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Dynamic self-organisation of haematopoiesis and (a)symmetric cell division

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ABSTRACT

A model of haematopoiesis that links self-organisation with symmetric and asymmetric cell division is presented in this paper. It is assumed that all cell divisions are completely random events, and that the daughter cells resulting from symmetric and asymmetric stem cell divisions are, in general, phenotypically identical, and still, the haematopoietic system has the flexibility to self-renew, produce mature cells by differentiation, and regenerate undifferentiated and differentiated cells when necessary, due to self-organisation. As far as we know, no previous model implements symmetric and asymmetric division as the result of self-organisation. The model presented in this paper is inspired by experiments on the *Drosophila* germline stem cell, which imply that under normal conditions, the stem cells typically divide asymmetrically, whereas during regeneration, the rate of symmetric division increases. Moreover, the model can reproduce several of the results from experiments on female Safari cats. In particular, the model can explain why significant fluctuation in the phenotypes of haematopoietic cells was observed in some cats, when the haematopoietic system had reached normal population level after regeneration. To our knowledge, no previous model of haematopoiesis in Safari cats has captured this phenomenon.

1. Introduction

Haematopoiesis is the generation of the blood-forming system. At the root of this process is a small group of slowly replicating cells, the *haematopoietic stem cells*, which are undifferentiated cells with the capacity to both *self-renew* and generate all types of blood cells (Baum et al., 1992; Morrison and Weissman, 1994). The haematopoietic stem cells are located within the bone marrow and segregated among different bones throughout the body. Through sequential division, the haematopoietic stem cells differentiate into *progenitor cells*, which in turn differentiate into red blood cells, white blood cells or platelets. Since the number of haematopoietic stem cells is much smaller than the number of more differentiated blood cells, the haematopoietic stem cells must be protected and tightly regulated. *Haematopoietic bone marrow niches*, which are restricted regions in the bone marrow that contain undifferentiated cells and support stem cell behaviour, may be crucial in both aspects (Wineman et al., 1996; Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998; Verfaillie, 1998; Koller et al., 1999; Yin and Li, 2006; Zhang and Li, 2008; Cheng et al., 2014). Since it is not possible to reconstruct a niche experimentally, it is difficult to maintain haematopoietic stem cells in vitro, because signals from the niche affect stem cell survival, self-renewal, and differentiation. This is one of the reasons why relatively little is known about the exact behaviour of haematopoietic stem cells. On the other hand, haematopoietic progenitors have been studied both in vivo and in vitro (Abkowitz et al., 1988, 1990, 1993; Gehling et al., 2000; Akita et al., 2013; Herrmann et al., 2014). A set of experiments was designed by Abkowitz et al., using female *Safari cats*, in order to get an idea of the contribution of haematopoietic stem cells to progenitor cells (Abkowitz et al., 1988, 1990, 1993). The Safari cat is a hybrid of the Geoffroy cat (a South American wildcat) and a domestic cat (which is of Eurasian origin). These two species have evolved independently for twelve million years, and have distinct phenotypes of the X chromosome-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) (Molecular genetics in the domestic cat and its relatives, 1986). Female Safari cats have some cells that contain Geoffroy-type G6PD (G G6PD) and other cells that contain domestic-type G6PD (d G6PD). The G6PD phenotype is retained after replication and differentiation, and is functionally neutral. Therefore, it provides a binary marker of each cell and its offspring. In particular, this means that a progenitor cell that expresses G G6PD is the daughter of a stem cell that expresses G G6PD, and likewise, a progenitor cell that is d G6PD-positive is the daughter of a stem cell that is d G6PD-positive. Abkowitz et al. (1988), Abkowitz et al. (1990), Abkowitz et al. (1993) tracked the contributions of haematopoietic stem cells to the progenitor cells by observing the G6PD phenotype of haematopoietic progenitor cells. In the first trials,

