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The population genetics of multistage carcinogenesis

Leonard Nunney

Department of Biology, University of California, Riverside, CA 92521, USA (nunney@citrus.ucr.edu)

Despite the many successes of cancer research, we lack the framework necessary to predict the ratio of familial (inherited) to sporadic (non-inherited) cancers. An evolutionary model of multistage carcinogenesis provides this framework by demonstrating that the number of tumour suppressor loci (TSLs) preventing cancer in a given tissue is expected to depend upon the tissue's vulnerability to pre-reproductive somatic mutation. Since this vulnerability increases with tissue size, single gene control of human cancer may be restricted to retinoblastoma, a cancer of the tiny embryonic retina. The model is used to estimate the frequency of mutant alleles causing inherited cancers, based on the population genetics of the mutation–selection balance between new mutations arising and selection that eliminates them. For each specific cancer, this balance is determined by the effectiveness with which pre-reproductive cancer is suppressed in the non-mutant genotype characteristic of that population. Effectiveness depends on an interaction between the number of TSLs suppressing the cancer and factors determining the tissue-wide somatic mutation rate, such as tissue size and number of pre-reproductive cell divisions. The model predicts that the commonest pre-reproductive cancers will have the lowest proportion of familial cases, and that cancers associated with the most TSLs will have the highest post-reproductive incidence but no elevated pre-reproductive risk (a pattern seen in human epithelial cancers).

Keywords: cancer; evolution; mutation–selection balance; lineage selection; tumour suppressor; genetic redundancy

1. INTRODUCTION

Cancer is a disease of multicellular animals. It occurs when one or more cells within an individual begin to divide uncontrollably, usually as a result of somatic mutation. A variety of genes normally prevent such unregulated division; however, these genes are vulnerable to both inherited and somatic mutation and if mutation knocks out these genetic controls then cancer will be initiated (see Hanahan & Weinberg 2000; Ponder 2001). This progression was first suggested in the 1950s (Nordling 1953; Armitage & Doll 1954) and is now recognized as the mutation-driven process of multistage carcinogenesis (Yokota 1994; Boland & Ricciardiello 1999). We usually think of mutations as rare events, occurring in the order of once per locus per million to ten million cell divisions. However, if we consider that large multicellular organisms such as humans consist of more than 10^{13} cells, some of which divide continuously throughout life, then what is rare on a per cell basis becomes inevitable in whole organisms. Almost any mutation that can occur will occur at some point during the lifetime of the individual. However, if mutations are so common, why is cancer so rare? The question of interest thus becomes 'What prevents cancer?' rather than 'What causes cancer?'

In multistage carcinogenesis, each type of cancer is due to the accumulation of mutations in a specific set of genes, so a cancer is familial if at least one of the necessary multistage mutations is inherited. Familial cancers can also result from an inherited mutation that raises the somatic mutation rate (Hoeijmakers 2001). However, even when an individual has no inherited predisposition to cancer, the critical set of mutations can accumulate somatically throughout life, leading to non-familial (sporadic) cancer. Such cancers can have clear environmental origins due to carcinogens that elevate the somatic mutation rate and drive the accumulation of cancer-causing mutations. It is even possible for carcinogens to act indirectly on the somatic mutation rate by favouring cells with specific types of genetic instability (Bardelli *et al.* 2001). However, an evolutionary perspective predicts that many sporadic cancers will have no identifiable cause, genetic or environmental.

Natural selection does not promote the immortality of individuals (Williams 1957). As a result, cancer need not be the result of inherited mutations or elevated exposure to carcinogens. Population genetic theory predicts that our baseline somatic mutation rate will sometimes result in the cancer-causing mutations accumulating by chance (Nunney 1999*a*). Natural selection favours the suppression of most pre-reproductive cancers, but we are not perfectly adapted to suppress all cancers, particularly those originating late in life when natural selection is weak or absent.

Many different approaches have been employed to determine the ratio of familial to sporadic cancers, but there is currently no model for predicting the frequency of inherited cancers. I use a population genetic approach in combination with an evolutionary model of cancer suppression. The ideas of evolutionary biology have long been productively applied in the study of cancer at the level of the cell with the recognition that the development of an invasive tumour is a dynamic process of mutation and selection (see Nowell 1976; Tomlinson *et al.* 1996). However, evolutionary ideas have rarely been exploited in understanding cancer at the level of the individual (Greaves 2000), even though population genetic models of mutation–selection balance have been used extensively to predict the frequency of single-locus genetic diseases. I extend this mutation–selection approach to multistage

carcinogenesis. I use an evolutionary model to investigate how relatively large-scale changes in tissue characteristics (such as size) affect the number of genes involved in cancer suppression, and how this gene number interacts with smaller-scale variation in tissue characteristics to influence the frequency of mutant alleles. These mutant alleles are the source of familial (inherited) cancers, and predicting their abundance is crucial to resolving the medical debate concerning how much of our cancer is due to our genotype.

