

# Redundant gene functions and natural selection

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## Abstract

Redundant gene functions are ubiquitous, and they are a potentially important source of evolutionary innovations on the biochemical level. It is therefore highly desirable to understand the mechanisms governing their evolution. Gene duplication is clearly a prominent mechanism generating redundant genes. However, because redundancy provides a protective effect against deleterious mutations, natural selection might be involved in generating and maintaining partial redundancy. Although much experimental data on redundant genes have accumulated, no data are available that could elucidate what role selection has in their evolution. As a first step towards answering this question, a conceptually simple mathematical model for the evolution of redundancy is introduced. Its main result is that selection cannot only maintain but also increase redundancy among genes in a population provided (i) that mutation generates sufficient variation in redundancy and (ii) that populations are large. The population biological process at work is somewhat unusual. Selection does not act on the (nonexisting) differential fitness between individuals with different degrees of redundancy. Rather, it acts through the low number of offspring with deleterious mutations that individuals with redundant genes will generate. Moreover, even if populations are small and variation in redundancy is low, selection will substantially slow the 'decay' of redundancy caused by mutation and genetic drift. Methodological problems in determining degrees of redundancy experimentally are discussed, as well as issues concerning the relation of redundancy to genetic canalization. The latter two phenomena necessitate a differentiated view of neutral mutations, where some neutral mutations are only neutral because their effects on gene products are absorbed by the epigenetic system.

## Introduction

Genes with redundant functions are ubiquitous. Numerous examples have been found in invertebrates (Hoffman, 1991; Cadigan *et al.*, 1994; González-Gaitán *et al.*, 1994; Li & Noll, 1994a,b), vertebrates (Joyner *et al.*, 1991; Higashijima *et al.*, 1992; Saga *et al.*, 1992; Lohnes *et al.*, 1993; Lufkin *et al.*, 1993; Condie & Capecchi, 1995; Hanks *et al.*, 1995) and micro-organisms (Kataoka *et al.*, 1984; Basson *et al.*, 1986; Lundgren *et al.*, 1991; Goldstein, 1993; Goodson & Spudich, 1995; for reviews

see Tautz, 1992; Thomas, 1993). Among these, no obvious commonalities exist with respect to the biochemical role of the redundant genes. They include transcriptional regulators (González-Gaitán *et al.*, 1994; Li & Noll, 1994a,b; Condie & Capecchi, 1995; Hanks *et al.*, 1995), extracellular matrix proteins (Saga *et al.*, 1992), protein kinases (Hoffmann, 1991; Lundgren *et al.*, 1991), genes potentially involved in intracellular force generation (Goldstein, 1993; Goodson & Spudich, 1995) and enzymes of metabolic pathways (Basson *et al.*, 1986). Despite this functional diversity, all genes with redundant functions share one property. The loss-of-phenotype of the gene is absent, or it is much weaker than expected from independent lines of evidence. Such lines of evidence include strong sequence conservation (Kataoka *et al.*, 1984; Joyner *et al.*, 1991; Goldstein, 1993) or

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the relation of a morphological defect to the size of the expression domain for gene products that presumably act in a cell-autonomous way (Joyner *et al.*, 1991; Higashijima, 1992; Condie & Capecchi, 1995). Sometimes the expression of such genes might serve no biological purpose at all. In general, however, one or more other genes exist with functions indistinguishable from (e.g. Basson *et al.*, 1986; Higashijima *et al.*, 1992) but at least overlapping with (e.g. Joyner *et al.*, 1991; Lufkin *et al.*, 1993; Condie & Capecchi, 1995) that of the presumably redundant gene. These two cases are often referred to as full and partial redundancy. The strong phenotype of double (triple etc.) mutations in the respective genes then serves as proof that they do fulfil a biological function.

The available evidence suggests that gene duplication events are the main source of redundant gene functions (e.g. Basson *et al.*, 1986; Joyner *et al.*, 1991; Higashijima *et al.*, 1992). Immediately after a duplication becomes fixed in a population, the two copies (original/duplicate) are completely redundant. Subsequent mutations either cause the silencing of one of the two copies or cause a functional diversification between them. In the latter case, redundancy will decrease over time. However, detailed genetic studies on the early development of *Drosophila* (Hülkamp & Tautz, 1991; Schulz & Tautz, 1993) have led to the suggestion (Tautz, 1992) that previously dissimilar gene functions may have been recruited into redundant control mechanisms of developmental pattern formation events. This would imply that redundancy can somehow be selected for because it 'masks' mutations that would otherwise be deleterious. A similar masking of deleterious mutations has been involved in models for the evolution of diploid life cycles (Bengtsson, 1992; Otto & Goldstein, 1992) and of dominance modifiers (e.g. Wagner & Bürger, 1985). The idea is attractive not only because of the potential 'buffering' effect of redundancy, but also because redundant gene functions may be an important source of evolutionary novelty on the biochemical level (Walsh, 1995).

The subject of this contribution is to determine what role natural selection might play in the evolution of redundancy. To this end, a conceptually simple mathematical model is introduced. In similar contexts, e.g. in studies regarding the evolution of diploidy or dominance (Wagner & Bürger, 1985; Bengtsson, 1992; Otto & Goldstein, 1992), modifier models are quite popular. However, a modifier approach has been deliberately avoided in this case. The reason is that nothing in the available evidence supports the existence of modifier loci of redundancy. The assumption of modifiers would be quite unnatural in this context. Instead, redundancy can be easily understood on the basis of functionally similar gene products alone (see also below), and the structure of the model presented here takes this into account. Its only essential ingredients are the following three assumptions. First, it is assumed that the functional similarity among

two or more genes can be quantified in some way. The appropriate measure of redundancy will clearly depend on the type of genes under consideration. Two examples may help to illustrate what form such a measure could take. Consider first genes encoding transcriptional regulators. Their products frequently regulate the expression of a large number of genes. Functional differences among transcriptional regulators with similar protein sequences may exist, among other reasons, through differences in their DNA binding domain oligomerization domain or transcriptional activation domains (Ptashne, 1988; Lamb & McKnight, 1991; Tjian & Maniatis, 1994). If two similar transcriptional regulators influence the expression of a nonidentical, but overlapping, set of genes, then the extent of overlap, i.e. the fraction of genes that they both regulate in the same way, could be taken as a measure of functional redundancy. As a second example, in organismal development often two or more genes with apparently identical biochemical functions show a non-congruent, but overlapping, spatiotemporal gene expression pattern. Here, partial redundancy arises through the divergence of *cis*-acting (enhancer or promoter) regions which drive the genes' expression patterns (Cadigan *et al.*, 1994; Li & Noll, 1994a,b; Hanks *et al.*, 1995; Wang *et al.*, 1996). It is well known that the expression of a gene in different tissues is frequently driven by different enhancer elements (Carroll, 1990). Thus, mutations in the regulatory region of a gene may abolish the expression of a gene (i) in a region where it is coexpressed with its partially redundant partner, (ii) in a region where it is expressed by itself or (iii) in both regions. The experimental evidence suggests that phenotypic effects of mutations are likely to occur only in regions where the genes are not coexpressed (for genes acting cell-autonomously). Clearly, the smaller the overlap of the expression domains of such partially redundant genes, the more likely it is that a regulatory mutation will abolish the expression of a gene in a region where it is not coexpressed with the redundant partner. Thus, the extent of overlap in expression patterns can serve as a measure of redundancy among the genes. Quantitative experimental data on such expression patterns are still sparse. However, gene expression studies involving large numbers of genes that will provide such data are becoming increasingly feasible as various genome projects are advancing rapidly (e.g. Nowak, 1995).

The second central assumption of the model is that mutations can change the functional overlap or redundancy among genes. Third, it is assumed that mutations in genes with low redundancy are more likely to have deleterious effects than mutations in genes with high redundancy. The last assumption deserves further comment. Virtually none of the available studies of redundant genes explicitly deals with fitness effects of loss-of-function mutations in redundant genes. Such effects may be profound even if morphological effects are weak or nonexistent (e.g. Lufkin *et al.*, 1993). Also, most

cases reported in the literature concern the effects of loss-of-function mutations. Naturally occurring mutations will cover a much wider spectrum of effects. Assumption three is essentially an extrapolation from weak morphological effects to weak fitness effects of mutations. It says that compared to a strong (morphological) effect of a loss-of-function mutation, a weaker (morphological) effect of a loss-of-function mutation due to redundancy will cause a lower probability of *some* mutation having a deleterious fitness effect.

The model is used to assess the influence that selection, mutation, drift and linkage may have on the evolution of partial redundancy. Questions regarding the rate of gene silencing and on the evolution of families of fully redundant genes could easily be incorporated. However, they will not be addressed here, because a sizable literature exists already in this area (e.g. Ohno, 1970; Nei & Roychoudhury, 1973; Kimura & King, 1979; Maruyama & Takahata, 1981; Watterson, 1983; Marshall *et al.*, 1994; Walsh, 1995).

## Model and results

### Selection and mutation

The model is concerned with an infinite population of haploid, dioecious, randomly mating organisms. The genic mutation rate is denoted as  $\mu$  ( $\mu \ll 1$ ), and it is assumed that all mutations that are not neutral on the phenotypic level are effectively lethal. Neutral mutations that do not affect any aspect of the function of a gene product are not considered. Both the assumption of lethality and the assumption of haploidy are used merely because they best illustrate the central principles involved.

The model will be developed in two steps. First, a simplified 'symmetric' model for redundancy among two genes is introduced. Second, a more general model for redundancy among two genes, suitable to describe the evolution of redundancy among many genes, is developed. The merit of the first model lies in the fact that important properties of the second model can be described in terms of the formally much simpler first model.

