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Is carcinogenesis a form of speciation?

Peter Duesberg,[1,*] Daniele Mandrioli,[1] Amanda McCormack[1] and Joshua M. Nicholson[2]

[1]Department of Molecular and Cell Biology; Donner Laboratory; UC Berkeley; Berkeley, CA USA; [2]Department of Biological Sciences; Virginia Tech, Blacksburg, VA USA

Since cancers have individual clonal karyotypes, are immortal and evolve from normal cells treated by carcinogens only after exceedingly long latencies of many months to decades—we deduce that carcinogenesis may be a form of speciation. This theory proposes that carcinogens initiate carcinogenesis by causing aneuploidy, i.e., losses or gains of chromosomes. Aneuploidy destabilizes the karyotype, because it unbalances thousands of collaborating genes including those that synthesize, segregate and repair chromosomes. Driven by this inherent instability aneuploid cells evolve ever-more random karyotypes automatically. Most of these perish, but a very small minority acquires reproductive autonomy—the primary characteristic of cancer cells and species. Selection for autonomy stabilizes new cancer species against the inherent instability of aneuploidy within specific margins of variation. The speciation theory explains five common characteristics of cancers: (1) species-specific autonomy; (2) karyotypic and phenotypic individuality; (3) flexibility by karyotypic variations within stable margins of autonomy; (4) immortality by replacing defective karyotypes from constitutive pools of competent variants or subspecies generated by this flexibility; and (5) long neoplastic latencies by the low probability that random karyotypic alterations generate new autonomous species. Moreover, the theory explains phylogenetic relations between cancers of the same tissue, because carcinogenesis is restricted by tissue-specific transcriptomes. The theory also solves paradoxes of other cancer theories. For example, "aneuploidy" of cancers is now said to be a "paradox" or "cancer's fatal flaw," because aneuploidy impairs normal growth and development. But if the "aneuploidies" of cancers are in effect the karyotypes of new species, this paradox is solved.

Key words: evolution, karyotype, instability of aneuploidy, reproductive autonomy, immortality, long neoplastic latency, individuality of cancer, mutation, marker chromosomes

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*Correspondence to: Peter Duesberg; Email: Duesberg@berkeley.edu

Introduction

Cancers are clones of autonomous cells, which are cytogenetically defined by individual clonal karyotypes.[1-4] Accordingly the Mitelman-NCI data base currently lists over 57,000 human cancers with individual clonal karyotypes.[4] Because of their individuality the karyotypes and chromosomes of cancers are typically described as "abnormal" or "aberrant," compared to those of the normal cells from which they originated.[3-10]

In view of the ubiquity of "abnormal" karyotypes in cancers it is, however, tempting to think that this abnormality could be normal. Following this theory cancers could be species of their own and carcinogenesis could be a form of speciation.[11-15] Consequently the individual karyotypes of cancers would be normal for each cancer species. They would only be "abnormal" relative to those of their non-cancerous progenitors—much like the karyotype of a cat would be abnormal compared to that of a dog.

The speciation theory could also explain the "conspicuously" long latent periods from carcinogen to cancer,[16] which range from many months to decades.[2,16-20] Such long latent periods would reflect the exceedingly low probability of generating a new autonomous karyotype by random alterations of the karyotype of a precursor.

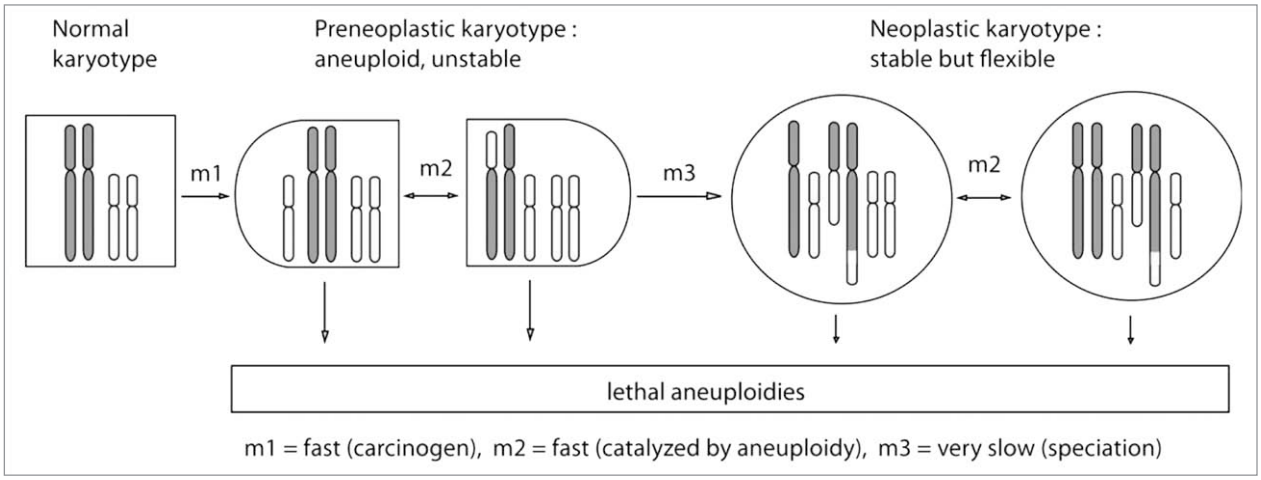


Figure 1. Carcinogenesis by speciation. Stage 1, Generation of the neoplastic karyotype. Carcinogens or spontaneous accidents induce aneuploidy, i.e., losses or gains of chromosomes, at high rates (m1). Since aneuploidy unbalances thousands of collaborating genes, including those that synthesize, segregate and repair chromosomes, it destabilizes the karyotype. Driven by this inherent instability aneuploid cells evolve ever-more random karyotypes automatically, again at high rates (m2). Most of these perish, but a very small minority acquires at very low rates (m3) reproductive autonomy—the primary characteristic of cancer cells and of species. Since aneuploidy changes the normal phenotype (squares), aneuploid cells are shown as half round-half squares, while cancer cells are shown as circles. Stage 2, Spontaneous evolutions of subspecies or progressions of the neoplastic karyotype. Selection for autonomy stabilizes the new cancer karyotypes against randomization by aneuploidy within karyotype-specific margins of variation. Depending on the degree of aneuploidy, these variations occur at rates from 3% to 100% per cell generation (m2). These high rates of variation thus generate large constitutive pools of variants, which allow rapid adaptations and further evolutions of malignancy, termed progressions.

By contrast, the competing mutation theory predicts that carcinogenesis would follow the last of several collaborating mutations without delay, and would depend on these mutations rather than on "abnormal" karyotypes.[21-23]

With these considerations in mind we advance here a new speciation theory of carcinogenesis, which draws on scattered precursors from others and us, particularly Van Valen and Vincent,[6,11,24-28] and on recent experimental tests from our lab.[15,29-31]

The Speciation Theory of Carcinogenesis

Our cancer theory proposes that carcinogens initiate carcinogenesis by inducing aneuploidy, i.e., losses or gains of chromosomes—a function that all carcinogens have in common.[15,25,32-34] Since cancers arise only many months to decades after initiation by carcinogens the question is: How do cancers evolve from aneuploid cells during those long latent periods? In the following we suggest a mechanism that could answer this question.

Stage 1: Generation of the neoplastic karyotype. According to the speciation theory carcinogens initiate carcinogenesis by causing aneuploidy. Aneuploidy destabilizes the karyotype, because it unbalances thousands of collaborating genes including those that synthesize, segregate and repair chromosomes.[35-39] This instability is inseparable from aneuploidy and is proportional to the degree of aneuploidy.[28,36,38,40,41] Driven by this instability aneuploid cells evolve ever-more random karyotypes automatically. Most of these perish because of nullisomies or other karyotypic defects,[5,6,42] but a very small minority acquires reproductive autonomy—the primary characteristic of cancers and of species (**Fig. 1**).

Selection for autonomy stabilizes the new cancer species against randomization by the inherent instability of aneuploidy within narrow margins of variation, termed cancer "heterogeneity."[43-45] Within this dynamic equilibrium cancer species form quasi-stable clones of neoplastic variants or subspecies (**Fig. 1**). See examples below in **Figures 3–7**.

The very low frequencies of carcinogenesis thus reflect the very low probability of generating reproductive autonomy by random karyotypic variations.[30] And this very low probability of speciation predicts the "conspicuously long"[16] neoplastic latencies of carcinogenesis[2,18,46] (see also below). As pointed out by Hauschka, "a karyotypic mutation-selection scheme… requires a long latent period."[6]

Stage 2: Spontaneous evolution of subspecies or progressions of the neoplastic karyotype. At any given time cancer species are a cohort of related variants or subspecies held together by this dynamic equilibrium between destabilizing aneuploidy and selection for autonomy. Variants within the margins of this cohort are more or less oncogenic and have therefore been described as "cancer heterogeneity."[43-45] Given their inherent instability cancer karyotypes would in fact be suicidal without a certain range of karyotypic-neoplastic flexibility. The tolerance for this variability probably reflects the low functional complexity of cancer species. According to Van Valen and Vincent cancer cells use only a minute fraction of the information of their normal predecessors to establish a primitive form of autonomy similar to that of microbes.[11,26]

Karyotypic outliers from without these margins of cancer-specific autonomy get lost in subsequent generations—just like those of other species. See previous studies in reference 30 and 31 and **Figures 3–7 and 9** below for examples of outliers,

which were not stable in clonal passages and selections.

The rates at which cancer karyotypes and phenotypes vary within their margins of autonomy are much higher than variations of conventional karyotypes.[47,48] Depending on the degree of aneuploidy, they range from 3% to almost 100% per cell generation.[30,36,38,40,41,49] These high rates of variation generate large constitutive cohorts of variants or subspecies, which allow rapid adaptations and phenotypic "progressions."[2,45,50,51] Examples are rapid acquisitions of resistance to chemotherapy and for metastatic growth[2,49,52,53] (**Figs. 1 and 3–7** below).