2. EVOLUTION OF CANCER SUPPRESSION

An evolutionary perspective predicts that the number of loci suppressing a given cancer can vary over evolutionary time and across taxa (Nunney 1999*a*,*b*). Any evolutionary trend that increases the level of pre-reproductive somatic mutation, such as increasing size or lengthening juvenile period, will increase the risk of any specific cancer. As a result, one or more cancers may become significant sources of early mortality and natural selection will promote the spread of a resistant genotype.

The genes recruited for added cancer suppression could be duplicate copies of genes already involved, or could be other pre-existing loci adopting a new role. In either event, the new activity is likely to be tissue specific, influencing primarily the particular cancer under selection. However, changes that have a more general effect could also be selected, so that selection for the suppression of a single cancer could enhance the suppression of all cancers. A possible example of general suppression is the decay of telomeres with cell division (Stewart & Weinberg 2000).

But what level of mortality triggers the successful selection of increased cancer suppression? Wright (1931) demonstrated that selection is deterministic only if $N_e s \gg 1$, where N_e is the effective population size and s is the selection coefficient. The point $N_e s = 1$ is generally used as a rough guide to the point of transition from selection being dominated by genetic drift (i.e. selection too weak to overcome random sampling) to selection overcoming drift. In the context of cancer, any increase in the pre-reproductive death rate (s) will increase the product $N_e s$. Once the inequality $N_e s > 1$ is satisfied, the chance of recruiting additional cancer suppression rapidly increases. When this occurs, the incidence of the cancer drops. Thus, there is no expectation that a very large long-lived animal will have a higher frequency of early-onset cancer than a small, short-lived one. Instead, the expected outcome is that the large animal will have more genes involved in cancer suppression.

The type of genetic change necessary to augment cancer suppression differs from 'typical' adaptive change. It requires the expression of new genes in the target tissue, rather than the allelic modification of pre-existing ones. Furthermore, the selection driving change originates from within the genome of each individual, because the evolution of cancer suppression is driven by an internal 'levels of selection' conflict. The conflict is between the short-term success of cancer cells (proliferation) and the long-term success of the individual containing them (sexual reproduction) (Michod 1996; Nunney 1999*b*). This conflict reflects the problem that is at the very core of the evolution of multicellularity (see Maynard Smith & Szathmáry 1995; Michod 1999*a*).

Cancer cells are evolutionarily successful when viewed at the time-scale of cells: they can be seen as 'cheats' that efficiently exploit the available resources within an individual and produce many copies of themselves. Obviously, they are doomed to extinction when viewed at the time-scale of individuals, but unfortunately, as we know all too well, their future fate does not influence their short-term success. Thus, the success of cancer cells reflects the general rule that natural selection acting at the shortest time-scale is the most effective; however, there is an exception. Lineage selection favours long-term benefit whenever there is a conflict between the short- and the long-term effects of selection, provided long-term lineages exist (in this case individuals; Nunney (1999*a*,*b*)). In relation to cancer suppression, lineage selection protects the individual level by favouring genotypes that have an enhanced ability to suppress cell-level cheats.

Lineage selection also favours the evolution of policing strategies (Frank 1995). Michod (1996) introduced a model to explore the transition to multicellularity that incorporates the evolution of such policing (see Michod 1999*a*,*b*). The problem of selfish mutants increases rapidly with size (Roze & Michod 2001), and analysis of a two-locus model (one locus determining cooperation versus selfishness; the other determining the policing activity of cooperating cells) supports the view that the recognition and destruction of cancer cells may have been an important factor in the evolution of the immune system (McKean & Zuk 1995).

It is clear that genotypes with too little regulation over cellular proliferation will be selected against. Perhaps less obvious is the fate of genotypes that over-regulate. Over-regulation could occur if, for example, selection favoured a smaller body size, or if some general cancer suppression mechanism was recruited, as outlined above. Genotypes with over-regulation avoid cancer, but may be at a disadvantage if they expend significant energy on maintaining unnecessary regulation. Even without this disadvantage, mutation–selection balance comes into play to erode redundancy. Mutations that knock out part of such a system will be effectively neutral and will accumulate in the population. Consequently, over-regulation will decay until the appropriate mutation–selection balance is achieved, as defined below.

3. A MULTISTAGE MODEL

Nunney (1999*a*) developed a model of multistage carcinogenesis in which each type of cancer is regulated by a set of n tumour suppressor loci (TSLs). Thus, the 'best' genotype has $2n$ functioning gene copies acting to prevent unregulated cell division. However, owing to the effects of recurrent germline mutation, other genotypes will exist in the population that carry j functioning gene copies, where $2n \geqslant j \geqslant 0$, and i non-functioning copies, so that $i + j = 2n$. Assuming the cancer is lethal or prevents reproduction, then the fitness (w_i) of a genotype already carrying i cancer-susceptibility mutations is the probability that no cell lineage accumulates, before reproduction, the j additional mutations that lead to cancer. Thus $w_i = 1 - s_i$,

where s_i is the probability of contracting and dying of pre-reproductive cancer.

