Body mass is related to age using data from McCammon (17), where body mass (in kg) at a given timepoint is described by Eq. (1)

$$body\ mass = -1.03e^{-7}x^4 + 4.63e^{-5}x^3 - 5.94e^{-3}x^2 + 0.46x + 4.44 \quad (1)$$

where x is the age in months. At each timepoint, the loads to be applied are scaled according to the proportion of the current body mass of the body mass at maturity, which is set at 70kg (17).

The loads applied during the lifetime of each individual depend on the type of physical activity engaged in during the particular stage of evolution. During the hunter-gatherer phase, the high intensity and prolonged duration of habitual physical activity was represented by increasing the loads during growth to 120% of modern walking loads (as detailed in Table 2). During the agricultural phase, it was considered that the daily physical loading regime would be intermediate to those of the hunter-gatherer and modern phases, and therefore the loads during growth were set at 110% of modern walking loads. The loads described in Table 2 are used in the modern phase. An element of random variation is included to represent non-genetic environmental influences on growth, such as nutrition and sunlight. The variation is implemented as an increase or decrease of up to 10% in applied loads throughout growth, as implemented previously (10), which affects the growth of the radii and the ash content adaptation. A random number between 0.9 and 1.1 is assigned to each individual, and this number is then applied as a scaling factor to the applied loads through the growth of the individual. The final applied loads at each timepoint are described by Eq. (2)

$$applied\ load = variation * load * \frac{body\ mass}{70} \quad (2)$$

where *applied load* is the axial load, torque or bending moment applied at a particular timestep, *variation* is the random number between 0.9 and 1.1 assigned to the individual, *load* is the axial load, torque or bending moment at maturity for a particular subsistence strategy as defined in Table 3, and *body mass* is the mass at that timepoint as calculated from Eq. (1).

Bone Growth and Adaptation

Calculation of Strain

At each time step, the growth of an individual's bone depends on the values of their genetic variables, and the strain present in the bone due to applied loads. The maximum principal strain is calculated at the outer circumference of the circular cross-section using the plane strain assumption as detailed in Eq. (3).

$$\varepsilon_{max,prin} = \frac{\varepsilon_{axial} + \varepsilon_{bending}}{2} + \sqrt{\left(\frac{\varepsilon_{axial} + \varepsilon_{bending}}{2}\right)^2 + \left(\frac{\varepsilon_{shear}}{2}\right)^2} \quad (3)$$

In order to calculate the axial strain, the Young's modulus of the bone is necessary. At each timepoint, the Young's modulus is calculated based upon a relationship between Young's modulus and ash content derived from Courtland et al. (18):

$$E = 1.0706 * \%ash - 54.518 \quad (4)$$

where E is in GPa. For calculating the shear strain, a value of 3.5GPa was used for shear modulus (19), and no dependence of the shear modulus on the ash content was implemented in the model (20).

During growth, bones can adapt their size and ash content in the presence of high or low strains. An upper limit on the 'lazy zone' (21, 22) was set at 1500 μ strain (23), and the lower limit of the lazy zone at 1000 μ strain (24). At any given timestep, if the calculated maximum principal strain in the bone is outside the lazy zone (i.e., greater than the upper limit, or lower than the lower limit), adaptation of the bone will occur in an attempt to bring the strain levels back within the lazy zone by modifying the radii and/or ash content, as detailed below.

Baseline Apposition Rates (g)

Bones are initialised as a post-natal collar with periosteal radius 3.65 mm and endosteal radius 2.0 mm (17, 25). Subperiosteal growth is simulated by expanding the outer radius following the growth curve for the outer diameter of the male femur reported by McCammon (17). This is implemented as an age-dependent monthly periosteal apposition rate ($baseline_{R_P}$) in metres/month, as shown in Figure 4, and as given by a polynomial equation, Eq. (5)

$$baseline_{R_P} = -4.5e^{-8} x^3 + 1.7e^{-5} x^2 - 2.1e^{-3} x + 0.11 \quad (5)$$

where x is the age in months. We were unable to find detailed data on the growth of the femoral endosteum, but as Garn (25) has described subperiosteal and endosteal growth for the second metacarpal, it was assumed that the relationship between subperiosteal and endosteal expansion in the second metacarpal would be the same as in the femur. By combining data from studies by McCammon (17) and Garn (25), the growth of the male femoral endosteal surface was derived. From this data, an average monthly endosteal expansion rate was calculated ($baseline_{R_E}$, (metres/month)), which can be positive or negative, as shown in Figure 4, and as given by Eq. (6)

$$baseline_{R_E} = -2.3e^{-8} x^3 + 8.5e^{-6} x^2 - 9.7e^{-4} x + 4.2e^{-2} \quad (6)$$

where x is the age in months. Apposition at the periosteal and endosteal surfaces is mediated by a genetically controlled, dimensionless factor, g . For each individual, the value of the growth parameter g is calculated as the sum of the average of the two genes at each locus in the relevant chromosome, as illustrated for another genetic variable p in Figure 2. At the start of the simulation, each bone is assigned a gene set that will yield a value of g between 0.8 and 1.2, which scales the apposition rates of the bone between 80% and 120% of the average rate, giving the individual's baseline apposition rate. At each time step, an individual's periosteal and endosteal growth rates were calculated as defined in Eq. (7) and (8)

$$growth_{R_P} = g * baseline_{R_P} \quad (7)$$

$$growth_{R_E} = g * baseline_{R_E} \quad (8)$$

where $baseline_{R_P}$ is calculated from Eq. 5 and $baseline_{R_E}$ is calculated from Eq. 6.