For two genes with overlapping functions, the central concept of the model lies in the notion that this overlap can be quantified by some measure, which will be called the 'redundancy' between these genes. More specifically, the variable  $r$ ,  $0 \leq r \leq 1$ , denotes the probability that a mutation in either gene has no deleterious phenotypic effect. If  $r = 0$ , any mutation will have a deleterious effect, and the respective organism will not survive to the next generation. For  $r > 0$ , if one of the two genes is affected by a mutation, it is assumed that the effect of the mutation will be strongly deleterious with probability  $1 - r$ , such that the respective organism will not survive to the next generation. With probability  $r$ , the organism will survive, in which case the mutation is phenotypi-

cally neutral despite the fact that it affected the function of one of the genes. The effect of the mutations was 'buffered' by the other gene. In terms of one of the concrete examples discussed in the introduction, one might think of  $r$  as a measure of the spatiotemporal overlap in expression pattern between two developmental genes whose products have identical biochemical functions. Redundancy  $r$  will be high if their spatiotemporal expression domains are congruent; it will be zero if they are expressed in disjoint body regions or nonoverlapping time-intervals. While not affecting the fitness of an organism, a mutation may change  $r$  itself. This will be modelled by a (conditional) probability density  $m(r^*/r)$ , where

$$m(r^*/r)dr^*$$

denotes the probability that the redundancy of a gene pair with redundancy  $r$  before mutation lies in  $(r^*, r^* + dr^*)$  after mutation. For now, only the minimal assumption is made that mutation changes redundancy on average by a factor  $\lambda$  ( $0 < \lambda \leq 1$ ), i.e.

$$\int_0^1 r^* m(r^*/r) dr^* = \lambda r. \quad (1)$$

$\lambda = 1$  implies that some mutations increase  $r$ , while others decrease it, but that mutations leave  $r$  on average unchanged. More realistic may be the case of  $\lambda < 1$ , where mutations cause  $r$  to decrease on average, i.e. they are responsible for the divergence of two genes' functions. In this scenario, mean redundancy in a population would 'decay' exponentially in the absence of natural selection on the two genes' function. Here, it will be assumed that  $\lambda > 0.5$ . Notice that a value of  $\lambda$  as small as 0.5 implies that each mutation reduces  $r$  to half of its value before mutation, i.e. a very rapid decay of  $r$  through mutation.

On the level of a population of organisms, mutation and selection have opposing effects on the distribution of  $r$  within the population. While mutation tends to reduce  $r$ , selection preferentially eliminates organisms with low  $r$  because they have a higher probability of generating mutants with deleterious phenotypic effects. Denote as  $p_t(r)$  the probability density of the distribution of  $r$  within the population at time  $t$ . With the notation introduced thus far, the following integral equation describes the evolution of redundancy in the population, assuming that generations are nonoverlapping:

$$p_{t+1}(r) = \frac{(1 - 2\mu)p_t(r) + 2\mu \int_0^1 zp_t(z)m(r|z)dz}{(1 - 2\mu) + 2\mu \int_0^1 zp_t(z)dz}. \quad (2)$$

The left-hand term of the numerator is the contribution to the next generation of the fraction  $1 - 2\mu$  of the population that was not affected by mutations. The right-hand term of the numerator represents the fraction of the population that was affected by neutral mutations changing redundancy. The integrand of this term,

$zp_t(z)m(r|z)dz$ , represents the probability that the redundancy of a mutated individual before mutation was  $z$ , that the mutation was neutral and that the mutation changed redundancy from  $z$  to  $r$ . The denominator is a normalization factor representing the fraction of organisms that survive in generation  $t$ . It is simply the fraction of the population in which no mutation (left-hand term) or only neutral mutations occurred (right-hand term). The factors 2 in front of  $\mu$  are a consequence of the two-gene system considered, and of the assumption that  $\mu^2 \approx 0$ . Note that the mean fitness of the population 'after selection', i.e. the mean fitness of those individuals that bear no lethal mutations, will be equal to one because all phenotypically deleterious mutants are eliminated. Only the distribution of redundancy values in the population changes via mutation and natural selection. Two initial conditions  $p_0(r)$  are of special importance. First, two genes may have very little functional overlap in all organisms of the population. In this case  $p_0(r) \approx \delta(r - \epsilon)$  will be used, where  $0 \leq \epsilon \ll 1$  and  $\delta$  denotes the Dirac delta function (Arfken, 1985). Second, and perhaps more importantly, one may study the evolution of redundancy as a result of a recent gene duplication event, after which the duplication has become fixed in the population. For this case,  $p_0(r) \approx \delta(r - 1 + \epsilon)$  will be used. The fixation of the duplication is of no concern here. Even if gene duplications do not confer a selective advantage in and of themselves, they will become fixed in a population provided that they occur at a finite rate, as Clark (1994) has shown.

The accumulation of pseudogenes by null-mutations shortly after gene duplication events could easily be incorporated into the model, because null-mutations are a special case of mutation events reducing  $r$  from 1 to 0. However, here only the gradual evolution  $r$ , and not the complete loss of gene functions (gene silencing) will be studied, because a large body of literature already exists in the latter area (e.g. Ohno, 1970; Nei & Roychoudhury, 1973; Kimura & King, 1979; Maruyama & Takahata, 1981; Watterson, 1983; Ohta, 1987; Marshall *et al.*, 1994; Walsh, 1995).

Denote the  $k$ th moment of  $r$  in generation  $t$  as  $\bar{r}_t^k = \int_0^1 r^k p_t(r) dr$ , and write  $\bar{r}$  for  $\bar{r}^1$ . Equations 1 and 2 then result in the following two recursion relations for the first- and second-order moments:

$$\bar{r}_{t+1} = \frac{(1 - 2\mu)\bar{r}_t + 2\mu\lambda\bar{r}_t^2}{(1 - 2\mu) + 2\mu\bar{r}_t}, \quad (3a)$$

and

$$\bar{r}_{t+1}^2 = \frac{(1 - 2\mu)\bar{r}_t^2 + 2\mu \int_0^1 \int_0^1 r^2 zp_t(z)m(r|z)dzdr}{(1 - 2\mu) + 2\mu\bar{r}_t}. \quad (3b)$$

Denote as  $p_\infty(r)$  any equilibrium distribution of  $p_r(t)$  attained in the limit as  $t \rightarrow \infty$ . A simple manipulation of eqn 2 shows that  $p_\infty(r)$  can be expressed as

$$p_\infty(r) = \frac{\int_0^1 zp_\infty(z)m(r|z)dz}{\int_0^1 zp_\infty(z)dz}, \quad (4)$$

and that it is therefore independent of the mutation rate. This holds *a fortiori* for all moments at equilibrium. Equation 3 thus becomes

$$\bar{r}_\infty = \frac{\lambda\bar{r}_\infty^2}{\bar{r}_\infty}, \quad (5a)$$

and

$$\bar{r}_\infty^2 = \frac{\int_0^1 \int_0^1 r^2 zp_\infty(z)m(r|z)dzdr}{\bar{r}_\infty}. \quad (5b)$$

Equation 5a shows that the relation

$$\frac{\bar{r}_\infty^2}{\bar{r}_\infty^2} = \lambda \quad (6)$$

holds in equilibrium. Note the generality of this relation. It holds regardless of any specific assumptions about the mutation process other than eqn 2, regardless of the initial distribution of redundancy  $p_0(r)$ , and regardless of the particular nature of the equilibrium distribution  $p_\infty(r)$ . The left-hand side can also be written in terms of the coefficient of variation of  $r$ , yielding

$$\frac{\sigma_r}{\bar{r}_\infty} = \sqrt{\frac{1 - \lambda}{\lambda}} \quad (7)$$

where  $\sigma_r$  is the standard deviation of  $r$  in equilibrium. Figure 1 shows a plot of this analytical prediction together with the results of Monte Carlo simulations of the underlying selection-mutation process for  $0.5 < \lambda < 0.99$ , and for a distribution of mutation effects that is

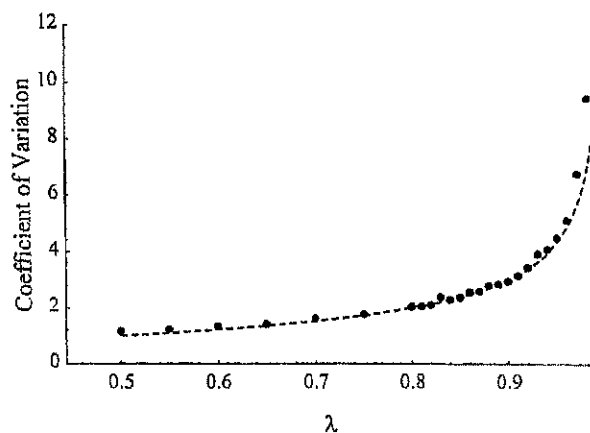


Fig. 1 Coefficient of variation  $\sigma_r/\bar{r}_\infty$  in mutation-selection equilibrium. Shown are the analytical prediction (eqn 7) (dashed line) along with results of Monte-Carlo simulations (dots) of the underlying selection-mutation process. Simulations were carried out as described in the appendix. Simulation parameters:  $N = 10^4$ ,  $\mu = 1$ ,  $c = 0.5$ ,  $\bar{r}_0 = 1$ .

Gaussian in the interior of (0, 1). A description of the numerical methods used in these simulations is given in the appendix. It follows from eqn 7 that mean equilibrium redundancy  $r$  approaches zero as  $\lambda \rightarrow 0$ , i.e. for small  $\lambda$ , redundancy decays so rapidly that selection cannot effectively counteract that decay. For  $\lambda \rightarrow 1$ , eqn 7 implies that  $\sigma_r \rightarrow 0$ . In this case, the only candidates for equilibrium distributions are those described by Dirac delta distributions,  $p_\infty(r) = \delta(r - r_0)$ , for some  $r_0$ ,  $0 \leq r_0 \leq 1$ . However, while the mean of such distributions is invariant under the evolution eqn 2 for  $\lambda = 1$ , the second moment,  $\int_0^1 r^2 \delta(r - r_0) dr = r_0^2$  is in general not. Substituting  $\delta(r - r_0)$  into the right-hand side of eqn 2 and calculating the second moment of the resulting distribution yields

$$\bar{r}^2 = \frac{(1 - 2\mu)r_0^2 + 2\mu r_0 \overline{m^2}(r_0)}{(1 - 2\mu) + 2\mu r_0},$$

where  $\overline{m^2}(r_0) = \int_0^1 r^2 m(r|r_0) dr$ . The right-hand side is equal to  $r_0^2$  only if  $m(r|r_0) = \delta(r - r_0)$ . Thus,  $\delta(r - r_0)$  is a candidate for an equilibrium distribution in this case. However, it is shown in the appendix that  $\delta(r - r_0)$  is not a stable equilibrium distribution for any  $r_0 < 1$  and  $\lambda = 1$ . Therefore,  $\delta(1 - r)$  is the only possible candidate for a stable solution to the long-term behaviour of the system. In other words, if mutations do leave  $r$ , on average, unchanged, the only possible equilibrium distribution of  $r$  is one in which all organisms have redundancy  $r = 1$ , because mutation does not 'resist' the force of selection which drives the population towards high mean redundancy.