To calculate the s_i, we need to calculate the probability that the j functioning alleles all accumulate somatic mutations in at least one cell. For simplicity, it is assumed that a single functioning copy of any one of these TSLs is sufficient to prevent a cell from becoming cancerous (but see below). In this context, somatic mutation includes anything that directly disrupts these gene copies and includes, in addition to DNA base mutation, insertions/deletions and larger-scale chromosomal abnormalities resulting from genetic instability (Cahill *et al.* 1999) and epigenetic gene silencing (Jones & Laird 1999).

It is useful to distinguish two kinds of tissue, representing opposite ends of a continuum. At one extreme, some tissues have negligible post-embryonic cell division and are primarily susceptible to somatic mutation during the developmental period of geometric growth. An example of such tissue is the embryonic retina, the retinoblast, in which the cancer retinoblastoma originates (see below). At the other extreme, some tissues divide regularly throughout life, so that the relatively short growth phase can be ignored. Examples include stem cell populations such as the crypt cells of the small intestine (Cairns 1975).

In tissues with negligible post-growth division, the risk of cancer (s_i) for a genotype with i non-functioning alleles (out of a possible $2n$) is approximately

$$s_i = 2(2n - i)(k - 1)^{(2n-i-1)}u^{(2n-i)}C \qquad (3.1)$$

when s_i is small (Nunney 1999*a*). The tissue grows to C cells, so that the number of divisions required is k ($= \ln(C)/\ln(2)$). The risk of somatic mutation causing loss of function is u per allele per daughter cell, and back mutation is ignored.

In tissues where the primary risk of somatic mutation is in the frequent post-growth cell divisions, the tissue size can be considered constant and the risk of cancer is defined by

$$s_i = 1 - \{1 - [1 - \exp(-uk)]^{(2n-i)}\}^C, \qquad (3.2a)$$

where k is the number of pre-reproductive cell divisions (Nunney 1999*a*). If cancer is very rare (s_i small), then

$$s_i = C(uk)^{(2n-i)}. \qquad (3.2b)$$

In deriving equations (3.1) and (3.2), it was assumed that somatic mutation is correlated with division rate, i.e. that some combination of division and the other transcriptional activity of dividing cells results in an elevated somatic mutation rate. There is some evidence that this may not always be the case when comparisons are made among different tissues (Dolle *et al.* 2000; Huttley *et al.* 2000). The assumption is not critical, but serves to simplify the parameterization of the model and facilitates comparisons of the same tissue type.

A second assumption was that unregulated cell division is completely inhibited provided at least one functioning allele remains. This assumption has been used to argue that the rates of somatic mutation are insufficient to drive multistage carcinogenesis (Orr-Weaver & Weinberg 1998). In fact, some clonal proliferation may occur as somatic mutations accumulate, an idea originally incorporated in the two-stage, one-locus model of Moolgavkar & Venzon (1979). Such stepwise clonal proliferation has the effect of increasing the likelihood that a cell lineage will accumulate the complete set of somatic mutations (Tomlinson & Bodmer 1999). The resulting increased risk of cancer occurs because stepwise proliferation effectively increases the somatic mutation rate by increasing the size of the population of vulnerable cells. Thus, it increases the likelihood of all mutations except the first. As a result, cell proliferation following somatic mutation can be incorporated easily into the model by using an increased 'effective' somatic mutation rate, i.e. the somatic mutation rate multiplied by the extent of cell proliferation.

The use of an effective somatic mutation rate does not precisely incorporate all possible scenarios in which pre-cancerous cells have enhanced proliferation (e.g. Tomlinson *et al.* 1996). However, the possibility of complex clonal selection following each mutational step of the multistage process does not qualitatively alter the conclusions of the model. It does, however, have one very important effect—the expected number of TSLs necessary for cancer suppression is always increased.

The fitness equations (3.1) and (3.2) can be used to predict the evolutionarily stable number of TSLs. For example, in a continuously dividing tissue, selection will act until (approximately) $N_e s < 1$, so the expected number of TSLs will be the smallest integer value of n greater than n_{min}, where

$$n_{min} = \frac{\ln(C) + \ln(N_e)}{-2\ln(uk)} \qquad (3.3)$$

(substituting $s = 1/N_e$ in equation (3.2*b*)). Of course, selection for further protection also acts on the more cancer-prone genotypes carrying one or more mutant alleles; however, this effect can be ignored because close to points of evolutionary transition these genotypes are infinitesimally rare (see below).

The expectation of $n \geqslant n_{min}$ assumes evolutionary equilibrium. In most cases, this is probably a reasonable assumption, since there is no *a priori* reason to believe that cancer suppression lags behind other adaptations. However, any rapid change leading to increased risk could lead to higher than expected levels of cancer persisting for a significant period of time until the appropriate level of cancer suppression evolves.

