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Age-dependent mutational effects curtail the evolution of senescence by antagonistic pleiotropy

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Abstract

One of the two main hypotheses to account for ageing is antagonistic pleiotropy (AP). This model requires alleles that increase vital rates (reproduction or survival) at early age at the expense of vital rates at late age. An important focus of evolutionary studies has been to assess the relative abundance of AP-type aging alleles that arise through mutation. Here, we develop theory that predicts that senescence *per se* reduces the probability that these alleles arise by mutation. A direct result is that these mutations should arise with extremely low frequencies in already senescing populations. This has profound implications for the evolution of life histories because it implies that the adaptive evolution of aging *via* AP will experience negative feedback. This theory also clarifies the previously inexplicable epistatic patterns of genetic covariance across age-specific vital rates that are observed in mutation accumulation experiments. We show that this epistasis is an emergent property of aging.

Introduction

Adaptation can occur from standing genetic variation or from newly arising mutations. In the latter case, the rate of adaptation will depend on both the rate at which beneficial mutations arise and the magnitude of their fitness effect. Adaptation theory predicts that there will be a strong negative relationship between the magnitude of a mutation's effect on phenotype and the probability that it is beneficial, suggesting that most beneficial mutations will be of small effect (reviewed in Orr, 2005). Adaptation can also be affected by deleterious mutations. Deleterious alleles can occasionally go to fixation in small populations if their effects on fitness are small relative to the effects of drift (Lande, 1994). Fixation of such mutations will cause a population to become maladapted.

One characteristic of organisms that seems at first glance to be maladaptive is senescence, an organisms' physiological deterioration with age. In terms of fitness, senescence is a reduction in vital rates, which includes both reproduction and survival, with age. One possible explanation for senescence is that natural selection actually favours genes that reduce survival and reproduction later in life, implying that senescence is a result of adaptation. Alternatively, senescence may truly be a maladaptation that is caused by unavoidable, deleterious mutations.

These two alternatives are at the heart of the best known evolutionary theories for senescence. The mutation accumulation (MA) model imagines that mutations can impact vital rates independently at different ages. Because selection against vital rate reducing mutations is expected to decline with age, deleterious mutations are more likely to accumulate if they affect vital rates at late age rather than early age. The result will be that populations are expected to have many more mutations that decrease survival and reproduction with advancing age (Fisher, 1930; Medawar, 1946, 1952; Hamilton, 1966; Charlesworth, 1994, 2001; but see Baudisch, 2005). The antagonistic pleiotropy (AP) model, on the other hand, is an adaptive theory of aging. It posits the existence of single mutations that have effects at more than one age. As the strength of selection is stronger at an early age relative to later ages, AP mutations that increase early-age vital rates but reduce late-age vital

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rates may spread (Williams, 1957). These adaptive mutations may fix in the population and contribute to aging in all individuals or, under certain conditions, segregate at intermediate frequencies (Hughes & Charlesworth, 1994; Charlesworth & Hughes, 1996; Moorad & Promislow, 2009). Such mutations will cause an overall increase in the fitness of their carriers due to their effects on early-age vital rates, but will also cause senescence due to their effects on late-age vital rates. Although these models are often kept separate, the genetic basis of senescence in a particular population may be due to either MA or AP or a mixture of both of these mechanisms.

Assessing the pleiotropic effects of single mutations on vital rates over multiple ages is critical to understanding the relative importance of these putative evolutionary mechanisms. If mutations are not pleiotropic or if single pleiotropic mutations never both increase early-age vital rates and decrease late-age vital rates, then the AP model for senescence would not be viable. Surprisingly, only a few studies have characterized the effects of new mutations over multiple ages (Pletcher *et al.*, 1998, 1999; Mack *et al.*, 2000; Yampolsky *et al.*, 2000; Gong *et al.*, 2006). These experiments (performed exclusively on *Drosophila*) examine the effects of new mutations on vital rates. Collectively, they show several interesting patterns of mutational effects:

1 Pleiotropy (as inferred by mutational correlations across mutation accumulation lines) is overwhelmingly positive over ages, causing declines in mortality at multiple ages.
2 Mutations tend to increase mean mortality more at early ages than at late ages.
3 The variation for mortality that is generated by mutations decreases with age.
4 The degree of pleiotropy seems to be linked to the number of new mutations that are carried by a line. Mutations have effects that are restricted to narrow ranges of ages when only few new mutations are present. However, the effects of new mutations apparently become more general and affect a wider range of ages as more mutations are accumulated.

Finding 1 argues against the frequent emergence of aging-type AP mutations (however, these mutations should have out-sized importance when they do arise). Previous demographic theory (Vaupel & Yashin, 1985; Yashin *et al.*, 1985; Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Vaupel *et al.*, 1998) and population genetic theory (Charlesworth, 2001) explore the evolutionary implications of mutations with positive pleiotropic effects on mortality at multiple ages. Explaining findings 2–4 have been problematic, however, because until recently there has been no theoretical explanation for why mutational effects should depend upon age of expression. Nor have we understood how this age dependency should affect the evolution of senescence. For example, population genetic theory assumes that the distributional properties of mutations are independent of the vital rates that they affect, meaning that the mean and variance of the mutational effects on mortality at late age are assumed to be the same as those that affect mortality at early age (Hamilton, 1966; Hughes & Charlesworth, 1994; Charlesworth & Hughes, 1996; Charlesworth, 2001).