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the percentage of committed progenitor cells expressing d G6PD was observed over a period of almost six years in normal female Safari cats, and Abkowitz et al. found that the percentage remained relatively constant (Abkowitz et al., 1988, 1990). On the contrary, the G6PD phenotype of haematopoietic progenitors varied extensively when six Safari cats were lethally irradiated, in order to kill the cells in their bone marrow, and a small number of bone marrow cells, collected prior to the radiation, were transplanted back (Abkowitz et al., 1990, 1993). Abkowitz et al. observed the percentage of progenitor cells expressing d G6PD while the cells in the bone marrow regenerated, and they found that the pattern of clonal contribution to haematopoiesis in each cat was unique. For instance, some of the cats that both had cells expressing d G6PD and cells expressing D G6PD when the regeneration started, had only cells expressing either d G6PD or D G6PD when the production of bone marrow cells stabilised after regeneration. Thus, one of the phenotypes had got extinct during the regeneration. On the contrary, in other cats, the percentage of cells expressing d G6PD and D G6PD remained on average relatively constant. Moreover, in some cats, significant variation in the percentage extended for years after the number of cells reached normal population levels, whereas in other cats, the percentage remained approximately constant. Several mathematical models (Guttorp et al., 1990; Newton et al., 1995; Abkowitz et al., 1996; Golinelli et al., 2006; Fong et al., 2009) have been proposed to explain the results from the experiments on female Safari cats (Abkowitz et al., 1988, 1990, 1993). These models are discussed in Section 1.4.

1.1. Symmetric and asymmetric stem cell division

Stem cells are, in general, undifferentiated cells that can both self-renew and generate differentiated progeny required by a given tissue (Morrison et al., 1997; Reya et al., 2001). An important aspect is the fate of the two daughter cells when a stem cell divides (Yamashita et al., 2003; Morrison and Kimble, 2006; McKenzie et al., 2006; Dingli et al., 2007). If one daughter cell has stem cell identity and the other daughter cell commits to differentiation and loses the stem cell identity, it is called as an *asymmetric stem cell division* or *asymmetric self-renewal*. Under normal conditions, the number of cells in a given tissue is approximately constant. It is generally believed that the number of stem cells is also approximately constant under normal conditions, and that they differentiate and self-renew at relatively constant rates to replace mature cells and to keep the stem cell number at a certain normal level (Wichmann et al., 1988; Shortman and Naik, 2007). By dividing asymmetrically, the stem cells manage to both self-renew and produce differentiated cells in a single division. The experiments by Abkowitz et al. indicate that haematopoietic cells divide asymmetrically under normal conditions, because the percentage of cells expressing d G6PD remained relatively constant when normal female Safari cats were observed over a period of almost six years (Abkowitz et al., 1988, 1990). However, a disadvantage of asymmetric stem cell division is that it leaves stem cells unable to expand in number. It is, in general, believed that the stem cells can regenerate (Morrison et al., 1997; Reya et al., 2001; Yamashita et al., 2003; Morrison and Kimble, 2006; McKenzie et al., 2006; Dingli et al., 2007). In particular, haematopoietic stem cells can expand rapidly in response to injury to the bone marrow, such as stem cell transplantation (Abkowitz et al., 1990, 1993; McKenzie et al., 2006). Hence, asymmetric self-renewal cannot be the complete story, since it leaves stem cells unable to expand in number.