4. IS ONE GENE ENOUGH?

The occurrence of any genetically based disease (including familial cancer) depends upon the frequency of deleterious mutant alleles in the population. These alleles reach an equilibrium at mutation–selection balance, when the addition of deleterious alleles through germline mutation is balanced by their loss through natural selection due to the disease. We have an accurate population genetic model for defining the allele frequency at mutation–selection equilibrium for inherited genetic disorders caused by a single gene. Thus, given a dominant disadvantageous allele

$$\hat{q} = u_{germ}/s, \qquad (4.1)$$

where $\hat{q}$ is the equilibrium frequency of the mutant allele, s is the selective disadvantage (where the relative fitness, w, of a genotype carrying the allele is $1 - s$), and u_{germ} is

Table 1. Simulation of the transition from one- to two-locus cancer suppression in response to an increase in tissue size, using parameter values from retinoblastoma and introducing a hypothetical second TSL, *RB*2, to supplement *RB*1.
(Simulation model: *RB*1 was initially fixed in the population, with *RB*2 switched off in the retinoblast (−). Functional ($RB2^+$) alleles were introduced at the start of the simulations at a frequency of 1%. I estimated the percentage of times (out of 50 runs) that $RB2^+$ became established for a given tissue size. Establishment was defined by three criteria: increasing to a frequency greater than 10%, 50% or 90% in 5000 generations. Mutation from functional (+) to non-functional alleles (−) occurred in both loci at a germline rate of $u_{germ} = 10^{-5}$ and a somatic rate $u = 4 \times 10^{-7}$. The model population, with $N = 7500$ adults, was ideal except that fecundity was exactly four per female (raising N_e; Nunney (1991)) and the mating system was lottery polygyny with females mating only once (lowering N_e; Nunney (1993)) giving $N_e = 6000$. Among the 15 000 offspring, cancer risk was assessed according to each individual's genotype, and the adults were chosen randomly from the juveniles that avoided cancer.)

tissue size ($\times 10^6$ cells)	$N_e s$	cancer incidence in one-locus genotype $RB1^+/RB1^+$; $RB2^-/RB2^-$	percentage successful spread of $RB2^+$ allele from 1% to:			expected number of TSLs
			> 10%	> 50%	> 90%	
4	0.3	0.000 05 = 1/20 000	0	0	0	1
10	0.8	0.000 14 = 1/7140	0	0	0	1
20	1.7	0.000 29 = 1/3450	8	2	0	2
40	3.7	0.000 61 = 1/1640	8	4	0	2
100	10.0	0.001 66 = 1/600	28	24	2	2
400	41.5	0.007 17 = 1/140	48	48	36	2

the germline mutation rate to that allele per generation. The frequency of the disorder in the population is $2\hat{q}$ (given that $\hat{q} \ll 1$).

Retinoblastoma is a cancer known to fit a single-gene model (Knudson 1971). However, it is likely that retinoblastoma is unique in this respect, with all other human cancers regulated by more than one gene. This conclusion follows from the relationship between tissue size and the somatic mutation rate: the known regulation of retinoblastoma by the gene *RB*1 is precisely the level expected for an embryonic tissue of only a few million cells (Nunney 1999*a*). However, compared with other tissues, the retinoblast has a minimal tissue-wide somatic mutation rate, since it is both small and lacks post-embryonic division. Even so, heterozygotes for *RB*1, with only a single functioning copy of the gene, almost invariably lose that copy due to somatic mutation in at least one cell of the retinoblast (Hethcote & Knudson 1978). We also know that this occurs primarily during tissue growth so that the average age of diagnosis of bilateral (and hence familial) retinoblastoma is 12 months of age (Newsham *et al.* 1998). Hethcote & Knudson (1978) demonstrated that the incidence of familial cancer is consistent with a somatic mutation rate of 4×10^{-7} per daughter cell given that the pair of retinoblasts reach a combined size of 4×10^6 cells.

We can use the example of retinoblastoma to examine the effect of increasing the incidence of pre-reproductive somatic mutation by increasing tissue size. The expectation is that the recruitment of a second gene becomes rapidly more probable once $N_e s > 1$. To test this prediction, I simulated a population ($N_e = 6000$) using equation (3.1) to define the risk of retinoblastoma as the size (C) of the retinoblasts was varied. At the beginning of each simulation, functioning alleles of a new hypothetical TSL (*RB*2) were introduced at low frequency into the population and the fate of the new TSL was recorded (table 1). The transition from one- to two-locus regulation was never observed using the human tissue size estimate of 4×10^6 cells or using 10^7 cells ($N_e s = 0.8$). However, for tissue of 2×10^7 cells ($N_e s = 1.7$), the hypothetical $RB2^+$ (functioning) allele was able to spread in 8% of the simulations. These simulations, which are conservative because they lack recurrent mutation of $RB2^-$ alleles (those not expressed in the retinoblast) to $RB2^+$, are in excellent agreement with the theoretical expectation. We can confidently predict that tissues more than approximately five times larger than the retinoblasts would evolve two-locus cancer suppression, even under the conservative condition of $N_e = 6000$. Such tissues would only be about the size of five fruitflies, and any post-growth division in the tissue would promote two-locus control in even smaller tissues. Hence, it appears that one-locus control is insufficient in any human tissue except in the uniquely small retinoblast.