To permit analysis of mutation-selection equilibria in the interior of (0, 1) for  $0 < \lambda < 1$ , one merely has to assume that the variance of redundancy caused by mutations is approximately constant for values of  $r$  sufficiently distant from the boundary  $r = 0$ , i.e.  $\sigma_m^2 = \int_0^1 (r^* - \lambda r)^2 m(r^*|r) dr^* \approx \text{const}$ . An intuitive argument can be made to suggest that there exists an equilibrium distribution  $p_\infty(r)$  whose mean is independent of  $\bar{r}_0$ . The absolute difference in mean redundancy before and after mutation decreases with decreasing  $\bar{r}$ , because the mean difference in redundancy before and after mutation of a gene pair with redundancy  $r$  is  $(1 - \lambda)r$ . At some value of  $\bar{r}$ , the selective pressure increasing redundancy and the mutational pressure decreasing redundancy will exactly balance and  $\bar{r}$  will cease to change.

For constant mutation variance, eqn 5b simplifies to

$$\bar{r}_\infty^2 = \frac{\sigma_m^2 \bar{r}_\infty + \lambda^2 \bar{r}_\infty^3}{\bar{r}_\infty}. \quad (8)$$

Neglecting central moments of higher than second order in  $r$  yields  $\bar{r}_\infty^3 \approx \bar{r}_\infty (3\bar{r}_\infty^2 - 2\bar{r}_\infty^2)$  (note that  $\int_0^1 (r - \bar{r})^k p(r) dr \rightarrow 0$  as  $k \rightarrow \infty$ ), and thus

$$\bar{r}_\infty^2 = \sigma_m^2 + \lambda^2 (3\bar{r}_\infty^2 - 2\bar{r}_\infty^2). \quad (9)$$

The two equations in two variables eqns 5a and 9 can be solved for  $\bar{r}_\infty$ , yielding

$$\bar{r}_\infty = \sigma_m \sqrt{\frac{\lambda}{2\lambda^3 - 3\lambda^2 + 1}}. \quad (10)$$

The approximations leading to eqn 10 are only sensible for intermediate values of  $\lambda$  and  $\sigma_m$ , i.e. values for which  $\bar{r}_\infty$  is sufficiently far away from the boundary  $r = 1$ . This is not only because  $p_\infty(r)$  is likely to be skewed close to the boundary, but also because if  $\bar{r}_\infty = 1 - \epsilon$  ( $\epsilon \ll 1$ ), then  $\bar{r}_\infty^2 - (\bar{r}_\infty)^2 < \epsilon$ , and as a consequence  $\sigma_m^2$  needs to be of order  $\epsilon$  as well. The pole of eqn 10 at  $\lambda = 1$  reflects the fact that eqn 10 is valid only for a limited parameter range.

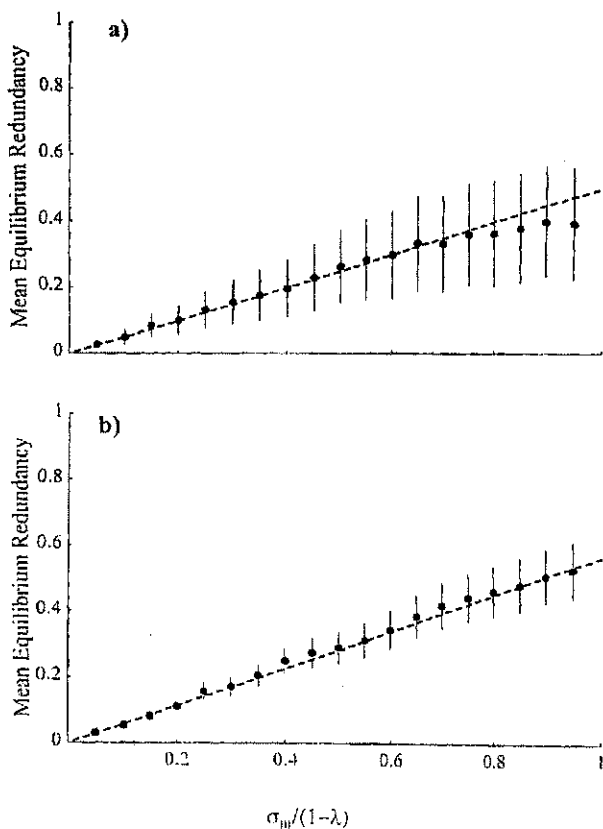
Because both  $\bar{r}_\infty$  and  $\bar{r}_\infty^2$  are uniquely specified, a distribution with mean eqn 10 is the only possible equilibrium distribution in the interior of (0, 1). This distribution is also stable to small perturbations in its first two moments, as is shown in the appendix. Distributions at either boundary,  $\delta(1 - r)$  or  $\delta(r)$ , are no candidates for (stable) equilibria for  $0 < \lambda < 1$ .  $\delta(1 - r)$  is not an equilibrium distribution, as can be seen from substituting it into the right-hand side of eqn 3b.  $\delta(r)$ , on the other hand, is an equilibrium distribution, but it is not stable, as is shown in the appendix.

If  $\sigma_m$  is much smaller than  $1 - \lambda$  (an upper bound for the mean mutational change in redundancy of any individual), then insufficient variation will be generated to drive the selection process.  $\bar{r}_t$  will 'ratchet' towards zero. On the other hand, the effect of selection will be less obvious if  $\sigma_m$  is very large, e.g. if large values of  $r$  are created from small values by mutation alone. For these reasons, it is sensible to scale  $\sigma_m$  in units of  $1 - \lambda$ . More precisely, to validate the accuracy of eqn 10 by comparing it with the result of Monte Carlo simulations of the underlying mutation-selection process,  $\sigma_m = c(1 - \lambda)$  will be used, where  $c$  is some real number in (0, 1). Equation 10 then becomes

$$\bar{r}_\infty = c \sqrt{\frac{\lambda}{2\lambda + 1}}. \quad (11)$$

The example in Fig. 2 shows that eqn 11 agrees well with the result of Monte-Carlo simulations over a range of mutation variances (values of  $c$ ), and both for slow ( $\lambda = 0.9$ ) and fast ( $\lambda = 0.5$ ) mutational decay of redundancy.

In summary, if mutations diversify gene functions (i.e. if  $\lambda < 1$ ), the amount of variation in redundancy generated by mutations determines whether significant amounts of redundancy can be maintained by natural selection. If mutation does not have an intrinsic tendency to diversify the functions of two genes (i.e. if  $\lambda = 1$ ), natural selection drives the population to a situation of complete redundancy for all genes. The mutation rate itself, however, does not influence equilibrium mean redundancy.

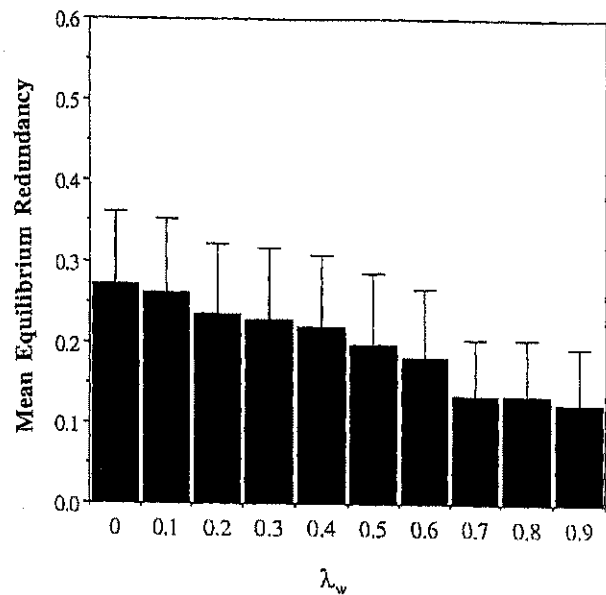


**Fig. 2** Mean redundancy in mutation-selection equilibrium. Shown are the analytical prediction (eqn 11) for equilibrium redundancy (dashed line), as well as results of Monte-Carlo simulations (dots) of the underlying selection-mutation process. The length of each bar corresponds to one standard deviation of redundancy. (a)  $\lambda = 0.5$ ; (b)  $\lambda = 0.9$ . Simulations were carried out as described in the appendix. The deviation of the means for large values of  $\sigma_m$  in (a) is due to a significant deviation of the simulated distribution of mutation effects from a Gaussian distribution for large  $\sigma_m$ . Further simulation parameters:  $N = 10^6$ ,  $\mu = 1$ ,  $\bar{r}_0 = 1$ .

For reasons of tractability, it is assumed throughout this paper that deleterious mutations are lethal. Relaxing this assumption would result in a much more involved formalism. This is because the coevolution of redundancy and genetic load can become quite complex, as is evident from related models on the evolution of ploidy (Bengtsson, 1992; Otto & Goldstein, 1992) and reproductive isolation (Bengtsson & Christiansen, 1983; Turelli & Orr, 1995; Orr & Turelli, 1996; Gavrillets & Gravner, 1997). Although a more elaborate treatment of this issue will be deferred to a forthcoming publication, it shall be briefly mentioned here that the assumption of lethality is not likely to affect the reported results qualitatively. Figure 3 shows mean redundancy in mutation-selection equilibrium, as obtained by Monte-Carlo simulations of the above model, with the exception that non-neutral mutations are not lethal, but reduce fitness, on average,

by a factor  $\lambda_w < 1$ , as detailed in the figure legend. The case where all non-neutral mutations are lethal is shown as  $\lambda_w = 0$ . The figure demonstrates that mean equilibrium redundancy decreases only modestly as the effects of non-neutral mutations become weaker.

In the model eqn 2, redundancy is 'symmetric', i.e. the probability that a mutation has no phenotypic effect does not depend on which of two genes is mutated. However, numerous examples in the experimental literature show that this is not necessarily the case (Lundgren *et al.*, 1991; Li & Noll, 1994a,b; Cadigan *et al.*, 1994; González-Gaitán *et al.*, 1994; Hanks *et al.*, 1995). The effects of loss-of-function mutations in one of two partially redundant genes may very well depend on which gene is mutated. Such observations call for an extension of the above model, in which each gene pair ( $G_1$ ,  $G_2$ ) is characterized by two values of redundancy ( $r_1$ ,  $r_2$ ).  $r_i$  is the probability that



**Fig. 3** Mean equilibrium redundancy depends on fitness effects of mutations. The figure shows mean (bars) and 1/2 standard deviation (error bars) of redundancy in mutation-selection equilibrium, as estimated from Monte-Carlo simulations of the symmetric two-gene model. Importantly, non-neutral mutations are not lethal here, but they reduce fitness on average by a factor  $\lambda_w$  ( $0 < \lambda_w < 1$ ). More specifically, if an individual with fitness  $w$  is affected by a non-neutral mutation, the expected fitness after mutation is  $\int_0^1 w^* m_w(w^*|w) dw^* = \lambda_w w$  ( $0 < \lambda_w < 1$ ), a formalism completely analogous to that used for  $r$  in eqn 1. The probability distribution  $m_w(w^*|w)$  is a Gaussian with mean  $\lambda_w w$ , and standard deviation  $\sigma_w = 0.05$ . Boundary effects are treated for  $w$  exactly as described for  $r$  in the appendix. The closer  $\lambda_w$  is to one, the smaller the average deleterious effect of a mutation.  $\lambda_w = 0$  corresponds to the limiting case of lethal mutations discussed throughout the rest of the paper. Notice the only moderate decrease in equilibrium redundancy as the mutational effect is reduced from the maximal value  $\lambda_w = 0$ . Further simulation parameters:  $\lambda = 0.7$ ,  $\sigma_m = 0.15$ ,  $N = 5 \times 10^3$ ,  $\mu = 1$ ,  $\bar{r}_0 = 1$ .

the organism survives if  $G_i$  is mutated. Such a phenotypically neutral mutation is assumed to change  $r_i$  in the same way as in the simpler model. Because this concept generalizes in a straightforward way to a model for redundancy among  $n$  genes, an  $n$ -gene model is given here.

