



Age- and sex-distribution of the mutation load

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Abstract

We investigate the age and sex distribution of genetic fitness under mutation–selection balance by means of simple one-locus two-allele models. We find that the extent of age and sex variation in the mutation load is very dependent on the average effect of new mutations. If the average heterozygote selective effect of new mutations is large, then age and sex differences may constitute a significant fraction of the total load, and be significant as compared to standing genetic variation. Whether the mutation load will increase or decrease with age depends on the age- and sex-specific effects of the new mutations, and on the rate of accumulation of mutations in the germ line as individuals age. We argue that the load will most likely increase with age in animals with continuous germ-cell division throughout life, and that this will occur even when mutations have unconditionally deleterious effects. We show that a male-biased mutation rate is likely to result in both a male-biased mutation load and a load that increases with male age.

Introduction

As a source of genetic variation, mutation is a fundamental prerequisite for evolutionary change. Still, the immediate effect of mutation on the organism is almost always disadvantageous. Every organism carries a load of mutations, a reduction of fitness due to segregation of deleterious mutations (Haldane, 1937; Muller, 1950). Estimates of the total genomic mutation rate indicate that the number of new deleterious mutations may average more than one per individual per generation (Crow & Simmons, 1983; Kondrashov, 1988; Crow, 1993a; Eyre-Walker & Keightley, 1999; Lynch et al., 1999). Under mutation–selection balance this translates into a large mutational load which may have a variety of evolutionary implications. First, the mutation load may play a crucial role in the evolution and maintenance of recombination and sexual reproduction (Kondrashov, 1988). Second, it may be important for the evolution of senescence (Rose, 1991; Partridge & Barton, 1993), and may strongly affect the evolution of reproductive effort in the later parts of life

(Charlesworth, 1990a). Third, due to partial recessivity of many mutations, the load may cause inbreeding depression and be a crucial factor in the evolution of inbreeding avoidance (Charlesworth, Charlesworth & Morgan, 1990). Finally, variation due to deleterious mutations may be an important factor in the evolution of mate choice (Heisler, 1984; Manning, 1985; Charlesworth, 1987; Pomiankowski, 1988; Rice, 1988; Pomiankowski, Iwasa & Nee, 1991; Hansen & Price, 1995; Rowe & Houle, 1996).

In this paper we investigate the distribution of the mutation load among the various life-history stages. Studies of mutation–selection balance have rarely taken age or sex structure into consideration. Charlesworth (1990a, 1994) provides some basic results for the zygotic load in an age-structured population, but does not directly address the question of how the load may distribute through the life cycle.

There are several mechanisms that may produce age and sex differences in the mutation load. First, mutations do not, as is often assumed, suddenly arise in the zygote. The majority of mutations seem to occur

during mitotic cell divisions in the germ line. In many animals, notably male mammals, the mitotic activity continues throughout life and new mutations will therefore accumulate in the germ line as the organisms age (Crow, 1993b; Hansen & Price, 1995). This is supported by the increased incidence of genetically defective children produced by older human fathers (Haldane, 1947; Parsons, 1964; Modell & Kuliev, 1990; Crow, 1993b). Furthermore, as males produce more gametes than do females, the sperm germ line must go through more cell divisions than the egg germ line. The result is that the male mutation rate may be much higher than the female rate; an hypothesis that has solid empirical support (e.g. Crow, 1993b; Shimmin, Chang & Li, 1993; Chang et al., 1994; Ellegren & Fridolfson, 1997). If mutation rates are sufficiently high, this may produce significant age and sex differences in genetic quality. Second, selection is far from uniformly distributed over a life cycle. The effect of a mutation on fitness decreases sharply with the age of expression (Hamilton, 1966), and mutations may have a variety of age- and sex-specific effects that make their frequency rise or fall during particular parts of the life cycle.

Theories of mate-choice evolution have commonly assumed that older individuals are genetically superior (Trivers, 1972; Halliday, 1978, 1983; Alcock, 1984; Manning, 1985, 1989; Heisler et al., 1987; Kirkpatrick, 1987; Cote & Hunte, 1993; Andersson, 1994; Kokko & Lindström, 1996; Kokko, 1998). This is based on the idea that older individuals have proven their genetic fitness through survival. Individuals with a high load of deleterious mutations are weeded out and only the fittest are able to survive into old age (Manning, 1985; Kokko & Lindström, 1996). Hansen and Price (1995) argued that this is not necessarily true for a number of reasons including the accumulation of mutations in the germ line, negative genetic correlations between early and late fitness components, and ongoing adaptation in the population. From our review of the relevant data, we conjectured that genetic quality would often peak relatively early in the reproductive life of an organism. However, this is sensitive to the life history, and on the distribution of genetic variation and covariation in fitness components among age groups. In particular, genetic fitness may improve with age if survival and fertility or their variances are increasing strongly with age.

The Hansen and Price (1995) model was based on quantitative genetics and did not consider genetic details. In this paper we extend that analysis to an

explicit model assuming that genetic variation in fitness is maintained by a balance between mutation and selection. We assume that mutations have an overall deleterious effect on total fitness, but not necessarily on any age-specific fitness components. In this way we can compute the effects of mutations with a wide variety of different trade-offs and age- and sex-specific expression patterns. We find that the distribution of the mutation load can be markedly affected by the exact properties of new mutations. We relate this to data on mutation rates and mutational effects on fitness. From this we address two main questions: (1) Can age and sex variation in the mutation load be an important cause of genetic variation? (2) Does the mutational load increase or decrease with age and do males have a significantly higher mutational load than females? Finally, we discuss some evolutionary consequences of the results.

Model

Total fitness is a measure of the expected contribution of a newly fertilized zygote to future generations. As males and females must contribute equally, fitness can be written as

$$W = \frac{1}{2}W_f + \frac{1}{2}W_m, \quad (1)$$

where W_f and W_m are the female and male components of fitness. Consider an infinitely large population of constant size and with a constant age and sex distribution. Let l_x be the probability that a male survive to age x and let m_x be the average male fertility at age x . Then the appropriate measure of male fitness is the life-time reproductive success

$$W_m = \int l_x m_x dx, \quad (2)$$

where integration extends over the life span. Female fitness can be expressed similarly in terms of the female life-history schedule. As results for males and females are symmetric, we shall only show details for males and treat female fitness as an aggregate parameter.