Other possible candidates for single-gene regulation have been suggested, including von Hippel–Landau renal carcinoma and neurofibromatosis I and II. These cancers produce their potentially lethal symptoms much later (*ca.* 15–20 years of age; see Linehan & Klausner 1998; Gutmann & Collins 1998; MacCollin & Gusella 1998), and involve much larger populations of cells. All are inherited as dominant disorders (like retinoblastoma), but the markedly later onset, plus the much larger population of target cells, makes a single-gene model untenable.

The prediction that multigenic cancer suppression is the rule in humans conflicts with the successful application of the two-stage model of Moolgavkar & Venzon (1979). Their model assumed that only two mutations are necessary for cancer development and refined the two-hit hypothesis of Knudson (1971) by including clonal proliferation. While this elegant four-parameter model can be statistically fitted to incidence data (Moolgavkar 1986), the parameter estimates for large tissues are inevitably constrained to define the very slow dynamics necessary for one-locus suppression; specifically, unrealistically low rates of cell division and/or somatic mutation (see Moolgavkar & Luebeck 1992). For example, Gregori *et al.* (2002) estimated parameters for breast cancer from six cohorts. Using their estimates, and assuming (conservatively) that breast tissue has only 10^9 cells that divide once per month, the somatic mutation rates for the first

Table 2. Per-generation loss of a cancer-causing allele (m) at a single locus, when cancer suppression is mediated by n identical TSLs.
(The frequency of the m alleles is q at all loci, and frequency of the normal allele (+) is p. The fitness disadvantage (s_i) of genotypes is defined by the number of m alleles (= i) that it carries over all n loci.)

number of alleles (i)	genotype at target locus	genotype at $(n-1)$ other loci	frequency	summed loss of m allele at target due to cancer (L_i)
1	$+/m$	+/+	$2p^{(2n-1)}q$	$L_1 = s_1 p^{(2n-1)}q$
2	m/m	+/+	$p^{(2n-2)}q^2$	$L_2 = (2n-1)s_2 p^{(2n-2)}q^2$
2	$+/m$	+/+ except one locus $+/m$	$4(n-1)p^{(2n-2)}q^2$	
3	m/m	+/+ except one locus $+/m$	$2(n-1)p^{(2n-3)}q^3$	$L_3 = (2n-1)(n-1)s_3 p^{(2n-3)}q^3$
3	$+/m$	+/+ except one locus m/m	$2(n-1)p^{(2n-3)}q^3$	
3	$+/m$	+/+ except two loci $+/m$	$4(n-1)(n-2)p^{(2n-3)}q^3$	

and second TSL mutations are unacceptably low at roughly 4×10^{-9} and 10^{-11} per division.

5. MUTATION–SELECTION BALANCE

Given that cancer suppression is generally multigenic, we need to extend the mutation–selection balance equation (4.1) to predict the frequency of non-functioning TSL alleles segregating in a population. Unfortunately, we have a very limited understanding of mutation–selection balance when the genetic systems involve more than one gene (Phillips & Johnson 1998). To alleviate this problem, we can use multistage cancer suppression as the genetic model. The calculation was simplified by assuming that all of the suppressor loci have the same germline mutation rate (u_{germ}) and that the effects of linkage can be ignored. This makes all of the loci equivalent, and the expected frequency of the non-functioning mutant allele is the same for each locus (= q). To calculate this frequency, we need expressions for both the gain and loss of mutant alleles at each locus per generation.

The gain per generation in mutant alleles is $u_{\mathrm{germ}}\,p$ at each locus, where p is the frequency of wild-type alleles (= $1 - q$). The loss is due to natural selection acting against any genotype carrying less than $2n$ functioning alleles. To estimate the loss precisely, we need to estimate s_i, the risk of pre-reproductive cancer, and g_i, the frequency of genotypes carrying i mutant alleles, for $0 < i \leqslant 2n$. However, g_i becomes very small as i increases and we need consider only $i = 1$, 2 and 3 (an assumption verified by simulation; see below). The loss of mutant alleles per generation due to cancer is calculated by first grouping genotypes according to the number (i) of mutant alleles that they carry across all of the TSLs regulating the cancer ($i = 0$, 1, 2, 3, etc.). Each genotype with the same value of i has the same fitness. Next, the loss (L_i) of mutant alleles from a single locus is calculated using these fitness values, the frequency of each genotype, and whether the genotype carries 0, 1 or 2 mutant alleles at the target locus (see table 2). This summed loss is equated to the mutational gain ($u_{\mathrm{germ}}p$). The relationship is simplified by dividing through by p and ignoring terms of order q^4 or greater (i.e. assuming $q^4 \approx 0$), giving

$$[(n-1)(2n-3)s_1 - (2n-1)(2n-3)s_2 + (2n-1)(n-1)s_3]q^3 + [(2n-1)s_2 - (2n-2)s_1]q^2 + s_1 q - u_{\mathrm{germ}} = 0. \quad (5.1)$$

The solution of equation (5.1) defines the equilibrium frequency ($\hat{q}$) of the mutant alleles. It can be applied to continuously dividing tissues using s_i derived from equation (3.2) or to non-dividing tissues using equation (3.1).

