**Modelling the evolution of genetic instability during tumour progression**

Ruchira S. Datta, Alice Gutteridge, Charles Swanton, Carlo C. Maley and Trevor A. Graham

1 Center for Evolution and Cancer, University of California San Francisco, San Francisco, CA, USA
2 Center of Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPELX), University College London, London, UK
3 Translation Cancer Therapeutics Laboratory, Cancer Research UK London Research Institute, London, UK

**Keywords**
carcinogenesis, clonal expansion, genetic instability, mathematical biology, selection

**Abstract**
The role of genetic instability in driving carcinogenesis remains controversial. Genetic instability should accelerate carcinogenesis by increasing the rate of advantageous driver mutations; however, genetic instability can also potentially retard tumour growth by increasing the rate of deleterious mutation. As such, it is unclear whether genetically unstable clones would tend to be more selectively advantageous than their genetically stable counterparts within a growing tumour. Here, we show the circumstances where genetic instability evolves during tumour progression towards cancer. We employ a Wright–Fisher type model that describes the evolution of tumour subclones. Clones can acquire both advantageous and deleterious mutations, and mutator mutations that increase a cell's intrinsic mutation rate. Within the model, cancers evolve with a mutator phenotype when driver mutations bestow only moderate increases in fitness: very strong or weak selection for driver mutations suppresses the evolution of a mutator phenotype. Genetic instability occurs secondarily to selectively advantageous driver mutations. Deleterious mutations have relatively little effect on the evolution of genetic instability unless selection for additional driver mutations is very weak or if deleterious mutations are very common. Our model provides a framework for studying the evolution of genetic instability in tumour progression. Our analysis highlights the central role of selection in shaping patterns of mutation in carcinogenesis.

**Introduction**
The role of genetic instability as a driver of tumorigenesis remains controversial. Recent whole-genome sequencing studies show that cancers frequently contain hundreds of putative driver mutations that are suggested to play a causative role in tumour development (Loeb 2011). Given the impressive replication fidelity of normal cells [estimated mutation rate of between $10^{-9}$ and $10^{-10}$ per base pair per division (Salk et al. 2010)], it has been argued that an elevated mutation rate is required to acquire this large number of carcinogenic mutations within a reasonable time (Loeb 2001). However, whilst this intuitive argument for a central role of genetic instability in tumorigenesis is appealing, it is likely to represent an oversimplification and as such may be misleading, for three key reasons. First, whilst an increase in the mutation rate accelerates the accumulation of tumour-causing mutations, it will also accelerate the accumulation of mutations deleterious for tumour growth (Cahill et al. 1999); therefore, an increase in the mutation rate could potentially cause retardation of tumour growth, a phenomenon termed negative clonal selection (Beckman and Loeb 2005). Indeed, breast cancers with the most extreme levels of chromosomal instability (CIN) have a better prognosis than cancers with more moderate levels of genetic instability (Birkbak et al. 2011; Roylance et al. 2011). Second, the development of a genetically unstable cancer, rather than a genetically stable cancer, requires that the tumour acquires additional (epi-) mutations in genome-destabilising genes (Tomlinson et al.
Evolution of genetic instability

1996); if genetic instability is an initiating event, rate-limiting disruption to these instability genes must occur prior to rate-limiting driver gene mutations. Third, the argument neglects the effects of clonal expansion as a means to drive malignant progression; clonal expansion increases the number of cells susceptible to further mutations and so accelerates carcinogenesis (Tomlinson and Bodmer 1999; Luebeck and Moolgavkar 2002). Here, we investigate the evolution of an increased mutation rate during tumour progression using a mathematical model of tumour progression. We determine the circumstances that select for an increased mutation rate and the circumstances when an increased mutation rate accelerates tumour progression.

There is extensive and conflicting literature about the role of genetic instability in both the initiation of tumour growth (hereafter termed initiation) and in the progression of established tumours towards cancer (hereafter termed progression). Herein, we focus on the role of genetic instability in tumour progression. Marked genetic instability is certainly found in many cancer types: for example, 85% of colorectal cancers (CRCs) show CIN, and the remaining 15% have microsatellite instability (MSI) (Fearon 2011). Furthermore, chromosomally unstable CRCs typically have a worse prognosis than non-CIN tumours (Walther et al. 2008) and are more likely to be intrinsically multidrug resistant (Lee et al. 2011). Moreover, in the premalignant conditions Barrett’s oesophagus and ulcerative colitis, aneuploidy is evident in nontumour tissue and is strongly associated with later tumour development (Rabinovich et al. 1999; Maley et al. 2004). It is tempting to conclude a causative role for genetic instability in CRC progression from these data. However, it remains unclear whether or not CIN per se is selected in aggressive tumours, or if CIN tends to be associated with another highly selected phenotype, such as faster replication in the absence of error correcting, or some kind of intrinsic cellular hardiness, for example through a tolerance of abnormal gene dosing that is the consequence of aneuploidy. Interestingly, in evolving bacterial populations, sporadic evolving mutator phenotype strains can occasionally outcompete resident nonmutator strains (Sniegowski et al. 1997) producing a population of rapidly evolving cells (Barrick et al. 2009). Indeed, theoretical models show that mutator phenotypes can reach fixation in a population by ‘hitchhiking’ along with a selectively advantageous mutation (Taddei et al. 1997). Here, we examine the evolution of an increased mutation rate in a growing population of tumour cells, taking into account that additional mutations can be either advantageous or deleterious for the newly generated clone.