Mechanoregulation of periosteum & endosteum (p, e)

The growth of the periosteal and endosteal radii are also dependent on genetically determined, dimensionless mechanoregulatory factors, p and e . Depending on the strain level experienced by the bone, the p and e mechanoregulatory factors mediate expansion or contraction of the radii. If the strain is greater than the upper limit of the lazy zone (1500 μ strain), a bone will increase its outer radius by $(1 + p)$ multiplied by the individual's baseline apposition rate. If the strain in the bone is within the lazy zone, the periosteum is increased by the individual's baseline apposition rate, while if the strain is less than the lower limit of the lazy zone, the periosteal diameter is not increased for that

timepoint. The equation for apposition at the periosteum (Δr_p) at a specific timepoint is therefore given by Eq. (9–11)

$$\text{if } \varepsilon \geq \varepsilon_{upper\ limit} \text{ then } \Delta r_p = (1 + p) * growth_{R_P} \quad (9)$$

$$\text{if } \varepsilon_{lower\ limit} \leq \varepsilon \leq \varepsilon_{upper\ limit} \text{ then } \Delta r_p = growth_{R_P} \quad (10)$$

$$\text{if } \varepsilon \leq \varepsilon_{lower\ limit} \text{ then } \Delta r_p = 0 \quad (11)$$

where ε is the maximum principal strain experienced at that timepoint, $\varepsilon_{upper\ limit}$ is 1500 μ strain, $\varepsilon_{lower\ limit}$ is 1000 μ strain, p is the mechanoregulatory factor governing adaptation of the periosteum, and $growth_{R_P}$ is calculated from Eq. 7.

A mechanobiological influence on the endosteum is also included, controlled by the genetically determined factor e , which modulates the influence of an individual's baseline apposition rate on the endosteal radius depending on the strains acting on the bone, as illustrated in Figure 5. Within the lazy zone, the endosteal radius changes by the baseline endosteal growth rate, which may be positive or negative (Eq. 11). If the baseline endosteal adaptation rate is positive (endosteum is expanding), and the calculated strain is greater than the upper limit of the lazy zone, the endosteal adaptation factor e has leads to reduced expansion of the endosteal radius, while if strains are lower than the lower limit, increased expansion of the endosteum will occur. In the presence of a positive endosteal baseline rate, the change in the endosteal radius (Δr_e) at a specific timepoint is determined by Eq. (12–14)

$$\text{if } \varepsilon \geq \varepsilon_{upper\ limit} \text{ then } \Delta r_e = (1 - e) * growth_{R_E} \quad (12)$$

$$\text{if } \varepsilon_{lower\ limit} \leq \varepsilon \leq \varepsilon_{upper\ limit} \text{ then } \Delta r_e = growth_{R_E} \quad (13)$$

$$\text{if } \varepsilon \leq \varepsilon_{lower\ limit} \text{ then } \Delta r_e = (1 + e) * growth_{R_E} \quad (14)$$

where $\varepsilon_{upper\ limit}$ is 1500 μ strain and $\varepsilon_{lower\ limit}$ is 1000 μ strain as defined above, e is the mechanoregulatory factor governing adaptation of the periosteum, and $growth_{R_E}$ is calculated from Eq. 8. If the baseline endosteal rate is negative (endosteum is infilling), the endosteal radius will decrease by a greater amount when strains are high and by a smaller amount when strains are low, as detailed in Eq. (15) and (16)

$$\text{if } \varepsilon \geq \varepsilon_{upper\ limit} \text{ then } \Delta r_e = (1 + e) * growth_{R_E} \quad (15)$$

$$\text{if } \varepsilon \leq \varepsilon_{lower\ limit} \text{ then } \Delta r_e = (1 - e) * growth_{R_E} . \quad (16)$$

The p and e values are calculated by summing the average of the two genes at each locus in the relevant chromosomes, as shown for p in Figure 2. At the start of the simulation, each bone is assigned gene sets that will yield p and e values between 0.0 and 0.4.

Mechanoregulation of ash content (ac)

Each bone is assigned an initial ash content value of 55% to represent the ash content at birth, a value that was extrapolated from data on the ash content of children's bones from Currey & Butler (26). A baseline age-dependent monthly accretion of ash content (in % increase/month) was calculated from the same data (26) and is described the exponentially decaying function in Eq. (17)

$$baseline_{ash\ content} = 1.52 * x^{-1.02} \quad (17)$$

where x is the age in months. A fourth genetically determined dimensionless variable, ac , modulates the adaptation of the ash content based on the strain levels during growth. Between the lower and upper bounds of the lazy zone, the ash content is increased by the baseline rate. At strains greater than the upper limit, the ash content is increased by $(1+ac)$ times the baseline rate, while at strains below the lower limit of the lazy zone, the ash content is increased by $(1-ac)$ times the baseline rate. Therefore, a decrease in ash content will only occur if strain levels for an individual were less than the lower limit of the lazy zone, and if that individual had an ash content adaptation factor of greater than 1.0. Initial ash content adaptation factors at the start of the simulation are set at between 0.0 and 0.4, as detailed in Table 1. The percentage increase in ash content (Δa_c) is therefore described by Eq. (16–18)

$$if\ \varepsilon \geq \varepsilon_{upper\ limit}\ then\ \Delta a_c \% = (1 + ac) * baseline_{ash\ content} \quad (18)$$

$$if\ \varepsilon_{lower\ limit} \leq \varepsilon \leq \varepsilon_{upper\ limit}\ then\ \Delta a_c \% = baseline_{ash\ content} \quad (19)$$

$$if\ \varepsilon \leq \varepsilon_{lower\ limit}\ then\ \Delta a_c \% = (1 - ac) * baseline_{ash\ content} \quad (20)$$

where ac is the mechanoregulatory factor governing adaptation of the ash content, and $baseline_{ash\ content}$ is calculated from Eq. (17). At each timestep, the ash content is updated across the whole bone cross section. The value of the ac parameter for each individual is calculated as the sum of the average of the two genes at each locus, as shown for another genetic variable p in Figure 2.