Consider now a single autosomal locus with two alleles A and a, where the deleterious a-allele is kept in the population through a balance between mutation and selection. Let the equilibrium frequency of this allele be q_0 among zygotes and q_x among males of age x . To account for fertility selection we also define q'_x , the frequency of the a-allele among successful gametes produced by males of age x . The

frequency among all gametes produced by males is then $q_m = \int l_x m_x q'_x dx$. Similarly, let q_f denote the frequency of the a-allele among gametes contributed by females such that $q_0 = (q_f + q_m)/2$.

New mutation from the A-allele to the a-allele appears in the zygotes with probability u per allele. This mutation rate is a composite of mutations accumulating throughout the life of the parents to the zygotes. Specifically,

$$u = \frac{u_m + u_f}{2}, \quad u_m = \int l_x m_x u_x dx \quad (3)$$

where u_m and u_f are the male and female mutation rates and u_x is the probability that an A-allele has mutated in a male of age x . We have used the fact that $\int l_x m_x dx = 1$ in a stationary population. We ignore back mutation. New mutations can affect any life-history parameter in males or females in any combination. The only constraint is that their net effect on fitness is negative.

Deleterious non-recessive mutations with small effects

Assume that the wild type (AA) has life-history parameters l_x, m_x and W_f . In the Aa-heterozygote, these life-history parameters are changed to $l_x(1 - s_x)$, $m_x(1 - s'_x)$ and $W_f(1 - s_f)$, respectively. Hence s_x is the selection coefficient due to male viability selection until age x , s'_x is due to male fertility selection at age x and s_f is due to selection in females. We assume that selection against the aa-homozygote is as strong or stronger than against the heterozygote. In the following, we use standard weak-selection approximations to the effect that the s 's are sufficiently small for any second-order terms to be ignored. We also assume that mutation is much weaker than selection so that quadratic terms of the u/s -type can be ignored. This is a reasonable assumption for typical mutation rates on a single locus.

In Appendix A we derive equations for the equilibrium gene frequencies in the different age classes. Among zygotes the equilibrium is, as also obtained in Charlesworth (1994, pp. 125–126),

$$q_0 = \frac{u}{s}, \quad (4)$$

where the age- and sex-weighted selection coefficient is

$$s = \frac{s_m + s_f}{2}, \quad s_m = \int l_x m_x (s_m + s'_x) dx. \quad (5)$$

Thus, the equilibrium gene frequency equals the mutation rate, u , over the age- and sex-weighted selection

coefficient, s , and is in this sense the age- and sex-structured analog of the standard first-order approximation for an allele in mutation–selection balance (Haldane, 1937; Bürger & Hofbauer, 1994). From (4), the mutation load ($L = 1 - W$) among zygotes can be computed as

$$L_0 = 2u, \quad (6)$$

again obtaining the age- and sex-structured version of a classical result (Haldane, 1937). The variance in fitness contributed by this locus is approximately $2us$.

In Appendix A we also show how the equilibrium gene frequencies are distributed through the life cycle of the organism.

$$q_x = (1 - s_x) \frac{u}{s} + u_x, \quad (7a)$$

$$q'_x = (1 - s_x - s'_x) \frac{u}{s} + u_x, \quad (7b)$$

$$q_m = (1 - s_m) \frac{u}{s} + u_m, \quad (7c)$$

$$q_f = (1 - s_f) \frac{u}{s} + u_f. \quad (7d)$$

From these we can derive the age- and sex-specific mutation loads as

$$L_x = L_0 + 2su_x - 2us_x, \quad (8a)$$

$$L'_x = L_x - 2us'_x, \quad (8b)$$

$$L_m = L_0 + 2su_m - 2us_m, \quad (8c)$$

$$L_f = L_0 + 2su_f - 2us_f. \quad (8d)$$

Hence, the difference in load between males of two different ages, x and y , is

$$\begin{aligned} L_y - L_x &= 2s(u_y - u_x) - 2u(s_y - s_x) \\ &= 2(su_{yx} - us_{yx}), \end{aligned} \quad (9)$$

where $u_{yx} = u_y - u_x$ is approximately the probability of a new mutation having happened between age x and y , and $s_{yx} = s_y - s_x$ is approximately the selection coefficient against the a-allele during this part of life. In (9), the first term, $2su_{yx}$ is the increase in total load due to new mutations that happens from age x to age y , and the second term, $2us_{yx}$, is the amount of load removed by selection in the same interval. To get the difference between the successful gametes produced by the two age classes, a term $-2u(s'_y - s'_x)$ must be added to account for a possible difference in the strength of fertility selection between the two age classes. The difference in the load between the sexes is

$$L_m - L_f = 2s(u_m - u_f) + 2u(s_f - s_m). \quad (10)$$

Note that the change in the load over any path in the life cycle is a sum of the decrease in load due to selection and the increase in the load due to mutation over that one path. As we are studying an equilibrium, a reproductively weighted sum of these changes must be zero. Hence, the load will increase along some paths and decrease along others. Where it will increase and where it will decrease depend on the relative strengths of selection and rates of mutation along the different paths.

Unconditionally deleterious viability mutations

Unconditionally deleterious mutations that only affect viability are the most likely candidates for decreasing the mutation load with age, as they are selected against throughout the life cycle. However, it is not necessarily true that the load due to such mutations decreases over the entire life span. From (9), the criterion for the load to decrease from age x to age y is $s_{yx}/s > u_{yx}/u$, or in words, the strength of selection during this interval relative to the average strength of selection over the entire life cycle must exceed the rate of mutation in the interval relative to the average rate of mutation over the entire life cycle. If the deleterious allele is sex specific so that only males are affected, the criterion for the load to decrease from age x to y becomes $s_{yx}/s_m > 2u_{yx}/u$, and it is more likely that the load will decrease in males. In this case, the load will necessarily increase among females. If the male mutation rate is higher than the female rate, stronger relative selection is necessary to make the load decrease in males. Age-specific mutations that primarily affect juvenile survival will make the load decrease early in life, but as they are not selected against later, they will increase the load during adulthood. Mutations that are only expressed late in life will accumulate and increase the load until they are expressed, and thereafter they will decrease the load as they are selected against.