There are a number of theoretical models concerning the evolution of genetic instability in tumour progression. Beckman and Loeb’s model suggests that carcinogenesis is more efficient with an elevated mutation rate, although the model does not consider the relative effects of clonal expansion of precancerous mutant clones (Beckman and Loeb 2006). Clonal expansion markedly alters the rate at which a tumour acquires driver mutations (Luebeck and Moolgavkar 2002; Hornsby et al. 2007), and so including this phenomenon is likely important. Furthermore, the prediction that an elevated mutation rate improves the efficiency of cancer production (Beckman 2010) (efficiency is defined in terms of the rate of carcinogenesis and incidence of cancer) suggests that a mutator phenotype can have a driving role in carcinogenesis, but does not address whether or not there is selection for a mutator phenotype per se. The Wright–Fisher type model of Beerenwinkel et al. described the acquisition of driver mutations that trigger clonal expansions within a tumour. They found that whilst an elevated mutation rate was not required for carcinogenesis to occur in a reasonable time, an increased mutation rate did reduce the waiting time to cancer (Beerenwinkel et al. 2007). A subsequent related analysis showed that selective advantage of driver mutations dominated the dynamics (Schollnberger et al. 2010). Beerenwinkel’s model considered only advantageous mutations and had a fixed mutation rate. Beckman and Loeb’s analysis of the effect of mutator phenotype-driven negative clonal selection in limiting tumorigenesis suggested negative selection was unlikely to be a limiting factor in the evolution of a mutator phenotype (Beckman and Loeb 2005); however, as this model did not simultaneously consider both advantageous and deleterious mutations, it remains unclear whether a mutator phenotype would be selected above a nonmutator phenotype when deleterious mutations can occur. Similarly, Beckman’s model that described both deleterious and advantageous mutations in relation to a tumour cell’s absolute fitness did not allow the mutation rate to naturally evolve (Beckman 2009). Komarova and Wodarz (2004) have investigated the role of a mutator phenotype in determining the efficiency of cancer growth that is initiated by a small number of mutations, whilst simultaneously considering deleterious mutation; they suggest that an initially high mutation rate that decreases later in progression increases the speed of carcinogenesis and make predictions as to the optimal mutation rate for carcinogenesis that balances deleterious versus advantageous mutation (Komarova et al. 2008). Komarova et al. models describe only the acquisition of an initial few driver mutations and do not describe intratumour clonal dynamics. Our model differs to these previous models by simultaneously considering both advantageous and deleterious mutations, whilst describing intratumour clonal dynamics and allowing the mutation rate to evolve.
Here, we address the role of genetic instability in driving tumour evolution using a model of clonal evolution within a growing tumour. Building upon previous modelling approaches, our model includes both ‘positive clonal selection’ that drives clonal expansions and ‘negative clonal selection’ that retards clonal expansion and permits the mutation rate to evolve during carcinogenesis. The model has a Wright–Fisher type construction, whereby the abundance of offspring from each intratumour clone is determined by the clone’s relative fitness within the tumour. A cell’s relative fitness is increased if it acquires additional driver mutations and is decreased by acquiring additional deleterious mutations. We use the model to consider how the strength of selection for driver mutations and against housekeeper mutations determines whether the genetic instability is required for efficient tumorigenesis. We also examine evidence for a selective pressure driving the evolution of an increased mutation rate in tumour progression, and so determine the circumstances and timing of switches to an elevated mutation rate.

Methods

We developed a mathematical model describing the accumulation of advantageous, deleterious and neutral mutations by clones within a growing tumour. Our model is an extension of the model first proposed by Beerenwinkel et al. (2007) that described the accumulation of driver mutations by intratumour clones during tumour progression. Here, we have extended the Beerenwinkel model to describe multiple classes of mutations; both deleterious and advantageous mutations, and other mutation classes that do not directly impact on a cell’s fitness. Following Beerenwinkel et al., our model has a Wright–Fisher type construction, wherein the number of offspring of each clone is determined by the clone’s relative fitness compared with all the other clones in the tumour. In this sense, the tumour is considered a ‘well-mixed’ population of cells, and spatial considerations, such as clonal interference (Martens et al. 2011), are ignored.

Fitness is determined by a cell’s genotype: accumulating driver gene mutations increases a cell’s fitness, whereas additional housekeeper gene mutations decrease a cell’s fitness. Additionally, cells can acquire mutator gene mutations that have no direct effect on fitness, but cause the cell to have decreased DNA replication fidelity (i.e. a higher intrinsic mutation rate). Finally, cells can acquire selectively neutral passenger mutations that have no effect on fitness. Driver genes represent the proto-oncogenes and haploinsufficient tumour suppressor genes, housekeeper genes the set of genes that is needed for cell survival, such as those required to maintain ribosomes, and mutator genes the set of genes involved in DNA replication fidelity, such as the mismatch repair family of proteins. Passenger mutations should be considered as mutations in nonfunctional regions of the genome, such as in nongenic nonregulatory regions, within nonexpressed genes or nonfunctional intronic mutations.

Tumour growth begins with a population of $10^6$ cells. The simulation ends when a tumour reaches a maximum size of $10^9$ cells (which we would expect to have an approximate size of between 1 and 10 cm$^3$) or when a cell has acquired $C$ driver mutations. The size of the tumour cell population, $N(t)$, is constrained to grow exponentially such that:

$$N(t + 1) = N(t) (1 + (w)\beta)$$  \hspace{1cm} (1)

where $\beta > 0$ is a constant that sets the speed of exponential tumour growth, and $<w>$ is the average fitness of the tumour population (defined below). To account for stochastic fluctuations in tumour size, in the simulations, the new size of the tumour at each timestep is sampled from a Poisson distribution with mean:

$$N(t) (1 + <w>\beta)$$

A tumour cell can acquire a maximum of $m_d$ driver gene mutations, $m_h$ housekeeper gene mutations, $m_p$ passenger mutations and $m_m$ mutator gene mutations. An additional driver gene mutation increases a cell’s fitness by a factor of $(1 + s_d)$, whereas an additional housekeeper gene mutation decreases fitness by a factor of $(1 - s_h)$ and the effect of multiple mutations is multiplicative. Therefore, the relative fitness of a cell with $i$ driver gene mutations, $j$ housekeeper gene mutations, $k$ passenger mutations and $l$ mutator gene mutations is defined as:

$$w_{ijkl}(t) = \frac{(1 + s_d)^i(1 - s_h)^j}{\sum_{p=0}^{m_d} \sum_{q=0}^{m_h} \sum_{r=0}^{m_p} \sum_{s=0}^{m_m}(1 + s_d)^p(1 - s_h)^q x_{pqrs}} x_{ijkl}$$  \hspace{1cm} (2)

where $x_{pqru} = \frac{n_{pqru}}{N}$ is the proportion of tumour cells that have $p$, $q$, $r$ and $u$ driver, housekeeper, passenger and mutator mutations, respectively. The average fitness of the tumour population as a whole is then:

$$<w(t)> = \sum_{i=0}^{m_d} \sum_{j=0}^{m_h} \sum_{k=0}^{m_p} \sum_{l=0}^{m_m}(1 + s_d)^i(1 - s_h)^j x_{ijkl}$$  \hspace{1cm} (3)

Mutator gene mutations act to increase a cell’s intrinsic mutation rate. We consider just two states for the mutation rate; the baseline (low) rate of $\mu_l$ per gene per division, and an elevated rate $\mu_{hi}$ that is, the consequence of mutator gene mutations. The switch from $\mu_l$ to $\mu_{hi}$ occurs when a cell has acquired $M$ mutator mutations.