One explanation for findings 2 and 3 was put forward by Moorad & Promislow (2008) who suggested extending Fisher's geometrical model of adaptation (1930) to age-structured populations. Fisher's model was originally intended to explain why adaptive change should involve many, small improvements rather than a few, large changes. The past decade has seen a resurgence of this powerful theory which has been used to investigate a broad range of evolutionary issues including the distribution of mutation size (Orr, 1998, 2006), the risk of extinction in small populations (Poon & Otto, 2000), hybridization (Barton, 2001) and the tempo of adaptive change (Orr, 2000; Welch & Waxman, 2003).

Fisher reasonably argued that fitness follows from the combination of numerous traits and that it is maximized at intermediate phenotypic values. So long as there is heritable trait variation in a population, selection will tend to drive the population along the multivariate fitness gradient towards the adaptive optimum. Fisher pointed out, however, that a mutation that changes an individual's phenotype in many trait dimensions will tend to be deleterious (so long as the direction of mutational effects are uniformly distributed in multivariate phenospace) because the local curvature of the multivariate fitness function presents more opportunities for movement away from the optimum than towards the optimum. There are two important implications of Fisher's geometric model. First is that the local curvature becomes more extreme for phenotypes that are closer to the optimum. As a result, mutations are more likely to be deleterious if the genotypes in which they occur are more fit (Fig. 1). Taken from the perspective of the marginal effects of mutations, Fisher's model predicts that new mutations should become less deleterious as more of them accumulate. The other implication of Fisher's model is that there is a cost to complexity (Orr, 2000; Welch & Waxman, 2003). Mutations are more likely to be deleterious as the number of traits (n) that determines fitness increases. The reason is that with more traits determining fitness, there are proportionally more directions in which a mutation can alter phenotype and still be deleterious.

Moorad & Promislow (2008) pointed out that because all vital rates are themselves determined by a multitude of traits, each could be assigned its own age-specific adaptive geometry characterized by its own adaptive peak. In this context, 'fitness' is given an age-specific meaning (e.g. Arnold & Wade, 1984). Regardless of its evolutionary mechanism (i.e. MA or AP), senescence is synonymous with a loss of adaptive fit between the traits expressed by an individual at late age and its late-age

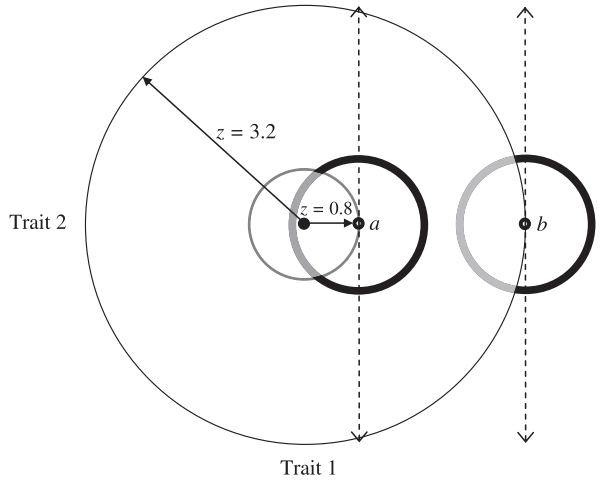

Fig. 1 Mutation and Fisher's geometric model of adaptation. The point at the centre represents the optimal combination of traits 1 and 2 for fitness. Each concentric circle represents a set of phenotypic values that are equidistant from the optimum value and, thus, have equal fitness. The larger concentric circle necessarily defines the phenotype values with lower fitness than those represented by the smaller circle. The two small hollow disks are two specific phenotypes '*a*' and '*b*' that are altered by mutations; these mutations have a constant effect magnitude but their angular distribution is uniform. When a large number of traits determine fitness ($n > 10$), then effective angular distributions are normally distributed around the dotted lines drawn tangentially to the two radii (see eqn 1). Note that the variance of this distribution will decrease as complexity, or the number of traits that contribute to vital rates, increase. The faded arcs represent the proportion of the mutational distributions that are beneficial; the dark arcs represent deleterious mutations. This simple, two-dimensional version of Fisher's model illustrates that mutations are more likely to be deleterious if they modify highly adapted phenotypes. The phenotypic distances z_a and z_b are scaled to the size of the mutation effect.

environment. This is a manifestation of the increased distance between the late-age phenotype expressed by an individual and the age-specific vital rate optimum, compared to the distance that characterizes early-age individuals (Fig. 2). Using this model, Moorad and Promislow showed analytically that the mean and variance of the effects of mutations on vital rates are greatest at early age, which helps to explain findings 2 and 3 above. Moreover, they found that this condition-dependent behaviour of mutations alters the evolution of senescence in ways that are unanticipated by classical evolutionary theory (e.g. Hamilton, 1966; Charlesworth, 1994).

Here, we apply this age-structured extension of Fisher's model to show that the aging-type AP mutations envisioned by Williams (1957) experience negative feedback; the likelihood that new aging-type AP mutations arise diminishes as populations evolve to exhibit senescence. We discuss the evolutionary implications of this tendency and reconcile these findings with the perception that artificial selection and classical quantitative

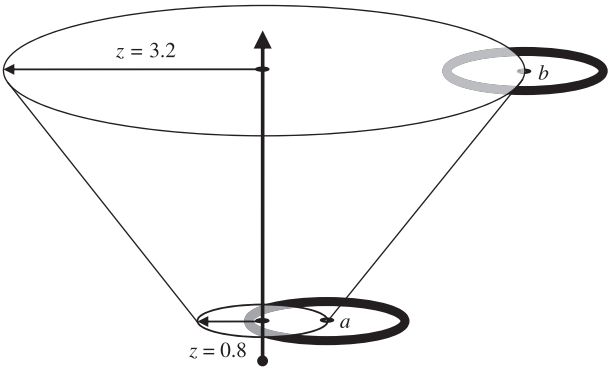

Fig. 2 Moorad and Promislow's model of age-specific adaptation. The age-specific phenotypic distances of individuals from their age-specific optima increase with age. The smaller proportion of mutations that decrease distance at early age *a* when compared with late age *b*, indicates that mutations that act at early age are more likely to be deleterious with respect to early-age vital rates.

genetic studies provide conclusive evidence for segregating aging-type AP alleles. We also demonstrate how senescence causes the seemingly inexplicable patterns of epistasis observed in the mutation accumulation studies.