By contrast, the karyotypes and phenotypes of sexually reproducing species are not flexible, because reproduction depends on karyotypic homology of the parents.[54,55]

In view of this we conclude that the notorious flexibility of cancers depends on the inherent instability of their aneuploid karyotypes. The rates of these karyotypic variations and subspeciations are proportional to the degree of aneuploidy: the higher the degrees of aneuploidy, the higher the rates of karyotypic variations and subspeciations.[28,36,38,40,41,53]

This conclusion is supported by the facts that the gene mutations rates of cancers are only about 10^{-6} per gene per generation and thus about the same as those of normal cells.[56-59] Since this mutation rate is orders lower than those of cancer-specific karyotypic alterations, mutations cannot play a major role in the variability of cancers. Indeed, if normal rates of mutations were sufficient to generate the new cancer-phenotypes of clinical progressions, normal cells would metastasize and acquire drug-resistance just like cancers cells.

In the following we test the ability of the speciation theory to predict and explain five common characteristics of cancer.

The Common Characteristics of Cancers in the Light of the Speciation Theory

Cancers share five common characteristics that a valid theory must be able to explain: (1) Autonomy,[6,11,24,25,52] (2) Karyotypic and phenotypic individuality including the many forms of anaplasia,[6,18,26,52,60] (3) Karyotypic and phenotypic flexibility within stable margins,[9,30,40,43,44,61] (4) Immortality[2,6,18,23,26,60,62] and (5) Exceedingly long latent periods from a carcinogen to cancer of many months to decades.[2,16-18,52] In the following we test whether the speciation theory explains these common characteristics of cancer.

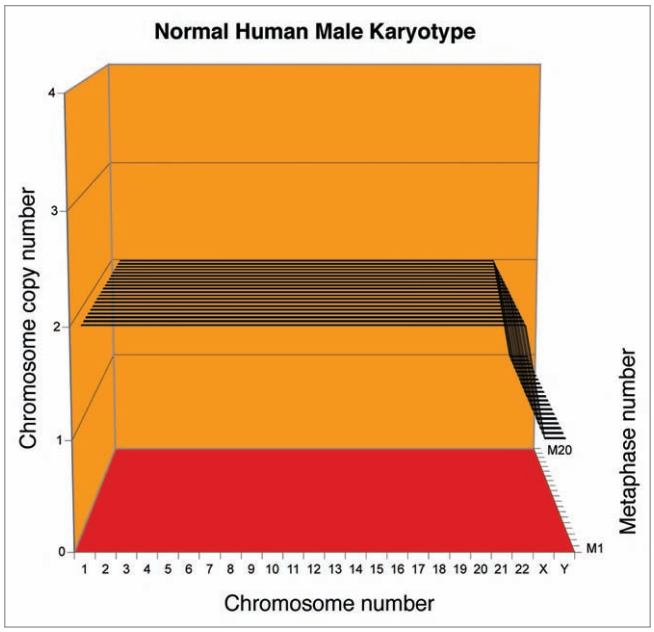


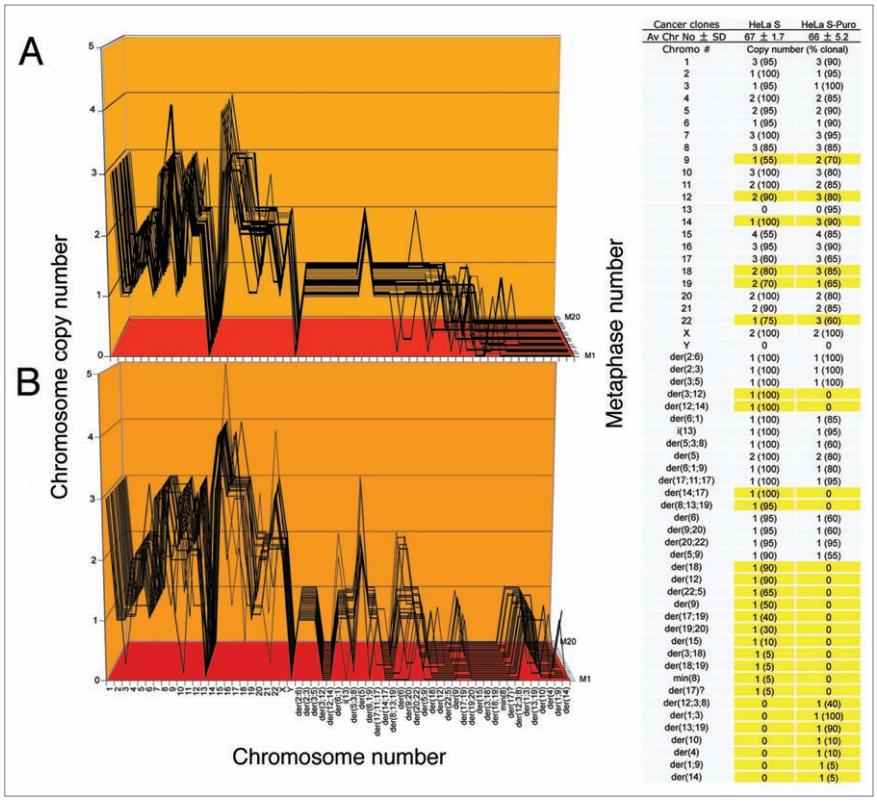
Figure 2. Karyograph of a diploid human male. Karyographs are 3-dimensional tables showing the chromosome numbers on the x-axis, the chromosome copy numbers on the y-axis and the numbers of metaphases arrayed for comparison to each other on the z-axis. As the karyograph shows, the 20 human fibroblast cells analyzed were 100% clonal. The cells were karyotyped from metaphases hybridized to chromosome-specific color-coded DNA probes, as described for **Figure 8A**.

(1) Autonomy. The biological equivalent of autonomy is the species. A species is defined by autonomous reproduction and an individual immortal karyotype.[12,14] Since cancers have been defined as autonomous, because they reproduce independently[6,24,26,52,63] and have individual immortal (see below) karyotypes[1-4] (rather than common gene mutations[64]), they fit the definition of species of their own.[11,25,26] This begs the question: Is there also a precedent for acquiring autonomy of growth and immortality (see below) by gene mutation, as is postulated by the competing mutation theory?

(2) Individuality. The speciation theory predicts that cancers have individual karyotypes and phenotypes, much like conventional species. This prediction is based on the exceedingly low probability that the same new, autonomous karyotype would evolve twice by random karyotypic variations of a given precursor species.[12-14]

Cancers defined by individual clonal karyotypes. Karyotypic individuality is apparently the rule for the over 57,000 human cancers listed in the NCI-Mitelman database,[4] as well as for all animal cancers that have been tested.[6,11,38,65,66] All of these are defined by individual clonal karyotypes with individual chromosome copy numbers and typically also with individual marker or rearranged chromosomes.

To test the predicted karyotypic individuality of cancers directly, we compare here the karyographs of a normal diploid male and of 5 distinct human cancers. Karyographs are three-dimensional arrays of metaphase chromosomes from about 20 cells that list the chromosome numbers and designations of marker chromosomes on the x-axis, the copy numbers of each chromosome on the y-axis, and the numbers of metaphases analyzed on the z-axis.[31] The karyograph of the diploid male is shown in **Figure 2**, and those of the



Cancer clones	HeLa S	HeLa S-Puro
Av Chr No ± SD	67 ± 1.7	66 ± 5.2
Chromo #	Copy number (% clonal)	
1	3 (95)	3 (90)
2	1 (100)	1 (95)
3	1 (95)	1 (100)
4	2 (100)	2 (85)
5	2 (95)	2 (90)
6	1 (95)	1 (90)
7	3 (100)	3 (95)
8	3 (85)	3 (85)
9	1 (55)	2 (70)
10	3 (100)	3 (80)
11	2 (100)	2 (85)
12	2 (90)	3 (80)
13	0	0 (95)
14	1 (100)	3 (90)
15	4 (55)	4 (85)
16	3 (95)	3 (90)
17	3 (80)	3 (65)
18	2 (80)	3 (85)
19	2 (70)	1 (65)
20	2 (100)	2 (80)
21	2 (90)	2 (85)
22	1 (75)	3 (60)
X	2 (100)	2 (100)
Y	0	0
der(2;6)	1 (100)	1 (100)
der(2;3)	1 (100)	1 (100)
der(3;5)	1 (100)	1 (100)
der(3;12)	1 (100)	0
der(12;14)	1 (100)	0
der(6;1)	1 (100)	1 (85)
i(13)	1 (100)	1 (95)
der(5;3;8)	1 (100)	1 (60)
der(5)	2 (100)	2 (80)
der(8;1;9)	1 (100)	1 (80)
der(17;11;17)	1 (100)	1 (95)
der(14;17)	1 (100)	0
der(8;13;19)	1 (95)	0
der(6)	1 (95)	1 (60)
der(9;20)	1 (95)	1 (60)
der(20;22)	1 (95)	1 (95)
der(5;9)	1 (90)	1 (55)
der(18)	1 (90)	0
der(12)	1 (90)	0
der(22;5)	1 (65)	0
der(9)	1 (50)	0
der(17;19)	1 (40)	0
der(19;20)	1 (30)	0
der(15)	1 (10)	0
der(3;18)	1 (5)	0
der(18;19)	1 (5)	0
min(8)	1 (5)	0
der(17)?	1 (5)	0
der(12;3;8)	0	1 (40)
der(1;3)	0	1 (100)
der(13;19)	0	1 (90)
der(10)	0	1 (10)
der(4)	0	1 (10)
der(1;9)	0	1 (5)
der(14)	0	1 (5)