In conclusion, even unconditionally deleterious male viability mutations may increase the load during adulthood in males provided they occur throughout life. Mutations that are expressed at the juvenile stage are most likely to make the load increase during adulthood, while mutations that are expressed only during adulthood are more likely to reduce the load as they are selected out.

Antagonistic pleiotropic mutations

Consider a mutation that has a positive effect on adult male viability ($s_{yx} < 0$), but an overall deleterious effect on total fitness ($s > 0$). The effect of a such

mutation is always to increase the load during adulthood (in (9), $s_{yx} < 0$ and $s > 0 \Rightarrow L_y > L_x$). The reason is that selection during this period will increase the frequency of a generally deleterious mutation. In this case it does not matter where the mutations arise or where the deleterious effects are expressed.

On the other hand, a mutation that has a negative effect on adult male viability may or may not increase the load during adulthood depending on the other parameters. If there is no negative effect elsewhere in the life cycle, it can be shown that the load must decrease. As an example, consider a mutation that has a positive effect on juvenile survival ($s_x < 0$), a negative effect on adult survival ($s_{yx} > 0$), and no effects on fertility, on females or in males after age y . Using this in (9), we see that the load must decrease during adulthood. If there is selection against the mutation also other places in the life cycle, this is no longer necessarily true.

It might be more common for an antagonistic pleiotropic mutation to have a positive effect late in life, as early fitness components can be expected to be more highly adapted making improvements less likely. In this case, antagonistic pleiotropic variation will have a tendency to increase the load during the later parts of life.

Mutations affecting fertility

The change in the load due to fertility selection at age x is given in (8b) as $-2us'_x$. The load is reduced more among gametes produced by age classes where fertility selection is strong. If mutations accumulate with age, this means that the strength of fertility selection must increase with age to stop the gametic load from increasing. Assuming that the mutation has no effect on male viability, the criterion for the load to decrease from age x to age y is $s'_y - s'_x > su_{yx}/u$, or the strength of fertility selection at the older age must exceed the strength of fertility selection at the younger age by an amount equal to the fraction of new mutational load that appears from age x to age y .

In general, we see no particular reason for selection against deleterious fertility mutations to be proportionally stronger among older individuals. Late in life we may in fact expect the opposite. Mutations with a deleterious effect on early reproduction may be more common than mutations with a late effect, as early life-history parameters are more important for total fitness and therefore likely to be more highly adapted.

Antagonistic pleiotropic mutations that enhance fertility at a survival cost will increase the load during fertility selection. If survival is enhanced at a fertility

cost, the load will tend to increase with age and then be reduced during fertility selection.

Effects of selection and mutation in females

Any overall deleterious effect of a mutation on female fitness ($s_f > 0$) will increase the load in males (relative to the changes we would expect if $s_f = 0$).

In mutation–selection balance the sum of the changes in the load in the two sexes must be zero, but the load may be reduced among average breeding individuals in one sex and elevated in the other. It is unknown whether there are any systematic differences between sexes in the strength of selection, but as mentioned in the introduction, there are both theoretical and empirical reasons to expect that the male mutation rate is higher than the female rate. From this we would, under mutation–selection balance, expect that the average breeding male carries an increased load of mutations as compared to both females and zygotes. A quantitative assessment of this effect will be given later.

Deleterious mutations with small recessive effects

Consider recessive mutations where AA and Aa genotypes have life-history parameters l_x , m_x and W_f , and the aa-homozygotes have life-history parameters $l_x(1 - s_x)$, $m_x(1 - s'_x)$ and $W_f(1 - s_f)$. As before we assume that the selection coefficients are sufficiently small to allow standard weak-selection approximations and that the total effect of the mutation is deleterious. At equilibrium, the frequency of the a-allele among zygotes is (Charlesworth, 1994)

$$q_0 = \sqrt{\frac{u}{s}}, \quad (11)$$

where $s = [\int l_x m_x (s_x + s'_x) dx + s_f]/2$. Among zygotes both the load and the variance in fitness contributed by this locus equals the mutation rate, u .

The changes in gene frequency through the paths of the life-cycle are identical to those given in (7) for a non-recessive gene (shown in Appendix A). From this it follows that the changes in the mutation load are exactly the same as for a non-recessive gene except for multiplication with a factor of $\sqrt{u/s}$. Unless selection is extremely weak, this means that the age and sex differences in the load are going to be orders of magnitude smaller when the mutation is recessive. Thus, recessive mutations have little effect on the age- and sex-distribution of genetic fitness.

Deleterious mutations of large effect

A classical result of population genetics is that the mutation load is independent of the mutational effect on fitness (Haldane, 1937; Muller, 1950). However, the changes in the load through the life cycle are proportional to this effect (Equation (8)). This means that the larger the effect of a mutation, the more important it becomes in creating age- and sex-based differences in genetic quality. Given this, it is important to investigate mutations of large effect.

A lethal mutation that kills before maturity leads to a subsequent increase in the load as the mutation accumulates in the germ-line of individuals. If the mutation is dominant, it is shown in Appendix B that the load among zygotes is approximately $L_0 = 2u$ and the load among males of age x will be $L_x = u_x$ if the mutation is neutral in females and $L_x = 2u_x$ if the mutation is also lethal among females. If most mutations occur in males and accumulate steadily throughout the males life, the difference between an old and a very young male will approach the total zygotic load. For a recessive lethal the zygotic load will be $L_0 = u$, but the age and sex differences will be of lower order (see Appendix B).

Mutations causing complete sterility in one or both sexes will create the same zygotic and gametic loads as lethals. If there is no viability selection, the zygotic load will be carried by all age classes, but the change in load with age will be solely due to accumulation of new mutations and therefore the same as for lethal mutations.

In conclusion both lethal and sterility mutations lead to an increase in the load with age, and the age effect may quantitatively approach the total zygotic load in size.

Effects on total fitness

So far, we have focused on a single locus in mutation–selection balance. In this section we ask what our results imply for additive genetic fitness as a whole by assuming that the total load is a sum of the load on individual loci with small effects. This ignores important factors such as epistasis and coupling disequilibrium, and should therefore be seen as a qualitative first approximation.