Denote as  $r_i$  the redundancy of gene  $i$  ( $i \in \{1, \dots, n\}, n \ll \mu^{-1}$ ), which is the probability that an organism survives if gene  $i$  undergoes a mutation. Let  $p_t(\mathbf{r}) = p_t(r_1, \dots, r_n)$  be the joint probability density describing the distribution of individual redundancies in the population at time  $t$ . Denote as  $r_1^{k_1} r_2^{k_2} \dots r_n^{k_n}$  the moments  $\int_{\mathbf{r}} r_1^{k_1} r_2^{k_2} \dots r_n^{k_n} p_t(\mathbf{r}) d\mathbf{r}$  where the subscript  $n$  under the integral is a symbol for the domain of integration, the  $n$ -fold Cartesian product of the interval  $(0, 1)$ .  $p_{i,t}(r_i) = \int_{(n-1)} p_t(\mathbf{r}) \prod_{j \neq i} dr_j$  is the (marginal) density of redundancy at locus  $i$ . Analogously to eqn 2, the joint probability distribution evolves as

$$p_{i,t+1}(\mathbf{r}) = (1/S_t) \left[ (1 - n\mu)p_t(\mathbf{r}) + \mu \sum_{j=1}^n \int_0^1 z p_t(r_1, \dots, r_{j-1}, z, r_{j+1}, \dots, r_n) m(r_j|z) dz \right], \quad (12)$$

where

$$S_t = (1 - n\mu) + \mu \sum_{i=1}^n \bar{r}_{i,t}$$

$m(r_i|z) dr_i$  is the probability that a mutation changes the redundancy of the mutated gene from  $z$  to  $r_i$ . For the marginal distributions, one finds

$$p_{i,t+1}(r_i) = (1/S_t) \left[ (1 - n\mu)p_{i,t} + \mu \left( \int_0^1 z p_{i,t}(z) m(r_i|z) dz + \sum_{j \neq i} \int_0^1 z p_{i,j,t}(r_i, z) dz \right) \right]. \quad (13)$$

Here,  $p_{i,j,t}(r_i, z)$  stands for  $\int_{(n-2)} p_t(r_1, \dots, r_{j-1}, z, r_{j+1}, \dots, r_n) \prod_{k \neq i,j} dr_k$ . Consider now the important special case where all loci are in linkage equilibrium, i.e. where  $p_t(\mathbf{r}) = \prod_{i=1}^n p_{i,t}(r_i)$ . Then the marginal distributions in mutation-selection equilibrium  $p_{i,\infty}$  are the solutions of the equation

$$p_{i,\infty}(r_i) = (1/S_\infty) \left[ (1 - n\mu)p_{i,\infty}(r_i) + \mu \left( \int_0^1 z p_{i,\infty}(z) m(r_i|z) dz + p_{i,\infty}(r_i) \sum_{j \neq i} \bar{r}_{j,\infty} \right) \right]. \quad (14)$$

Multiplying both sides by  $S_\infty$  shows that this is equivalent to

$$p_{i,\infty}(r_i) = \frac{\int_0^1 z p_{i,\infty}(z) m(r_i|z) dz}{\bar{r}_{i,\infty}}. \quad (15)$$

Thus, the marginal equilibrium distributions are not only independent of the mutation rate, they also take exactly the same form as those in the simpler symmetric redundancy model for two loci. In fact, any equilibrium distribution  $p_\infty(\mathbf{r})$  is independent of the mutation rate, i.e. even if it is not assumed that the population is in linkage equilibrium.

Would an assumption of negligible linkage disequilibrium be justified? For reasons of tractability, only the two-gene model, i.e. eqn 12 for  $n = 2$ , will be studied. One can show that

$$\bar{r}_{i,t+1} = (1/S_t) \left[ (1 - 2\mu)\bar{r}_{i,t} + \mu \lambda \bar{r}_{i,t}^2 + \mu \bar{r}_1 \bar{r}_{2,t} \right] \quad i = 1, 2 \quad (16)$$

and

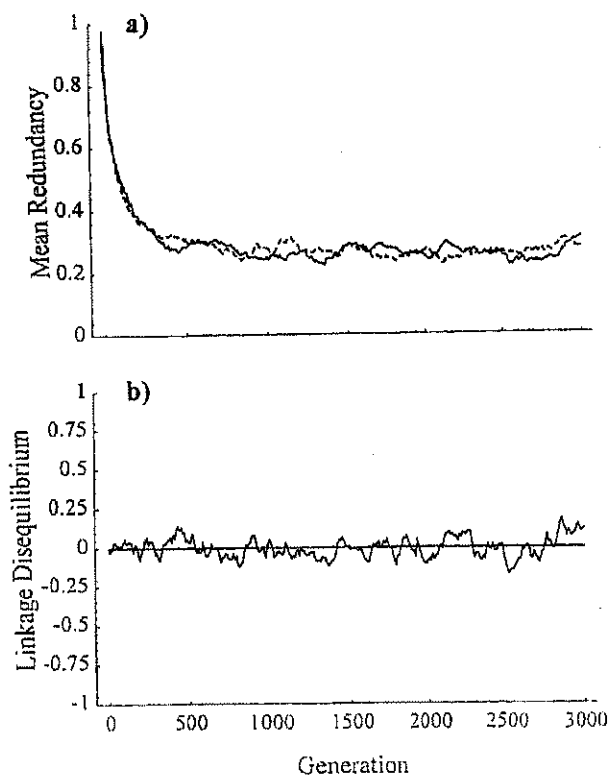
$$\bar{r}_1 \bar{r}_{2,t+1} = (1/S_t) \left[ (1 - 2\mu)\bar{r}_1 \bar{r}_{2,t} + \mu \lambda \bar{r}_1^2 \bar{r}_{2,t} + \mu \lambda \bar{r}_1 \bar{r}_{2,t}^2 \right].$$

Using  $\text{Cov}_t(r_1, r_2) = \bar{r}_1 \bar{r}_{2,t} - \bar{r}_{1,t} \bar{r}_{2,t}$  as a measure of linkage disequilibrium, it follows that

$$\text{Cov}_{t+1}(r_1, r_2) = (1/S_t^2) \left[ (1 - 4\mu)\text{Cov}_t(r_1, r_2) + \lambda \mu \text{Cov}_t(r_1^2, r_2) + \lambda \mu \text{Cov}_t(r_1, r_2^2) \right]. \quad (17)$$

In deriving eqn 17, all terms containing  $\mu^2$  were neglected. If redundancies at the two loci are stochastically independent in generation  $t$ , then all covariance terms on the right-hand side of eqn 17 are equal to zero, and linkage disequilibrium will be zero in generation  $t + 1$ . Thus, selection in and by itself does not cause linkage disequilibrium to deviate from zero. However, eqn 17 does not permit the conclusion that linkage disequilibrium will return to zero if it is different from zero. To explore whether systematic deviations from linkage equilibrium occur during evolution, Monte Carlo simulations of the selection-mutation process for a system of two loci were carried out. No recombination was allowed between loci, so that the reduction of disequilibrium by recombination would not obscure any increase in disequilibrium caused by selection (or drift). The qualitative result of these simulations was independent of the particular parameter values chosen. A representative example is shown in Fig. 4. The upper panel shows the evolution of mean redundancy for two tightly linked loci, and the lower panel shows the evolution of the Pearson product-moment correlation coefficient (Sokal & Rohlf, 1981) of  $r_1$  and  $r_2$  in the same population and over the same time interval. While substantial excursions from equilibrium occur in both directions around zero, disequilibrium does not show any obvious systematic deviations from zero. (Mean correlation coefficient over the 3000 generations shown:  $-3.4 \times 10^{-3}$ ; standard deviation:  $6.4 \times 10^{-2}$ ).

Analogous simulations for a larger number of genes suggest that even in the absence of recombination,

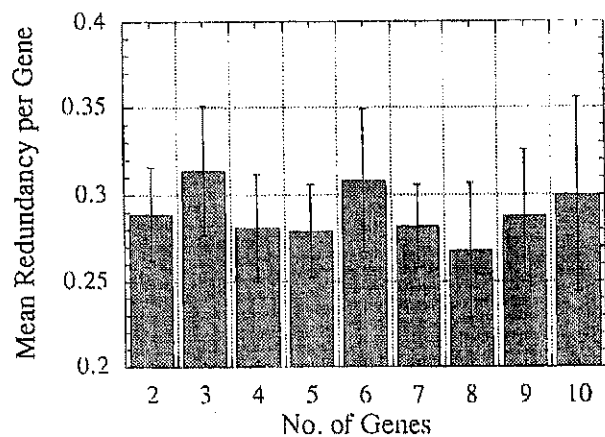


**Fig. 4** Evolution of linkage disequilibrium under mutation and selection. Shown is the result of a Monte-Carlo simulation of the evolution of redundancy for an organism with two tightly linked genes, i.e. in the absence of recombination. (a) The evolution of mean population redundancy for gene 1 and gene 2 by a solid and a dashed line, respectively ( $\bar{r}_0 = 1$  for both genes). (b) The evolution of linkage disequilibrium for the two genes shown in (a). The measure of disequilibrium is the Pearson product-moment correlation coefficient (Sokal & Rohlf, 1981) between redundancy at the two loci. Despite the absence of recombination, linkage disequilibrium shows no apparent systematic deviation from zero (mean over 3000 generations:  $-3.4 \times 10^{-3}$ ; standard deviation:  $6.4 \times 10^{-2}$ ). Simulation parameters:  $N = 10^4$ ,  $\mu = 5 \times 10^{-2}$ ,  $\lambda = 0.7$ ,  $\sigma_m = 0.15$ .

equilibrium redundancy  $r_i$  attained in a system of  $n$  genes has a value that is statistically indistinguishable from that predicted by eqn 11 for the simpler symmetric model. This is exemplified by Fig. 5, which shows population means of redundancy in mutation-selection balance averaged over the number of loci. It suggests that the polygenic model behaves much like  $n$  independent symmetric two-gene models. It will thus be the simpler two-gene model whose behaviour under the influence of genetic drift will be studied more closely in the next section.