6. FREQUENCY OF FAMILIAL CANCERS

Mutation–selection balance defines the frequency of inherited mutant alleles in a population. Knowing this frequency is essential in any search for predictable patterns in the incidence of familial cancer. Simulations were used to confirm that equation (5.1) accurately defines the mutation–selection balance, and to examine further the relationship between the incidence of cancer and the proportion attributable to inherited mutant alleles. The simulations modelled a population of constant adult size with non-overlapping generations (see figure 1 for details). The vulnerable tissue was assumed to be continuously dividing, and equation (3.2*a*) was used to calculate s_i. The results of the simulations verified the mutation–selection balance equation (5.1) (figure 1).

The overall pattern of the simulations is shown in figure 1, which shows the frequency of mutant alleles plotted as a function of n_{min} (equation (3.3)), which defines the idealized minimum level of cancer suppression for a given tissue. Increasing the risk of pre-reproductive cancer in the 'best' (mutation-free) genotype (s_0) (for example, by increasing tissue size) corresponds to an increase in n_{min}. The pattern has two components. First, for a fixed number of TSLs (n), increasing n_{min} increases selection against genotypes carrying mutant alleles and lowers their equilibrium frequency in a fashion predicted by equation (5.1). The result is a declining curve, a result repeated for TSL numbers of 1, 2, 3 and 4 (see figure 1). Second, increasing s_0 beyond the point $N_e s_0 = 1$ for a given n promotes the recruitment of an additional TSL (for example, see table 1). The point at which each transition from n- to $(n+1)$-locus regulation becomes likely is shown by a vertical line joining a lower and an upper curve for each of $n = 1$ to 3. The result is a saw-tooth pattern in the frequency of mutant alleles. At each transition point, the lower curve defines the mutation–selection balance for n loci while the upper curve defines the mutation–selection balance for $(n+1)$ loci. The transition occurs because, at that point, n loci provide incomplete protection against cancer.

After the recruitment of a new TSL, a new $(n+1)$-locus genotype carrying a single mutant allele has greater

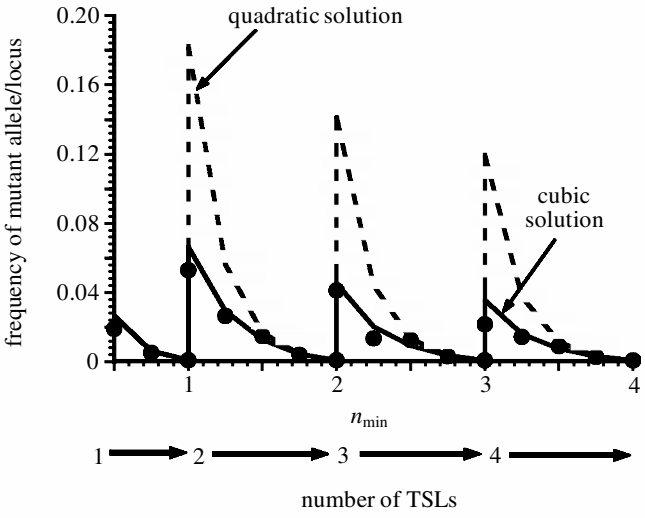

Figure 1. Mutation–selection balance defines the expected frequency of deleterious (mutant) alleles at TSLs. The solid circles are derived from simulations of the model and these values are accurately predicted by the solution of the cubic approximation (equation (5.1); solid line). The quadratic approximation (dashed line) has a poor fit to the data. The x-axis (n_{min}; see equation (3.3)) increases with tissue size, and reflects increases in the tissue's risk of pre-reproductive somatic mutation. The numbers below the x-axis are the expected evolutionarily stable number of TSLs for a given tissue size, defined by the smallest integer greater than n_{min}. The model population was as defined for table 1, except $N = 10\,000$ ($N_e = 8000$). All loci were unlinked. Each simulation was run for 5200 generations and parameters were estimated by averaging over the final 5000 generations of four replicate runs. Fixed parameter values: $uk = 0.01$, $u_{germ} = 10^{-5}$.

cancer protection than the best (old) n-locus genotype. Consequently, mutant alleles can accumulate to appreciable frequencies; hence the jump in the graph (figure 1). At this threshold tissue size, there is no effective selection for further increasing protection to $(n + 2)$ loci, since the extra fitness advantage of $(n + 2)$ loci over $(n + 1)$ would be infinitesimally small ($N_e s_0 \ll 1$). Natural selection cannot overcome random sampling to favour such small advantages, and the possibility is made even less likely if any costs are incurred by adding an additional layer of genetic regulation.