We need some definitions

$$U = \sum_i 2u_i, \quad (12a)$$

$$S_x = \sum_i 2u_i s_{xi}, \quad (12b)$$

$$S'_x = \sum_i 2u_i s'_{xi}, \quad (12c)$$

$$Z_x = \sum_i 2s_i u_{xi}, \quad (12d)$$

where i indexes loci and summation is over i . The parameter U is the total mutation rate of deleterious mutations per zygote. The parameter S_x is the total deleterious effect of new mutations on male viability to age x , and S'_x is the total deleterious effect of new mutation on male fertility at age x . The parameter Z_x is the total deleterious effect of new mutations that have appeared at age x in males. The values of these parameters for the average breeding male are $S_m = \int l_x m_x (S_x + S'_x) dx$ and $Z_m = \int l_x m_x Z_x dx$. With similar definitions for females, the total deleterious effect of new mutations in the zygote is $S = (S_m + S_f)/2$, and it can be verified that this must be equal to $Z = (Z_m + Z_f)/2$. Summing Equations (8) over loci and using (12) yield expressions for the total load

$$L_0 = U, \quad (13a)$$

$$L_x = U + Z_x - S_x, \quad (13b)$$

$$L'_x = U + Z_x - S_x - S'_x, \quad (13c)$$

$$L_m = U + Z_m - S_m, \quad (13d)$$

where L is to be interpreted as the total load carried by individuals. These equations allow us to discuss the quantitative aspects of load differences in terms of parameters that pertain to the whole organism rather than individual loci. The age and sex differences in the load are controlled by the S and Z parameters. The difference in load between males of two age classes x and y is

$$L_y - L_x = (Z_y - Z_x) - (S_y - S_x), \quad (14)$$

where $Z_y - Z_x$ is the deleterious effect of all new mutations that have accumulated in the germ line between age x and y and $S_y - S_x$ is the total effect of all alleles that have been removed by selection between age x and y .

Under mutation–selection balance the additive genetic variance in fitness among zygotes, V_A , must equal the deleterious additive effect of new mutations (e.g. Price, 1970)

$$V_A = S. \quad (15)$$

Using this relationship and estimates of S we may compare the potential for changes in the load to the standing genetic variation in fitness. In the discussion we review estimates of the total deleterious effect of new mutations and conclude that it is reasonable to expect S to be on the order of 1–10% of mean fitness. To make this comparison on the same scale, we should compare to the standard deviation of fitness, which is then on the scale of the square root of the total deleterious effect of new mutations. If the total deleterious effect of new mutations is as large as 10% of the mean, the standard deviation of fitness is about 30% of the mean, and the age and sex component of variation may be significant. If the total deleterious effect is 1%, the standard deviation of fitness is roughly 10% of the mean and the age and sex variation significantly less important.

Consider the difference between the loads of average breeding males and females. From (13d) and its female analog we get

$$L_m - L_f = Z_m - Z_f + S_f - S_m. \quad (16)$$

Assuming, from lack of information, that the total mutational effect on male fitness (S_m) is equal to the total effect on female fitness (S_f), we find that the difference in load is equal to the difference in the average deleterious effect of new mutations in the two sexes. Estimates of the ratio of male to female mutation rates range from 2 to infinity. Some recent estimates that are also consistent with what is known about differences in number of germ-cell division between the sexes are 2 for rodents, 6 for primates and 10 for humans (Montadon et al., 1992; Shimmin, Chang & Li, 1993; Chang et al., 1994). If the male mutation rate is twice the female rate, a simple computation shows that $Z_m - Z_f = 2S/3$. Hence, the difference between average breeding individual of the two sexes is conservatively a full two thirds of the average deleterious effect of new mutations as they appear in a zygote. If the male mutation rate is much higher than this, $Z_m - Z_f$ will approach $2S$. Hence, if S , is large, a question we consider in the discussion, we expect a substantially different mutation load in the two sexes.

Discussion

Under mutation–selection balance the net force of mutation must equal the net force of selection when averaged over the life cycle of an organism. The mutation load must be the same in the average breeding

Table 1. Qualitative summary of the effect of type of mutation on the change in mutation load with age in reproductively mature males (based on Equations (9) and (14)). The columns describe different types of mutational effects on male fitness, and the rows indicate where the mutations occur. 'Germ line' means that mutations accumulate in the germ line, 'male biased' means that mutations accumulate in the male germ line only, and 'in zygote' means that there is no accumulation of mutations in the germ line after maturation. A plus (+) indicates that the load is likely to increase with age, a minus (−) that the load is likely to decrease with age, a plus-minus (±) that either outcome is likely, and a zero (0) that the effect is likely to be small. More plusses or minuses indicate strength of effect. We are assessing the change in load (in gametes produced) from young, but reproductively mature, adults to old adults. We assume that the antagonistic pleiotropic mutations have a net deleterious effect, and that the deleterious part of their effect acts to decrease the load if mutations appear in the zygote, to increase the load in case of male biased mutations, and is equivocal in case of ordinary germ line mutations. Note that the quantitative effects will be roughly proportional to the average deleterious effect of new mutations, and may also depend on the life history of the organism

Mutation type	Unconditionally deleterious mutation with main effect on					Antagonistic pleiotropic mutation with positive effect on			
	Uniform viability	Juvenile viability	Adult viability	Young adult fertility	Old adult fertility	Juvenile viability	Adult viability	Young adult fertility	Old adult fertility
Germ line	±	+	−	++	−	±	++	±	++
Male biased	+	++	±	+++	±	+	+++	+	+++
In zygote	−	0	--	+	--	−	+	−	+

individual as it was in the zygote. However, the load may rise or fall through any part of the life cycle as the rate of mutation and strength of selection rise and fall. We have shown how this pattern of rise and fall is dependent on the exact properties of new mutations. We have argued that most antagonistically pleiotropic mutations and mutations that affect fertility will have a net tendency to make the load increase with age. The effect of unconditionally deleterious viability mutations depends on their age-specific expression pattern and on when in life they arise. If mutations accumulate continuously in the germ line as the individuals age, mutations with a deleterious effect on juvenile fitness will tend to elevate the load as the individuals age. If the rate of mutation is male biased, the load will also be male biased, and the average breeding male will have an elevated load as compared to the zygote. A rough qualitative summary of these results is given in Table 1.