Assessment of Fitness

At maturity (age 20), growth is stopped and each individual is assessed for fitness. During all phases of the model, bones must be functional, i.e., capable of supporting weight and locomotion. The criterion selected for functionality was that the maximum principal strain active in the bone under walking loads at maturity must be equal to or below the upper limit of the lazy zone, set at 1500 μ strain. This threshold was set because if an adult bone is under high strain during low intensity physical activity, the bone is likely to fail under high intensity physical activity such as hill running or jumping. It is considered likely that during the hunter-gatherer and agricultural phases, individuals with lighter bones and lower strains were more likely to survive. A lighter bone is metabolically cheaper to maintain than a heavier one, and a bone with low strain levels is less likely to fail than a bone with high strain levels. Therefore, during the hunter-gatherer and agricultural phases of the model, an evolutionary disadvantage is attributed to those with heavy bones with high strain levels where individuals which fall within the 'least fit' 0.5% of the population are not considered for reproduction. The other 99.5% of the population have an equal likelihood of passing on their genes to the next generation.

The calculation of the fitness of individuals is based on a ranking system, where each individual receives a rank for their strain and another for their mass relative to the rest of the population. Strain and mass are equally important to fitness during the hunter-gatherer phase. The scores are calculated on an exponential scale and then summed, meaning that an individual with the median strain and median mass will be more likely to be considered for selection than an individual with the lowest strain and the highest mass. The practical implementation involves ranking the population by mass (lowest first) and assigning a score to each individual which is the exponential of their rank in the population, then re-ordering according to strain (again, lowest first), and augmenting the previous score by the exponential of their current rank. The lower the final score, the more fit the bone is. During the agricultural phase, evolutionary pressure is assumed to act on both strain and mass, with a preferential pressure on minimizing mass due to the scarcity of resources common with this subsistence strategy (15). During this phase, the individuals are ranked by mass and then by strain, but with the importance of the strain ranking being less important than the mass ranking. This is done by scaling the

magnitude of the rank when ordered for strain by a factor of 0.5. The equation for the score for a hunter-gatherer individual is detailed in Eq. (21) and that for an individual during the agricultural phase in Eq. (22)

$$score_{hunter-gatherer} = e^{rank, mass} + e^{rank, strain} \quad (21)$$

$$score_{agriculture} = e^{rank, mass} + e^{0.5 * rank, strain} \quad (22)$$

where *rank, mass* and *rank, strain* are measures of how low the individual's mass and strain are in comparison to the rest of the population. In developed countries, availability of food and access to medical facilities mean that light or low strain bones are unlikely to give any evolutionary advantage for passing on genes to the next generation. Therefore, during the modern phase, no evolutionary pressure is enforced, apart from the necessity of a bone to be functional (as detailed above).

In order to calculate the mass of each bone, a relationship between ash content and tissue density was derived from data from Spatz et al., 1996 (20) and Tommasini et al., 2008 (27), for a range of bones and species. The density of the bone tissue in kg/m³ is calculated for each individual bone at maturity as described in Eq. (23)

$$density = 56.12 * (\%ash\ content) - 1874 \quad (23)$$

where the *%ash content* is that present in the bone at maturity. The mass of the bone tissue is calculated as density multiplied by cortical area and length, where a standard length of 0.4873m, from McCammon (17) is assumed for every individual. The mass of the marrow area is added to the mass of the bone tissue, giving the total mass. The density of the marrow tissue (930 kg/m³) is taken from Currey & Alexander, 1985 (13).

Outcome measures are also calculated for each population at maturity to chart the evolution of bone over the course of the simulation. The polar section modulus, a measure of structural strength, and maximum compressive strain, an indicator of stiffness, are calculated for each bone. In order to compare the stiffness of the bones (taken as the reciprocal of the maximum compressive strain) between generations, independently of the varying loads applied, the maximum compressive strain under a constant load is also calculated for each bone. The values of the *g*, *p*, *e* and *ac* genetic variables present in the population are recorded, as is the cortical area and ash content of each individual.

Simulation Execution

Each simulation incorporated the three subsistence strategies/ lifestyles; hunter-gatherer, agricultural and modern using the phase-specific loading scheme and selection criteria detailed in Table 3. Simulations were run four times to examine the influences of the initial conditions (the initial gene pool) and the random processes within the course of the simulation (e.g., recombination events, random element of selection, mutations). Two different gene pools (gene pool A and gene pool B) were created in order to test if the results of the simulation were dependant on the initial gene pool, and simulations were run twice for each gene pool, to test if the results depend on the initial gene pool. Therefore, simulations were run twice for each of two gene pools, leading to four simulations in total. In order to hypothesize what might happen to today's population in the future, the effects of further decreasing, maintaining or increasing modern loads in the modern phase were examined. Applied loads were set at 90% (decreased), 100% (standard) or 110% (increased) of modern loads in distinct simulations taking the gene data at generation 1600 as a starting point.

As we cannot be sure how loading magnitudes and selection pressures have changed over time, alternative loading regimes and selection criteria were examined in further repetitions of the simulation. An alternative loading regime where loads were held constant throughout the hunter-gatherer, agricultural and modern phases ("constant loads") was examined. Two alternative selection criteria were also examined, one where fitness in the agricultural phase was kept the same as that of the hunter-gatherer phase ("equal selection") and another when fitness was based on low mass ("mass selection"). Combinations of the standard and alternative loading regimes and selection criteria were simulated, leading to a total number of 4 types of 'alternative' simulation (constant loads + standard selection; standard loads + equal selection; constant loads + equal selection; mass selection + standard loads), which were then compared to the 'standard' simulation (standard loads + standard selection, as detailed in Table 3). Each type of simulation was repeated twice for each of two gene pools, so therefore four times in total for each simulation type.