The model

The goal of our model is to elucidate the effects of pleiotropic mutations on age-specific vital rates. Our model relaxes Moorad and Promislow's assumption of age-independent mutations by imagining that a single mutation will affect phenotypes at two different ages, an early age *a* and a late age *b*. For simplicity, we assume that age-specific phenotypes differ only in their distance from their optima. Each mutation moves individuals in multivariate phenospace some Euclidean distance *r* away from each of its age-specific positions. At age *x*, a mutation moves individuals from some initial distance z_x to some new distance z'_x. We assume that $z_b > z_a$ because selection declines with age, causing distances from age-specific optima to increase regardless of the evolutionary mechanism of aging. The change in phenotype (from z_x to z'_x) caused by mutation can be in any direction in the *n* dimensional phenospace (for now we assume nothing about how these directions are associated across ages). If a mutation causes z'_x to exceed z_x, then the vital rate at age *x* for mutant individuals will decrease because this phenotype will be further away from the age-specific optimum. If $z'_x < z_x$, then the vital rate will increase at *x*.

First, we ask how a change of length *r* in multivariate phenospace affects the distance between an individual's phenotype and the age-appropriate optimum. The new distance z'_x will depend on both the original distance and the direction of the vector of length *r*, which can be represented by an *n*-element vector of angles. If we knew the distribution function of all of these angles, then, in

principle, we could calculate a distribution function of the new distances z'_x caused by single mutations. We can simplify this distribution by assuming that a reasonably large number of traits ($n \geq 10$) determine the vital rate of interest and that there is a uniform probability distribution of angles in each dimension over $\{0 \leq \theta \leq \pi\}$. No matter the number of dimensions, there will always be some single angle θ^* that describes the relationship between lines drawn from (1) the phenotypic optimum to the current phenotype and (2) the current phenotype and the phenotype after mutation (Poon & Otto, 2000). We can then approximate the distribution of θ^* (we refer to this quantity as the *effective* angle) with a standardized normal curve on a transformed scale,

$$p(\theta^*) = \frac{1}{\sqrt{2\pi}} e^{-\theta^{*2}/2}, \quad (1)$$

where $\theta^* = \cos\theta\sqrt{n}$ (Fisher, 1930; Leigh, 1987; Hartl & Taubes, 1996). Mutations in highly complex phenospace (high n) will tend to move phenotypes away from the optimum along the line drawn tangentially from the isoclines drawn in Fig. 1. Note that normality arises as a property of the Central Limit Theorem and follows from a large number of dimensions n; it does not depend upon the shape of the vital rate function of multivariate phenotypes. On this transformed scale, these angular displacements can be translated into changes in the distances of z_x from the optimum using the Law of Cosines (Poon & Otto, 2000),

$$\Delta z_x(\theta^*) = \sqrt{z_x^2 + r^2 - 2zr\cos\theta^*} - z_x. \quad (2)$$

Change can occur in any of the n dimensions independently for two or more traits, although we expect that the angular changes are correlated in at least some dimensions. In general, the joint distribution of effective angular changes will be well approximated by the uniform multivariate Gaussian distribution with characteristic correlation matrix ρ, where the effective angular correlation, $\rho_{ab,}$ indicates the correlation between the angular effects θ^* of mutations on phenotypes at age a and b (throughout most of this paper we drop the subscripts because we are concerned with only two age classes). This correlation is important because it captures the biological essence of mutational correlations on the scale of phenotypes. When mutations tend to do the same thing to physiological traits at two different ages, then ρ will tend to be positive for these two ages. We can visualize this sort of mutation in Fig. 3 as parallel displacements away from the original phenotypic locations of the individual. A mutation that reduces body size at 4 years of age may tend also to reduce body size at 5 years, for example. Although our model does not require it, we can reasonably assume that the pleiotropic effects of mutations on multivariate phenotypes are more similar at ages that are closer together. If so, then ρ between two ages will be reduced as the two ages become more temporally distant. In other words, the maps of phenotypes to genotypes are expected to diverge with age.

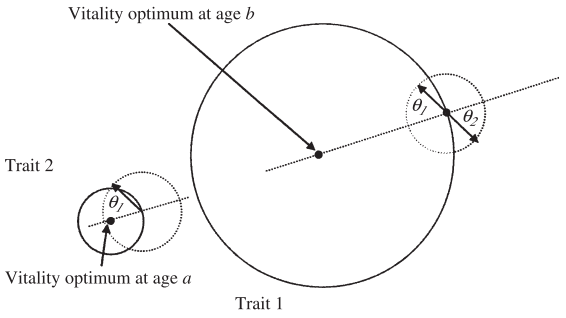

Fig. 3 Mutations can act at more than one age. Age-specific vital rates are maximized at age-specific combinations of two different traits. A pleiotropic mutation that shifts both age-specific bivariate phenotypes away from those optima by the same angle θ_1 at both ages will increase vital rates at late age but decrease it at early age. A mutation that shifts the phenotypes at angle θ_1 at a but θ_2 at b will decrease vital rates at both ages.