#### Genetic drift

In a population with mean redundancy  $\bar{r}$  a fraction  $2\mu(1 - \bar{r})$  of individuals is subject to deleterious muta-



**Fig. 5** Mean redundancy per gene in mutation-selection equilibrium. Results of Monte-Carlo simulations of the evolution of redundancy for a varying number of tightly linked genes under the influence of mutation and selection. For each number of loci given on the abscissa, the ordinate corresponds to the average mean population redundancy over all loci in mutation-selection equilibrium. Lengths of error bars correspond to one standard deviation of the mean over loci. Despite the absence of recombination, mean redundancy per locus is statistically indistinguishable from the predicted equilibrium redundancy  $\bar{r}_\infty = 0.28$  for the 'symmetric' two-locus model. Simulation parameters:  $N = 10^4$ ,  $\mu = 10^{-2}$ ,  $\lambda = 0.5$ ,  $\sigma_m = 0.05$ .

tions. This quantity could be viewed as a measure of the genetic load (Crow & Kimura, 1970, p. 298) due to incomplete redundancy. It is of the order of the mutation rate, i.e. very small, suggesting that the selection process outlined above will only be effective in very large populations. The subject of this section is to analyse the evolution of redundancy for small populations. The key parameter in this regard is  $N\mu$  or  $N_e\mu$ , where  $N_e$  is the effective population size. It will be varied through changes in  $\mu$ , which is possible here because the distribution of redundancy in mutation-selection equilibrium is independent of  $\mu$ . Instead of studying the evolution of mean redundancy in one population, one has to study the evolution of mean redundancy in an infinite ensemble of populations (Crow & Kimura, 1970). This ensemble mean of individual population means at time  $t$  is denoted by  $\langle r_t \rangle$ . As  $N_e \rightarrow \infty$ ,  $\langle r_\infty \rangle \rightarrow \bar{r}_\infty$ .

First it will be studied how redundancy evolves if  $N_e\mu \ll 1$  and if all individuals initially have redundancy equal to one ( $\langle r_0 \rangle = 1$ ), e.g. shortly after a gene duplication has become fixed in the population. If  $N_e\mu$  is small, the model shares important features with an infinite allele model of neutral alleles (Crow & Kimura, 1970). The population is monomorphic for redundancy most of the time. However, on average every  $1/\mu$  generations mutations will occur that sweep to fixation in  $2N_e$  generations (on average), sufficiently fast that their spreading will not be appreciably slowed by the few



mutations that may occur during the process. As an approximation for the dynamics of  $\langle r_t \rangle$  the difference equation

$$\langle r_{t+1} \rangle = \lambda^{2\mu\langle r_t \rangle} / [(1-2\mu) + 2\mu\langle r_t \rangle] \langle r_t \rangle \quad (18a)$$

is used. It can be viewed as having two components that correspond to two different processes. The first component, represented by  $\langle r_{t+1} \rangle = \lambda^{2\mu\langle r_t \rangle}$  would in and of itself cause an exponential 'decay' of redundancy. This component reflects the reduction of  $\langle r_t \rangle$  through mutations whose effects are distributed as given by eqn 1. However, for  $\langle r_t \rangle < 1$ , the mutation rate per two genes,  $2\mu$ , will overestimate the rate at which mutations occur that might spread in the population. The reason is that if an individual with redundancy  $r$  undergoes a mutation, only a fraction of  $r$  of the mutants will be phenotypically neutral. The remaining fraction,  $1 - r$ , will die. This leads to an 'effective' rate of (neutral) mutations, represented by  $2\mu\langle r_t \rangle / [(1-2\mu) + 2\mu\langle r_t \rangle]$  in eqn 18a. It is the contribution to generation  $t+1$  of individuals that were mutated in generation  $t$  and that survived. This fraction decreases as the mean ensemble redundancy decreases, such that the decay of redundancy is much slower than exponential for  $\langle r \rangle < 1$ . A number of implicit simplifying assumptions are hidden in eqn 18a, e.g. that properties of the distribution of mutation effects other than eqn 1 are irrelevant to the evolution of  $\langle r \rangle$ , that no higher-order ensemble moments are necessary to describe the evolution of  $\langle r \rangle$ , and that the ensemble average of the effective (population) mutation rate is given by the above form.

For reasons of tractability, the differential equation

$$\frac{d\langle r_t \rangle}{dt} = \ln \lambda \frac{2\mu\langle r_t \rangle^2}{(1-2\mu) + 2\mu\langle r_t \rangle} \quad (18b)$$

which is analogous to eqn 18a, will be analysed. The unit of time is identical to one generation of the discrete time

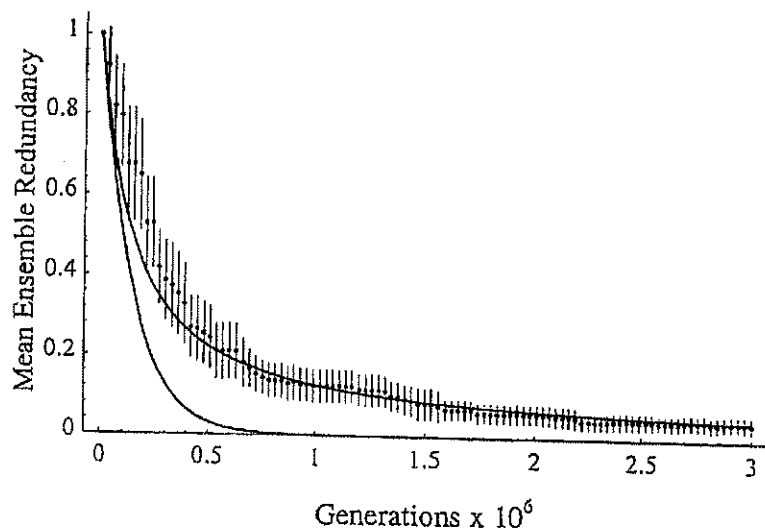
model. Let  $t_k$  denote the time necessary for  $\langle r \rangle$  to decay from one to  $1/2^k$ . Then

$$t_{k+1} - t_k = \left( -\frac{1}{\ln \lambda} \right) \left[ \left( \frac{1-2\mu}{2\mu} \right) 2^k + \ln 2 \right] \propto 2^k.$$

Thus, the 'half-life' of redundancy increases with decreasing  $\langle r \rangle$ . Figure 6 shows a comparison of the analytical prediction eqn 18b and of a series of stochastic simulations for  $N_e\mu = N\mu = 10^{-3}$ . The upper solid line shows the analytical prediction (18b) for  $\lambda = 0.5$  and  $\mu = 5 \times 10^{-6}$ . The lower solid line shows, for comparison, a purely exponential decay for the same parameters  $\mu$  and  $\lambda$ . The dots represent the results of Monte-Carlo simulations. They are the averages over mean population redundancies  $\bar{r}_t$  of 20 independent population simulations, demonstrating that eqn 18b is in qualitative agreement with the stochastic simulations.

Thus, redundancy decays to  $\langle r_\infty \rangle = 0$  under the influence of genetic drift if  $N_e\mu \ll 1$ , albeit increasingly slowly for large  $t$ . On the other hand, in very large populations ( $N_e\mu \gg 1$ ), selection-mutation balance causes mean ensemble redundancy to attain the finite equilibrium  $\langle r_\infty \rangle = \bar{r}_\infty$  given by eqn 11. The question thus arises of how large  $N_e\mu$  has to be for the decay of redundancy to be appreciably slowed or reversed by selection. This question was addressed by stochastic simulations. In such a numerical analysis, initial conditions  $\langle r_0 \rangle > \bar{r}_\infty$  are not likely to be very informative, because both selection and drift will reduce the ensemble mean. But if  $\langle r_0 \rangle < \bar{r}_\infty$ , the influence of drift will tend to decrease  $\langle r_t \rangle$ , whereas the influence of selection will cause an increase of  $\langle r_t \rangle$ . Because of the large computational cost required to analyse the evolution of ensemble means, only a crude approach was taken, in which several populations of size  $n$  (an ensemble sample) were initialized at a mean redundancy of  $\bar{r}_\infty$  for some value of the parameters  $\lambda$  and  $\sigma_m^2$ . The evolution of these populations was then simu-

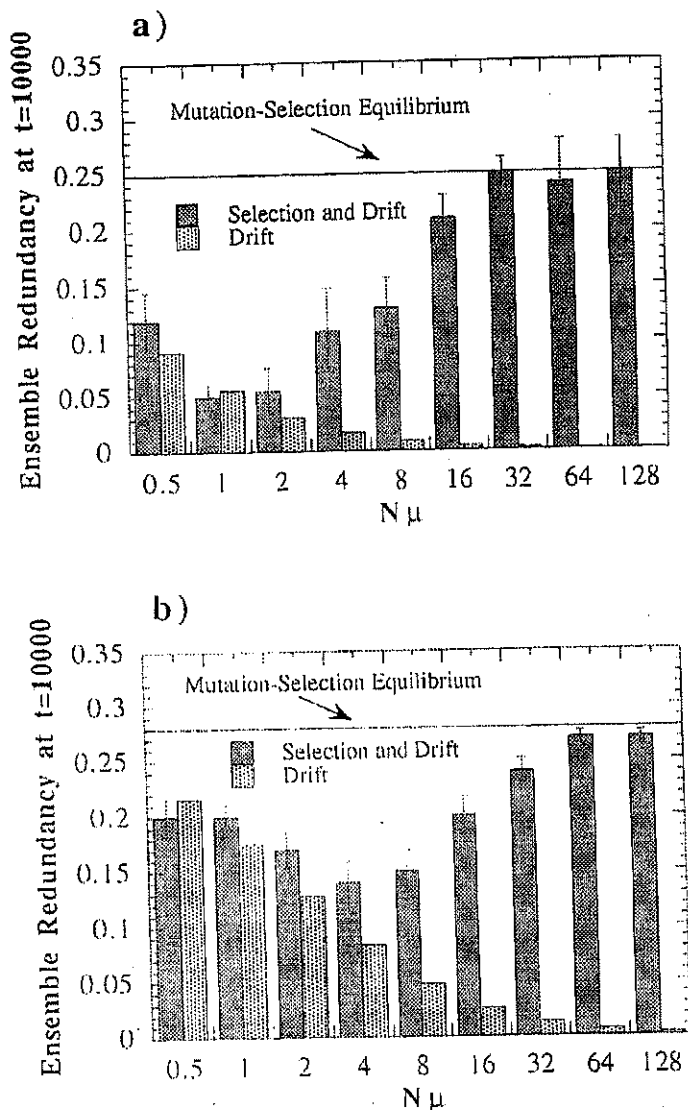
**Fig. 6** Evolution of mean ensemble redundancy under the influence of genetic drift. The upper solid line shows the analytical prediction (eqn 18b) for the evolution of the ensemble mean redundancy  $\langle r_t \rangle$  for  $\lambda = 0.5$ , and  $\mu = 5 \times 10^{-6}$ . Equation 18b was solved numerically. The lower solid line shows the predicted evolution of  $\langle r_t \rangle$  for the same parameters if the decay of redundancy was purely exponential, as explained in the text. Dots represent the results of Monte-Carlo simulations of the underlying process with  $N = 10^2$  (i.e.  $N\mu = 5 \times 10^{-4}$ ), and  $\sigma_m = 0.05$ . More precisely, each dot is the mean over the mean population redundancies of 20 independent population simulations at the respective time point on the abscissa. Length of lines crossing the dots correspond to one standard deviation over 20 populations.