The probability of sampling a cell with $i$ driver gene mutations, $j$ housekeeper gene mutations, $k$ passenger
mutations, irrespective of the number of mutator gene
mutations is:

\[ \dot{\theta}_{ijkl}(t) = \sum_{p=0}^{l} \sum_{q=0}^{k} \sum_{r=0}^{j} \left( \begin{array}{c} m_d - p \\ i - p \\ j - q \\ k - r \\ m_h - q \\ \end{array} \right) \left( \begin{array}{c} m_p - r \\ k - r \\ \end{array} \right) \]

\[ \mu_i \mu_j \mu_k \mu_l \left( 1 - \mu_l \right)^{m_p - i + m_m - j + m_p - k + m_m - h_{ppq}q_{pq}u} \]

Consideration of the mutator genes leads to the probability
of sampling a cell with \( i, j, k, l \) mutations in the
respective classes as: If \( l \leq M \) then:

\[ \theta_{ijkl}(t) = \sum_{p=0}^{l} \sum_{q=0}^{k} \sum_{r=0}^{j} \sum_{u=0}^{M} \left( \begin{array}{c} m_d - p \\ i - p \\ j - q \\ k - r \\ m_h - q \\ \end{array} \right) \]

\[ \left( \begin{array}{c} m_p - r \\ k - r \\ \end{array} \right) \left( \begin{array}{c} m_m - u \\ l - u \\ \end{array} \right) \mu_i \mu_j \mu_k \mu_l \mu_m \mu_p \mu_q \mu_u \]

\[ \left( \begin{array}{c} 1 - \mu_l \end{array} \right)^{m_p - i + m_m - j + m_p - k + m_m - h_{ppq}q_{pq}u} \]

and if \( l \geq M \) then:

\[ \theta_{ijkl}(t) = \sum_{p=0}^{l} \sum_{q=0}^{k} \sum_{r=0}^{j} \sum_{u=0}^{M} \left( \begin{array}{c} m_d - p \\ i - p \\ j - q \\ k - r \\ m_h - q \\ \end{array} \right) \]

\[ \left( \begin{array}{c} m_p - r \\ k - r \\ \end{array} \right) \left( \begin{array}{c} m_m - u \\ l - u \\ \end{array} \right) \mu_i \mu_j \mu_k \mu_l \mu_m \mu_p \mu_q \mu_u \]

\[ \left( \begin{array}{c} 1 - \mu_l \end{array} \right)^{m_p - i + m_m - j + m_p - k + m_m - h_{ppq}q_{pq}u} \]

To simulate the model, \( \theta_{ijkl} \) are used to form a multi-
nomial distribution describing the probability of sampling
a mutant bearing \( i, j, k \) and \( l \) mutations in the respective
classes in the next generation. The probability mass function
is:

\[ \left( n_{0000}(t + 1), \ldots, n_{m_i m_j m_k m_l}(t + 1) \right) \sim \]

\[ \frac{N(t)!}{n_{0000}(t)! \cdots n_{m_i m_j m_k m_l}(t)!} \prod_{i=0}^{m_i} \prod_{j=0}^{m_j} \prod_{k=0}^{m_k} \prod_{l=0}^{m_l} q_{ijkl}^{n_{ijkl}} \]

A sample from this distribution defines \( n_{ijkl}(t + 1) \).

For computational efficiency, a maximum of four muta-
tions are permitted to occur in a single cell per division. To
implement a maximum number of mutations per cell, the
summations in eqns (4) and (5) begin at \( p = i - 4, q = j - 4, \)
etc. The \( \theta_{ijkl} \) are then normalised to sum to 1.

\[ \text{C code that simulates the model is available from the} \]

\[ \text{corresponding author on request.} \]

**Results**

A Wright–Wright type model of intratumour clonal evolu-
tion was constructed. The model described the genetic pro-
geression of tumour cells, defined as non-invasive neoplastic
cells, to cancer cells, defined as the cells capable of local
invasion and metastasis. Within the model, cells could
acquire selectively advantageous driver mutations, that
increased the relative fitness of the cell by a factor of \((1 + s_d)\),
selectively disadvantageous housekeeper mutations that
decreased fitness by a factor of \((1 - s_h)\) and selectively neu-
tral passenger mutations (Fig. 1). Cells could also acquire
mutator mutations that led to an increase in the cell’s
mutation rate; after \( M \) mutator mutations occurred in a
single cell, the cell’s intrinsic per-locus mutation rate
increased from \( \mu_l \) to \( \mu_{ij} \) mutations per division. Mutations
at each locus within a particular class (driver, housekeeper,
passerger and mutator, respectively) were assumed to be
equivalent, so it sufficed to track only the total number of
mutations borne by each cell in each class of genes. A
‘clone’ was defined as the population of cells with a partic-
ular number \((i, j, k, l)\) of mutations in the respective classes.
The tumour population was assumed to grow expen-
tially. The number of offspring of each intratumour clone
was a function of the clone’s relative fitness within the
tumour, as determined by the number of driver and house-
keeper mutations borne by the clone; this gave the model a
Wright–Wright type construction. The tumour population
was allowed to evolve under this model and the clonal
structure and efficiency of tumorigenesis was examined
under a range of model parameters.

The model exhibited ‘travelling waves’ of the growth and
then subsequent extinction of clones bearing increasing
numbers of driver mutations (Fig. 2A,D), as had been pre-
viously reported by Beerenwinkel et al. (2007). The wave
patterns were due to clonal competition; each new driver
mutation triggered clonal expansion and drove the pre-
viously resident cells to extinction. Deleterious mutations did
not accumulate; cells with a large number of driver mutations
could tolerate only a few deleterious (housekeeper) muta-
tions without being outcompeted in the tumour by clones
without deleterious mutations (Fig. 2B,E). Passenger muta-
tions accrued in a linear fashion with respect to time but
typically remained at low numbers in the tumour (Fig. 2C,
F). Stochastic fluctuations were observed in passenger
mutation numbers (Fig. 2C), as clonal expansion of pas-
sengers was caused by their hitchhiking along with a clone
that had acquired a new driver mutation.
An increased mutation rate markedly accelerates tumorigenesis only when selection for driver mutations is weak

The effect of an increased mutation rate on the rate of cancer evolution was examined. Mutator mutations were not considered here, so that the mutation rate remained fixed throughout the simulation; hereafter, the model with a fixed mutation rate is referred to as ‘model 1’. A cancer was defined as having grown when at least 10% of the tumour was composed of cells with at least 20 driver mutations. The number of driver mutations is likely to differ significantly between tumours; the requirement for 20 driver mutations represented the estimated maximal driver mutation burden in colorectal cancer (Sjöblom et al. 2006) and is in accordance with previous models (Beerenwinkel et al. 2007). Runs of the model were terminated when the tumour population exceeded $10^9$ cells; the probability of developing cancer was defined as the proportion of runs (with a particular set of parameters) that led to the development of cancer before the tumour as a whole was composed of more than $10^9$ cells.