The frequency of mutant alleles in a population drives the occurrence of inherited forms of cancer. However, it does not tell us directly what fraction of cases of any given cancer can be attributed to the inheritance of one or more deleterious alleles. To calculate this fraction we need to know the total number of cases. The simulations provided the initially unexpected answer: the higher the incidence of a cancer, the lower the proportion that is familial (figure 2). The negative correlation arises because cancer suppression is controlled by a relatively small number of genes, so that cancer cannot be regulated precisely. When the best genotype is very effective, the cancer will be rare and usually only an individual carrying an inherited mutation will succumb to the disease. When the best genotype is less effective, the cancer will be more common and many occurrences will have no inherited cause because they will be in individuals with the best genotype.

The negative correlation between overall incidence and percentage familial incidence makes the prediction that rare cancers are expected to be almost entirely genetic in causation, unless, of course, some novel environmental factor (e.g. a mutagen) is introduced. Conversely, commoner early-onset cancers will be more often sporadic (non-inherited) than rarer cancers. Data on childhood cancers are generally consistent with this prediction. The commonest solid tumour of childhood is neuroblastoma (with a frequency of *ca.* 1/8000) and has been estimated at 22% familial (Knudson & Strong 1972). Retinoblastoma has an incidence of *ca.* 1/20 000, and is *ca.* 40% familial (Newsham *et al.* 1998). By contrast, some other cancers of early childhood are much rarer and are close to 100% familial (e.g. Li-Fraumeni syndrome; Malkin (1998)).

7. LATE-ONSET CANCER

The incidence of cancer generally increases exponentially with age. Armitage & Doll (1954) used a multistage model to show that this rate of increase is proportional to the number of TSLs involved. However, lacking an evolutionary perspective, they had no basis for establishing how the frequency of a cancer would be related to the number of TSLs. We can resolve this problem by recognizing that the effect of natural selection declines to zero late in life. As a result, there is an important qualitative difference between the frequency of early- and late-onset cancer (figure 3). The incidence of pre-reproductive cancer shows no strong relationship with TSL number, whereas the incidence of post-reproductive cancers increases dramatically. For cancers controlled by a single TSL, the difference in frequency between early- and late-onset cancers is quite small; however, for cancers controlled by four TSLs, the difference in frequency is 10–100-fold (figure 3).

This relationship suggests a simple explanation for the observed age-related shift in the proportion of human cancers that are epithelial, mesenchymal or haemopoietic. Specifically, the proportion of epithelial cancers increases markedly with age and it has been argued that explaining this shift is an important prerequisite for understanding cancer suppression (DePinho 2000). The multistage model predicts that it occurs as a direct consequence of a larger number of genes involved in the regulation of epithelial cancers, which in turn is the result of a large tissue size (C) and a high cell turnover (k).

8. DISCUSSION

Multigenic cancer suppression can be viewed as a form of genetic redundancy since several genes are maintained where, under ideal conditions, only one gene is needed (see Tautz 1992). However, this 'redundancy' is actively maintained by selection. Nowak *et al.* (1997) developed a haploid model of such redundancy in genes controlling an essential developmental step. They assumed that a single gene could perform the task, but due to a constant probability of gene failure, several apparently redundant genes were maintained. Using a clonal model, they demonstrated that, even in an infinitely large population, selection could maintain a fixed maximum number of

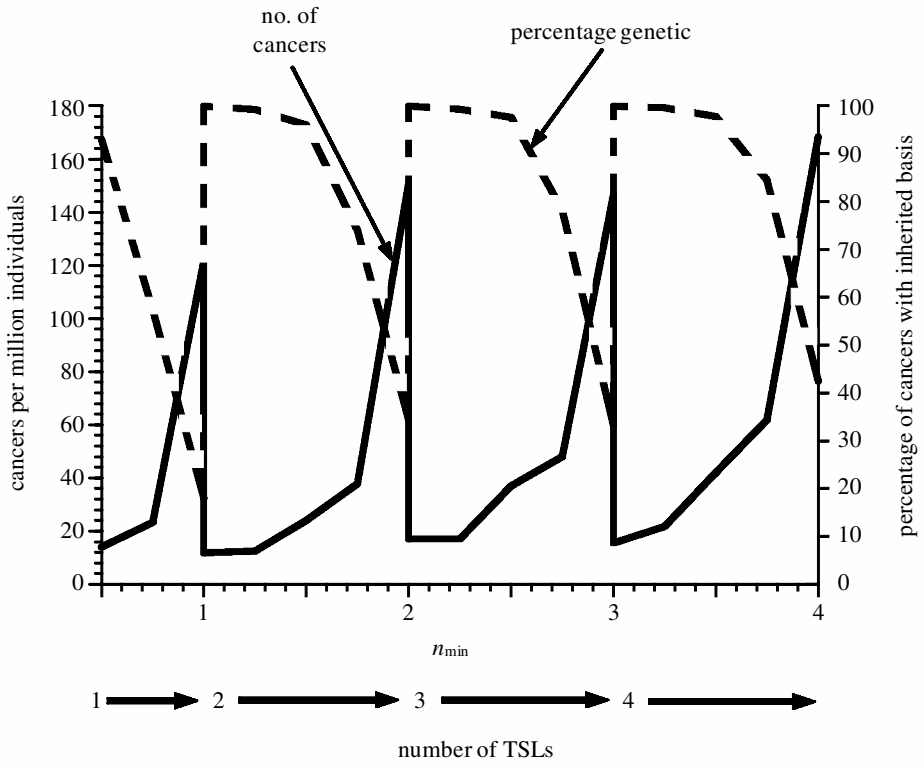

Figure 2. The negative association between the frequency of early-onset cancer (number of cancers) and the proportion of these cases that has some inherited cause (percentage genetic). The *x*-axis and simulations were as defined in figure 1.