Figure 3. Karyographs and karyotypic parameters of the HeLa cervical cancer line and of a puromycin-resistant variant. Karyotypic parameters including the total chromosome numbers per metaphase and the copy numbers of normal and marker chromosomes, were obtained from metaphase chromosomes, which were hybridized in situ with chromosome-specific color-coded DNA probes and karyotyped as described previously in reference 30 and 40. Non-clonal marker chromosomes associated with less than 3 in 10 HeLa cells are not listed here (see text). The karyographs were then assembled from the karyotypic data of 20 metaphases listed in the attached tables. They were prepared to determine the degrees of clonality and variability of the chromosomes of a clonal cancer by comparing the karyotypic parameters of these metaphases to each other. (A) Karyograph of 20 representative metaphases of HeLa cells. (B) The karyograph of a puromycin-resistant (1.5 μg per 3 ml medium) derivative of the HeLa line, prepared by published procedures.[40] The karyotypic parameters of the resistant strain are tabled next to those of the drug-sensitive strain. The karyotypic differences between A and B are visible by comparison of the karyographs or the tables, in which they are marked yellow. It can be seen that the drug-resistant variant differs from the parental strain in the clonal copy numbers of several normal chromosomes, and in the loss of parental and gain of derivative-specific marker chromosomes.

five cancers maintained as lines in culture, namely the cervical cancer line HeLa,[67] the colon cancer lines HT29 and SW480,[40] the breast cancer line MDA231,[40] and the bladder cancer LD583 together with a metastatic derivative, LD611 [68] are shown in **Figures 3–7.**

As can be seen in these figures the karyographs of the human species and of each of the five cancers form indeed unique patterns, which can be seen as signatures of their individuality.[31] Notably even the two colon cancers HT29 and SW480 have individual karyographs.

The one-to-one correlations between individual cancers and individual karyographs confirm the prediction of the speciation theory that cancers have individual karyotypes, "one cancer one karyotype"—just like ordinary species.

The cancer karyographs also show clonal heterogeneities, which reflect the characteristic flexibility of cancer karyotypes that is discussed in the next section. Thus karyographs show clonality and variability at once, whereas conventional karyograms only show karyotypes of individual cells.

Figure 8A and B shows two representative karyograms from which these karyographs were constructed, (A) karyogram of a fibroblast from a normal diploid male, (B) karyogram a cell from the human bladder cancer LD583 whose karyograph is shown in **Figure** 7. These karyograms were prepared from metaphase chromosomes hybridized with chromosome-specific colors as described by us previously in reference 15 and 31. The bottom lines in **Figure 8B** show the 26 clonal and 7 non-clonal marker chromosomes of the bladder cancer.

Karyotype-transcriptome correlations in cancers and in non-canerous aneuploidies. In further support for a genetic basis of the phenotype-karyotype correlations of cancers, several researchers have recently found that the gene expression profiles of thousands of normal genes are directly proportional to the copy numbers of the respective aneuploid chromosomes.[69-77] In other words the individual karyotypes determine the individual transcriptomes of cancers.

Moreover, comparisons between different cancers have shown, "Numerous associations between genomic abnormalities and clinical behavior."[9] The more aneuploid the karyotype the more malignant is the cancer.[20,43,52,74]

These cause-effect relations between cancer karyotypes and phenotypes are also supported by the specific phenotype-karyotype relations of (1) normal species[13] and of (2) non-cancerous individuals with congenital aneuploidies. The best-known human examples of congenital aneuploidies are Down syndrome or trisomy 21 [78,79] and trisomy 13.[80,81] For example, although "chromosome 21 represents (only) about 1% of the human genome," trisomy 21 (Down Syndrome) generates "more than 80 mental and physical disorders."[82] Abnormal phenotypes induced by experimental aneuploidy have also been described in animal species such as sea urchins,[5] Drosophila,[42] plants[37] and yeast[83-85] for over 100 years. Likewise the copy number of bacterial plasmids with drug-resistance genes determines the level of resistance against toxic drugs. All of these examples revealed numerous dominant phenotypes based solely on gene dosage that could not be reduced to any consistent mutations.[78,85,86]

It follows that the individual transcriptomes and phenotypes of cancers correlate directly with individual copy numbers of their aneuploid chromosomes. The same is true for the transcriptomes and phenotypes of aneuploid non-cancerous cells.

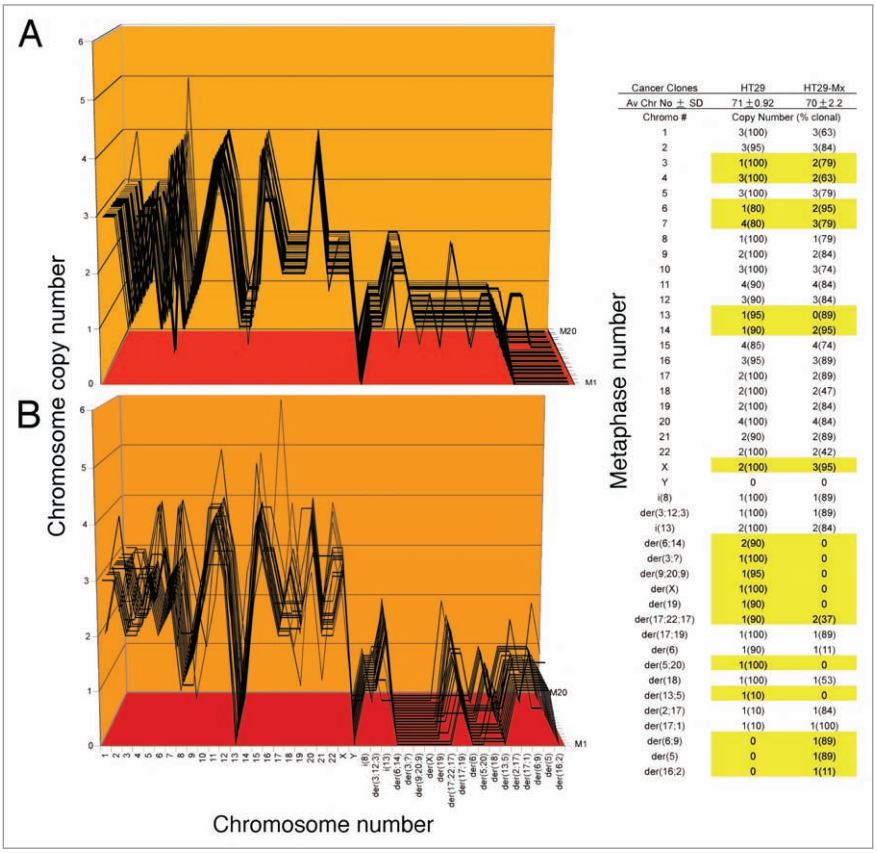
A role for "specific" mutations in cancers with individual karyotypes? In view of the karyotypic and phenotypic individualities of cancers, and the correlations of these individual karyotypes with the abnormal expressions of thousands of normal genes,[71,74-77] it is unclear what role mutations of 3–6 "specific" oncogenes play in carcinogenesis (see also Section 5 below).

This uncertainty is based on two consistent problems with oncogenes:

(1) *The low levels of expression of hypothetical cellular oncogenes*. For instance, mRNAs of cellular oncogenes are typically undetectable in cancers without artificial amplification[87] (and Zhang and Vogelstein, personal communication).[49] It is probably for this reason that the expressions of oncogenes are rarely even mentioned or specifically discussed in gene-expression studies of cancers.[70-77] By contrast, viral sequence-homologs of cellular oncogenes derive their carcinogenic function from transcriptional over-activation by viral promoters,[15,20,88-91] with or without gene mutations.[88,90,92,93] See Klein et al. for a recent discussion.[15]

(2) *The independence of cancers from known oncogenes*. Cancers of the same kind do not consistently share the same mutated oncogenes.[58,94-97] According to Vogelstein, "There are a few genes that are commonly mutated—we call these mountains—but the landscape is dominated by hills."[64] It could thus be argued that the mutations of oncogenes are either not necessary to maintain transformation or that other genes can take their place. The first alternative is supported by *ras*-positive human fibrosarcomas, colon cancers and melanomas, which retained oncogenicity after spontaneous losses of their *ras*-genes,[98,99] and by jak-negative acute myeloid leukemias derived from jak-positive precursors.[64,100] The same observations have been made with animal cancers that retained tumorigenicity after losing their presumed oncogenes.[15,97,101]

Thus the consistently low levels of oncogene-expression, and the independence of cancers of oncogenes suggest that cancers derive carcinogenicity from their individual karyotypes with their massive individual transcriptomes.