It is important to note that mutational changes that occur in the same direction in phenospace need not have similar effects on vital rates, which scale to the distances from the optima. Figure 3 illustrates how differential curvature (which necessarily follows from different initial distances) can cause the same movement in multivariate phenospace to decrease the distance to the optima at late age and increase it at early age. It is also possible that changes that occur in opposite directions at different ages can cause the same direction of change in age-specific distances. Finally, we note that our assumption that age-specific phenotypes differ only in their distance from their respective optima does not need to be true. In principle, the age-specific optima can lie in different directions in multivariate phenospace away from the age-specific phenotypes (following our example above, an increase in body weight might be beneficial at age 5 but deleterious at age 4). Our model can account for this contingency if we modify our meaning of ρ to include both the pleiotropic effects of mutations on phenotypes and the differences among the angular positions of age-specific phenotypes and optima. Thus, the strength of this angular correlation ρ corresponds to among-age similarities in the direction of selection on phenotypes and in the manner that mutations change these phenotypes.

Pleiotropic effects of age-specific mutations

A mutation will have one of four possible patterns of pleiotropic effects on vital rates at two different ages. It may be

1 universally beneficial because it decreases distances at both ages ($z'_a < z_a$, $z'_b < z_b$),

2 antagonistically pleiotropic because it decreases the age-specific distance at early age but increases it at late age ($z'_a < z_a$, $z'_b > z_b$),
3 antagonistically pleiotropic because it decreases the age-specific distance at late age but increases it at early age ($z'_a > z_a$, $z'_b < z_b$), or
4 universally deleterious because it increases both age-specific distances ($z'_a > z_a$, $z'_b > z_b$).

All else equal, mutations showing the pleiotropic patterns of type 1 are always adaptive. Mutations of types 2 and 3 may be adaptive, depending upon the magnitudes of changes. Of these, type 2 mutations are aging-type alleles and will contribute to senescence (Williams, 1957). Type 3 mutations, although still AP, will reverse senescence. Type 4 mutations are always deleterious.

Our first result is to show that the probability that a mutation will be an aging-type AP mutation (type 2) decreases with senescence. Using eqns 1 and 2 above (and applying their associated assumptions), we calculate the probability that a mutation that causes the same *magnitude* of multivariate phenotypic change at two different ages (r is invariant and age independent) will be beneficial at early age and deleterious at late age. The late age phenotype is set at 10 standardized mutational units away from its optimum (i.e. $z_b = 10r$). The initial early-age phenotype is assumed to be between 1 and 10 units from its optimum. In this way, we explore a continuum of senescent patterns, from nonsenescent ($z_a = z_b$) to highly senescent ($z_a = 0.1z_b$). We have assumed that r is invariant and age independent for the sake of simplicity. This need not be the case but different values of r can be accommodated by re-standardizing the diameters in Fig. 2 by the age-specific values of r (following Fisher, 1930; Orr, 1998). The qualitative results discussed below will not be affected by age-related changes in r unless the effects of age-specific mutation on multivariate phenotypes increase faster than senescence increases the age-specific diameters. We have no reason to expect this to happen. If it did, then we would expect mutations to be more severe at late age than ate early age – a pattern opposite to what has been observed (Moorad & Promislow, 2008). We explore treatments of low and high complexity (n = 10, 50) and various values of effective angular correlations (ρ = −0.9, 0, +0.9).

Mutation accumulation experiments and correlations across ages

Mutation accumulation experiments are performed to characterize the combined effects of many mutations. Replicate lines sampled from the same population are allowed to accumulate mutations by mitigating purifying selection by various means (Mukai *et al.*, 1972; Houle *et al.*, 1994; Shabalina *et al.*, 1997; Vassilieva & Lynch, 1999; Moorad & Hall, 2009). Ideally, there is no selection acting in these experiments and the fixation of new mutations in mutation accumulation lines reflects the frequencies of mutation that arise in these lines. Applied to age-specific vital rates, vital rate correlations among mutation accumulation line means should indicate the relative frequency of mutations with the pleiotropic patterns given above: mutations of types 1 and 4 generate positive covariance and types 2 and 3 contribute to negative covariance in line means.

We explored the effects of mutation accumulation on the correlation between age-specific distances by simulation (distance correlations). We simulated 10 000 independent lines that were allowed to accumulate mutations over 1000 time intervals. At each interval, each line fixed some number of mutations. This number was Poisson distributed with mean and variance equal to one (changing this parameter to one-half or two had no discernible effect other than changing the scaling of the time intervals – data not shown). This value is in accordance with mutational rates inferred from studies of *Drosophila* (Haag-Liautard *et al.*, 2007). As above, we considered treatments with varying complexity (n = 10, 50) and effective angular correlations (ρ = −0.9, 0, +0.9). We assumed that senescence existed prior to mutation accumulation ($z_a = 0.1z_b$). We measured distance correlations once mutations had accumulated.

Results

Figure 4 shows how the probability that a new mutation is an aging-type AP (type 2) mutation increases as a function of the initial distance of the younger age-specific multivariate phenotype (while holding the older-age phenotypic distance constant). More extreme senescence implies smaller distances at early age. Thus, this figure shows that senescence reduces the relative frequency of new aging-type AP mutations. This tendency is affected by the complexity of the vital rate; a higher trait number causes more dramatic changes in the log-transformed frequencies of aging-type AP mutants (steeper curves). In general, greater complexity reduces the frequency of aging-type AP mutations regardless of initial distance (lower curves). The effective angular correlation ρ is also important. Aging-type AP mutations are more frequent when effective angular correlations are negative and most rare when these correlations are positive. The most profound effect of senescence on the frequency of aging-type AP mutations is observed with positive correlations. With high complexity, in fact, changing the angular correlation from −0.9 to 0 reduced the frequency far less (on its log scale) than changing it from 0 to +0.9.