Carcinogenesis was faster (Fig. 3A) when the basal mutation rate was higher. However, the relative increase in efficiency of tumorigenesis due to an elevated mutation rate was reduced by strong selection for additional driver mutations (Fig. 3A). In other words, when additional driver mutations caused a strong selective advantage, a mutator phenotype was not necessary for efficient tumorigenesis. Relatedly, an increased selective advantage for driver mutations caused a bigger reduction in the waiting time to cancer when the mutation rate was lower.

The simulations here were performed with equal numbers of housekeeper and driver mutations. In this case, deleterious housekeeper mutations had little effect on the rate or likelihood of carcinogenesis (Fig. 3B). Cells acquiring a deleterious housekeeper mutation were rapidly outcompeted and driven to extinction by more fit clones. As the probability of a cell acquiring a deleterious mutation was comparable to that of the cell acquiring an advantageous mutation, negative clonal selection impeded the growth of only rare cells within the most aggressive clone, and so failed to impede tumour growth. This result highlights that clonal selection was the dominant force within the model.

The selective advantage of driver mutations determines whether a mutator phenotype evolves

The evolution of a mutator phenotype within a growing cancer was examined. The model where the mutation rate could vary is referred to as model 2. The switch to a mutator phenotype, whereby a cell’s intrinsic mutation rate increased from $\mu_L = 10^{-7}$ to $\mu_H = 10^{-5}$ per-locus per division, occurred when a cell had acquired at least $M$ mutator mutations. The model was simulated for a range of values of $M$, and for a range of values of $s_d$, the fitness increment bestowed by an additional driver mutation. For these simulations, the number of driver and housekeeper loci was set to $m_d = m_h = 100$.

Cancers were more likely to have evolved a mutator phenotype when selection for additional driver mutations
was moderate $s_d \sim 0.01$; strong selection for additional driver mutations suppressed the evolution of a mutator phenotype (Fig. 4A). Requiring more mutator mutations to develop a mutator phenotype (increasing $M$) prevented the evolution of genetic instability as cells had to acquire more intrinsically selectively neutral mutator mutations through genetic drift (Fig. 4A). Notably, with $M = 1$ cancers always had a mutator phenotype, whereas with $M = 8$ cancers never had a mutator phenotype. Being able to develop a mutator phenotype accelerated tumorigenesis (Fig. 4B), although the accelerating effect was reduced when selection for additional driver mutations was strong. For $s_d \geq 0.01$, deleterious selection against housekeeper mutations had negligible impact on the likelihood of evolving a cancer with a mutator phenotype or on the waiting time to cancer (Fig. S1).

Requiring fewer mutations for cancer development reduced the waiting time to cancer (Fig. S2A) as fewer driver mutations needed to accrue. Correspondingly, the likelihood of the cancer having evolved a mutator phenotype decreased when fewer driver mutations were required for cancer (Fig. S2B). This was because the likelihood of a mutator clone hitchhiking to clonal dominance on a selective sweep of a driver mutation was reduced when there were fewer selective sweeps.

When selection for additional driver mutations was very weak (regions where $s_d < 0.01$; whereby an additional dri-
Evolution of genetic instability

Figure 3 The effect of an increased mutation rate as a function of selection. (A) Waiting time to cancer for $\mu = 10^{-7}$ compared with $\mu = 10^{-5}$ for a range of driver mutation advantages (values of $s_D$). The deleterious effect of housekeeper mutations was set at $s_H = 0.01$ for all values of $s_D$. The waiting time to cancer was reduced by an elevated mutation rate. The mutation rate has the greatest effect on the waiting time to cancer when selection for new driver mutations was weak. Increased selection for new driver mutations caused a more pronounced reduction in the waiting time to cancer when the mutation rate was lower. Simulations for $m_d = m_h = m_p = 100$, averaged over 10,000 runs. (B) Waiting time to cancer for a variety of housekeeper selective disadvantages. When the number of driver and housekeeper loci was equal, deleterious housekeeper mutations had minimal effect on the waiting time to cancer, and the effect was independent of the strength of deleterious selection.

Figure 4 Selection and the evolution of an increased mutation rate. (A) Probability that cancer will have evolved a mutator phenotype as a function of the relative fitness benefits of additional driver mutations. Strong selection for additional driver mutations suppressed the evolution of a mutator phenotype, and this effect was exaggerated when multiple mutator gene mutations are required to develop genetic instability. $s_H = 0.01$, $m_d = m_h = m_p = 100$, $\mu_d = 10^{-7}$ and $\mu_p = 10^{-5}$. Simulations averaged over 10,000 runs. (B) Mean waiting time to cancer as a function of the relative fitness benefits of additional driver mutations (averaged over 10,000 runs of the model). Tumorigenesis tended to be faster when a mutator phenotype could readily evolve, or when selection for driver mutations was strong. Dashed lines represent 95% quantiles of the simulated values. (C) Probability of cancer for very weak selective advantages of driver mutations. Simulations averaged over 750 runs. Cancer was more likely when a mutator phenotype could evolve. Selection against housekeeper mutations reduced the incidence of cancer (solid lines: $s_H = 0.01$; dashed lines: $s_H = 0$). (D) Probability that a cancer will have evolved a mutator phenotype as a function of selection. For very weak or very strong driver gene selective advantage, a mutator phenotype is less likely to evolve.
ver mutation had less than a 1% effect on the probability of the cell producing surviving offspring), the role of the mutator phenotype in carcinogenesis was altered. In this case, deleterious mutations reduced the likelihood of carcinogenesis (Fig. 4C); in regimes of very weak selection, clonal expansion was slow and so housekeeper mutations could significantly impede clone growth. Indeed, cancers were more likely to have evolved a mutator phenotype when $s_h = 0$ than when $s_h = 0.01$ (Fig. 4D). This was a consequence of the rate at which deleterious mutations accrued in these two parameter regimes: the increased probability of acquiring deleterious mutations appeared to reduce the advantage of the mutator phenotype when $s_h > 0$. As the selective benefit of an additional driver mutation was increased from very low $s_d$ to high $s_h$, the proportion of cancers evolving a mutator phenotype increased to a peak value and then decreased (Fig. 4A,D), reflecting the likelihood of sufficient mutator mutations accruing by drift in the most aggressive clone; with $M = 1$ cancers always evolved a mutator phenotype.