If the changes in the load are compared to the zygotic load on a per locus basis, they may seem rather small. The zygotic load is proportional to the total mutation rate while the relative age and sex differences in the load are roughly proportional to the total deleterious effect of non-recessive mutations (see Equations (9), (10), (14) and (16)). Estimates of the typical heterozygotic effect of a new viability mutation, excluding lethals and sublethals, are often given as 1% or a little more (Mukai et al., 1972; Simmons & Crow, 1977; Lynch et al., 1999). Thus, the changes in the load are potentially only about 1% of the total load. However, this value is likely an underestimate. First, mutations of large effect are excluded from such

estimates, and as much as 2–5% of all *Drosophila* zygotes may carry a new lethal mutation (Crow & Simmons, 1983; Fry et al., 1999). Second, viability is not the only component of total fitness. Third, the estimates of effects are done under benign conditions. Kondrashov and Houle (1994) have shown that the deleterious effects of mutations can be elevated in harsh environments. Fourth, the majority of mutations in *Drosophila* may be caused by transposable elements (Green, 1988) and these mutations may have atypically weak deleterious effects (Keightley, 1996). In other organisms the transposition rate may be smaller (Favor, 1994). Fifth, reanalyses of some of the classical mutation–accumulation experiments with different statistical models (Keightley, 1996; Garcia-Dorado, 1997) indicate that the average deleterious effect of new mutations may be much larger than previously assumed, and also include a sizeable fraction of dominant lethals. Correspondingly, the rate of deleterious mutation, U , is found to be smaller than previously assumed. A similar result was found in a recent mutation–accumulation experiment (Fry et al., 1999). If true, these new estimates mean that the age and sex differences in the load will be much higher relative to the total load, but see Lynch et al. (1999) for defense of the classical estimates.

In conclusion, in an organism like *Drosophila*, and presumably in many other organisms as well, it is a real possibility that new mutations have sufficiently large effects to produce fitness differences between life-history stages of at least 1–10% of the total load. Indeed, the mutation–accumulation experiments are consistent with a per generation reduction in viab-

ility on the order of 1–10% or more (Kondrashov, 1988; Crow, 1993a, Charlesworth, 1994; Kondrashov & Houle, 1994; Burt, 1995; Lynch et al., 1999). Changes in genetic fitness through life on the order of 1–10% of mean fitness thus also seem possible under mutation–selection balance.

Epistasis may influence these considerations. Synergistic epistasis will reduce the load in a sexual population (Kondrashov, 1988; Charlesworth, 1990b), but it will also accelerate the effects of mutations accumulating in the germ line of an individual. If synergistic epistasis is important, we predict that the relative age and sex differences in the load will be elevated over the predictions from the single-locus model in this paper.

Our results are relevant for the lek paradox, the question of whether there can be enough genetic variation in fitness to make mate choice for good genes a viable strategy. The realization that the mutational variance for fitness may be high (Houle, Morikawa & Lynch, 1996 and refs above), and direct estimates of high levels of additive genetic variation in fitness components (Houle, 1992) and sexually-selected characters (Pomiankowski & Møller, 1995) have more or less settled this question in the affirmative. However, this does not mean that mate choice based on a surrogate trait, such as age, would be a good strategy (Burt, 1995). Assuming that age can be assessed without error, it still remains to show that there is significant age variation in genetic fitness. Our results show that age variation under mutation–selection balance can, at least potentially, constitute a substantial fraction of the total genetic fitness variation in the population provided the total deleterious mutation rate is sufficiently high.

In a very comprehensive mutation–accumulation experiment, Houle et al. (1994) found an overwhelming pattern of positive correlations between several life-history traits in *Drosophila*. They looked at male and female longevity (\approx late viability), early and late fecundity, and two measures of overall fitness. Simmons, Preston and Engels (1980) found a similar pattern by showing that new mutations with a deleterious effect on viability must also have deleterious effects on other components of fitness in *Drosophila*. Although other studies in *Drosophila* and other species show a less convincing pattern (Crow & Simmons, 1983; Lynch, 1985; Fernandez & Lopez-Fanjul, 1996), it is possible that many new mutations have a generally deleterious effect on the organism. This result seems contrary to the negative genetic correlations that are often found in artificial selection and breeding studies.

There are three possible resolutions of this discrepancy. The first is that the genetic correlations are not primarily due to mutation–selection balance. In this case the results presented in this paper have little relevance and add nothing to the arguments in Hansen and Price (1995). The second is that there usually are more antagonistically pleiotropic and specifically late-acting mutations than indicated by the Houle et al. (1994) experiment. The third possibility is that antagonistically pleiotropic mutations are indeed rare, but have individually large effects on fitness components thereby producing a significant element of negative genetic correlations. Such mutations need not have a large effect on total fitness, as opposed to individual fitness components, and may be swamped by the more frequent unconditionally deleterious mutations.

If unconditionally deleterious mutations that affect all ages are the most common, the distribution of the load becomes very sensitive to when in life they appear. If new mutations are evenly distributed through life, as may be the case in male mammals, we suggest that the load increases with age, and if new mutations occur mainly early in life, as may be the case in female mammals, the load may decrease with age. A male-biased mutation rate will reinforce this pattern by making the male load increase and the female load decrease from zygote to average breeding age. In terms of mate choice we thus reach the somewhat counterintuitive prediction that males should prefer old females while females should prefer young males.

Why then do females often prefer old mates? The simplest explanation is that such preference is not a preference for genetic quality (Hansen & Price, 1995).

Kondrashov (1988) has argued that the sexual recombination of deleterious mutations can overcome the two-fold cost of sex if there is substantial synergistic epistasis and if the deleterious mutation rate, U , is very high. However, a very high mutation rate may also produce a substantial sex difference in the mutation load, thereby introducing an additional cost of sex. Redfield (1994) has shown through simulations that this cost may be a very significant problem in the maintenance of sex, and favor the evolution of parthenogenetic females. Our analysis adds to hers by quantifying the relationship between the sex difference in the load and mutation rates. Assume as an illustration that the total deleterious effect of new mutations, S , is on the order of 10% of mean fitness. If the male mutation rate is twice the female rate and the strength of selection the same in the two sexes, this means that the male load will be about 6.67% higher than the

female load (see Equation (16)). Maximally the difference will approach 20%. Clearly, this is a major cost of mating for females. The benefits of sex depends on the mutation rate U (Kondrashov, 1988), while the cost of mating depends on the total deleterious effect, S . This indicates that the benefits of sex are dependent on the average deleterious effects of new mutations, s , being relatively small. These relationships need further investigation. In particular, the strength and pattern of epistasis are of crucial importance.