Results

Consequences of changing loads and selection pressures on bone evolution

The initial gene pool was found not to have any consistent effect on the outcome of the simulation, such as the range or convergence of a variable. The results from two populations with different initial conditions are shown in Figure 6, and the results presented are representative of the complete dataset unless specified otherwise. All populations decreased in average cortical area (by an average of 21% between generations 0 and 2000, Figure 6A) and section modulus (by an average of 28%, Figure 6B) over the course of the simulation. After the evolutionary pressures for mass and strain were removed during the modern phase, cortical areas continued to decrease slightly (by an average of 2% between generations 1600 and 2000, Figure 6A). Variability in the population tended to decrease after the end of the hunter-gatherer phase (Figure 6A). All populations showed an increase in maximum compressive strain (decrease in stiffness) of 30% on average over the course of the simulation (Figure 6C). However, in three out of four simulations, a relatively constant average strain was maintained during the hunter-gatherer stage, as shown for population A in Figure 6C. In the modern phase, all populations showed a small increase of between 1.5-3.5% in average strain levels by the end of the simulation (Figure 6C). During the hunter-gatherer phase, the average ash content increased by between 0.7-1.4% (Figure 6D), due to a concurrent average increase of 63% in the mechanoregulatory ash content adaptation factor between generations 0 and 1000 (Figure 6E). Populations evolved an average baseline growth rate close to one by the end of the simulations, and tended to converge to a single growth rate during the simulation, as shown in Figure 6F. All populations showed decreases in periosteal and endosteal mechano-adaptation over the course of the simulation (Figure 6G, H), with adaptation at the endosteum (average 0.16) evolving to be higher than at the periosteum (average 0.05) by the end of the simulation. Populations maintained a range of endosteal adaptation values, while periosteal adaptation rates tended to converge by the end of the simulation (Figure 6G, H).

Effect of increasing or decreasing loads at generation 1600

Decreasing the loads to 90% of modern loads at generation 1600 resulted in further decreases in cortical area (average -1.5%) and section modulus (average -2%) and

increases in maximum compressive strain (average 2.4%) in all four populations as compared to populations under standard loads (Figure 6A-C, panels). With decreased loads, three out of four populations evolved a lower ash content than in standard simulations (Figure 6D, panel), but the difference was small- a 0.1% decrease in ash content on average. The changes in size with decreased loads were mainly due to mechanoadaptation of the individuals in the population rather than due to further evolution of the genetic variables controlling growth (g , p and e ; Figure 6F-H, panels). However, with decreased loads, the ash content adaptation factor was lower by an average of 2% compared to populations with standard loads. Increasing the loads to 110% of modern loads had a small but favourable effect on bone size and material properties, with a slightly increased cortical area (0.05%) and section modulus (0.1%), and a small decrease in maximum compressive strain (-0.03%) compared to bones obtained with standard loads (Figure 6A-C, panels). The ash content of the populations with increased loads either increased or stayed the same as the populations with standard loads (Figure 6D, panel).

Comparison of alternative loading regimes and selection criteria

A number of alternative loading regimes and selection criteria options were examined: 1. constant loads, 2. equal selection for mass and strain, and 3. selection for mass alone. Once again, four iterations of each simulation type were run, with two different gene pools. For clarity, only one outcome from each simulation type is presented, and the results shown in Figure 7 are representative of the complete data set. Simulations in which ‘equal selection’ for strain and mass was applied, (no preferential selection for low mass over low strain in the agricultural phase), when standard loads were applied did not show dramatic differences from standard simulations. However, there was a trend towards lower strains in simulations with equal selection (Figure 7C), with an average decrease of 9% from strain levels in the standard simulations. Selecting for mass alone (‘mass selection’) led to lower ash content values (decreased by an average of 1.4%), as shown in Figure 7D, due to lower ash content adaptation factors (Figure 7E), which were, on average, 50% lower than values found in standard simulations. Strain levels were higher (and therefore stiffness levels lower) by an average

of 5% when bones were selected based on mass alone (Figure 7C), and this selection criterion also led to quicker convergence of bone size (cortical area) as shown by the diminishing standard deviations in Figure 7A. Applying constant loads throughout the simulation, instead of decreasing loads between changes in lifestyle, did not yield dramatically different results when standard evolutionary pressures were applied, as shown in Figure 7. However, a dramatic change in bone evolution was seen when loads were held constant (at modern loads) and equal selection for mass and strain was maintained throughout the first 1500 generations. Although slight decreases in cortical area and section modulus still occurred in these populations (roughly 5% on average over the course of the simulation, Figure 7A, B), all four populations maintained the same maximum compressive strain levels throughout the simulation (Figure 7C), in sharp contrast to all other simulation types. In these populations, growth rates tended to be greater than in other types of simulation (10% greater on average than in a standard simulation), and always evolved to numbers greater than 1 (Figure 7F). Finally, in these simulations, the average periosteal adaptation factor was maintained or increased from the initial value, in contrast to other simulations (Figure 7G).

Discussion

Our results predict that smaller, less stiff and less structurally strong bones have evolved due to changes from hunter-gatherer to agricultural to sedentary lifestyles. Regardless of how changes in lifestyle were implemented, whether by decreasing the loads over time, or by changing the selection criteria, or by combining these measures, the population average evolved towards decreased cortical areas and section moduli and increased maximum compressive strain. Changes in bone size and material properties occurred due to evolution of genes governing the age-dependent growth rate of bone, and genes governing the mechanosensitive adaptation of the periosteal and endosteal surfaces and of ash content accretion. The model predicts further decreases in size, stiffness and structural strength during the modern phase, even in the absence of strong evolutionary pressures on bones in this phase. Furthermore, if habitual loads on bone were to be reduced further (by 10%) in a modern population, bones may become even smaller and

weaker. As the average bone size decreases in the population, a greater proportion of individuals may exceed a 'fracture threshold' due to normal age-related bone loss.

The decreases in cortical area and section modulus predicted by our model correlate with those found by Ruff (1) in comparing early prehistoric *Homo* to modern human femora. Our results suggest that the transition from a hunter-gatherer to agricultural lifestyle is likely to have resulted in decreased bone size, mirroring the findings of Ruff and colleagues (2) who found decreases in cortical, marrow and total bone areas in the femora of agricultural groups compared to those of hunter-gatherer groups. The model predicts that more robust bones are maintained in populations engaged in high levels of physical activity, a result corroborated by archaeological data from Stock & Pfeiffer (8) who found more robust lower limb bones in highly mobile foragers from the African Late Stone Age and more robust forelimbs in 19th century Andaman Islanders, a population accustomed to swimming and canoeing (8). However, our model also suggests that changes in selection criteria between lifestyles, even in the absence of changes in levels of physical activity, will affect the evolution of bone size and material properties.