The development of a mutator phenotype was frequently not an early event in tumour progression. Requiring a greater number of mutator mutations to switch to a mutator phenotype delayed the establishment of a clone with a mutator phenotype, both in absolute numbers of generations (Fig. 5A), and in terms of the proportion of the time taken for carcinogenesis that had elapsed before the mutator clone appeared (Fig. 5B). The strength of selection had a less-marked effect on the time at which a mutator clone formed. The clone with the most driver mutations (termed

![Figure 5 Timing of mutator phenotype evolution. (A) Generation when a mutator clone is established in the tumour (composes more than 10% of the tumour), as function of the strength of selection for driver mutations, for different numbers of mutator mutations required for a mutator phenotype. Requiring more mutator mutations to switch to a mutator phenotype increased the generations elapsed before a mutator clone is established. Stronger selection for driver mutations made a mutator clone evolve in fewer generations. Solid lines are simulation means, dashed lines represent 95% quantiles of the simulations. (B) Proportion of the time taken for a cancer to evolve before a mutator clone is established. Requiring more mutator mutations for a mutator phenotype caused the mutator phenotype to develop later in progression. For moderate to strong selection, increasing the strength of selection for driver mutations caused only marginal change in the relative timing of a mutator clone. Solid lines are simulation means, dashed lines represent 95% quantiles of the simulations. (C) Number of driver mutations in the most aggressive clone (clone with most driver mutations) at the time that it acquires a mutator phenotype. Clones acquired significant numbers of driver mutations before switching to a mutator phenotype. Dashed lines are 95% quantiles of the simulations. (D) The accumulation of driver mutations versus mutator mutations in the most aggressive clone. Clones acquired driver mutations at a much faster rate than mutator mutations. When selection for driver mutations was weak, driver mutations accrued at a more comparable rate to mutator mutations, indicating that genetic drift became a stronger influence in the clonal dynamics. Solid lines represent strong selection for additional driver mutations ($s_d = 0.05$); dotted lines moderate selection ($s_d = 0.01$); dashed lines weak selection ($s_d = 10^{-5}$). $m_d = m_h = m_p = 100, \mu_d = 10^{-7}, \mu_h = 10^{-5}$ for all simulations. Data reported from aggregate measures of at least 100 runs.© 2012 The Authors. Evolutionary Applications published by Blackwell Publishing Ltd 6 (2013) 20–33
the most aggressive clone) accrued a significant number of driver mutations prior to developing a mutator phenotype; when additional driver mutations caused large increases in fitness, the number of driver mutations accrued prior to a mutator phenotype increased (Fig. 5C). Indeed, driver mutations always accrued more quickly than mutator mutations, and the difference in rate was increased as the selective benefit of additional driver mutations increased (Fig. 5D). These data reflect that mutator mutations were selectively neutral, and consequently that mutator clones formed only after mutator mutations had hitchhiked to prominence on the back of clonal expansions driven by the selected driver mutations.

Abundant deleterious mutations slow carcinogenesis
To look more closely at the impact of deleterious housekeeper mutations on carcinogenesis (negative clonal selection), simulations were performed with large numbers of housekeeper loci \((m_d \ll m_h)\). This represented the situation where most mutations where likely to be deleterious, and where driver mutations were relatively rare. In model 1 (fixed mutation rate), increasing the number of housekeeper loci increased the waiting time to cancer (Fig. 6A, B). The longer waiting time for cancer due to increasing numbers of housekeeper genes was reduced as the selective advantage of driver mutations increased (Fig. 6A) as deleterious mutations impeded clone growth, and so in turn reduced the effective rate at which driver mutations accrued (Fig. 6C).

Interestingly, for a given number of housekeeper genes, increasing the strength of deleterious selection against housekeeper mutations led to a slight decrease in the waiting time to cancer (Fig. 6B). When deleterious mutations were particularly lethal for a cell, the fitness differential between deleteriously mutated and nondeleteriously
mutated cells was marked. As such, cells that avoided deleterious mutations were relatively very fit within the tumour where deleterious mutations were abundant – the nondeleteriously mutated clones effectively received a fitness boost – and so grew to the detriment of deleteriously mutated clones. Thus, driver mutations accrued faster as the deleteriousness of housekeeper mutations was increased (Fig. 6D).

Using model 2, the effect of abundant deleterious mutations on the evolution of an increased mutation rate was examined. In this case, increasing the abundance of housekeeper loci decreased the likelihood that the tumour would evolve a mutator phenotype (Fig. S3). The strength of selection for new driver mutations determined whether a mutator phenotype would evolve; very strong or very weak selection for driver mutations suppressed the evolution of a mutator phenotype (Fig. S3A). Strong negative clonal selection against deleterious housekeeper mutations suppressed the evolution of a mutator phenotype (Fig. S3B).

The evolution of genetic diversity

Genetic diversity within the tumour was quantified using three ecological measures: the Shannon index (a measure of the uncertainty in predicting the genotype of a sampled cell), the Simpson index (approximates the probability of drawing two cells that have the same genotype; smaller values indicate a more diverse population) and the richness (the number of different clones). Model 1 (fixed mutation rate) was used for the simulations of genetic diversity, with the mutation rate $\mu = 10^{-5}$ or $\mu = 10^{-7}$ and various values of the selective advantage of driver mutations $s_d$.

For all parameter values, diversity initially increased sharply before reaching a pseudo-plateau where diversity increased only slowly over time (Fig. 7). The pseudo-plateau level of diversity was defined both by the fitness gain of additional driver mutations $s_d$ and by the basal mutation rate $\mu$. Strong selection for additional driver mutations reduced the pseudo-plateau levels of genetic diversity, whilst higher mutation rates increased the pseudo-plateau level of diversity. Strong selection caused clonal expansions to happen faster, causing the tumour to more rapidly become homogeneous for the new mutation and so suppressing diversity. A higher mutation rate led to the more rapid generation of new, genetically diverse, mutants. The mutation rate had a marked effect on the clonal diversity of a tumour; for instance, when measured by the Shannon index, the diversity measure at $\mu = 10^{-5}$ was approximately sixfold higher than the diversity at $\mu = 10^{-7}$. In comparison, a fivefold increase in the selective benefit of a new driver mutation caused about a 1.5-fold decrease in the final diversity of the tumour. In the initial phase of tumour growth, diversity increased more quickly for higher values of $s_d$ likely reflecting the more rapid subclone growth for these parameter regimes.