Perhaps a surprising result of our modeling is that recessive mutations have almost no effect on the distribution of the load. Age and sex may be unimportant as factors in strategies of outbreeding.

The present analysis is in broad agreement with the results of Hansen and Price (1995) in showing that an increase in the mutation load with age is a realistic possibility. However, the consideration of detailed genetic mechanisms have added several insights. In our previous paper we suggested that negative genetic correlations between early- and late-expressed fitness components may make genetic fitness decrease with age. Such correlations are probably due to the segregation of antagonistically pleiotropic alleles, but the effect these have on the load can vary, depending strongly, for example, on whether the mutations are advantageous early and disadvantageous later or vice versa. It is also possible that antagonistically pleiotropic mutations play a small role in determining the distribution of the load at the same time as they have a strong effect on the genetic variation in the population. The above evidence on mutational effects indicate that unconditionally deleterious mutations may be more important for the distribution of the load. Furthermore, under mutation–selection balance the decrease in the sensitivity to fitness with age is counteracted by the increased variation in the trait. Indeed, the Haldane–Muller principle states that the strength of selection has no effect on the load. It is not clear that an increasing variance with age is borne out by data. Hughes and Charlesworth (1994) found that the variance in viability increased with age in *D. melanogaster*; but the coefficient of variation did not show a similar increase, and Promislow et al. (1996) found that the variance in viability first increased, but then decreased with age. Hence, it is not obvious which analysis is the most appropriate.

Finally, the distribution of the age- and sex-specific genetic fitness is foremost an empirical question for which there are little direct data. In a small experiment, we estimated the effect of father's age on several fit-

ness components in *D. melanogaster* (Price & Hansen, 1998). We found very little difference between two-day old and 14-day old fathers, but 33–34 day old fathers produced offspring with a performance reduction of 3–4% in larval viability and son mating ability, but there was little effect on daughter fecundity. These relatively small, inconsistent and late-acting effects do not point to a strong age effect, but more data are certainly needed before firm conclusions can be drawn.

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Appendix A. Mutations of small effects

At equilibrium

$$q_0 = \frac{1}{2} \int l_x m_x q'_x dx + \frac{1}{2} q_f, \quad (\text{A1})$$

To solve this we need to compute q'_x and q_f from q_0 . These are changed by selection and mutation occurring through the appropriate paths of the life cycle. Viability selection to an age x is determined by the marginal viability of the a-allele

$$\begin{aligned} & l_x(1 - s_x) \frac{H_0}{2q_0} + l_x(1 - d_x) \frac{R_0}{q_0} \\ & = l_x \left(1 - s_x + d_x \frac{R_0}{q_0} \right) \approx l_x(1 - s_x), \end{aligned} \quad (\text{A2})$$

where H_0 and R_0 are the frequencies of heterozygotes and aa-homozygotes, respectively, among the zygotes, and d_x is the selection coefficient against the aa-homozygote. We assume that the cumulative selection coefficient against the aa-homozygote is not smaller than s . Initially, we have to consider the genotype frequencies, as the unequal gene frequencies in males and females will make the population depart from Hardy–Weinberg equilibrium. In (A2), $H_0/2q_0$ is the probability that a given a-allele is associated with a A-allele, and R_0/q_0 the probability that it is associated with an a-allele. As $R_0 = q_m q_f = q_0^2$, we see that the last approximation is of the order of $d_x q_0$.

The mean viability is approximately l_x (to the order of $s_x q_0$). Adding mutation we get

$$\begin{aligned} q_x &= q_0(1 - s_x) + u_x(1 - q_0) \\ &\approx q_0(1 - s_x) + u_x. \end{aligned} \quad (\text{A3a})$$

Note that mutations accumulate independently of selection as they sit in the germ line and cannot affect the phenotype. The gene frequency after fertility selection can similarly be shown to be

$$q'_x \approx (1 - s_x - s'_x)q_0 + u_x, \quad (\text{A3b})$$

where we have used the approximation $(1 - s_x)(1 - s'_x) = 1 - s_x - s'_x$. The gene frequency among gametes produced by females is

$$q_f = (1 - s_f)q_0 + u_f, \quad (\text{A3c})$$

with s_f and u_f defined in the main text. Using (A3) in (A1) and solving for q_0 , we get

$$q_0 = \frac{u}{s}, \quad (\text{A4})$$

with $u = (u_m + u_f)/2$ and $s = (s_m + s_f)/2$. In deriving (A4), we used the assumption that $\int l_x m_x dx = 1$. This is a stable equilibrium. Using (A4) in (A3) we can get expressions for the equilibrium gene frequencies in the various stages of the life cycle. For example, the expression for the change in equilibrium gene frequency between two ages x and y is

$$q_y - q_x = \frac{u(s_x - s_y) + s(u_y - u_x)}{s}. \quad (\text{A5})$$

The mutation load corresponding to a certain frequency of the deleterious allele, q , is computed as $L(q) = 1 - W(q) \approx 2sq$. The variance in fitness is approximately $2s^2q$.

For recessive mutations (parameters defined in the main text) we get

$$q_x = q_0(1 - q_0 s_x) + u_x, \quad (\text{A6a})$$

$$q'_x = q_0[1 - q_0(s_x + s'_x)] + u_x, \quad (\text{A6b})$$

$$q_f = (1 - q_0 s_f)q_0 + u_f. \quad (\text{A6c})$$

Using this in (A1) we obtain $q_0 = \sqrt{u/s}$, and, by combining this with (A6), we can show that the changes in gene frequency through the paths of the life-cycle graph are identical to those given in (A3) for a non-recessive gene except that the s_x 's are now interpreted as the deleterious effect of the aa-homozygote. For a recessive gene, the mutation load is computed as $L(q) = 1 - W(q) \approx sq^2$. The variance in fitness is also sq^2 .