Many populations in this study maintained a range of values in the variables controlling adaptation, which corroborates the conclusion of Nowlan & Prendergast (10) that there is likely to be a range of values in the parameters governing mechanoregulation in a given population. In accordance with the recommendations of Currey and colleagues (11), stochastic representations of growth and loading parameters have been used in our model. In support of our implementation of distinct gene sets controlling growth and mechanoregulation of bone, data from inbred mouse strains (28) and human tibiae (27) have suggested that bones are able to coadapt their morphology and material properties to satisfy the demands of the loading environment. Quantitative trait loci (QTL) have been identified in mice that separately control morphological compensation and mineralization (29) similar to the genetic factors proposed in our model that control the adaptation of the periosteal and endosteal surfaces and of the ash content. Our model predicts an increase in average ash content values over the hunter-gatherer period, and either a level or decreasing trend over the agricultural and modern phases, but there is limited data on bone quality in prehistoric and historic populations to compare our findings with, perhaps

due to the potential alteration in the composition of the material during internment or fossilization (30). However, there is evidence to suggest that bone mineral content values may have evolved since the early Neolithic age (31) and since the middle ages (32).

The bone growth model used in the simulations is a simple 2-D cross-section, and so the simulation cannot predict changes in the distribution of bone about an axis, as reported by Trinkhaus et al., 1999 (33) and Ruff et al., 1984 (2). Despite the simplified cross-section, the model does corroborate other changes reported by osteo-archaeological data such as decreases in cortical and total areas and section moduli (1). Changes in loading regimes between lifestyles were represented by scaling modern walking loads, and the higher loads applied during the hunter-gatherer and agricultural phases in the model are intended to represent the higher intensity and increased duration of physical activity during these phases. While this may have been better represented by a so-called “stress-stimulus” (10, 34), we wanted to include physiologically realistic loads (for modern humans) and complex loading regimes that were specific to the femur. Although it is not possible to calculate how much higher the stress stimulus would have been in a hunter-gatherer population, we selected a 20% increase on modern loads as a reasonable representation of the more demanding physical lifestyle with this subsistence strategy, and we made the assumption that both the intensity and the duration of physical activity during the agricultural phase are intermediate to the hunter-gatherer and modern phases, and therefore applied a 10% increase to the loads during the agricultural phase. There is no gender distinction in the model, and therefore we did not represent sex-specific differences in growth rates (17), or investigate how sexual dimorphism may develop or evolve with changes in lifestyle. It has been proposed that sexual dimorphism in bone geometric properties decreased with the change from hunter-gatherer to agriculture due to a more equal division of physical activities post hunter-gatherer (35). As our model predicts that different phenotypes evolve with different loading regimes (for example, maintenance of strain levels with constant loads, as shown in Figure 7C), we believe that if gender-specific loading regimes or selection criteria were included in the model, sexual dimorphism would evolve. Another important aspect of human evolution has been the splitting and migration of populations, which we have not included in the model. As we ran several models with the same loading regimes, selection criteria and initial gene

pools, and found different results, we believe that if the splitting and migration of populations were included in the model, divergence of the split populations would result (assuming gene pool convergence had not occurred). We chose to assess the evolution of slender or robust bones independently of changes in height, as it has been shown that height is heavily dependent on environmental factors. It has been shown that ethnic (genetic) differences account for only 3% of the difference in height in preschool children, while differences in socio-economic status can account for a 12% decrease in height in children from less affluent backgrounds (36). It has also been proposed that the reason that people of the U.S., once the tallest nation worldwide, have been surpassed in height by European people is due to sub-optimal nutrition and more limited access to healthcare in early life in the U.S. (37).

The results of our model suggest that our bones have evolved so that a genetically controlled growth rate is supplemented by adaptation at the periosteal and endosteal surfaces in response to mechanical forces. In many cases, the model predicted a convergence of the genetically controlled growth rate in the population, indicating that individuals of the same height may have the same baseline growth rate, but different adaptation parameters, leading to different bone phenotypes. Based upon the results of our model, we propose that it was advantageous to evolve genes that can adapt the ash content of the bone in response to the mechanical environment. The results suggest that it may be more advantageous to adapt the endosteal surface rather than the periosteal surface in response to mechanical forces, as most populations maintained a higher endosteal adaptation ability than periosteal adaptation ability. The exception to the overall trends of the model was during the ‘extended’ hunter gatherer phase, when loads and selection criteria were held constant for 1500 generations. These populations maintained their stiffness, despite a slight decrease in cortical area, by maintaining high ash content, periosteal and endosteal adaptation abilities in response to mechanical stimulus. These results would therefore suggest that bone is likely to have evolved during the hunter-gatherer phase to become more efficient by decreasing its size while maintaining structural strength through increased morphological and ash content adaptability. Our findings echo the proposal of Martin (3), that the evolutionary pressure towards minimizing bone mass is compensated for by shape and material properties

adaptive mechanisms. Variability in the population tended to be higher during the hunter-gatherer phase, perhaps due to the balance between two opposing pressures; minimizing mass while keeping strains low. After the end of the hunter-gatherer phase, the preference towards minimizing mass rather than strain may have increased the likelihood of convergence. Our results have shown that human bones are unlikely to have been selected for mass alone, as homogeneous populations of weaker (lower section modulus) bones evolved when this selection pressure was applied.