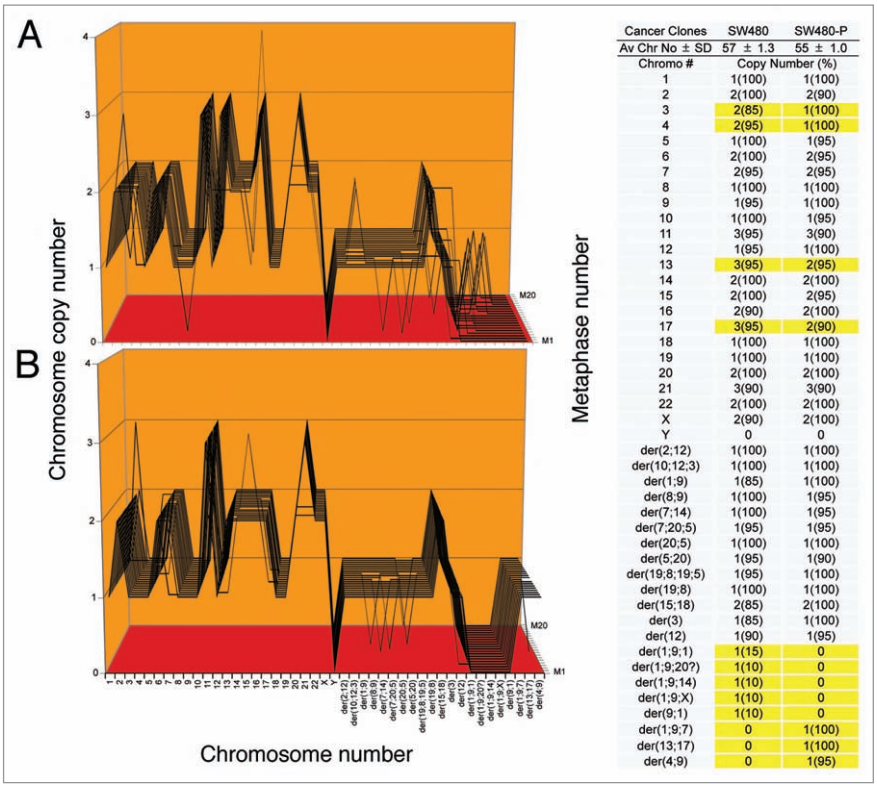


Cancer Clones	HT29	HT29-Mx
Av Chr No ± SD	71 ± 0.92	70 ± 2.2
Chromo #	Copy Number (% clonal)	
1	3(100)	3(63)
2	3(95)	3(84)
3	1(100)	2(79)
4	3(100)	2(63)
5	3(100)	3(79)
6	1(80)	2(95)
7	4(80)	3(79)
8	1(100)	1(79)
9	2(100)	2(84)
10	3(100)	3(74)
11	4(90)	4(84)
12	3(90)	3(84)
13	1(95)	0(89)
14	1(90)	2(95)
15	4(85)	4(74)
16	3(95)	3(89)
17	2(100)	2(89)
18	2(100)	2(47)
19	2(100)	2(84)
20	4(100)	4(84)
21	2(90)	2(89)
22	2(100)	2(42)
X	2(100)	3(95)
Y	0	0
i(8)	1(100)	1(89)
der(3;12;3)	1(100)	1(89)
i(13)	2(100)	2(84)
der(6;14)	2(90)	0
der(3;?)	1(100)	0
der(9;20;9)	1(95)	0
der(X)	1(100)	0
der(19)	1(90)	0
der(17;22;17)	1(90)	2(37)
der(17;19)	1(100)	1(89)
der(6)	1(90)	1(11)
der(5;20)	1(100)	0
der(18)	1(100)	1(53)
der(13;5)	1(10)	0
der(2;17)	1(10)	1(84)
der(17;1)	1(10)	1(100)
der(6;9)	0	1(89)
der(5)	0	1(89)
der(16;2)	0	1(11)

Figure 4. Karyographs and tables of karyotypic parameters of (A) the colon cancer cell line HT29 and (B) a methotrexate-resistant HT29 variant. The karyographs and tables were prepared as described for Figure 3 and in the text. The methotrexate-resistant HT29 variant shown in (B) was selected for resistance against 1.5 μg methotrexate per 3 ml medium following published procedures.[40] It can be seen that the drug-resistant variant differs from the parental strain in the clonal copy numbers of several normal chromosomes, and in the loss of parental and gain of drug resistance-specific marker chromosomes. Differences are marked yellow.

In view of this it seems not surprising that there is no clear evidence for a consistent oncogenic phenotype setting apart cancers with mutations of specific oncogenes from cancers without those mutations. Even the textbook, *The Biology of Cancer*, states on page 459, "a one-to-one mapping between genes and cancer-associated phenotypes is not possible."[23] Concordantly, McCormick pointed out recently that pancreatic cancers with *ras* mutations do not differ from pancreatic cancers without *ras* mutations in any consistent cancer-specific phenotype (Frank McCormick, "New Approaches to Targeting Ras," seminar at the Lawrence Berkeley Lab, March 29, 2011). Likewise a recent review on this issue points out that "K-*ras* mutation may complement… the diagnosis of PC [pancreatic cancer] in spite of its limited contribution to clinical decision making. The presence of K-*ras* in chronic [non-cancerous] pancreatitis classifies a subgroup of PC risk patients…."[102] Thus there is no consistent evidence for the neoplastic function of mutated oncogenes like *ras* (See also Section 5).

Conclusion. We conclude that the one-cancer-one-karyotype correlations, described here by us, are genetic evidence for the speciation theory. This and the proportionalities between chromosome copy numbers and copy numbers of corresponding mRNAs indicate that cancer karyotypes as a whole determine cancer phenotypes, much like karyotypes



Cancer Clones	SW480	SW480-P
Av Chr No ± SD	57 ± 1.3	55 ± 1.0
Chromo #	Copy Number (%)	
1	1(100)	1(100)
2	2(100)	2(90)
3	2(85)	1(100)
4	2(95)	1(100)
5	1(100)	1(95)
6	2(100)	2(95)
7	2(95)	2(95)
8	1(100)	1(100)
9	1(95)	1(100)
10	1(100)	1(95)
11	3(95)	3(90)
12	1(95)	1(100)
13	3(95)	2(95)
14	2(100)	2(100)
15	2(100)	2(95)
16	2(90)	2(100)
17	3(95)	2(90)
18	1(100)	1(100)
19	1(100)	1(100)
20	2(100)	2(100)
21	3(90)	3(90)
22	2(100)	2(100)
X	2(90)	2(100)
Y	0	0
der(2;12)	1(100)	1(100)
der(10;12;3)	1(100)	1(100)
der(1;9)	1(85)	1(100)
der(8;9)	1(100)	1(95)
der(7;14)	1(100)	1(95)
der(7;20;5)	1(95)	1(95)
der(20;5)	1(100)	1(100)
der(5;20)	1(95)	1(90)
der(19;8;19;5)	1(95)	1(100)
der(19;8)	1(100)	1(100)
der(15;18)	2(85)	2(100)
der(3)	1(85)	1(100)
der(12)	1(90)	1(95)
der(1;9;1)	1(15)	0
der(1;9;20?)	1(10)	0
der(1;9;14)	1(10)	0
der(1;9;X)	1(10)	0
der(9;1)	1(10)	0
der(1;9;7)	0	1(100)
der(13;17)	0	1(100)
der(4;9)	0	1(95)

Figure 5. Karyographs and tables of karyotypic parameters of (A) the colon cancer cell line SW480 and (B) a puromycin-resistant variant. The karyographs and tables were prepared as described for Figure 3 and in the text. The puromycin-resistant SW480 variant shown in (B) was selected for resistance against 6 μg puromycin per 3 ml medium following published procedures.[40] As can be seen in the karyographs and tables, the drug-sensitive and resistant variants are closely related, but differed from each other in the clonal copy numbers of several normal chromosomes and in individual sets of marker chromosomes.

as a whole determine the phenotypes of conventional species. By contrast, the evidence for the neoplastic function of common oncogenes shared by many but not all individual cancers is still uncertain.

(3) **Karyotypic flexibility.** The speciation theory holds that the karyotypes of cancers are flexible within stable karyotype-specific margins, based on an equilibrium between the inherent instability of aneuploidy and selection for autonomy. This predicts (a) that the cancer karyotype will be heterogeneous at any time and (b) that the karyotype or the respective subspecies will vary with different selective conditions. To test these predictions we have analyzed the karyotypes of several cancers for clonal heterogeneity at a given time, and compared the karyotypes of the same cancers growing under different selective conditions (drug-resistance and metastasis).

(a) Heterogeneity of clonal neoplastic karyotypes. As can be seen in **Figures 3–7** and the underlying Tables, the total chromosome numbers of individual cells of the five human cancer lines HeLa, HT29, SW480, MDA231 and the bladder cancer LD583/611 vary a few % around clonal averages. The cancer-specific chromosome copy numbers typically oscillate ±1 and rarely ±2 around modal values. Occasionally there are also polyploidizations of the whole cancer karyotype, as for example in the case of the bladder cancer shown here in **Figure** 7.

In addition there are a few outliers from without the apparent equilibrium of autonomous variants in any condition, which are typically not conserved in further passages in the same conditions[30,31] or under different selective conditions (Compare **Figs. 3–7A to 3–7B** for examples). Other outliers show up as non-clonal marker chromosomes in minorities of cells of otherwise clonal cancers. For example, 2 in 10 HeLa cells, 3 in 10 HT29 cells, 4 in 10 SW480 cells, 1 in 20 MDA231 cells, and almost every LD583 bladder cancer cell contained nonclonal marker chromosomes (see for example, **Fig. 8B**). Again these outliers were not seen in subsequent passages, but were replaced by others, indicating ongoing variability (not shown in **Figs. 3–7**).[28,30,36,38,40,41,103]

Further work analyzing single cell-derived clones of these cancers would be necessary to determine which outliers are viable, i.e., clonable and which are not.[40]

(b) Selection of new clonal phenotypes. Next, we have asked, whether the acquisition of new cancer-specific phenotypes, such as resistance to the cytotoxic drugs puromycin and methotrexate or adaptation to a new habitat, i.e., metastasis, correlates with clonal karyotypic alterations.

For this purpose, puromycin- and methotrexate-resistant variants of the 4 cancer lines, HeLa, HT29, SW480 and MDA231 were prepared as described briefly in the legends of **Figures 3–6** and previously in reference 15, 30 and 40. The karyographs of each drug-resistant variant were then compared to the corresponding drug-sensitive precursor. It can be seen in **Figures 3A and B–6A and B** that each drug-resistant variant of the four cancer lines differed from the drug-sensitive precursor in the modal or clonal copy numbers of 2 to 7 normal and marker chromosomes (yellow highlights in Tables). In addition, the drug-resistant variants differed from parental lines in the loss of parental and gain of new clonal marker chromosomes, totaling between 1 and 20 different markers per variant line.