Moorad & Promislow (2008) suggested that complexity might decrease with age. We can account for age-related changes in complexity by changing n in eqn 1. These changes alter the way that distances are standardized, causing the 'r' in eqn 2 to change with age. In these cases, our predictions of the relative frequencies of

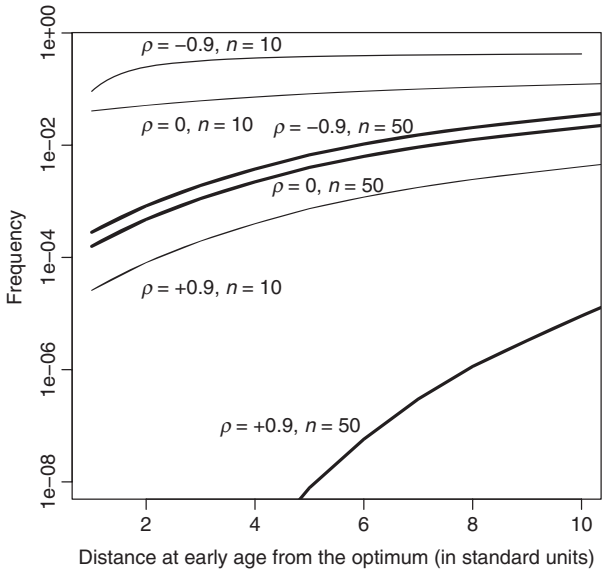

Fig. 4 The probability of aging-type AP mutations decreases with early-age adaptation. Each line represents the probability that a new mutation will contribute to senescence by antagonistic pleiotropy. This probability increases as the phenotypic distance of individuals from the early-age adaptive optimum increases. Light and heavy lines correspond to low and high complexity treatments, respectively. The probability of aging-type AP mutations with high effective angular correlations (ρ = +0.9) and high complexity (n = 50) were too rare to appear on this graph for early-age distances of less than five standard units. This frequency dropped rapidly with decreased early-age distance (leftward along the abscissa), reaching approximately 2.6×10^{-15} when individuals' phenotypes are one standard unit from their early-age optimum.

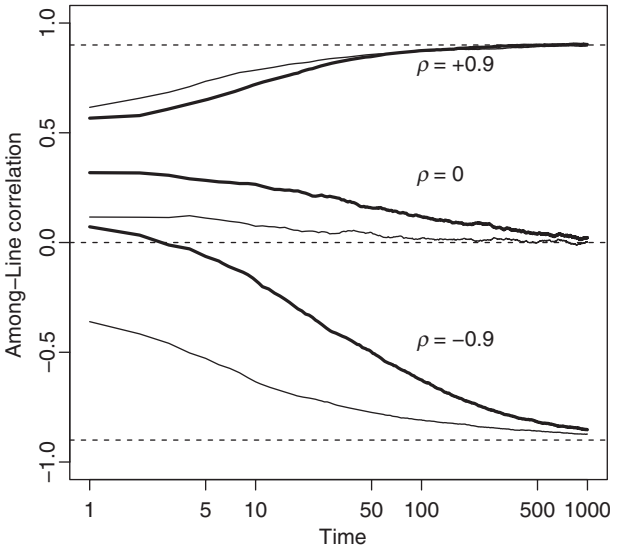

Fig. 5 Distance correlations change with mutation accumulation. Each mutation accumulation line experiences changes in multivariate distances from age-specific optima at both ages. The correlation among age-specific distances (distance correlation) changes as a function of the number of opportunities for mutation (time). Light lines are low complexity treatments (n = 10) and heavy lines are high complexity treatments (n = 50). High and low complexity trajectories are paired into high effective angular correlation treatments (ρ = +0.9), no correlation treatments (ρ = 0) and low effective angular correlation treatments (ρ = −0.9).

aging-type AP mutations change. If early-age vital rates are more complex than late-age vital rates, then the curves in Fig. 4 will be shifted downwards. Aging-type AP mutations will become even rarer, just as if the initial distance at early age was reduced. The curves in Fig. 4 will shift upwards (increasing the relative frequency of aging-type AP mutations) if complexity increases with age.

Figure 5 shows the effect of mutation accumulation on the correlations among phenotypic distances from age-specific optima. These represent the correlations among mutational effects on vital rates at early and late ages. Several interesting patterns emerge. Initially, distance correlations are biased away from the effective angular correlations (where they eventually equilibrate) in the direction of low to moderate, positive values: bias is positive for the zero and high, negative correlation treatments but negative for the high, positive treatment. Early on, distance correlations appear to be poor indicators of effective angular correlations and increased complexity makes them even less so. Eventually, they tend to converge with angular correlations if given enough time, although that can take hundreds of mutational events. For the highly positive angular correlation, ρ = +0.9, the distance correlations begin around 0.5–0.6 (in both complexity treatments) and increased to +0.9 over time. This last pattern is reminiscent of the increases in mutational correlations over time reported by Pletcher *et al.* (1999).