Our findings indicate that the processes that have influenced the evolution of the genes governing the growth and adaptation of our bones are likely to continue influencing the evolution of our bones, even with a sedentary lifestyle and without strong selection pressure on bones. In practical terms, this means that our bones may continue to reduce in size and to decline in mechanical competence, and potentially reduce their adaptability, thereby decreasing their ability to respond to a change in the mechanical environment. Continued decrease in bone size from the present day could lead to increased severity of osteoporosis (7) and a higher incidence rate of fractures due to increased slenderness (4). The results indicate that a further decrease in physical activity levels to 90% of modern day loads may exacerbate the situation, but that increasing levels of physical activity by 10% could mitigate the decrease in bone size and structural strength. The simulations predict that increasing or decreasing loads in the future will not have a large effect on the genetic variables contributing to bone size, but rather that the change in loads will affect bone growth through the mechanoregulatory processes active in each individual. Somewhat worryingly, the model predicts that the adaptability of the mineral content of bone may decline in the future, which may have consequences for bone aging and obesity. It has been shown that morbidly obese patients have higher bone mineral content than people with normal weight, and that mineral levels drop and then stabilise after weight loss (38). If the adaptability of bone were to decrease in future populations, bones may not respond as well to the mechanical demands placed upon them, compromising their functionality.

In conclusion, we have shown that changing loading regimes and selection criteria are likely to have influenced the evolution of bone growth and mechanoregulation. When selection acts to minimize the mass and strain in a bone, population averages evolve

towards smaller, weaker and less stiff bones. In an environment with high levels of physical activity, such as the hunter-gatherer period, bones can maintain their stiffness by evolving genes which increase their ability to increase their size and enhance their material properties in response to mechanical forces. Finally, our model has predicted that, unless exercise and physical activity during growth are increased in future generations, average bone size, structural strength and stiffness may continue to decrease, bringing increased risk of fracture and increased severity of osteoporosis.

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Table 1. Minimum and maximum variable and gene values. Gene values are calculated based on the variable values and the number of loci (five)

	Minimum (variable)	Maximum (variable)	Minimum (gene)	Maximum (gene)
g	0.8	1.2	0.16	0.24
p, e, ac	0.0	0.4	0.0	0.08

Table 2. Values for axial force, bending moment and axial torque at maturity used in the model, as reported by Taylor & Walker (2001) for the mid-stance phase of the gait cycle.

	Reported value (walking)
Axial force	$1.96 \times \text{Bodyweight}$
Bending moment	$6.25 \times \text{Bodyweight cm}$
Axial torque	$0.8 \times \text{Bodyweight cm}$

Table 3. Loading regimes and selection criteria for the three subsistence strategies.

	Hunter-gatherer	Agricultural	Modern
Loads	1.2 * modern loads	1.1 * modern loads	1.0 * modern loads
Selection	Strain < 1500 μ strain Fitness based on mass & strain (1:1 ratio)	Strain < 1500 μ strain Fitness based on mass & strain (2:1 ratio)	Strain < 1500 μ strain

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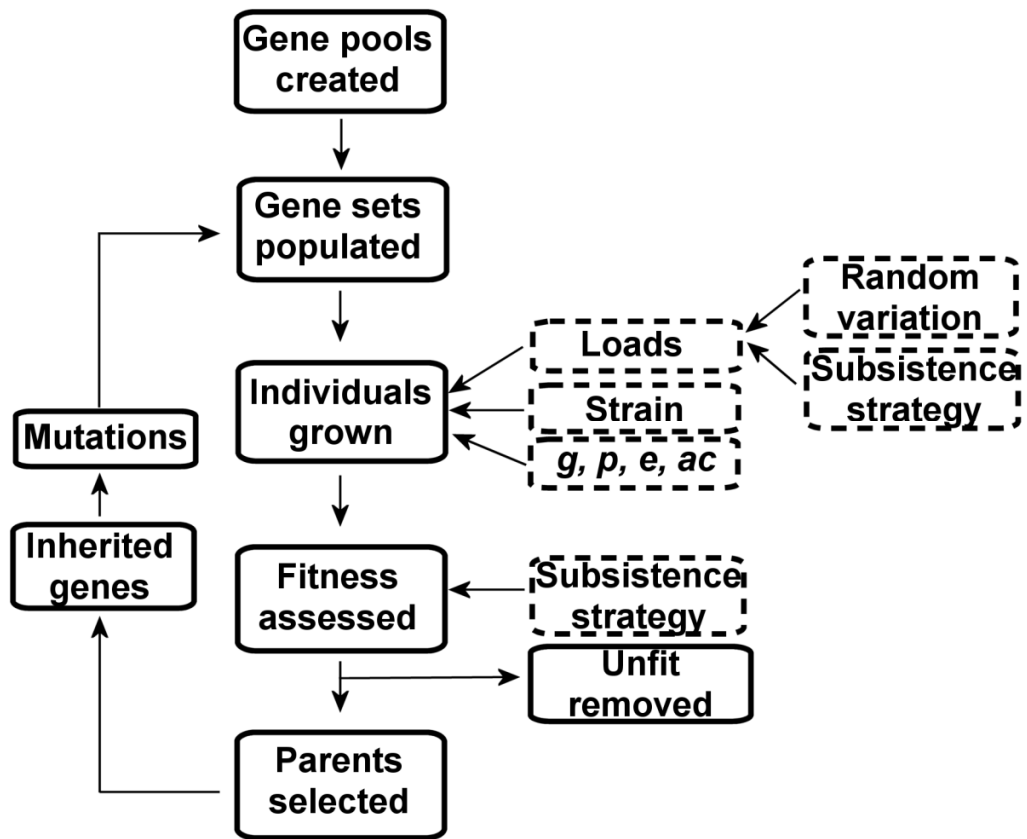


Figure 1. Schematic of the computational implementation

$$p = \sum_{i=1}^n \frac{P_{ia} + P_{ib}}{2}$$

a	P_{1a}	P_{2a}	P_{3a}	P_{4a}	P_{5a}
b	P_{1b}	P_{2b}	P_{3b}	P_{4b}	P_{5b}

Figure 2: Calculation of p variable from gene set, $n = 5$ in chromosome with five loci

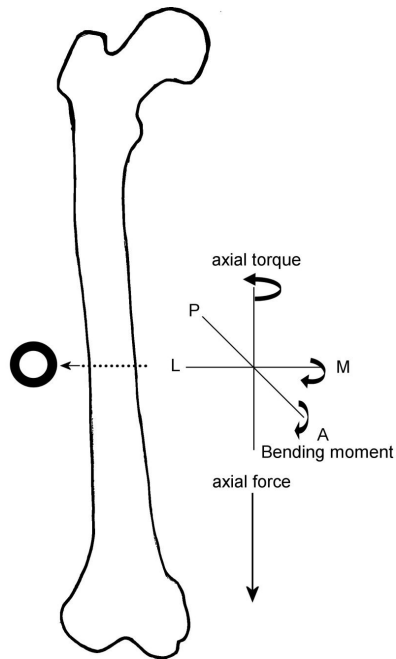


Figure 3. Schematic of loads applied to section of femur at mid-diaphysis. Magnitudes of adult loads during walking taken from Taylor and Walker (16) as detailed in Table 2.