Further, we compared the karyograph of a metastatic bladder cancer to the karyograph of the corresponding primary cancer for metastasis-specific karyotype alterations. Tsao et al. have isolated this metastasis from a patient 9 months after the removal of a primary cancer and adjuvant chemotherapy.[68] It can be seen in **Figure 7A–C** that the near tetraploid karyotypes of the primary cancer and of the metastasis are closely related species. But, the metastatic bladder cancer differed from the primary in 17 clonal copy number changes of normal chromosomes

and 4 of shared marker chromosomes. In addition the primary cancer contained 16 individual markers and the metastasis contained 32. Moreover, the metastasis contained a second karyotype, an apparent duplication of the primary near-tetraploid karyotype to a new near-octaploid karyotype (**Fig. 7C**).

Such polyploidizations of cancer karyotypes confirm earlier observations of others. For example, Hauschka wrote in 1961, "Besides the principal mitotic errors of lagging and nondisjunction, which cause minor numerical departures from diploidy, polyploidization through endomitotic mechanisms plays a prominent role in tumor evolution."[6]

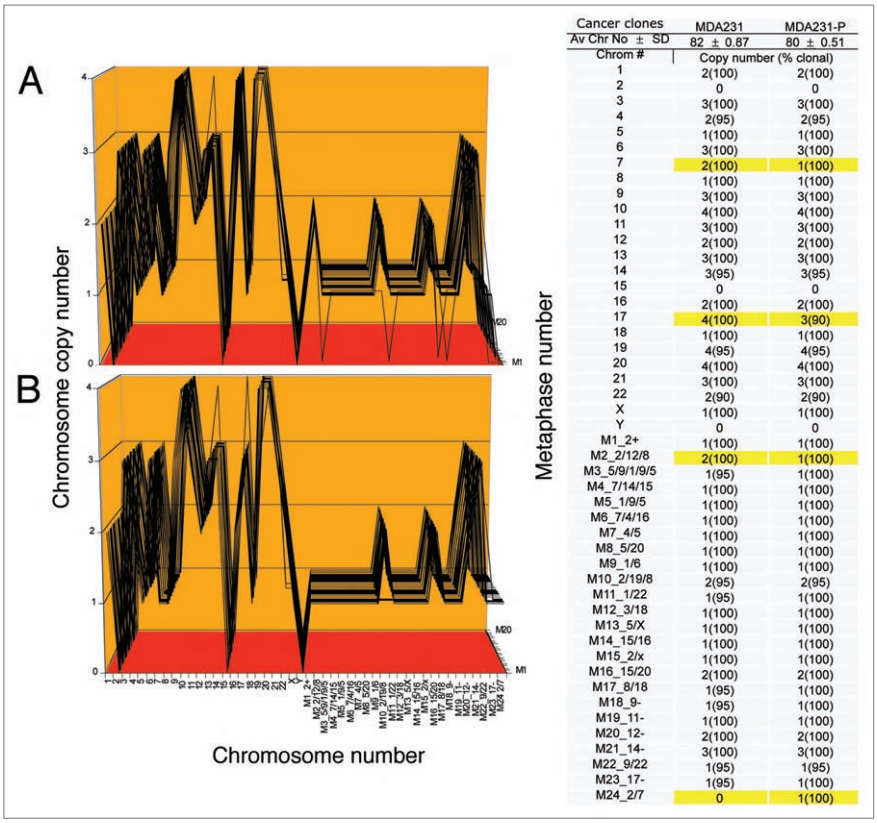
Rates of cancer-specific karyotypic variations. The times during which these cancers evolved such complex new phenotypes as drug-resistance and metastasis were only several weeks to months. At the same time these cancers vary their karyotypes within specific margins at rates of several to over 50% of karyotypes per cell generation as described above. Thus, these rates are several orders faster than conventional mutations (see, *The speciation theory of cancer* and **Fig. 1**, above). As predicted by our theory, these high rates of variation alone distinguish the cancer-specific mechanism of phenotypic variations from the much lower rates of conventional mutation.[40,58,59]

We conclude that aneuploidy-catalyzed karyotypic variation buffered by selection for autonomy provides a cancer-specific explanation for the characteristic variability of cancers, for the genetic complexity of these variations and for the high rates at which variations occur compared to conventional mutations in normal and cancer cells.[40,58,59]

(4) **Immortality.** Early on researchers have called cancers immortal, because they could be transplanted indefinitely from animal to animal or cultivated in vitro.[11,18,104,105] According to Hauschka in 1961, "tumor karyotypes have competitive survival value and will be constant for thousands of cell generations."[6] In 1965 Hayflick also connected immortality with the karyotype, "Lacking any evidence on this point, it could be argued that escape from the inevitability of aging of cells in vivo and in vitro… is heteroploidy (usually modally distributed)."[104] Heteroploidy is synonymous with aneuploidy. In 1972 the British cancer researcher Koller linked immortality specifically to the variability of the cancer karyotype, "It seems that malignant growth is composed of competing clones of cells with different and continuously changing genotypes, conferring the tumor with an adaptable plasticity against the environment. The bewildering karyotypic patterns reveal the multi-potentiality of the neoplastic cell; while normal cells and tissues age and die, through their inherent variability, tumor cells proliferate and survive."[62] But a consistent theory connecting the cancer karyotype with immortalization did not emerge.

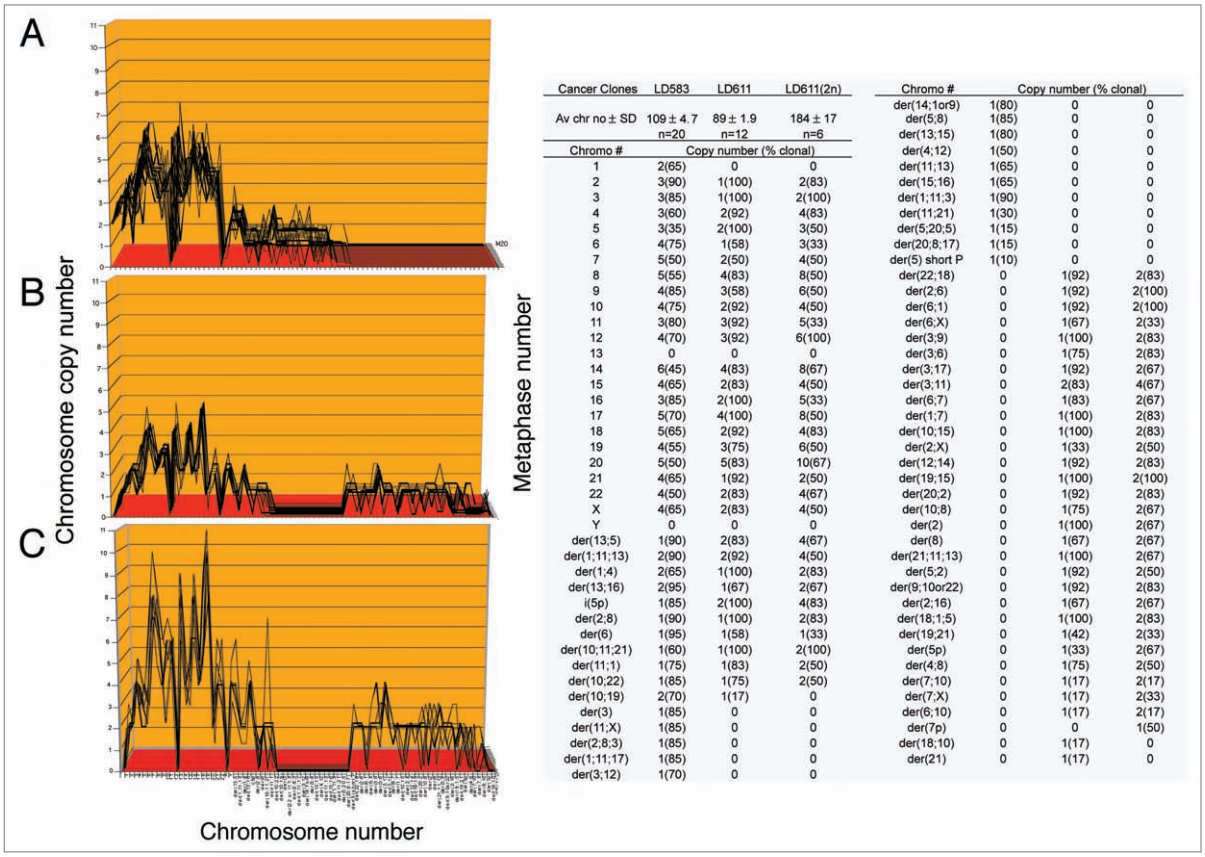
Adaptations via karyotypic flexibility as mechanism of immortalization. The speciation theory proposes that the "bewildering" karyotypic variability buffered by selection for autonomy generates immorality. The theory holds that the equilibrium between the inherent instability of aneuploidy and selection for autonomy generates a steady pool of variants or subspecies that immortalize cancers against toxic drugs or non-native habitats as in metastasis and experimental transplantations (see also Section 3). Normal species also derive immortality by selections against abnormal karyotypes or other genetic defects from pools of normal individuals.