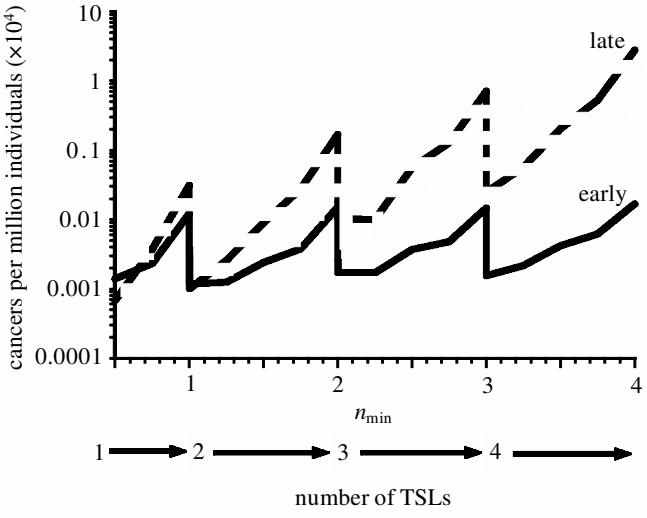

Figure 3. The incidence of late- and early-onset cancers, as a function of a tissue's risk of somatic mutation (*x*-axis). The *x*-axis is defined as in figure 1. Note that the incidence is plotted on a log scale. The data are from simulations as defined in figure 1, with late-onset cancers defined as those occurring during a post-reproductive period identical in duration to the pre-reproductive period. Late-onset cancers are caused by the somatic mutations occurring late in life adding to those already accumulated during the pre-reproductive period; however, post-reproductive cancer has no effect on fitness and cannot drive natural selection for increased cancer suppression.

functioning genes. Beyond that number, selection was too weak to replace the 'perfect' wild-type genotypes lost by germline mutation. However, this limit does not apply if recombination occurs, because the fully wild-type genotype can increase in frequency due to recombination as well as by selection. Given recombination, the present analysis has shown that the number of genes maintained depends on the strength of selection versus genetic drift (equation (3.3)), although the frequency of mutant alleles depends strongly on the balance of selection and mutation (equation (5.1)).

The complex interaction of selection, drift and mutation in the model of multistage carcinogenesis has revealed three robust predictions. The first is the prediction that all lethal (or sterilizing) human cancers, with the exception of retinoblastoma, are regulated by more than one gene. This is based on estimates of tissue-wide somatic mutation rates. Second, it is predicted that the commonest pre-reproductive cancers will have the lowest percentage of familial causation. This prediction depends primarily on the well-supported assumption that cancer suppression does not fit into the classic continuous quantitative genetic mould. It is a trait determined by a relatively small number of loci of large effect, rather than a large number of loci each of small effect. As a result, each additional TSL has a discontinuous influence (see figure 1) and the predicted pattern follows.

The third prediction, that cancers regulated by many TSLs will become disproportionately more abundant late in life, depends on the truism that natural selection acts to minimize fitness loss, and as such it has no direct effect on post-reproductive patterns of survival. Natural selection acts to prevent cancer up to the age at which reproductive investment ceases. As a result, we can expect that, at that age, some cell lineages will be close to becoming cancerous (e.g. just one mutational event away). However, tissues with large numbers of TSLs are those with high somatic mutation rates (i.e. they are large or rapidly

dividing) and, assuming that these high rates continue into post-reproductive life, these tissues will be very vulnerable to accumulating the few additional somatic mutations necessary to induce cancer.

The model can also be used to speculate on interspecific effects. Animals that are large and long-lived are intrinsically more vulnerable to cancer; however, we do not expect them to exhibit a higher frequency of pre-reproductive cancers. Instead, we expect them to have more TSLs involved in cancer suppression than very small, short-lived animals (Nunney 1999*a*). Beyond this qualitative expectation, our understanding of the genetic suppression of cancer is insufficient to make more specific predictions, with the exception of retinoblastoma. Assuming that the relevant developmental trajectory of the retina is evolutionarily conserved, we can confidently predict that animals markedly larger than humans have a second gene to suppress this cancer. Without a second gene, retinoblastoma would occur at high enough levels to significantly depress average fitness.

Finally, while there are good reasons to believe that the predictions from the evolutionary model are robust, a model of multistage carcinogenesis based on TSLs is, of course, a great simplification. Including more complexity in the model, particularly the addition of genes influencing somatic mutation (Loeb 1991) and of clonal expansion and selection following each mutation (Tomlinson & Bodmer 1999), will undoubtedly provide useful additional insight into the population genetics of cancer.

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