lated for  $10^4$  generations. For a given mutation rate,  $\mu$  (eqn 18b) provides an estimate of how much redundancy would decline in  $10^4$  generations if the evolutionary dynamics was dominated by drift, and this estimate can be used to assess the effect of selection. This approach does not yield information regarding an equilibrium ensemble mean  $\langle r_\infty \rangle$ , but it provides an estimate for the influence of selection relative to drift. Figure 7 shows examples for  $\lambda = 0.5$  and  $\lambda = 0.9$ , where values for  $\sigma_m$  were chosen such that the respective mutation-selection equilibria were of similar magnitude in the two cases. Populations were initialized with uniform redundancy close to the mutation-selection equilibrium indicated by the horizontal line in both panels. For small values of  $N\mu - N\mu_c$ ,  $\langle r \rangle$  declines at a rate similar to that predicted for  $N\mu \ll 1$ . But for  $N\mu \approx 2$ , selection already slows the decay of redundancy considerably, and for  $N\mu > 50$ , the

ensemble means do not decrease appreciably from the initial condition. Because the decrease of redundancy per mutation event is less for  $\lambda = 0.9$  than for  $\lambda = 0.5$ , the change in redundancy is in general slower for  $\lambda = 0.9$ . The neutral theory predicts that the expected number of alleles in a population is  $2N_e\mu + 1$ . Not all mutations are neutral here, but the fact that the decay of redundancy is slowed by selection for values of  $N\mu$  that are of the order of unity implies that selection already has an effect if merely two alleles are present in the population.

Does it take equally long for redundancy to reach an intermediate value of  $\bar{r}$  if a population starts from high rather than from low initial redundancy? The following approach was taken to address this question. For a given  $N\mu$ , 20 populations were initialized at values of high ( $\bar{r}_0 = 0.99$ ) redundancy, and the time was recorded when individual population means  $\bar{r}_t$  first had reached



**Fig. 7** Evolution of mean ensemble redundancy under the influence of drift and selection. Dark bars represent means over mean population redundancies of 20 independent population simulations after  $t = 10^4$  generations. Simulation parameters: (a)  $N = 10^3$ ,  $\lambda = 0.5$ ,  $\sigma_m = 0.25$ ,  $5 \times 10^{-4} < \mu < 0.128$ ; (b)  $N = 10^3$ ,  $\lambda = 0.9$ ,  $\sigma_m = 0.05$ ,  $5 \times 10^{-4} < \mu < 0.128$ . In both panels, all populations within an ensemble were started with the same initial condition  $\bar{r}_0$  close to the respective mutation-selection equilibrium. In (a)  $\bar{r}_0 = 0.26$ , in (b)  $\bar{r}_0 = 0.28$ . Lengths of error bars correspond to one standard error over the 20 population simulations. Light bars show, for comparison, the analytical prediction (eqn 18b) for the same mutation rates as used for the neighbouring dark bars, but assuming that  $N\mu \ll 1$ . Both panels show that selection starts to be effective for  $N\mu \geq 1$ . Note that  $N = N_c$  in the simulations carried out here (see also appendix).

**Table 1** Time (generations  $\times 10^3$ ) until crossing the mutation-selection equilibrium eqn 11 for different initial values of  $r$ ,  $\sigma_m = (1 - \lambda)/2$ , and  $N = 10^3$ . Individual entries represent mean and one standard deviation over 20 independent population simulations, except where noted otherwise. A simulation was stopped if the mutation-selection equilibrium was not reached after  $5 \times 10^4$  generations. Initial values of  $r$  were the same for all individuals in a population.

	$N\mu = 25$		$N\mu = 50$		$N\mu = 100$	
	$r_0 = 0.99$	$r_0 = 0.01$	$r_0 = 0.99$	$r_0 = 0.01$	$r_0 = 0.99$	$r_0 = 0.01$
$\lambda = 0.95$	$3.04 \pm 0.64$	$> 45.78$ (14)*	$1.64 \pm 0.32$	$> 28.3$ (7)*	$0.93 \pm 0.13$	$> 8.7$ (1)*
$\lambda = 0.75$	$0.87 \pm 0.35$	$> 10.6$ (3)*	$0.42 \pm 0.14$	$1.56 \pm 0.89$	$0.26 \pm 0.09$	$0.6 \pm 0.2$
$\lambda = 0.55$	$0.36 \pm 0.12$	$> 6.97$ (2)*	$0.3 \pm 0.11$	$1.02 \pm 0.6$	$0.17 \pm 0.07$	$0.35 \pm 0.12$

\* In these simulations the predicted mutation-selection equilibrium had not been crossed after  $5 \times 10^4$  generations in one or more of the 20 independent population simulations, as indicated by the number in parentheses. The averages given here include the number of generations until the population mean first crossed the deterministic equilibrium for those populations that crossed equilibrium, and the value  $5 \times 10^4$  for each population that did not cross equilibrium. The symbol '>' indicates that the average crossing time to equilibrium is greater than the value shown.

a value lower than that given by the mutation-selection equilibrium eqn 11. Conversely, the time was recorded at which  $\bar{r}_t$  first exceeded the mutation-selection equilibrium in each of 20 populations initialized at low redundancy ( $\bar{r}_0 = 0.01$ ). These 'crossing times' averaged over the 20 populations are given in Table 1 for different values of  $N\mu$ . For each  $\lambda$ ,  $\sigma_m$  values were chosen such that the deterministic equilibria were of comparable magnitude (between 0.25 and 0.3). For a given  $N\mu$ , crossing times increase with decreasing  $\lambda$ , which is not surprising for  $\bar{r}_0 = 0.99$ . For  $\bar{r}_0 = 0.01$  this holds because of the larger  $\sigma_m$ , emphasizing the important role of mutational variation in the selection process for increased redundancy. More importantly, although the chosen mutation-selection equilibria were closer to  $r = 0$  than to  $r = 1$ , it took longer to cross them if redundancy was initially low. In addition, it took much longer (a factor 10–20) to reach  $\bar{r}_\infty$  from low initial redundancy if  $N\mu$  was small than if it was large. For small  $N\mu$ , selection not only has to overcome the effects of mutation, but also the effects of genetic drift for initially low redundancy.

## Discussion

In the model considered here, redundancy is conceptualized as some measure of overlap in two genes' biological function. This measure will depend on the types of genes and biological contexts considered. For example, in the case of two genes with similar biochemical functions, the extent of overlap in their spatiotemporal expression pattern throughout organismal development can serve as a measure of redundancy. The greater this overlap, the more redundantly specified is the biological function of these genes. The model is most general in the sense that it does not restrict itself to a particular measure of redundancy.

Two roles of natural selection in the evolution of redundancy can be distinguished. First, natural selection will lead to the stable maintenance of partial redundancy, i.e. overlapping functions between genes, provided that (i) mutation generates a sufficient amount of variation in

redundancy and that (ii) population sizes are sufficiently large ( $N_e\mu > 1$ ). Thus, genetic redundancy can increase through the action of natural selection. This holds even though mutations tend to reduce functional similarity among gene products. Moreover, the level of redundancy maintained in mutation-selection equilibrium is independent of the mutation rate. Selection cannot act on fitness differences among organisms carrying genes with different degrees of redundancy, because such differences do not exist. Rather, mutations in individuals carrying genes with low redundancy produce larger numbers of offspring with low fitness. Thus, individuals carrying genes with high redundancy contribute more offspring to subsequent generations than those with low redundancy. The large population sizes required are unrealistic for most vertebrates, but not for micro-organisms and possibly some small invertebrates. The second role of natural selection is independent of population size and of the amount of variation generated by mutation. Selection will always slow the mutational 'decay' of redundancy because it eliminates the deleterious mutations invariably generated by genes with redundancy  $r < 1$ . This is illustrated by the solid lines in Fig. 6, which represent the expected 'decay' rate of redundancy by mutational pressure alone (lower curve), as well as by mutation and selective elimination of deleterious mutants (upper curve). Clearly, the decay rate is much lower if selection acts, despite the very small population sizes ( $N_e\mu \ll 1$ ).

In this model, selection among two or multiple partially redundant genes does not lead to a build-up of linkage disequilibrium,  $D$ , in redundancy. Linkage relations among genes are therefore not likely to influence the evolution of redundancy. It means also that the fitness effect of a mutation in one redundant gene is not necessarily similar ( $D > 0$ ) or dissimilar ( $D < 0$ ) to that of a mutation in other genes. Clearly, the available experimental data do not provide the level of resolution necessary to corroborate this statement. However, one might naively assume that redundancy is often 'symmetric' between genes, i.e. that the effect of a (loss-of-

function) mutation among partially redundant genes is similar. As a corollary of the theoretical result obtained here, such symmetry is not to be expected, because it would imply large amounts of linkage disequilibrium. This is consistent with available experimental evidence. Most often, loss-of-function mutations in partially redundant gene pairs have different effects depending on which gene was mutated (Lundgren *et al.*, 1991; Cadigan *et al.*, 1994; González-Gaitán *et al.*, 1994; Li & Noll, 1994a,b; Hanks *et al.*, 1995).