Discussion

Here, we have examined the evolution of a mutator phenotype during tumour progression from a benign lesion to a cancer. We have determined circumstances when a mutator phenotype evolves during carcinogenesis, and looked at the effect of an evolving mutator phenotype on the efficiency of tumorigenesis.

Our model predicts that the strength of selection for driver mutations determines whether a cancer is likely to evolve a mutator phenotype: in situations where selection for driver mutations is very weak, or very strong, a mutator
phenotype is unlikely to evolve. Previous studies have noted the central role of selection, predominating over the mutation rate, in driving the speed of carcinogenesis (Schollnberger et al. 2010). Indeed, an agent-based modelling study found that when both neutral and driver mutations were necessary for cancer formation, requiring more driver mutations to form a cancer actually increased the probability of cancer formation, as the rapid clonal expansions induced by the extra driver mutations made it more likely that a neutral mutation would be able to hitchhike to dominance within the neoplasm (Maley and Forrest 2000). Our study illustrates how the fitness advantage of driver mutations determines whether a mutator phenotype is likely to evolve at all. In the case of strong selection for additional driver mutations, the growth of the most aggressive clone (the clone with the most driver mutations) is very rapid, so that many cells that are ‘susceptible’ to acquiring the next driver mutation are rapidly produced. Mutator mutations are assumed to be selectively neutral in the model; mutator mutations therefore accrue only through genetic drift or by hitchhiking on the most aggressive clones. When two or more mutator mutations are required to generate the mutator phenotype [as would be the case for recessive mismatch repair (MMR) genes (Markowitz and Bertagnolli 2009)], the rate at which mutator genes drift to dominance within the most aggressive clone is much slower than the rate at which the most aggressive clone acquires the next driver mutation. In this way, mutator mutations are effectively removed from the tumour population as a result of their being outcompeted by the most aggressive clones. When selection for additional driver mutations is moderate, cells carrying mutator gene mutations are not outcompeted so rapidly, and so a mutator phenotype can evolve. A mutator cell then acquires driver mutations faster than its nonmutator counterparts, and so the mutator phenotype indirectly bestows a selective advantage. When selection for additional driver mutations is very weak, intratumour clone dynamics are driven nearly entirely by drift (not clonal selection). In this case, acquiring a mutator phenotype, which still accelerates the rate of driver gene mutation accumulation, does little to accelerate the rate of clone growth, and so selection for mutator cells is weak.

In the light of this result, it is instructive to consider what order of fitness advantage is bestowed by driver mutations within tumours. There is a lack of empirical measurements of the relative fitness of different tumour clones, although modelling efforts have suggested that drivers may cause only slight increases in fitness. Beerenwinkel et al. (2007), using their mathematical model of clonal evolution, suggested a relative fitness advantage of about 1% per driver mutation (i.e. $s_d = 0.01$; making cells 1% more likely to have surviving offspring) was sufficient to cause progression of colorectal adenomas to cancers in a reasonable time. Similarly, in their study of subclone expansion, Siegmund et al. (2011) saw only evidence for weak selection in CRCs. Bozic et al. (2010) estimated a fitness advantage of only about 0.4% per driver mutation for glioblastoma and pancreatic cancers. These predictions are in the ‘moderate’ range of selective advantages in our model. Furthermore, given the disparity in the number of putative driver mutations found in different types of cancer (Salk et al. 2010), it is likely that the fitness advantage of driver mutations differs significantly between cancer types. Thus, whether or not genetic instability is a hallmark of a particular cancer type may reflect, to some degree, the strength of selection for new driver mutations in the cancer type. Of course, fitness advantages will also differ between mutations at different loci, even within the same cancer type.

We have assumed that all driver mutations have the same consequence for a cell’s fitness. In reality, mutations of different genes will have different consequences for fitness; for example, that distinct mutations of the SETD2 gene are found in different intratumour clones in the same renal carcinoma (Gerlinger et al. 2012) indicates a strong selective pressure for inactivation of this particular gene. Consequently, the rate of clonal evolution within an individual tumour may change over time, and so the likelihood of evolving a mutator phenotype may change over time. For example, it is likely that the driver mutations with the largest selective effects would expand initially (as they would outcompete the clones with weaker driver mutations), leading to weaker driver mutations later in progression. In this case, genetic instability would be unlikely to evolve at the onset of tumour growth and would likely feature in the later stages of carcinogenesis. Indeed, this appears to be the case in Barrett’s oesophagus where the detection of aneuploidy is a strong predictor of cancer development risk (Maley et al. 2004, 2006).

Unless deleterious mutations were very common, they did little to impede the progression of the most aggressive clones, supporting Beckman and Loeb’s previous conclusion (Beckman and Loeb 2005). In fact, we observed that carcinogenesis could be actually accelerated by increasing the strength of selection against deleterious mutations, as strong selection against deleterious mutations served to increase the relative fitness of clones without deleterious mutations. This observation highlights how competition within tumours likely influences the pattern of clonal evolution. We had initially hypothesised that the rapid accrual of deleterious mutations would inhibit the growth of mutator clones, as have others previously (Cahill et al. 1999; Tomlinson and Bodmer 1999; Komarova and Wodarz 2004; Komarova et al. 2008). Whilst this was the case when selection for additional driver mutations was very weak, or
if deleterious mutations were very common, moderate to
strong selection for driver mutations caused cells that had
acquired deleterious mutations to be rapidly outcompeted
and removed from the tumour, particularly in the case
where driver and housekeeper loci were of equal
abundance. Increasing the likelihood of a cell acquiring a
deleterious mutation (by increasing the abundance of
housekeeper loci in each cell) did slow tumorigenesis, and
indeed suppress the evolution of a mutator phenotype,
indicating that deleterious mutations can act as a brake on
carcinogenesis. However, the strength of selection for
driver mutations dominated the dynamics of the time to
cancer and the emergence of a mutator phenotype. Our
model predicts that deleterious mutations will not accrue
within tumours. This hypothesis could be tested by exam-
ing ‘cancer genomes’ for evidence of clonal mutations
(high frequency within the tumour) that are predicted to
impede cell growth or survival, or genetically engineering
them into cells.