Natural examples of immortal cancer karyotypes. The karyotypes of all clinical cancers have been clonal and thus stable, despite clonal heterogeneity, for at least 30 generations by the time they are typically



Cancer clones	MDA231	MDA231-P
Av Chr No ± SD	82 ± 0.87	80 ± 0.51
Chrom #	Copy number (% clonal)	
1	2(100)	2(100)
2	0	0
3	3(100)	3(100)
4	2(95)	2(95)
5	1(100)	1(100)
6	3(100)	3(100)
7	2(100)	1(100)
8	1(100)	1(100)
9	3(100)	3(100)
10	4(100)	4(100)
11	3(100)	3(100)
12	2(100)	2(100)
13	3(100)	3(100)
14	3(95)	3(95)
15	0	0
16	2(100)	2(100)
17	4(100)	3(90)
18	1(100)	1(100)
19	4(95)	4(95)
20	4(100)	4(100)
21	3(100)	3(100)
22	2(90)	2(90)
X	1(100)	1(100)
Y	0	0
M1_2+	1(100)	1(100)
M2_2/12/8	2(100)	1(100)
M3_5/9/1/9/5	1(95)	1(100)
M4_7/14/15	1(100)	1(100)
M5_1/9/5	1(100)	1(100)
M6_7/4/16	1(100)	1(100)
M7_4/5	1(100)	1(100)
M8_5/20	1(100)	1(100)
M9_1/6	1(100)	1(100)
M10_2/19/8	2(95)	2(95)
M11_1/22	1(95)	1(100)
M12_3/18	1(100)	1(100)
M13_5/X	1(100)	1(100)
M14_15/16	1(100)	1(100)
M15_2/x	1(100)	1(100)
M16_15/20	2(100)	2(100)
M17_8/18	1(95)	1(100)
M18_9-	1(95)	1(100)
M19_11-	1(100)	1(100)
M20_12-	2(100)	2(100)
M21_14-	3(100)	3(100)
M22_9/22	1(95)	1(95)
M23_17-	1(95)	1(100)
M24_2/7	0	1(100)

Figure 6. Karyographs and tables of karyotypic parameters of (A) the breast cancer cell line MDA231 and (B) a puromycin-resistant variant. The karyographs were prepared as described for Figure 3 and in the text. Markers were labeled M1 to M24, followed by chromosomal constituents. The puromycin-resistant variant shown in (B) was selected for resistance against 6 μg puromycin per 3 ml medium following published procedures.[40] As can be seen in the karyographs and tables, the drug-sensitive and resistant variants are closely related, but differed from each other in the copy numbers of several normal and marker chromosomes and in one resistance-specific marker chromosome.



Cancer Clones	LD583	LD611	LD611(2n)
Av chr no ± SD	109 ± 4.7 n=20	89 ± 1.9 n=12	184 ± 17 n=6
Chromo #	Copy number (% clonal)		
1	2(65)	0	0
2	3(90)	1(100)	2(83)
3	3(85)	1(100)	2(100)
4	3(60)	2(92)	4(83)
5	3(35)	2(100)	3(50)
6	4(75)	1(58)	3(33)
7	5(50)	2(50)	4(50)
8	5(55)	4(83)	8(50)
9	4(85)	3(58)	6(50)
10	4(75)	2(92)	4(50)
11	3(80)	3(92)	5(33)
12	4(70)	3(92)	6(100)
13	0	0	0
14	6(45)	4(83)	8(67)
15	4(65)	2(83)	4(50)
16	3(85)	2(100)	5(33)
17	5(70)	4(100)	8(50)
18	5(65)	2(92)	4(83)
19	4(55)	3(75)	6(50)
20	5(50)	5(83)	10(67)
21	4(65)	1(92)	2(50)
22	4(50)	2(83)	4(67)
X	4(65)	2(83)	4(50)
Y	0	0	0
der(13;5)	1(90)	2(83)	4(67)
der(1;11;13)	2(90)	2(92)	4(50)
der(1;4)	2(65)	1(100)	2(83)
der(13;16)	2(95)	1(67)	2(67)
i(5p)	1(85)	2(100)	4(83)
der(2;8)	1(90)	1(100)	2(83)
der(6)	1(95)	1(58)	1(33)
der(10;11;21)	1(60)	1(100)	2(100)
der(11;1)	1(75)	1(83)	2(50)
der(10;22)	1(85)	1(75)	2(50)
der(10;19)	2(70)	1(17)	0
der(3)	1(85)	0	0
der(11;X)	1(85)	0	0
der(2;8;3)	1(85)	0	0
der(1;11;17)	1(85)	0	0
der(3;12)	1(70)	0	0

Chromo #	Copy number (% clonal)		
der(14;1or9)	1(80)	0	0
der(5;8)	1(85)	0	0
der(13;15)	1(80)	0	0
der(4;12)	1(50)	0	0
der(11;13)	1(65)	0	0
der(15;16)	1(65)	0	0
der(1;11;3)	1(90)	0	0
der(11;21)	1(30)	0	0
der(5;20;5)	1(15)	0	0
der(20;8;17)	1(15)	0	0
der(5) short P	1(10)	0	0
der(22;18)	0	1(92)	2(83)
der(2;6)	0	1(92)	2(100)
der(6;1)	0	1(92)	2(100)
der(6;X)	0	1(67)	2(33)
der(3;9)	0	1(100)	2(83)
der(3;6)	0	1(75)	2(83)
der(3;17)	0	1(92)	2(67)
der(3;11)	0	2(83)	4(67)
der(6;7)	0	1(83)	2(67)
der(1;7)	0	1(100)	2(83)
der(10;15)	0	1(100)	2(83)
der(2;X)	0	1(33)	2(50)
der(12;14)	0	1(92)	2(83)
der(19;15)	0	1(100)	2(100)
der(20;2)	0	1(92)	2(83)
der(10;8)	0	1(75)	2(67)
der(2)	0	1(100)	2(67)
der(8)	0	1(67)	2(67)
der(21;11;13)	0	1(100)	2(67)
der(5;2)	0	1(92)	2(50)
der(9;10or22)	0	1(92)	2(83)
der(2;16)	0	1(67)	2(67)
der(18;1;5)	0	1(100)	2(83)
der(19;21)	0	1(42)	2(33)
der(5p)	0	1(33)	2(67)
der(4;8)	0	1(75)	2(50)
der(7;10)	0	1(17)	2(17)
der(7;X)	0	1(17)	2(33)
der(6;10)	0	1(17)	2(17)
der(7p)	0	0	1(50)
der(18;10)	0	1(17)	0
der(21)	0	1(17)	0

Figure 7. Karyographs and tables of karyotypic parameters of (A) the bladder cancer LD583 and (B and C) the metastasis LD611 that appeared 9 months after the removal of the primary cancer originally isolated by Tsao et al. in 2000.[68] The metastasis was a mixture of two karyotypic variants, a major near-tetraploid and a minor near-octaploid variant. As can be seen in the karyographs and tables, the primary and metastatic cancers were closely related, but differed in the copy numbers of several normal chromosomes and in individual sets of marker chromosomes. A representative metaphase karyotype of LD583 is shown in **Figure 8B**.

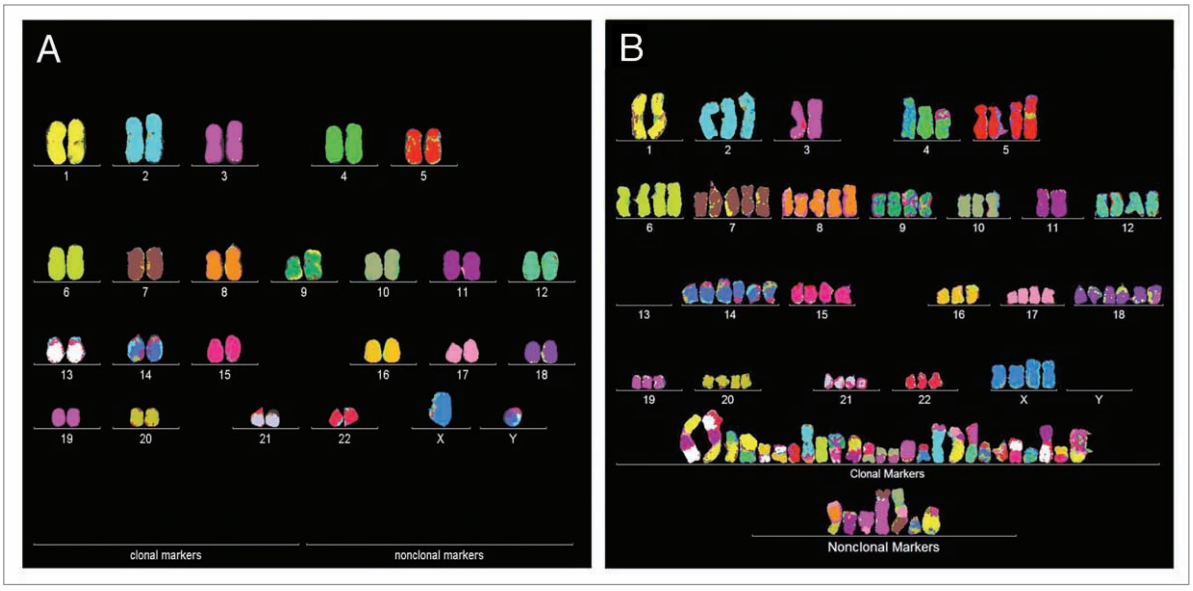


Figure 8 The karyograms of (A) the fibroblast of a diploid human male and (B) a cell from the bladder carcinoma LD583 (see text and Fig. 7). Metaphase chromosomes spread on a microscope slide were hybridized to chromosome-specific color-coded DNA probes to facilitate karyotyping following published procedures.[40]

first diagnosed as a mass of about 1 ml or 10^9 (=2^{30}) cells. They are thus at least "relatively" immortal at this stage.[4] Further, it was found that the original karyotypes of human cancers survive in patients over long time periods, often with variations induced by chemotherapy[9,30,57,106-110] (See also **Fig.** 7 for an example). The survival of the basic karyotype of a metastatic melanoma in a patient for 12 years maybe a record of its kind.[111]

Cancer karyotypes have also been immortal in thousands of experimental transplant generations[6,18,105,112] and in thousands of experimental passages in cell culture, despite inherent flexibility.[11,104] The immortality of cancer species is even dominant in fusions with normal cells, termed hybridomas.[113]

The "infectious cancers" are fascinating natural examples of the immortality of "fully speciated cancers" with individual clonal karyotypes.[26] These cancers are naturally passed from animal to animal such as the "canine venereal tumor"[60,114] and the facial cancer of the Tasmanian devil.[115] Apparently the karyotypes of the canine and Tasmanian tumors are basically the same in all cases that have been tested. They have thus been stable in countless natural transmissions—just like microbial parasites. According to Vincent, "The acquisition of germ line properties by cancer cells clearly indicates they have transcended the host and become something different."[26]

We conclude that the flexibility of cancer karyotypes within stable margins and the resulting constitutive pools of competent variants or subspecies confer immortality to cancers. Karyotypic immortality thus links cancer species once more with normal phylogenetic species.[12,14]

(5) Inevitably long latent periods from carcinogen exposure to cancer. No matter what carcinogen is used and how often it is applied, cancers only develop after "conspicuously"[16] long latent periods of many months to decades.[2,17,18,52] The speciation theory predicts that these long latent periods reflect the low probability of evolving the karyotype of a new autonomous species by random karyotypic variations of a precursor species (see above **Fig. 1** and text). This has been confirmed in several ways.