The number of fixed mutations varied among simulated mutation accumulation lines because mutation is a Poisson process. Among-line variation has been used previously to explain why mutational correlations can exceed genetic correlations in nematodes (Keightley *et al.*, 2000). To investigate whether among-line variance in the number of mutations contributed to the early differences between angular and distance correlations in our simulations, we repeated the simulations setting the mutation rate to exactly one per time period and re-examined the distance correlations over time. The results indicated that distance correlations agreed with effective angular correlations throughout the simulated experiments (data not shown), indicating that the variance among lines in the number of mutations was responsible for the differences between distance correlations and effective angular correlations.

We expect that among-line variance in numbers of mutations should cause correlations to be more positive than the underlying ρ because most mutations are

expected to be deleterious with respect to vital rates at all ages. Although this seems to be true for $\rho = 0.0$ and -0.9, this is not seen with $\rho = +0.9$. Furthermore, we expect intuitively that among-line variance should cause a similar bias regardless of the mean number of mutations across lines. Thus, it cannot explain the loss of bias as with prolonged mutation accumulation. Because Fisher's model of adaptive geometry (1930) predicts condition-dependent mutational distributions, we surmised that this model, coupled with among-line variance in numbers of mutations, might explain the patterns in Fig. 5 more completely. We asked if aging *per se* is required to generate the observed biases or if age-independent curvature of the adaptive surfaces was sufficient. We repeated the mutation accumulation, this time assuming that individuals were nonsenescent ($z_a = z_b$) and had age-specific phenotypes that were very close to the age-specific optima at both ages ($z_x = r$). These conditions can be visualized using Fig. 2. Instead of a funnel shape, the figure would appear as a narrow column. With this assumption, the distance correlations exceeded the effective angular correlations ρ for all combinations of ρ and complexity n (Fig. 6).

In every case, mutations tended to be deleterious with respect to vital rates. For this reason, variation among lines in the number of fixed mutations contributed positive distance covariance across ages, effectively causing distance correlations to become more positive. Even when effective angular correlations were negative, individuals from lines with many mutations tended to have greater distances at both ages than individuals with few mutations. Factors that increased the tendency for mutations to increases distance, such as complexity and proximity to the age-specific optima, exacerbated the disagreement between effective angular correlations and distance correlations. This explains the results from the nonsenescent treatments (Fig. 6), but it cannot explain the pattern observed in the senescent treatment where the bias was *negative* for $\rho = +0.9$ (Fig. 5) and the results from the Pletcher *et al.* (1999) study. Clearly, senescence is *necessary* to generate this particular pattern.

One explanation for this becomes clear if we re-orient our perspective of Moorad and Promislow's geometric model (Fig. 2) so that individuals' late-age phenotypes are superimposed on their early-age phenotypes (Fig. 7). Given our requirement that mutations have similar effects on phenotypes at both ages (high and positive ρ), we have shown that aging-type AP mutations (type 2) will arise with extremely low frequency. Senescence, or the increase in age-specific phenotypic distance with age, will make type 3 mutations (AP mutations that are deleterious at early age and beneficial at late age) arise with much greater frequency. So long as there is among-line variation in number of mutations, these mutations will contribute negatively to the among-line covariance in distance across ages. Meanwhile, mutations of types 1 and 4 will make positive contributions to this covariance.

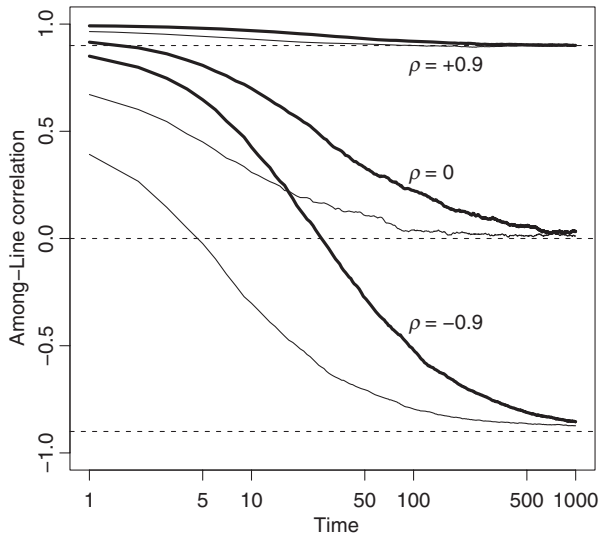

Fig. 6 Distance correlations with mutation accumulation in a highly adapted nonsenescing population. Mutation accumulation lines have adaptive distances that are more highly correlated across early and late ages than their effective angles (dotted lines). These differences disappear over time. See Fig. 5 description for legend.

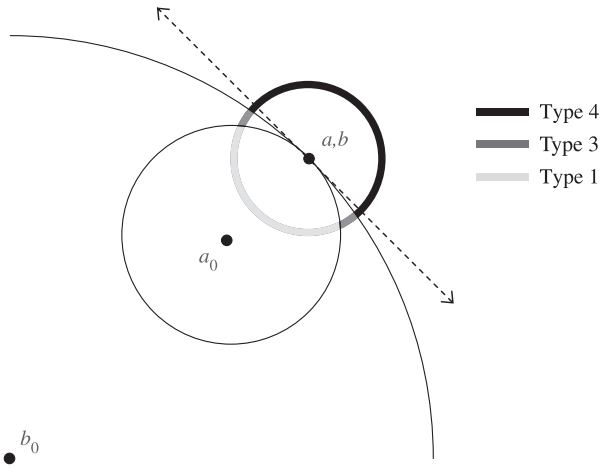

Fig. 7 Senescence causes mutations with highly correlated effective angular effects to have antagonistic fitness effects at different ages. We re-orient Fig. 2 such that we look down along the surface of the funnel through age-specific phenotypes b and a. For simplicity, we assume $\rho = +1$ so that a mutation of some length r has the same distribution of effective angles θ^* at both ages (we choose this extreme value for illustration only – the same concepts apply to any positive value). Most mutations are type 4, these will increase age-specific distances from age specific optima at both ages (a_0 and b_0). These mutations are indicated by the dark arcs of the thick circle. Some minority of mutations (type 1) will decreases distances from age-specific optima; these are indicated by the light gray arcs. Type 3 mutations increase distances at early age but decrease them at late age. Type 2 mutations are not included here because the high value of ρ makes them exceedingly rare.