Tumours that developed a mutator phenotype, when
more than one mutator mutation was required to switch to
a mutator phenotype, tended to develop it after the tumour
had acquired a significant driver mutation burden, which
was typically late in tumour progression. This time-delay
was due to the time taken for mutator mutations to accrue
by drift, or by hitchhiking, in the most aggressive subclones.
Therefore, our model predicts that if tumour growth is
initiated without an increased mutation rate (Bodmer et al.
2008), then a mutator phenotype is likely to feature only
later in progression, which is again consistent with the data
from Barrett’s oesophagus (Maley et al. 2004, 2006).

When only a single mutator mutation is required to
switch to a mutator phenotype (case $M = 1$), cancers
always evolved with a mutator phenotype and carcinogene-
sis was universally faster. Thus, our model predicts that
mutator phenotypes drive efficient carcinogenesis and that
mutator mutations should be selected in tumours. This
supports the conclusion of Beckman and Loeb (Beckman
and Loeb 2006). The case $M = 1$ represents the scenario
when mutator genes have a dominant effect; that is, mutat-
ing a single copy of the mutator gene causes a switch to a
mutator phenotype. However, whether or not mutator
genes act dominantly in cancers is uncertain. The mismatch
repair genes operate in a recessive fashion (Markowitz and
Bertagnolli 2009) and the genetic basis of other mutator
phenotypes seen in cancer is uncertain. It is noteworthy
that in model systems, there are examples of dominant neg-
avative mutations in $TP53$ that are associated with genetic
instability (Song et al. 2007).

The level of genetic diversity within a tumour was related
to both the selective advantage attributable to driver
mutations and the basal mutation rate. Thus, our model predicts
that genetic diversity within a tumour is a proxy measure
of both the mutation rate and strength of genetic instability.
Similarly, the model of Durrett et al. (2011) predicted that
the degree of genetic diversity within a tumour was largely
determined by the age of the tumour, and the stepwise fit-
ness gain of each additional driver mutation. These data
illustrate that genetic diversity within a tumour population
is not necessarily indicative of an elevated mutation rate,
but instead may signify that tumour clones are experienc-
ing only weak selection.

We note that our model makes a number of gross over-
simplifications in describing carcinogenesis that could
potentially impact the model dynamics. First, driver genes
in our model represented only proto-oncogenes or haploin-
sufficient tumour suppressor genes, that is, a cell acquiring
a mutation to a single driver gene locus gained an increase
in fitness. In reality, many driver genes are recessive tumour
suppressor genes, and so no increase in fitness is caused
until both a selectively neutral first hit, and a second hit are
acquired at the tumour suppressor locus. In this regard, a
mutator phenotype might cause particularly efficient
tumorigenesis if many selectively neutral first hits are
required for cancer production. Second, we assumed that
mutations accrue in a monotonic stepwise fashion. The
mutator phenotype CIN is a hallmark of many cancer types
(Rajagopalan et al. 2003): CIN tumours frequently show
whole- or part-chromosome arm aberrations, wherein
many genes located on a particular chromosome have been
‘mutated’ in a single mutational event. Also, copy number
gains are frequently observed in CIN; genetic gains are
potentially reversible events, as additional genic copies may
later be lost. Thus, CIN cancers are unlikely to accrue muta-
tions in the monotonic stepwise fashion we have described
in our model, and so, the dynamics of the evolution of CIN
may differ from our model’s predictions. Third, we have
assumed that mutator genes are themselves selectively neu-
ral, that is, that mutations of mutator genes do not cause
an increase in fitness. Some driver genes may double as
mutator genes; for example, mutation of the intestinal
tumour suppressor gene $APC$ is sufficient for tumorigenesis
(Lamlmum et al. 2000), but may also instigate CIN (Fodde
et al. 2001); similarly mutations to the $TP53$ tumour sup-
pressor are usually considered driver mutations and are
associated with genetic instability (Song et al. 2007). Selec-
tion dominates evolution in our model, and so direct selec-
tion for mutator genes would likely alter the dynamics.
Fourth, we have assumed that all driver genes have equiva-
Ient effects on fitness when mutated and, similarly, that all
genes are equally likely to be mutated. The patterns of
mutation and selection would likely alter if this unrealistic
assumption were relaxed. Fifth, we have neglected to
describe spatial heterogeneity within a tumour. Clonal
interference, whereby two spatially adjacent clones with
similar fitness impede each other’s growth, slows the rate of
tumour evolution (Martens et al. 2011). Such spatial considerations may also affect the evolution of mutator clones. Relatedly, we have assumed that the fitness of cells within the tumour is strictly relative; this leads to the least fit cells being rapidly outcompeted by fitter cells, and so clones carrying deleterious housekeeper mutations are rapidly driven to extinction. Relaxing this assumption, for instance by competing cells only with their ‘neighbours’, may alter the clonal dynamics (and indeed overall tumour dynamics), particularly for clones bearing deleterious mutations.

In summary, we have constructed a model of the evolution of genetic instability during tumour progression. Our model incorporates clonal expansions and deleterious mutations and allows the mutation rate to evolve, whereas previous models have considered only some of these issues. Our model predicts that the strength of selection for additional driver mutations determines whether or not a cancer is likely to evolve a mutator phenotype and suggests that future efforts should be devoted to measuring the degree of fitness effects of mutations in carcinogenesis.

Conflicts of interest

The authors have no conflicts of interest to declare.

Literature cited

Barrick, J. E., D. S. Yu, S. H. Yoon, H. Jeong, T. K. Oh, D. Schneider, R. E. Lenski et al. 2009. Genome evolution and adaptation in a long-term experiment with Escherichia coli. Nature 461:1243–1247.

Beckman, R. A. 2009. Mutator mutations enhance tumorigenic efficiency across fitness landscapes. PLoS ONE 4:e5860.

Beckman, R. A. 2010. Efficiency of carcinogenesis: is the mutator phenotype inevitable? Seminars in Cancer Biology 20:340–352.

Beckman, R. A., and L. A. Loeb 2005. Negative clonal selection in tumor evolution. Genetics 171:2123–2131.

Beckman, R. A., and L. A. Loeb 2006. Efficiency of carcinogenesis with and without a mutator mutation. Proceedings of National Academy of Science of the United States of America 103:14140–14145.

Beerenwinkel, N., T. Antal, D. Dingli, A. Traulsen, K. W. Kinzler, V. E. Velculescu, B. Vogelstein et al. 2007. Genetic progression and the waiting time to cancer. PLoS Computational Biology 3:e225.

Birkbak, N. J., A. C. Eklund, Q. Li, S. E. McClelland, D. Endesfelder, P. Tan, I. B. Tan et al. 2011. Paradoxical relationship between chromosomal instability and survival outcome in cancer. Cancer Research 71:3447–3452.