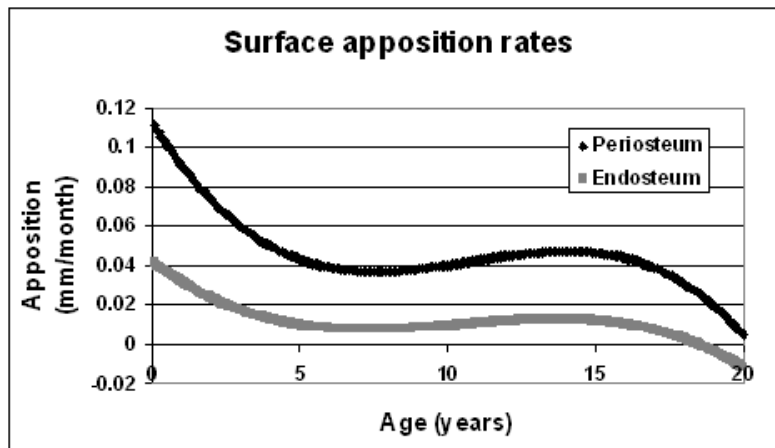


Figure 4. Equations describing the age-dependent monthly apposition rate for the femoral periosteum (derived from McCammon (16)) and endosteum (derived from McCammon (16) and Garn (15)). Lines shown are for baseline growth with a growth parameter $g=1.0$.

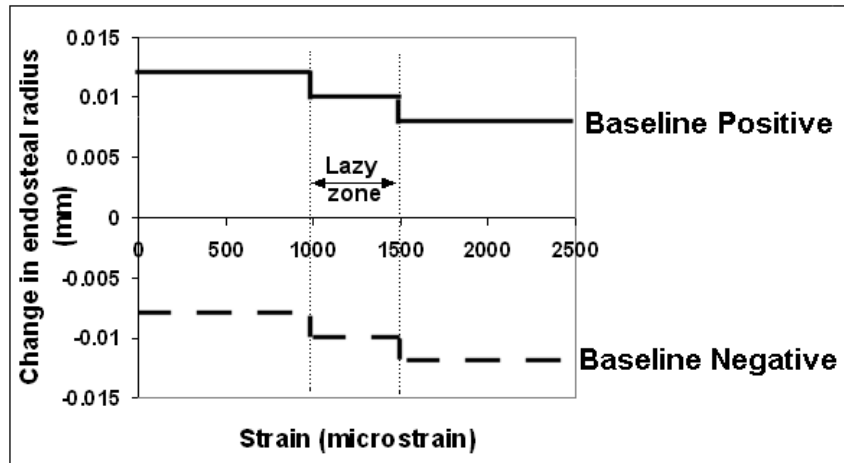


Figure 5. Change in endosteal radius when the baseline rate on a particular day is 0.01mm (solid line) or -0.01mm (dashed line) and the endosteal adaptation factor e is 0.2. A positive change in endosteal radius indicates resorption at the endosteal surface, while a negative change indicates apposition at this surface.