An important question regarding the evolution of redundancy is whether genes with originally dissimilar functions can evolve (converge) towards partial redundancy. In this case, selection for increased redundancy has to overcome the influence of both genetic drift and mutations. Not only is an increase of redundancy through selection only possible for large populations ( $N\mu$ ), this increase will be very slow if  $N\mu$  is not substantially greater than one, e.g.  $N\mu > 100$ , as shown by Table 1. For example, if  $N\mu = 10$ , it may take, on average, 15 times longer for a population to cross a specified threshold in redundancy when starting from low redundancy, than when starting from high redundancy (e.g. after a gene duplication event). Moreover, in this case mean redundancy in a population will not reach a quasi-stable equilibrium, but will constantly change through the strong influence of genetic drift. It thus seems that observed redundancy will most often be the result of functional diversification after gene duplication events, which is slowed or ultimately stopped by the action of natural selection, and more rarely the result of selection for *de novo* redundancy.

The model, as presented here, contains many simplifications that were mainly introduced to illustrate the relevant evolutionary mechanisms most clearly. None of these simplifications is likely to affect any of the qualitative conclusions made here. The assumptions of haploidy and of lethality of non-neutral mutations would lead only to a reduction in the advantage of redundancy. The extent of this reduction would depend on factors such as the degree of dominance, and the average fitness effect of mutations. Effects of relaxing these assumptions may be quite modest, as suggested by the numerical results presented in Fig. 3, in which the assumption of lethality was relaxed. A more detailed analysis of the potentially complicated coevolution between redundancy and genetic load will be presented elsewhere. The implicit assumption that effectively all non-neutral mutations are deleterious is also inconsequential. Assume that a selectively advantageous mutant occurs in a gene with redundancy  $r_0$ . If this mutant goes to fixation, the evolution of  $r$ , as described by eq 2, will continue with initial condition  $p_0(r) = \delta(r - r_0)$ . Since redundancy in mutation-selection equilibrium is independent of initial redundancy, the population will approach the equilibrium starting from  $r_0$ .

There is one assumption that is unrealistic but probably unavoidable if any level of generality of the model is to be maintained. It is assumed that a mutational change in redundancy at one locus does not affect redundancy at other loci. Envisage, for example, the overlap in the spatiotemporal expression pattern of two genes expressing identical products as a measure of functional redundancy, and assume that mutations in each gene change only the spatiotemporal extent of the expression pattern. It is clear that a phenotypically neutral mutation in one locus may well affect redundancy at both loci. To relax this assumption, one would probably need a biochemically based model of redundancy and very specific assumptions about mutational effects. One result of this assumption is that redundancy evolves independently at each of  $n$  loci. Relaxing it is likely to change the importance of selection for increased redundancy, in that selection might become effective at smaller values of  $N_e$ , depending on the number of genes involved.

The model uses a conceptually simple, universal measure of redundancy involving a reduction in the rate of phenotypically neutral mutations. This simplicity is unlikely to translate into mechanistic explanations of redundancy. The wide array of biochemical functions that can be redundantly specified suggests that no universal measure of overlap or similarity among gene functions will exist. Certainly not all cases will be reducible to similarity among biochemical functions of gene products (Kataoka *et al.*, 1984; Basson *et al.*, 1986). Differences in both spatial and temporal expression patterns will often contribute to partial redundancy. The striking example of the transcription factor genes *paired*, *gooseberry* and *gooseberry neuro* in *Drosophila* demonstrates that functionally identical gene products can lack complete redundancy because they lack identical *cis*-regulatory sequences (Li & Noll, 1994a,b). And even assuming that a particular notion of redundancy can be agreed upon for a particular biological question, a much deeper problem arises. It lies in the fact that redundancy results from the interaction of two or more genes, and that it cannot be reduced to properties of individual genes. To estimate redundancy, one has to know as a reference point the rate of (deleterious or lethal) mutations of that gene in the absence of its redundant partners. This would be very difficult for reasons aside from practical problems of assessing mutation rates (Hartl & Clark, 1989). Because one is usually confronted with partial redundancy, one cannot study the individual gene in isolation. Part of the biological function has to be provided by another gene. A remedy, although problematic, would consist in an evolutionary approach identifying and studying taxa closely related to the one under consideration in which the respective gene is not partially redundant (e.g. in which a gene duplication has not occurred). If the gene has a similar biological function in the related taxon, then that taxon might

provide the necessary reference point. The clusters of homeotic genes in chordates serve as an example. Multiple gene and cluster duplications occurred in this case (Carroll, 1995), which may have led to examples of partial redundancy (Condie & Capecchi, 1995). The genes seem to be used in a similar functional context, i.e. axial determination, throughout the chordates, and a reference taxon has been found that has at most one copy of each gene in the cluster (*Amphioxus*; Garcia-Fernández & Holland, 1994; Carroll, 1995). However, one is confronted with the deep problem of whether gene functions in different taxa can be meaningfully called homologous, and the greater the evolutionary distance between taxa, the more problematic such comparisons become. This holds even if the biochemical functions of the respective genes are well preserved, such as may be the case with the mouse *Engrailed* genes *En-1* and *En-2* (Hanks *et al.*, 1995) and their unique *Drosophila* counterpart *en*. Basic body plan features of these organisms cannot be homologized, and one can therefore not meaningfully compare degrees of redundancy.

Another empirical question concerns the amount of variation in redundancy generated by mutation, and, more precisely, whether mutation generates variants with increased redundancy. Only if this is the case would selection be able to increase mean redundancy. (In terms of the model, this translates into the question of whether  $\sigma_{ii}$  is of the same order of magnitude  $1 - \lambda$ .) The case of increasing functional similarity among genes is of special interest here. Relevant evidence comes from the evolution of (nonredundant) genes in different taxa. Functional convergence among genes must have occurred in numerous cases (e.g. haemocyanin and haemoglobin), including examples of directed homoplasy, where not only functions but also protein sequences may have converged (Stewart *et al.*, 1987; Swanson *et al.*, 1991). This suggests that mutations provide the variants necessary for functional convergence. Moreover, many redundant genes are closely linked, and in this case gene conversion may considerably increase structural and, by correlation, functional similarity. Thus, there is no reason to expect that mutation will always lead to decreased redundancy, although it is likely to do so on average.

The results presented here are a special case of a more general principle, namely that nonlinear (epistatic) gene interactions can profoundly influence the extent to which mutational effects propagate through the epigenetic system. Recent studies on the evolution of genetic canalization (A. Wagner, 1996a,b; G. P. Wagner *et al.*, 1996) have shown that many mutations that are not neutral on the molecular level may be neutral on the phenotypic level because their effects are 'absorbed' by the epigenetic system. Moreover, natural selection can increase the ability of the epigenetic system to act as a buffer against mutational effects. Redundancy is a special case of an epistatic interaction among two or more genes, and selection can affect redundancy among these genes.

A developmental pathway with many partially redundant genes might be more canalized than one with few such genes. This important role of epigenetic interactions also sheds a different light on the nature of neutral mutations. Phenotypically neutral mutations need not leave the biochemical function of a gene product unchanged. Instead, they may be neutral because their effects are buffered by the epigenetic system, such as in the case of partial redundancy. It remains to be seen what fraction of phenotypically neutral genetic variation is 'molecularly neutral' and what fraction might be 'epigenetically neutral'.

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## Appendix

### Stability analysis

To study the stability of the boundary solution  $p(r) = \delta(r)$  for  $0 < \lambda \leq 1$ , consider a small perturbation of this distribution, i.e. a  $p_i(r)$  with  $\bar{r}_i \ll 1$ ,  $\bar{r}_i^2 \ll 1$ . If these two moments are sufficiently small,  $\bar{r}_{i+1}^2 > \bar{r}_i^2$ , because

$$\bar{r}_{t+1}^2 - \bar{r}_t^2 = \frac{2\mu\bar{r}_t[\sigma_m^2 + (3\lambda^2 - 1)\bar{r}_t^2 - 2\lambda^2\bar{r}_t^2]}{(1 - 2\mu) + 2\mu\bar{r}_t}$$

It follows then that

$$\bar{r}_{t+2} - \bar{r}_t = \frac{2\mu\lambda\bar{r}_t[\bar{r}_{t+1}^2 - \bar{r}_t^2] + (1 - 2\mu)[\lambda(\bar{r}_{t+1}^2 + \bar{r}_t^2) - 2\bar{r}_t^2]}{(1 - 4\mu) + 2(1 - 2\mu)\bar{r}_t + 2\mu\lambda\bar{r}_t^2} > 0,$$

because  $\bar{r}_t$  can be made arbitrarily small. Thus, both  $\bar{r}$  and  $\bar{r}^2$  increase in the vicinity of the boundary solution  $\delta(r)$ .

Consider now the stability of the solutions  $p(r) = \delta(r - r_0)$  for  $0 < r_0 < 1$ , and for  $\lambda = 1$ . For any given  $r_0$ , the first and second moments of  $\delta(r - r_0)$  are  $r_0$  and  $r_0^2$ , respectively. Now perturb  $\delta(r - r_0)$  via these moments, i.e. consider a  $p_t(r)$  with  $\bar{r}_t = r_0 + \epsilon$  and  $\bar{r}_t^2 = r_0^2 + \delta$  ( $0 < \epsilon, \delta \ll 1$ ). Using eqn 3a, one obtains

$$\bar{r}_{t+1} = \frac{(1 - 2\mu)(r_0 + \epsilon) + 2\mu\lambda(r_0^2 + \delta)}{(1 - 2\mu) + 2\mu(r_0 + \epsilon)}$$

It follows that

$$\bar{r}_{t+1} - \bar{r}_t = \frac{2\mu(\delta - 2\epsilon r_0 - \epsilon^2)}{(1 - 2\mu) + 2\mu(r_0 + \epsilon)} > 0,$$

because  $\epsilon$  can be made arbitrarily small. Thus, the solution  $\delta(r - r_0)$  is not stable for any  $r_0 < 1$ .