Bodmer, W., J. H. Bielas, and R. A. Beckman 2008. Genetic instability is not a requirement for tumor development. Cancer Research 68:3558–3560; discussion 3560-1.

Bozic, I., T. Antal, H. Ohtsuki, H. Carter, D. Kim, S. Chen, R. Karchin et al. 2010. Accumulation of driver and passenger mutations during tumour progression. Proceedings of the National Academy of Sciences of the United States of America 107(43):18545–50.

Cahill, D. P., K. W. Kinzler, B. Vogelstein, and C. Lengauer 1999. Genetic instability and darwinian selection in tumours. Trends in Cell Biology 9:MS7–M60.

Durrett, R., J. Foo, K. Leder, J. Mayberry, and F. Michor 2011. Intratumor heterogeneity in evolutionary models of tumor progression. Genetics 188:461–477.

Fearon, E. R. 2011. Molecular genetics of colorectal cancer. Annual Review of Pathology: Mechanisms of Disease 6:479–507.

Fodde, R., J. Kuipers, C. Rosenberg, R. Smits, M. Kidman, C. Gaspar, J. H. van Es et al. 2001. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nature Cell Biology 3:433–438.

Gerlinger, M., A. J. Rowan, S. Horwell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez et al. 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. New England Journal of Medicine 366:883–892.

Hornsby, C., K. M. Page, and I. P. M. Tomlinson 2007. What can we learn from the population incidence of cancer? Armitage and Doll revisited. The lancet oncology 8:1030–1038.

Komarova, N. L., and D. Wodarz 2004. The optimal rate of chromosome loss for the inactivation of tumor suppressor genes in cancer. Proceedings of National Academy of Science of the United States of America 101:7017–7021.

Komarova, N. L., A. V. Sadovsky, and F. Y. Wan 2008. Selective pressures for and against genetic instability in cancer: a time-dependent problem. Journal of the Royal Society, Interface 5:105–121.

Lamlum, H., A. Papadopoulou, M. Ilyas, A. Rowan, C. Gillet, A. Hanby, I. Talbot et al. 2000. Adenomatous polyposis coli (APC) mutations are sufficient for the growth of early colorectal adenomas. Proceedings of the National Academy of Sciences of the United States of America 97:2225–2228.

Lee, A. J., D. Endesfelder, A. J. Rowan, A. Walther, N. J. Birkbak, P. A. Futreal, J. Downward et al. 2011. Chromosomal instability confers intrinsic multidrug resistance. Cancer Research 71:1858–1870.

Loeb, L. A. 2001. A mutator phenotype in cancer. Cancer Research 61:3230–3239.

Loeb, L. A. 2011. Human cancers express mutator phenotypes: origin, consequences and targeting. Nature Reviews Cancer 11:450–457.

Luebeck, E. G., and S. H. Moolgavkar 2002. Multistage carcinogenesis and the incidence of colorectal cancer. Proceedings of the National Academy of Science of the United States of America 99:15095–15100.

Maley, C. C., and S. Forrest 2000. Exploring the relationship between neutral and selective mutations in cancer. Artificial Life 6:325–345.

Maley, C. C., P. C. Galipeau, X. Li, C. A. Sanchez, T. G. Paulson, P. L. Blount, and B. J. Reid 2004. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Research 64:7629–7633.

Maley, C. C., P. C. Galipeau, J. C. Finley, V. J. Wongsurawat, X. Li, C. A. Sanchez, T. G. Paulson et al. 2006. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nature Genetics 38:468–473.

Markowitz, S. D., and M. M. Bertagnolli 2009. Molecular origins of cancer: molecular basis of colorectal cancer. New England Journal of Medicine 361:2449–2460.

Martens, E. A., R. Kostadinov, C. C. Maley, and O. Hallatschek 2011. Spatial structure increases the waiting time for cancer. New Journal of Physics 13:11504.

Rabinovitch, P. S., S. Dziadon, T. A. Brentnall, M. J. Emond, D. A. Crispin, R. C. Haggitt, and M. P. Bronner 1999. Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. Cancer Research 59:5148–5153.

Rajagopalan, H., M. A. Nowak, B. Vogelstein, and C. Lengauer 2003. The significance of unstable chromosomes in colorectal cancer. Nature Reviews Cancer 3:695–701.
Roylance, R., D. Endesfelder, P. Gorman, R. A. Burrell, J. Sander, I. Tomlinson, A. M. Hanby et al. 2011. Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. Cancer Epidemiology Biomarkers Prevention 20:2183–2194.

Salk, J. J., E. J. Fox, and L. A. Loeb 2010. Mutational heterogeneity in human cancers: origin and consequences. Annual Review of Pathology 5:51–75.

Schollnberger, H., N. Beerwenwinkel, R. Hoogenveen, and P. Vineis 2010. Cell selection as driving force in lung and colon carcinogenesis. Cancer Research 70:6797–6803.

Siegmund, K. D., P. Marjoram, S. Tavare, and D. Shibata 2011. High DNA methylation pattern intratumoral diversity implies weak selection in many human colorectal cancers. PLoS ONE 6: e21657.

Sjoblom, T., S. Jones, L. D. Wood, D. W. Parsons, J. Lin, T. D. Barber, D. Mandelker et al. 2006. The consensus coding sequences of human breast and colorectal cancers. Science 314:268–274.

Sniegowski, P. D., P. J. Gerrish, and R. E. Lenski 1997. Evolution of high mutation rates in experimental populations of E. coli. Nature 387:703–705.

Song, H., M. Hollstein, and Y. Xu 2007. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nature Cell Biology 9:373–380.

Taddei, F., M. Radman, J. Maynard-Smith, B. Toupance, P. H. Gouyon, and B. Godelle 1997. Role of mutator alleles in adaptive evolution. Nature 387:700–702.

Tomlinson, I., and W. Bodmer 1999. Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. Nature Medicine 5:11–12.

Tomlinson, I. P., M. R. Novelli, and W. F. Bodmer 1996. The mutation rate and cancer. Proceedings of the National Academy of Sciences of the United States of America 93:14800–14803.

Walther, A., R. Houlston, and I. Tomlinson 2008. Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. Gut 57:941–950.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

- Figure S1. Housekeeper mutations and an evolving mutation rate with moderate to strong selection.
- Figure S2. Requiring different numbers of driver mutations for cancer.
- Figure S3. Abundant deleterious mutations and the evolution of an increased mutation rate.