These positive and negative contributions will have antagonistic effects on the mutational correlations, causing it to tend towards intermediate values. As mutations accumulate, age-specific distances will both increase (mutations tend to be deleterious) and converge with one another (early-acting mutations increase distance more than late-acting mutations). As a result, there will be fewer type 3 mutations and less negative distance covariance available to counteract the positive covariance generated by the increasingly common mutations of types 1 and 4. The among-line distance correlation will increase with the number of mutations.

Discussion

The evolution of senescence has been explained using both MA and AP genetic models. The viability of each putative mechanism depends upon the availability of mutations with appropriate pleiotropic characteristics. Our model predicts that the frequency of new aging-type AP mutations is dependent upon the adaptive fit of phenotypes expressed early in life. When early-age survival or reproductive rates are high, novel aging-type AP mutations are expected to be very rare; extremely so if the direction of age-specific selection on phenotypes and the genotype-to-vital rate map is relatively constant over age (corresponding to high positive values of ρ). These mutations are still quite rare in senescing populations even when the actions of mutations on phenotypes are completely age dependent ($\rho = 0$). Part of this can be explained by the first requirement of aging-type AP mutations: they must be beneficial at early age. This is unlikely because age-specific distances to vital rate optima are small at early age. The second condition is that the mutation is deleterious at late age. Late-age distances will be great with highly senescent life histories making late-age beneficial mutations more common. In these cases, the relative frequency with which a new aging-type AP mutation arises can be as little as one-half that of that for a (very rare) early beneficial mutation.

This condition-dependent relationship between mutation and AP-facilitated aging has important evolutionary implications. AP may contribute significantly to the evolution of senescence only in its earliest stages because aging-type AP mutations are much more likely to arise in nonsenescing populations. If selection in a nonsenescent population favours vital rates at early age relative to late age, then aging-type AP alleles may become adaptive. Provided that mutations make these aging-type AP alleles available, they may spread through the population and fix, thereby contributing to aging. New aging-type AP mutations become rarer as populations are driven closer to their early-age phenotypic optima and further from their late-age optima (thereby causing senescence due to AP). Consequently, further adaptation involving AP aging mutations is expected to become less likely. If we measured the pleiotropic action of new mutations that arise *after* this evolution of senescence, we may find no evidence for new aging-type AP mutations because they have become too rare to detect. Senescence would still be free to evolve further by MA however.

A major goal of evolutionary genetic studies of aging has been to compare empirically the potential importance of MA vs. AP by characterizing the pleiotropic actions of vital rate genes across ages. Mutation accumulation studies offer little evidence for new AP alleles (Pletcher *et al.*, 1999). This is not surprising given our results. We can reasonably infer that positive mutational correlations can lead to positive genetic covariance across age-specific survival. This supports arguments that variation in age-*independent* mortality within populations may play an important role in determining patterns of aging (Vaupel & Yashin, 1985; Yashin *et al.*, 1985; Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Vaupel *et al.*, 1998).

In the largest such mutation accumulation study, Pletcher *et al.* (1999) measured the mutational correlations between mortality effects at different ages. Surprisingly, these correlations became more positive as the study progressed and the lines presumably accumulated more mutations. They admit to being perplexed by this strange form of epistasis and note that these results do not follow from any life-history model. Our model predicts that these patterns should emerge when the following biologically reasonable conditions are met. First, there must be among-line variation in the number of mutations. Second, individuals must senesce. These first two conditions are met for mutation accumulation studies in *Drosophila* and virtually any other organism. The third requirement is that the effective angular correlation ρ must be positive, which requires that mutations do similar things to phenotypes at different ages. We believe that Pletcher *et al.*'s results provide evidence that this final condition is met.

Our results indicate that estimates of vital rate correlations made late in these experiments (after the bias dissipates) best reflect the way that mutations affect phenotypes at different ages (ρ). These results appear counter intuitive; we usually think that mutations that accumulate on more natural genetic backgrounds are more informative (e.g. Shabalina *et al.*, 1997). The results from Pletcher *et al.* (1999) seem consistent with high values of effective angular correlations (corresponding to ρ between 0.5 and 1, depending upon the temporal separation of ages) for mutations that affect survival at multiple ages in *Drosophila*. For two ages separated by 2 weeks, the results suggest values of ρ to exceed 0.9, which corresponds to the high correlation treatment in our simulation.

Our model recognizes two important implications of Pletcher *et al.*'s (1999) results. First, the third necessary condition given in the previous paragraph is met (high and positive values of ρ). Second, the frequency of aging-type AP mutations should be extremely low in *Drosophila* when compared with the frequencies of mutations with

other pleiotropic patterns, especially between similar age classes (e.g. 2 weeks). For two ages with wider temporal separation (up to 6 weeks), this frequency may increase slightly. Nevertheless, our analysis suggests that aging-type AP mutations can be so rare in mutation accumulation experiments as to be practically undetectable.