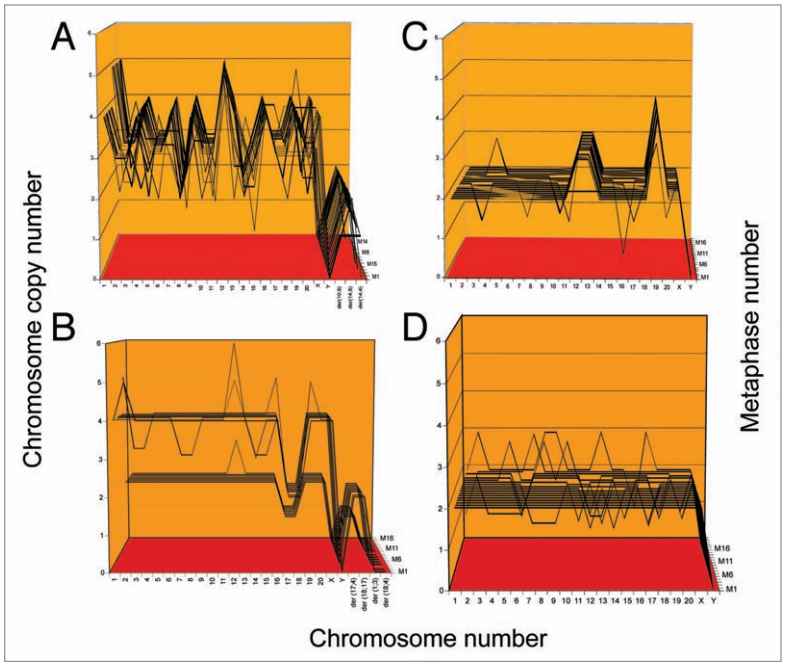


Figure 9. (A–C) Karyographs of 3 rat mammary tumors that appeared 6–12 months after a single injection with nitrosurea. It can be seen that each rat mammary tumor (RMT), RMT 58 (A), 61 (B) and 47 (C), had an individual, clonal karyotype, although they were generated in the same strain of rats with the same carcinogen.[130] (D) The karyotypes of the preneoplastic mammary hyperplasia or "tumor" from which RMT 47 (C) was derived. At the time of explantation the karyotype of RMT 47 was non-clonal as shown in (D). After a few weeks in culture, foci of morphologically transformed cells appeared with the clonal karyotype shown in (C). This result is consistent with the prediction of the speciation theory that a carcinogen induces random aneuploidy, which is followed, after long latencies, by cancers with clonal karyotypes (**Fig. 1**). The tumor RMT 47 was apparently explanted at the transition from preneoplastic to clonal neoplastic karyotypes.

Classical observations of long neoplastic latencies. Classical clinical observations[16,21,116] and animal experiments, beginning with Yamagiwa and Ishikawa in 1915,[46] have shown long ago that carcinogens cause cancer only after long neoplastic latencies of many months to decades, but the reason for the inevitable latencies remained unsolved.[2,16,18,20,21,46,116-118]

Neoplastic latenies setting apart initiation by carcinogens and cancer. More recent research has revealed that carcinogens induce random aneuploidy without delay, but cancers with clonal karyotypes only after long delays, as predicted by the speciation theory. Such preneoplastic aneuploidy has been observed in, (a) humans after exposure to atomic radiations,[119] (b) human cells in which "a surprisingly high proportion of T-cells with stable and often complex irradiation-induced chromosome aberrations are able to proliferate and form expanding cell clones in vitro."[120] (c) hyperplastic livers of mice fed butter yellow in 1957,[121] (d) "preneoplastic lesions" of mice treated with dimethylbenzanthracene either in the liver, spleen, thymus[122] or the skin in the form of precancerous papillomas,[123] (e) livers of rats treated with nitrosamine and other chemicals that induce liver cancer "to identify the importance of chromosome versus genome mutations,"[124] (f) hyperplastic mammary tissue of rats treated with dimethylbenzanthracene to induce mammary cancer,[125] (g) "transformed" Syrian hamsters cells treated with carcinogens in vitro,[34] and (h) spontaneously transformed mouse and Chinese hamster cells growing in vitro prior to acquiring tumorigenicity.[126,127]

New experimental tests of the long latencies between induction of aneuploidy and carcinogenesis. Here we have tested in Chinese hamsters and rats the predictions of our theory that carcinogens initiate carcinogenesis by random aneuploidy, but cause clonal cancers only after long latent

periods using nitrosourea as carcinogen. We considered the use of nitrosourea as carcinogen a particular challenge of our theory, because this carcinogen reportedly causes cancer by specific mutations of the *ras* gene, in which case carcinogenesis should be independent of aneuploidy.[128] But our experiments with Chinese hamsters and rats, summarized below, showed that even nitrosourea induced random aneuploidy long before the appearance of cancers with clonal karyotypes.

(1) Studying carcinogenesis in Chinese hamsters, we found that nitrosourea induces random aneuploidy in 80–90% of embryo cells within several weeks, and that such cells generated in syngeneic hamsters cancers with individual clonal karyotypes and phenotypes such as cell morphology and growth rates—but only after latencies of 4 to 7 months.[129]

(2) Studying mammary tumors of rats that appeared 6–12 months after injection with nitrosourea,[130] we found in collaboration with Aldaz that 9 out of 9 tumors had individual karyotypes, individual neoplastic phenotypes, individual cell morphologies and growth rates. We karyotyped these tumors with newly developed, color-coded chromosome-specific rat DNA probes, which greatly facilitate the identification of cancer karyotypes compared to the conventional techniques used in the original study. The karyographs of three of these rat mammary tumors (RMT), termed RMT 58, 61 and 47 are shown in **Figure 9A–C** respectively (others not shown).

In addition we found that some original explants of the rat mammary tumors were hyperplasias with non-clonal aneuploidies, which are the predicted precursor of clonal cancer karyotypes according to our theory (**Fig. 1**). An example of such a non-clonal, preneoplastic aneuploidy, i.e., tumor RMT 47 is shown in **Figure 9D**. A minor fraction of the apparently non-clonal aneuploidy of this tumor must have already included a neoplastic karyotype, because after a few weeks in cell culture foci of morphologically transformed cells appeared with the individual clonal karyotype shown in **Figure 9C**. This result confirmed earlier observations by Aldaz et al. that, "tumors showed coexistence of normal diploid clones with abnormal clones"[130] and by Nandi et al. who found even transplantable, nitrosourea-induced precancerous mammary hyperplasias in rats.[131]

Thus carcinogenesis by nitrosourea in Chinese hamsters and rats confirms the predictions of the speciation theory, namely early induction of random aneuploidy followed by cancers with individual clonal karyotypes and phenotypes after latencies of 4–12 months.

It might be argued, however, that these cancers also depended on common mutations of the *ras* gene, reportedly shared by most nitrosourea-induced tumors and by preneoplastic tissues.[128,130] But, (a) the individualities of the cancers Aldaz et al. and we analyzed and (b) non-correlations between *ras* mutations and cancers induced by nitrosourea call this view in question. Regarding *ras*-non-correlations, Aldaz et al. found that *ras* expression of the rat carcinomas studied by them was either higher or lower than in normal cells.[130] Other researchers have found that nitrosourea induces mammary carcinomas with and without *ras* mutations in rats[132,133] and pancreas cancer with and without *ras* mutations in hamsters.[134] Thus there is neither consistent correlative, nor phenotypic evidence that *ras* mutations are necessary to maintain cancers of rats induced by nitrosourea (see also Individuality, Section 2 above). We conclude, in accord with our theory, that mutation of *ras* genes is not necessary for carcinogenesis.