Appendix B. Lethal mutations

Consider first a dominant lethal allele that acts before reproduction and before age x in males. As every carrier dies, every occurrence of the allele in males must be due to new mutation. Hence, $q_x = u_x$. Using this in (A1) and assuming $q_f = q_0 + u_f$ yields

$$q_0 = 2u. \quad (\text{B1})$$

If the allele is neutral among females, $s = 1/2$ and the load among zygotes is $L_0 \approx 2sq_0 = 2u$. Among males of age x the load is $L_x \approx u_x$. If the mutation is also lethal in females, $s = 1$, $q_f = u_f$ and $q_0 = u$, so that $L_0 = 2u$ and $L_x = 2u_x$.

For a recessive male-lethal allele the marginal viability is $l_x H_0/2q_0$. Assuming mean viability is l_x , we get

$$q_x = \frac{H_0}{2} + u_x = \frac{q_m + q_f - 2q_m q_f}{2} + u_x. \quad (\text{B2})$$

Assuming $q_f = q_0 + u_f$, and using $q_m = 2q_0 - q_f = q_0 - u_f$ in (B2) and (A1), we get

$$q_0 = \sqrt{2u + u_f^2} \approx \sqrt{2u}, \quad (\text{B3a})$$

$$q_x = q_0 + u_x - 2u. \quad (\text{B3b})$$

And the load is $L_0 \approx sq_0^2 = q_0^2/2 = u$ and $L_x \approx L_0 + (u_x - 2u)\sqrt{u}$. If the allele is also recessive lethal in females, $q_f = H_0/2 + u_f$ and solving the equations

$$q_m = \frac{H_0}{2} + u_m = \frac{q_m + q_f - 2q_m q_f}{2} + u_m, \quad (\text{B4a})$$

$$q_f = \frac{H_0}{2} + u_f = \frac{q_m + q_f - 2q_m q_f}{2} + u_f, \quad (\text{B4b})$$

for q_m and q_f yields

$$q_0 = \sqrt{u + \frac{(u_m - u_f)^2}{4}} \approx \sqrt{u}, \quad (\text{B5a})$$

$$q_x = q_0 + u_x - u, \quad (\text{B5b})$$

$$q_f = q_0 + u_f - u. \quad (\text{B5c})$$

In this case $L_0 \approx q_0^2 \approx u$ and $L_x \approx L_0 + 2(u_x - u)\sqrt{u}$.

References

- Alcock, J., 1984. *Animal Behaviour: An Evolutionary Approach*, 3rd ed. Sinauer Assoc. Sunderland, Massachusetts.
 Andersson, M., 1994. *Sexual Selection*. Princeton Univ. Press, Princeton.