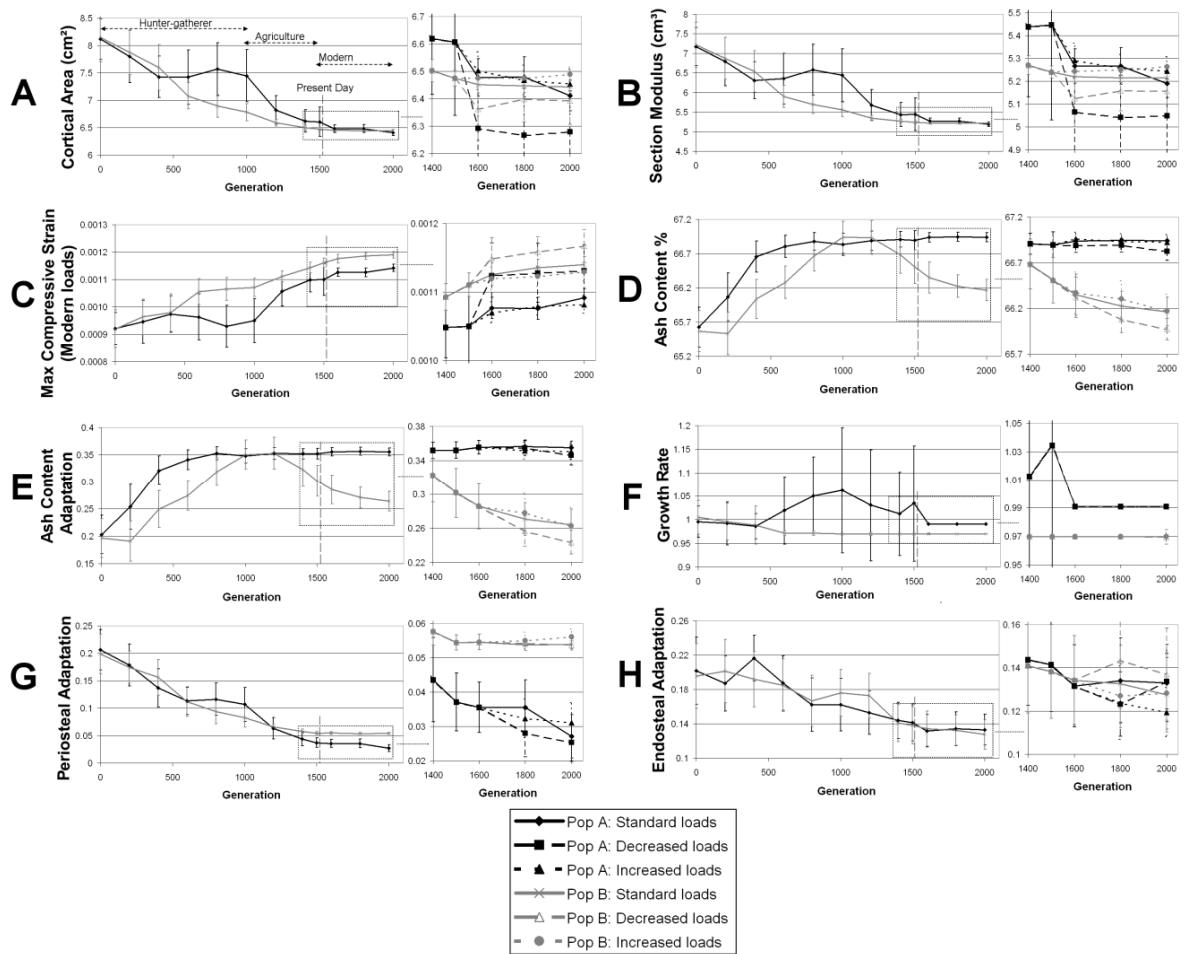


Figure 6. Evolution of two populations with two different initial gene pools. Standard selection pressure and loading as defined in Table 3. Average values with standard deviations are shown. Dashed vertical line marks the present day (generation 1510). A: Cortical Area, B: Section Modulus, C: Strain under modern-day loads, D: Ash content, E: Ash content adaptation factor (ac), F: Growth rate (g), G: Periosteal adaptation factor (p), H: Endosteal adaptation factor (e).

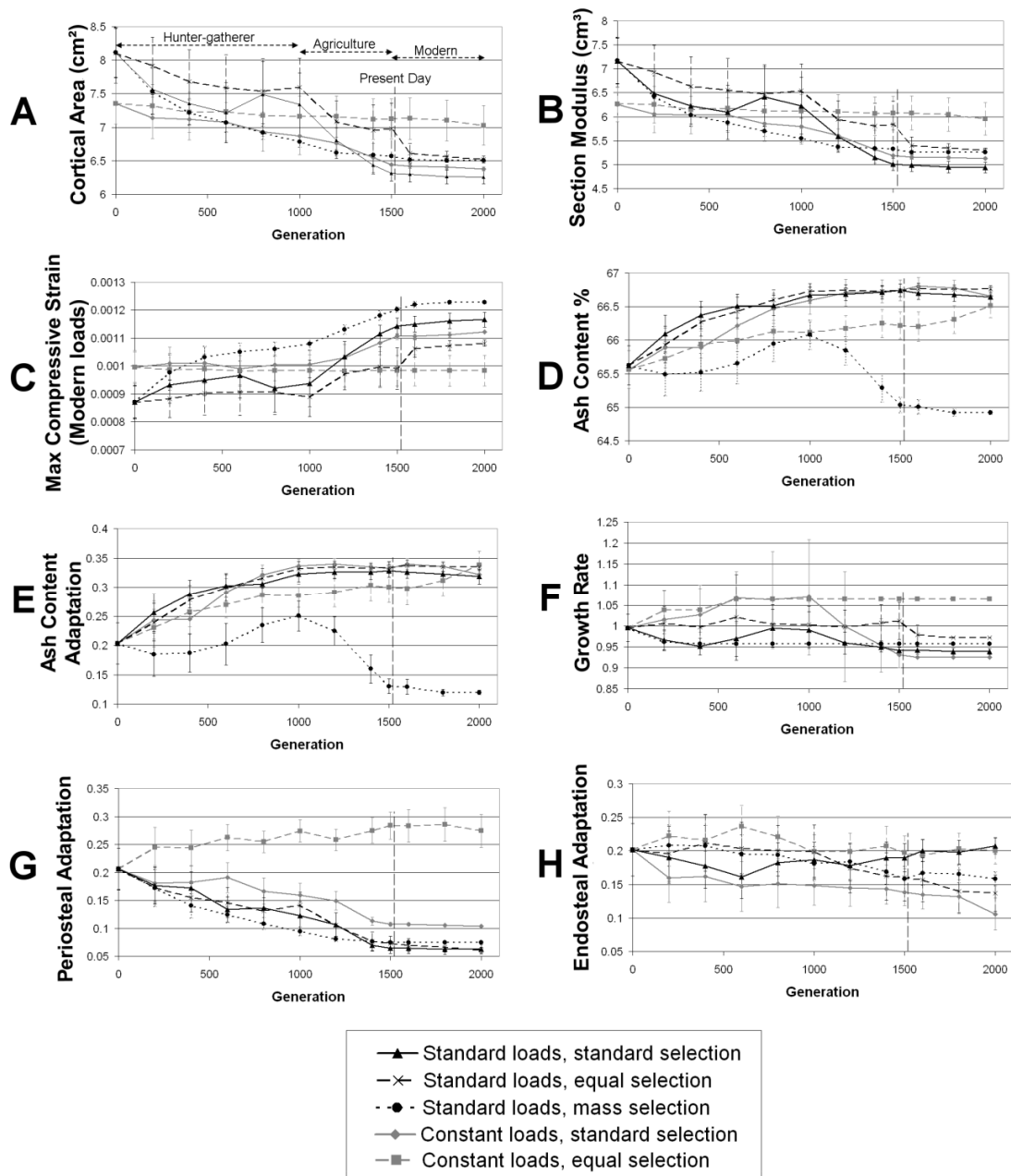


Figure 7. Evolution of populations with varying loading regimes and selection pressures. Dashed vertical line marks the present day (generation 1510). All results shown here are from simulations run with same initial gene pool. Population average values with standard deviations are shown.