Preneoplastic aneuploidy to forecast cancer. Ever since 1952 preneoplastic aneuploidy of hyperplastic, dysplastic and carcinogen-exposed cells has been used to forecast and prevent human cancer with remarkable clinical success, first by Papanicolau et al.[135] and then by several others.[28,136-144] Since this happened, although there was no consistent underlying theory, the success of these tests lends unbiased clinical proof to the speciation theory of carcinogenesis.[145,146]

In sum, (a) the early presence of random aneuploidy in hyperplastic or dysplastic or transformed cells exposed to carcinogens and (b) the late appearance of cancers with individual clonal karyotypes long after primary aneuploidization, confirm the speciation cancer theory. Thus the rate-limiting and time-consuming step in carcinogenesis is the evolution of a new autonomous karyotype by random karyotypic variations. This seems also the rate-limiting step in the evolution of normal species.[12,14,31]

So why is the evolution of cancers faster than that of normal species? Even though carcinogenesis is inevitably slow, cancer species evolve much faster than normal phylogenetic species did according to the fossil record—namely in many months to decades compared to millions of years. So is the analogy to speciation justified? We think the difference between the rates of the two kinds of speciation is a matter of complexity. Since the genetic complexity of normal sexual species consisting of many highly differentiated cells is orders higher than that of their corresponding asexual cancer progenies, the probability of forming new sexual species is much lower, and thus slower than forming a new cancer species by the same mechanism. It may be argued, however, that the genetic complexity of cancers is about the same as that of their sexually reproducing precursors. But this argument fails to consider that cancer cells use only a minute fraction, a microbe-equivalent of the eukaryotic genome.[11,26]

Phylogenetic Relationships between Cancers: Another Parallel with Speciation

Early cytogenetic comparisons between individual cancers from the same tissue of origin have revealed recurrent or "non-random" aneusomies.[3,129,147] More recently quantitative comparisons of the aneuploidies of different cancers by the technique of comparative genomic hybridization have confirmed and extended the early results: The individual chromosome copy numbers of a majority of cancers from a given tissue were closely related, although those of consistent minorities were not.[9,108,109,148-152] But, despite the many karyotypic similarities, "no completely specific primary or secondary karyotypic abnormality has been identified."[147]

In view of this most researchers suggested that these common aneusomies encode common genes that are necessary for carcinogenesis.[9,108,109,147-152] It remained

unexplained, however, what these common genes were and why consistent percentages of cancers from the same tissue of origin were not related to the rest.

The speciation cancer theory, however, proposes a coherent alternative explanation. It proposes that the generation of new autonomous cancer species from a given tissue is limited by the tissue-specific availability of active transcriptomes. This view is based on the facts that differentiation-specific transcriptomes and phenotypes are typically fixed for a lifetime, and even persist in cancer cells.[153,154] Nevertheless, more radical karyotypic innovations could occur in a minority of cases at lower rates.

This again is mirrored by limitations in conventional speciation, where new species typically look a lot like their immediate predecessors, e.g., rodents and primates,[13] although more radical innovations must have occurred at lower rates to generate the current diversity of species.

Speciation Theory Explains Paradoxes of Competing Mutation Theories

(1) Are cancers "specific" chromosomal mutations? Assuming a "unitary cause of malignant tumors," Boveri proposed in 1914 that "specific" gains or losses of chromosomes are the causes cancer.[5] But when the technology to test Boveri's theory became available in the 1950s, no "specific" aneusomies but individual karyotypes were found in all cancers tested.[3,4,9,10,43,110,129,155-158] As a result Boveri's theory was abandoned in favor of the now prevailing theory that 3–6 "specific mutations" cause cancer.[16,21-23,25,159,160] In contrast, the speciation theory predicts the individual karyotypes that have been found in all cancers. Accordingly the "unitary cause" of cancer must be speciation.

Boveri further expected in line with his theory that carcinogenesis "could be achieved by the loss of single chromosomes."[5] Accordingly he set out to induce cancer in a rabbit cornea by inducing chromosome non-disjunction. For this endeavor he induced tetraploidy with inhibitors of mitosis, which would then favor losses or gains of chromosomes by non-disjunctions in subsequent mitoses. But no tumors appeared "after some time" in his animals.[5]

The speciation theory predicts, however, that not enough time was allowed and the respective cells were probably not treated with a sufficient dose of carcinogen in Boveri's experiment for the evolution of a new autonomous cancer karyotype. Indeed, within a year after Boveri published his classic paper,[5] Yamagiwa and Yoshikawa demonstrated in 1915 the dependence of tar (a carcinogen)-induced carcinogenesis in rabbits on latent periods of over one year, and on tarring the prospective tissue 2 to 3 days per week for one year.[46]

(2) Aneuploidy thought to inhibit cancer. It is known since Boveri's discovery of the individuality of chromosomes, that aneuploidy typically inhibits and impairs growth and development of non-cancerous cells and organisms.[5,6,42] Recently the adverse effects of aneuploidy on normal growth and development have been reinvestigated and extended to genetically engineered animals.[84,85,161-165]

Because aneuploidy impairs normal growth and development, but is ubiquitous in cancer several researchers have recently concluded that aneuploidy must be incompatible with cancer, unless its adverse effects are buffered by aneuploidy-tolerating mutations.[162,163,165,166] Aneuploidy in cancer was thus called a "paradox,"[164] even "cancer's fatal flaw".[165] Accordingly it was suggested that "identifying genetic alterations that permit cells to tolerate aneuploidy… will provide important insights into tumor evolution."[165] This view thus assumes that the "aneuploidy" of cancers is equivalent to that of non-cancerous cells.

But if the "aneuploidies" of cancers were instead the karyotypes of new autonomous cancer species, the paradox would be solved. As species of their own cancers are no more aneuploid compared to the normal species from which they evolved, than one species is compared to another.

(3) Age bias of cancer due to postnatal mutations? In *Cancer Science and Society* Cairns introduces the age bias of cancer as a little known specialty of cancer research (much like other authors[2,21,23]): "It is not generally realized just how steeply cancer incidence rises with age. To take a typical example, the death rate of cancer from the large intestine increases more than one thousand fold between ages 30 and 80."[16] In view of this we show here in **Figure 10** the exponential increase of the cancer incidence with age of American males in 2001 from the US National Program of Cancer Registries (www.cdc.gov/cancer/npcr/index.htm).

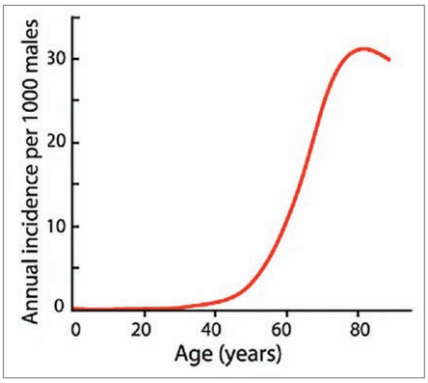


Figure 10. Age-specific incidence of invasive cancers of males in the United States in 2001. Data are from the US National Program of Cancer Registries.

To reconcile the exponential rise of the cancer incidence with age, the mutation theory postulates that an "intentionally vague" number[16] of 3–6 specific mutations is necessary for carcinogenesis.[21,23] Since the cancer incidence in newborns is statistically negligible, the mutation theory assumes that newborns are free of such mutations and that these mutations must be acquired after birth.

This assumption is, however, not supported by the mutation theory. Instead, the mutation theory postulates heritable subsets of mutated oncogenes, such as heritable retinoblastoma-, Wilms tumor-, adenomatous polyposis coli (APC)- and Xeroderma Pigmentosa-genes that are not sufficient to cause cancer.[21-23] Moreover, experimental evidence has shown that mutated oncogenes can be stably integrated into the germ line of numerous strains of mice, termed transgenic oncomice.[15,20,55]

The mutation theory thus predicts that subsets of oncogenes should accumulate in the germ line and that inheritance of complementary subsets of oncogenes should generate breast-, colon-, or lung cancers in newborn humans or animals. But this

has never been described in the literature (see **Figure 10**). The absence of cancers in newborns is thus a paradox in view of the mutation theory.

By contrast, the speciation theory predicts the age bias of cancer exactly: since congenital aneuploidies are typically lethal,[54,55] the speciation theory predicts normal karyotypes at birth and thus no cancers in newborns. So the clock for carcinogenesis is set at zero in newborns. The age bias of cancer is then a predictable consequence of time during which (1) the slow accumulations of spontaneous aneuploidies and (2) the subsequent very rare and thus very slow evolutions of autonomous cancer karyotypes eventually cause cancer at an advanced age (See Speciation theory and **Figure 1** above).

Conclusion: Speciation Emerges as the "Unitary Cause" of Cancer

Nature uses two alternative mechanisms to generate new phenotypes, (1) mutation of specific genes, which preserve the basic karyotype and thus the species; and (2) speciation by remodeling the karyotype as whole, which typically preserves the genes of the progenitor.

Given these potential alternatives to convert a normal cell to a cancer cell, the mutation theory attributes carcinogenesis to the mutation of specific genes, whereas the speciation theory attributes carcingenesis to the generation of new autonomous karyotypes. So how can we decide whether the "unitary cause" of cancer[5,167] is mutation or speciation?

Here we have tried to answer this question by comparing the abilities of the two competing theories to explain the common characteristics of cancers. These comparisons showed that the speciation theory has the potential to explain all five common characteristics of cancers, autonomy, individuality, flexibility, immortality and long latencies from carcinogen to cancer.

By contrast, the potential of the mutation theory to explain the five common characteristics of cancer is still unclear for several reasons: (1) Lacking functional proof for oncogenic mutations, the theory is still uncertain about the identity and the exact numbers of mutations that are necessary to transform a normal cell to a cancer cell.[55,94,145,168-170] Instead, estimates of "3–6" mutations are typically offered.[21,23,160] But recent "sequencing the genetic changes in different cancers has revealed what many feared [sic]"[64]—even more individual mutations.[95,171-173] (2) The theory does not identify mutations that determine the complex morphological and physiological individualities of cancers, e.g., the individual phenotypes of the over 57,000 human cancers with individual karyotypes listed in the NCI-Mitelman database.[4] (3) The mutation theory does also not offer an explanation for the extraordinary coincidence that every cancer would originate not only with a specific mutation but also with an individual karyotype that would be stable and thus clonal for thousands of generations.

In sum we conclude that the unitary cause of cancer is speciation, and that the speciation theory explains, why cancers are autonomous, have individual karyotypes and complex individual (rather than unitary) phenotypes, are flexible yet immortal, and why even the most potent carcinogens take many months to decades to cause cancer.

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