- Bürger, R. & J. Hofbauer, 1994. Mutation load and mutation-selection balance in quantitative genetic traits. *J. Math. Biol.* 32: 193–218.
- Burt, A., 1995. The evolution of fitness. *Evolution* 49: 1–8.
- Chang, B.H.-J., L.C. Shimmin, S.-K. Shyue, D. Hewett-Emmett & W.-H. Li, 1994. Weak male-driven molecular evolution in rodents. *PNAS* 91: 827–831.
- Charlesworth, B., 1987. The heritability of fitness, pp. 21–40 in *Sexual Selection: Testing the Alternatives*, edited by J.W. Bradbury and M. Andersson (eds), Wiley, NY.
- Charlesworth, B., 1990a. Optimization models, quantitative genetics, and mutation. *Evolution* 44: 520–538.
- Charlesworth, B., 1990b. Mutation-Selection balance and the evolutionary advantage of sex and recombination. *Genet. Res.* 55: 199–221.
- Charlesworth, B., 1994. *Evolution in age-structured populations*, 2nd ed. Cambridge University Press, Cambridge.
- Charlesworth, B., D. Charlesworth & M.T. Morgan, 1990. Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* 347: 380–382.
- Cote, I.M. & W. Hunte, 1993. Female redlip blennies prefer older males. *Anim. Behav.* 46: 203–205.
- Crow, J.F., 1993a. Mutation, mean fitness, and genetic load. *Oxford Surveys in Evol. Biol.* 9: 3–42.
- Crow, J.F., 1993b. How much do we know about spontaneous human mutation rates? *Env. Mol. Mutagen.* 21: 122–129.
- Crow, J.F. & M.J. Simmons, 1983. The mutation load in *Drosophila*, pp. 1–35 in *The Genetics and Biology of Drosophila*, vol 3c, edited by M. Ashburner, H.L. Carson, and J.N. Thompson. Academic Press, London.
- Ellegren, H. & A.-K. Fridolfson, 1997. Male-driven evolution of DNA sequences in birds. *Nat. Genet.* 17: 182–184.
- Eyre-Walker, A. & P. D. Keightley, 1999. High genomic deleterious mutation rates in hominids. *Nature* 397: 344–347.
- Favor, J., 1994. Spontaneous mutations in germ cells of the mouse: estimates of mutation frequencies and a molecular characterization of mutagenic events. *Mutation Res.* 304: 107–118.
- Fernandez, J. & C. Lopez-Fanjul, 1996. Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. *Genetics* 143: 829–837.
- Fry, J.D., P.D. Keightley, S.L. Heinsohn & S.V. Nuzhdin, 1999. New estimates of the rates and effects of mildly deleterious mutations in *Drosophila melanogaster*. *PNAS* 96: 574–579.
- Garcia-Dorado, A., 1997. The rate and effects distribution of viability mutation in *Drosophila*: minimum distance estimation. *Evolution* 51: 1130–1139.
- Green, M.M., 1988. Mobile DNA elements and spontaneous gene mutation, pp. 41–50 in *Eukaryotic Transposable Elements as Mutagenic Agents*, edited by M.E. Lambert, J.F. McDonald and I.B. Weinstein. Cold Spring Harbor Press, Cold Spring Harbor, NY.
- Halliday, T.R., 1978. *Sexual selection and mate choice*, in *Behavioral Ecology*. Blackwell Press, London, UK.
- Halliday, T.R., 1983. The study of mate choice, pp. 3–32 in P. Bateson (ed.), *Mate Choice*. Cambridge Univ. Press, Cambridge, UK.
- Haldane, J.B.S., 1937. The effect of variation on fitness. *Amer. Natur.* 71: 337–349.
- Haldane, J.B.S., 1947. The rate of mutation of the gene for hemophilia and its segregation ratios in males and females. *Ann. Eugen.* 13: 262–271.
- Hamilton, W.D., 1966. The moulding of senescence by natural selection. *J. Theor. Biol.* 12: 12–45.
- Hansen, T.F. & D.K. Price, 1995. Good genes and old age: Do old mates provide superior genes? *J. Evol. Biol.* 8: 759–778.
- Heisler, I. L., M.B. Andersson, S.J. Arnold, C.R. Boake, G. Borgia, G. Hausfater, M. Kirkpatrick, R. Lande, J. Maynard Smith, P. O'Donald, A.R. Thornhill & F.J. Weissing, 1987. The evolution of mating preferences and sexually selected traits. Group report, pp. 96–118 in *Sexual Selection: Testing the Alternatives*, edited by J.W. Bradbury and M.B. Andersson. Wiley, NY.
- Heisler, I.L., 1984. A quantitative genetic model for the origin of mating preferences. *Evolution* 38: 1283–1295.
- Houle, D., 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130: 195–204.
- Houle, D., K.A. Hughes, D.K. Hoffmaster, J. Ihara, S. Assimacopoulos, D. Canada & B. Charlesworth, 1994. The effects of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. *Genetics* 138: 773–785.
- Houle, D., B. Morikawa & M. Lynch, 1996. Comparing mutational variabilities. *Genetics* 143: 1467–1483.
- Hughes, K.A. & B. Charlesworth, 1994. A genetic analysis of senescence in *Drosophila*. *Nature* 367: 64–66.
- Keightley, P.D., 1996. Nature of deleterious mutation load in *Drosophila*. *Genetics* 144: 1993–1999.
- Kirkpatrick, M., 1987. Sexual selection by female choice in polygynous animals. *Ann. Rev. Ecol. Syst.* 18: 43–70.
- Kokko, H., 1998. Good genes, old age and life-history trade-offs. *Evol. Ecol.* 12: 739–750.
- Kokko, H. & J. Lindström, 1996. Evolution of female preference for old mates. *Proc. R. Soc. B.* 263: 1533–1538.
- Kondrashov, A.S., 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature*, 336, 435–440.
- Kondrashov, A.H. & D. Houle, 1994. Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* 258: 221–227.
- Lynch, M., 1985. Spontaneous mutations for life-history characters in an obligate parthenogen. *Evolution* 39: 804–818.
- Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva & J. Willis, 1999. Perspective: Spontaneous deleterious mutation. *Evolution* 53: 645–663.
- Manning, J.T., 1985. Choosy females and correlates of male age. *J. theor. Biol.* 116: 349–356.
- Manning, J.T., 1989. Age-advertisement and the evolution of the peacock's train. *J. Evol. Biol.* 2: 379–384.
- Modell, B. & A. Kuliev, 1990. Changing paternal age distribution in the human mutation rate in Europe. *Hum. Genet.* 86: 198–202.
- Montadon, A.J., P.M. Green, D.R. Bentley, R. Ljung, S. Kling, I.M. Nilsson & F. Giannelli, 1992. Direct estimate of the haemophilia B (factor IX deficiency) mutation rate and the ratio of the sex specific mutation rates in Sweden. *Hum. Genet.* 89: 319–322.
- Mukai, T., S.I. Chigusa, L.E. Metler & J.F. Crow, 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* 72: 335–355.
- Muller, H.J., 1950. Our load of mutations. *Amer. J. Hum. Genet.* 2: 111–176.
- Parsons, P.A., 1964. Parental age and the offspring. *Quart. Rev. Biol.* 39: 258–275.
- Partridge, L. & N.H. Barton, 1993. Optimality, mutation and the evolution of ageing. *Nature* 362: 305–311.
- Pomiankowski, A., 1988. The evolution of female mate preferences for male quality, in *Oxford Surveys in Evolutionary Biology*, vol 5, edited by P.H. Harvey and L. Partridge, Oxford Univ. Press, Oxford, UK.
- Pomiankowski, A. & A.P. Møller, 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* 260: 21–29.

- Pomiankowski, A., Y. Iwasa & S. Nee, 1991. The evolution of costly mate preferences: I. Fisher and biased mutation. *Evolution* 45: 1422–1430.
- Price, D.K. & T.F. Hansen, 1998. How does offspring quality change with age in male *Drosophila melanogaster*? *Behav. Genet.* 28: 395–402.
- Price, G.R., 1970. Selection and covariance. *Nature* 227: 520–521.
- Promislow, D.E.L., M. Tatar, A.A. Khazaeli & J.W. Curtsinger, 1996. Age specific patterns of genetic variance in *Drosophila melanogaster*. I. mortality. *Genetics* 143: 839–848.
- Redfield, R.J., 1994. Male mutation rates and the cost of sex for females. *Nature* 369: 145–147.
- Rice W.R., 1988. Heritable variation in fitness as a prerequisite for adaptive female choice: the effect of mutation–selection balance. *Evolution* 4: 817–820.
- Rose, M., 1991. *Evolutionary Biology of Aging*. Oxford Univ. Press, Oxford, UK.
- Rowe, L. & D. Houle, 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Genetics* 263: 1415–1421.
- Shimmin, L.C., B.H.-J. Chang & W.-H. Li, 1993. Male-driven evolution of DNA sequences. *Nature* 362: 745–747.
- Simmons, M.J. & J.F. Crow, 1977. Mutations affecting fitness in *Drosophila* populations. *Ann. Rev. Genet.* 11: 49–78.
- Simmons, M.J., C.R. Preston & W.R. Engels, 1980. Pleiotropic effects of mutations affecting viability in *Drosophila melanogaster*. *Genetics* 94: 467–475.
- Trivers, R., 1972. Parental investment and sexual selection, pp. 136–179 in *Sexual Selection and The Descent of Man 1871–1971*, edited by B. Campbell, Aldine Press, Chicago, IL.