To study the stability properties of the interior equilibrium in eqn 10, define, for notational convenience, the variables  $x_t := \bar{r}_t, y_t := \bar{r}_t^2$ , and  $\omega := (1 - 2\mu)/2\mu$ . Throughout,  $0 < \lambda < 1$ . Using eqn 1,  $\int_0^1 (r^* - \lambda r)^2 m(r^* r) dr^* = \sigma_m^2$ , and  $\int_0^1 (r - \bar{r}_t)^3 p_t(r) dr \approx 0$ , as in the main text, the following dynamical system in  $x_t$  and  $y_t$  follows from eqn 2:

$$x_{t+1} = \frac{\omega x_t + \lambda y_t}{\omega + x_t} \quad (\text{A1a})$$

$$y_{t+1} = \frac{\omega y_t + \sigma_m^2 x_t + \lambda^2 x_t (3y_t - 2x_t^2)}{\omega + x_t} \quad (\text{A1b})$$

The form

$$\Delta x_t = x_{t+1} - x_t = \frac{\lambda y_t - x_t^2}{\omega + x_t}$$

$$\Delta y_t = y_{t+1} - y_t = \frac{\sigma_m^2 x_t + \lambda^2 x_t (3y_t - 2x_t^2) - x_t y_t}{\omega + x_t}$$

shows that the mutation rate enters the rate of change in both variables only through the multiplicative factor  $\omega + x_t > 0$ . Thus,  $\mu$  determines only the rate, but not the direction, of change in the phase space of the two-dimensional dynamical system (eqn A1). Therefore, for the stability analysis of the interior equilibrium (eqn 10), the set of equations

$$x_{t+1} = \frac{\lambda y_t}{x_t} \quad (\text{A2a})$$

$$y_{t+1} = \sigma_m^2 + \lambda^2 (3y_t - 2x_t^2) \quad (\text{A2b})$$

can be used. The Jacobian of eqn A2 is

$$\lambda A := \lambda \begin{pmatrix} -y_t/x_t^2 & 1/x_t \\ -4\lambda x_t & 3\lambda \end{pmatrix}.$$

Using eqn 6, i.e.  $y/x^2 = 1/\lambda$  at equilibrium, the eigenvalues  $\gamma_{1,2}$  at equilibrium are given by

$$\gamma_{1,2} = \frac{1}{2} \left[ 3\lambda^2 - 1 \pm \sqrt{9\lambda^4 - 16\lambda^3 + 6\lambda^2 + 1} \right]. \quad (\text{A3})$$

An examination of eqn A3 shows that both eigenvalues are real and that

$$|\gamma_{1,2}| < 1 \quad \forall \lambda \in (0, 1). \quad (\text{A4})$$

Thus, eqn 10 is locally stable (e.g. Hofbauer & Sigmund, 1988, p. 55).

## Numerical methods

This section outlines the methods used for carrying out the Monte-Carlo simulations used to illustrate and validate the analytical predictions of the model. A population of  $n$  individuals  $\{I_1, \dots, I_N\}$  each with  $n$  genes is represented by (i)  $n$  arrays  $R_{I_k} = (r_{k1}, \dots, r_{kn})$  ( $k \in \{1, \dots, N\}$ ), where each entry  $r_{km}$  is restricted to the interval  $(0, 1)$ , and corresponds to the redundancy of gene  $m$  in individual  $I$ , and (ii) an array  $(w_1, \dots, w_N)$  whose entries  $w_k \in \{0, 1\}$  represent the fitness of individual  $I_k$ . All  $r_{ik}$ 's are set to the same value at the beginning of a simulation.

A simulated evolution process consisted in the iteration of two steps in the following order.

### 1. Mutation

The following procedure was carried out for each entry  $r_{kj}, 1 \leq k \leq N, 1 \leq j \leq n$  starting with  $r_{11}$ . First, a uniformly distributed pseudo-random variate  $v_1 \in (0, 1)$  was generated. If  $v_1 > \mu$ , redundancy  $r_{kj}$  and fitness  $w_k$  were left unchanged. If  $v_1 < \mu$ , another uniform random variate  $v_2 \in (0, 1)$  was generated. If  $v_2 > r_{kj}$ , then  $w_k$  was set to zero, if it was not already equal to zero. If  $v_2 < r_{kj}$ , then a Gaussian pseudo-random deviate  $v_3$  with mean  $\lambda r_{kj}$  and variance  $\sigma_m^2$  was generated. ( $\sigma_m^2$  was parameterized by a variable  $c$  such that  $\sigma_m = c(1 - \lambda)$ .) If  $0 < v_3 < 1$ , then  $r_{kj}$  was set to the value  $r_{kj}^* = v_3$ . If  $v_3 < 0$ ,  $r_{kj}$  was set to  $r_{kj}^* = 0$ , and if  $v_3 > 1$ ,  $r_{kj}$  was set to  $r_{kj}^* = 1$ . Random number generators used for uniform and Gaussian deviates were based on the routines 'ran1' and 'gasdev' from Press *et al.* (1992).  $r_{kj}^*$  follows a probability distribution with density  $\delta(r_{kj}^*) \int_{-\infty}^0 g(x) dx + g(r_{kj}^*) + \delta(1 - r_{kj}^*) \int_1^{\infty} g(x) dx$ , where  $0 < r_{kj}^* < 1$ , and  $g(x)$  is the density of a Gaussian distribution with the above parameters. For  $r_{kj}$  close to either boundary, this distribution will deviate from assumptions made in the text, e.g. eqn 1. However, for most of the parameter

$$\bar{r}_{t+1}^2 - \bar{r}_t^2 = \frac{2\mu\bar{r}_t[\sigma_m^2 + (3\lambda^2 - 1)\bar{r}_t^2 - 2\lambda^2\bar{r}_t^2]}{(1 - 2\mu) + 2\mu\bar{r}_t}$$

It follows then that

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because  $\bar{r}_t$  can be made arbitrarily small. Thus, both  $\bar{r}$  and  $\bar{r}^2$  increase in the vicinity of the boundary solution  $\delta(r)$ .

Consider now the stability of the solutions  $p(r) = \delta(r - r_0)$  for  $0 < r_0 < 1$ , and for  $\lambda = 1$ . For any given  $r_0$ , the first and second moments of  $\delta(r - r_0)$  are  $r_0$  and  $r_0^2$ , respectively. Now perturb  $\delta(r - r_0)$  via these moments, i.e. consider a  $p_t(r)$  with  $\bar{r}_t = r_0 + \epsilon$  and  $\bar{r}_t^2 = r_0^2 + \delta$  ( $0 < \epsilon, \delta \ll 1$ ). Using eqn 3a, one obtains

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The form

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can be used. The Jacobian of eqn A2 is

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Thus, eqn 10 is locally stable (e.g. Hofbauer & Sigmund, 1988, p. 55).

## Numerical methods

This section outlines the methods used for carrying out the Monte-Carlo simulations used to illustrate and validate the analytical predictions of the model. A population of  $n$  individuals  $\{I_1, \dots, I_N\}$  each with  $n$  genes is represented by (i)  $n$  arrays  $R_{ik} = (r_{k1}, \dots, r_{kn})$  ( $k \in \{1, \dots, N\}$ ), where each entry  $r_{lm}$  is restricted to the interval  $(0, 1)$ , and corresponds to the redundancy of gene  $m$  in individual  $l$ , and (ii) an array  $(w_1, \dots, w_N)$  whose entries  $w_k \in \{0, 1\}$  represent the fitness of individual  $I_k$ . All  $r_{ik}$ 's are set to the same value at the beginning of a simulation.

A simulated evolution process consisted in the iteration of two steps in the following order.

### 1. Mutation

The following procedure was carried out for each entry  $r_{kj}, 1 \leq k \leq N, 1 \leq j \leq n$  starting with  $r_{11}$ . First, a uniformly distributed pseudo-random variate  $v_1 \in (0, 1)$  was generated. If  $v_1 > \mu$ , redundancy  $r_{kj}$  and fitness  $w_k$  were left unchanged. If  $v_1 < \mu$ , another uniform random variate  $v_2 \in (0, 1)$  was generated. If  $v_2 > r_{kj}$ , then  $w_k$  was set to zero, if it was not already equal to zero. If  $v_2 < r_{kj}$ , then a Gaussian pseudo-random deviate  $v_3$  with mean  $\lambda r_{kj}$  and variance  $\sigma_m^2$  was generated. ( $\sigma_m^2$  was parameterized by a variable  $c$  such that  $\sigma_m = c(1 - \lambda)$ .) If  $0 < v_3 < 1$ , then  $r_{kj}$  was set to the value  $r_{kj}^* = v_3$ . If  $v_3 < 0$ ,  $r_{kj}$  was set to  $r_{kj}^* = 0$ , and if  $v_3 > 1$ ,  $r_{kj}$  was set to  $r_{kj}^* = 1$ . Random number generators used for uniform and Gaussian deviates were based on the routines 'ran1' and 'gasdev' from Press *et al.* (1992).  $r_{kj}^*$  follows a probability distribution with density  $\delta(r_{kj}^*) \int_{-\infty}^0 g(x) dx + g(r_{kj}^*) + \delta(1 - r_{kj}^*) \int_1^{\infty} g(x) dx$ , where  $0 < r_{kj}^* < 1$ , and  $g(x)$  is the density of a Gaussian distribution with the above parameters. For  $r_{kj}$  close to either boundary, this distribution will deviate from assumptions made in the text, e.g. eqn 1. However, for most of the parameter



combinations that were used here, mutation-selection equilibria lie at some distance from the boundaries, so that the dynamics close to equilibrium will conform to the assumptions made in the main text. For example, mean redundancy in mutation-selection equilibrium for  $0.5 < \lambda < 0.9$  and  $0.9 < c < 1$  lies between one and six standard deviations  $\sigma_m$  away from the boundary  $r = 0$ .

#### *Selection and reproduction*

From the population  $\{I_1, \dots, I_N\}$ , one member  $I_k$  was chosen at random with uniform distribution. If its fitness  $w_k$  was equal to one, it 'survived' into the next generation. If  $w_k = 0$ , another random member of the population was chosen at random and its fitness checked. This process was iterated using the entire population  $\{I_1, \dots, I_N\}$ , i.e. 'with replacement', until  $n$  members of the population of survivors had been found. The procedure corresponds to 'soft selection' (e.g. Hartl & Clark, 1989) which assures a constant number of  $n$  individuals every generation.

Each iteration of the above two steps was termed a 'generation'. If  $N\mu \gg 1$ , populations reached a (quasi)-

equilibrium of population mean redundancy. Whether such an equilibrium had been reached after a certain number of generations was judged by visual inspection (for an example of the dynamics see Fig. 5). For the 'crossing time' estimates of Table 1, a population was initialized such that all redundancies  $r_{kj}$  were equal to 0.99 (0.01) at time  $t = 0$ . It was then established how many generations elapsed before  $\bar{r}_t$  first became less than (greater than) the equilibrium value predicted by eqn 11.

Note that the case  $n = 1$  corresponds to the 'symmetric' redundancy model (2) for two genes with genic mutation rate  $\mu/2$ .

#### **Note added in proof**

Since the acceptance of this manuscript in July 1997, a conceptually quite different model for the evolution of redundancy was proposed (Nowak *et al.*, 1997, *Nature* 388: 161–171), arriving at similar conclusions for the case  $N\mu \gg 1$ .