Our model shows that senescence will cause the frequency of AP mutations to be biased towards those that do not increase senescence. This should not be interpreted as a rejection of Williams' model but it does challenge the interpretations of many experiments and observations used to support this evolutionary mechanism for senescence. Consider, for example, quantitative genetic tests that seek to detect signals of MA or AP. These tests take three forms. The first involves measuring age-specific variance components and inbreeding depression of vital rate traits. Population genetic models (Hughes & Charlesworth, 1994; Charlesworth & Hughes, 1996) have suggested that these quantities should de different under equilibrium conditions that have followed from the two mechanisms. Recently, however, Moorad & Promislow (2009) showed that the diagnostic properties of this test did not hold under more general population genetic models of aging. AP may cause segregating patterns of genetic variation that appear consistent with MA under the conditions of the older models.

Another quantitative genetic test seeks to estimate additive genetic correlations between early- and late-age traits (e.g. Rose & Charlesworth, 1981a; Tatar *et al.*, 1996). It is expected that negative correlations are evidence of AP. In fact, our results suggest that evidence for AP alleles that affect patterns of aging does not imply the presence of the adaptive AP mutations required by Williams' model (1957). The reason for this becomes clear if we imagine a population that has evolved senescence due to MA. Let us suppose that a new AP mutation arises. Our model predicts that this mutation is far more likely to be beneficial late and deleterious early (nonaging) than the other way around. Nevertheless, it will contribute to a negative segregating genetic covariance between vital rate traits at different ages. So, in this population we would find both senescence and negative genetic correlations, but no mutations with the pleiotropic characteristics envisioned by Williams. A third test for AP is similar in that it also seeks to identify negative genetic correlations. Here, selection is applied to a vital age at one age and opposite, indirect responses at other ages are taken as evidence for AP (e.g. Rose & Charlesworth, 1981b; Rose, 1984; Partridge & Fowler, 1992; Zwaan *et al.*, 1995; Partridge *et al.*, 1999). As before, however, it is important to understand that segregating negative genetic covariances can correctly identify the existence of AP alleles without requiring that they be aging-type AP mutations. The same principle applies to evidence for AP alleles inferred from QTL studies (e.g. Leips *et al.*, 2006). Ample genetic variation may exist because of recent mutations (fixed in some of the inbred lines used to generate linkage maps) that increase survival at late age at the expense of reproduction at early age. These may be nonaging type alleles that do not necessarily indicate either a past history of or the potential for the adaptive process described by Williams (1957). In fact, our study suggests that the overwhelming share of all AP mutations are adaptively incapable of contributing to increased aging.

Rose *et al.* (2007) have argued that quantitative genetic experiments are not effective for testing evolutionary theories of aging (e.g. MA or AP) based upon both the equivocal history of results and the sensitivity of genetic variance components to peculiarities relating to populations' population structure, breeding history and environment. We share their skepticism, but our reasons involve problems inherent in models that produce the quantitative genetic tests (Moorad & Promislow, 2009) and the dubious relationship between segregating genetic covariation and the availability of adaptive mutations that increase early fitness traits at the expense of late-acting traits. The evolution of aging by AP is an adaptive process. The presence of additive genetic correlations speaks to the potential for future genetic change, which is not necessarily the same thing. We believe that persuasive evidence for aging by AP must come from a better understanding of the fitness effects of novel mutations.

What about the identification of individual genes that increase lifespan – the so called 'longevity' genes (e.g. Kenyon *et al.*, 1993; Bartke *et al.*, 2001; Tatar *et al.*, 2001; Arantes-Oliveira *et al.*, 2002; Niemi *et al.*, 2003)? Are these evidence for a past adaptation of aging? Our results suggest that it is possible that they are not. The characteristic expansion of individuals' distance from adaptive optima with age (see Figs 2 and 3) can occur by either MA or AP. Regardless of which is responsible, a new AP mutation will most likely increase lifespan and qualify as a 'longevity gene'. Significantly, the greater the background senescence and the greater magnitude of phenotypic change, the more likely it is that a new AP allele is of this class (rather than the aging-type class). Quite sensibly, molecular biologists interested in aging tend to investigate organisms with life histories that exhibit pronounced senescence. Likewise, they also tend to focus on mutations with very large effects (corresponding to large values of '*r*' in our model). For these reasons, it should not surprise us to see a great number of mutations that increase longevity. However, these say nothing about the relative importance of MA and AP mechanisms in the past. A very different question is whether AP can cause *further* evolution of aging. Here, we are more hopeful that molecular biologists can provide answers. An important step here is to understand how longevity mutations affect fitness. This is a question that is seldom addressed (Leroi *et al.*, 2005) despite the obvious requirement that an adaptively relevant lifespan-increasing

mutation must also be the more fit allele. We are also hopeful that more mutation accumulation experiments similar to that of Pletcher *et al.* (1999) can be performed on species other than *Drosophila melanogaster*.

This study, along with Moorad & Promislow (2008), has applied Fisher's model of adaptive geometry to explain patterns of aging that have been problematic. By taking this approach, we have tried to integrate genetic models of aging into the genetic theory of adaptation. The evolutionary theory of aging explains senescence as a manifestation of decreased natural selection with age. Because natural selection drives adaptation, can aging contribute to our understanding of adaptation in general? Orr (2005) proposed four lines of evidence that argue for the explanatory power of the genetic theory of adaptation: there are more beneficial mutations that have small rather than large effects, QTL studies find that most substitutions are of small effect, early substitutions in microbes have larger effects than late substitutions, and parallel evolution is common at the DNA level. Our models suggest that age-dependent mutational distributions are a persuasive fifth category of supporting evidence.

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