Symmetric division is defined as generation of daughter cells destined to acquire the same fate. In this paper, symmetric stem cell division is defined as *symmetric self-renewal* if both daughter cells are stem cells and *symmetric commitment* if none of the daughters are stem cells. The number of stem cells increases by one after symmetric self-renewal. Hence, since the haematopoietic bone marrow can regenerate after injury (Abkowitz et al., 1990, 1993; McKenzie et al., 2006), it is likely that the rate of symmetric self-renewal depends on the number of haematopoietic stem cells. On the contrary, the number of stem cells decreases by one after a symmetric commitment. Thus, this type of division can cause the extinction of a stem cell phenotype. The experiments on female Safari cats indicate that both types of symmetric stem cell division occur when the haematopoietic bone marrow niche regenerates after injury (Abkowitz et al., 1990, 1993). Wide fluctuation in the percentage of progenitors with d G6PD was observed for one to four years, before the percentage stabilised and became relatively constant. This indicates that when there are significantly less haematopoietic stem cells in the niche than under normal conditions, the rate of symmetric self-renewal increases such that the number of haematopoietic stem cells also increases. When the number of haematopoietic stem cells reaches its normal population level, the rate of symmetric self-renewal decreases, and proliferation in the haematopoietic niche stabilises. Moreover, some of the cats that both had cells expressing d G6PD and D G6PD when the regeneration started, only had cells expressing either d G6PD or D G6PD when the production of bone marrow cell stabilised after regeneration. This indicates that the haematopoietic stem cells commit symmetrically to differentiation under regeneration, since this type of division can cause the extinction of a phenotype. Clearly, the rate of symmetric self-renewal must, on average, be higher than the rate of symmetric commitment when the haematopoietic niche regenerates, such that the number of stem cells increases. On the other hand, under normal conditions, the number of stem cells remains constant, and hence, the two types of symmetric division must occur at the same rate. Thus, the experiments by Abkowitz et al. indicate that haematopoietic stem cells divide mostly asymmetrically under normal conditions, whereas when the haematopoietic bone marrow niche regenerates after injury, the haematopoietic stem cells start to divide symmetrically (Abkowitz et al., 1988, 1990, 1993; McKenzie et al., 2006). Does this mean that a stem cell “knows” that it must divide asymmetrically under normal conditions and self-renew symmetrically when stem cells need to be replaced? This would also imply that the daughter cells inherit this “knowledge”. As discussed by Loeffler and Roeder (2002), the assumption that each cell “knows” how to behave in different situations is too rigorous and potentially misleading. In the next subsection, it is argued that each stem cell behaves completely random. However, the stem cells divide mostly asymmetrically under normal conditions and symmetrically under regeneration due to dynamic regulation and self-organisation in the haematopoietic bone marrow niche.

Several mathematical models that include symmetric and asymmetric stem cell division have been proposed (Abkowitz et al., 1988, 1990, 1993; Dingli et al., 2007; Wodarz and Komarova, 2005). Wodarz and Komarova (2005) present a model where the haematopoietic stem cells only divide asymmetrically under normal conditions, whereas during regeneration, the stem cells switch to symmetric division. On the contrary, in the model proposed by Abkowitz et al. (1996), the haematopoietic stem cells can only divide symmetrically: Under normal condition, the stem cells undergo symmetric self-renewal and symmetric commitment at the same, constant rate, and under regeneration, the rate of the former type of division increases. Even though all the models presented in Abkowitz et al. (1988), Abkowitz et al. (1990), Abkowitz et al. (1993), Dingli et al. (2007), Wodarz and Komarova (2005) capture important aspects related to stem cell behaviour, it is a drawback that stem cell self-renewal and differentiation do not depend on local growth conditions. The model proposed by Roeder and Loffler in Loeffler and Roeder (2002) and Roeder and Loeffler (2002) considers the dependence of proliferation control on the local growth conditions. However, no implications about symmetric or asymmetric stem cell division are included in this model.

1.2. Haematopoietic bone marrow niche

The haematopoietic bone marrow niche is composed of both localised signalling cells and an extracellular matrix that control the

Fig. 1. The population dynamics in the compartments of undifferentiated cells The bone marrow niche is represented as the compartment of stem cells and the compartment of undifferentiated cells committed to differentiation. In this figure, the former compartment is green, whereas the latter compartment is blue. Both the compartments have M sites. In this figure, $M = 100$, and each site is represented by a square. Each site can either be full, i.e. contain one cell, or be vacant, i.e. contain no cell. In this figure, the full sites are the squares that contain a circle, and the vacant sites are the squares that do not contain a circle. At each elementary event, a random site and a random stem cell are selected. In this figure, the selected stem cell is in the red box and the selected site is in the yellow box, in the two compartments to the left. (a) Asymmetric stem cell division: a site in the compartment of stem cells is selected, and hence, the selected stem cell, in the red box, divides. One of the daughter cells inherits the mother's site. Since the selected site, in the yellow box, is full, the second daughter cell migrates to the compartment of committed undifferentiated cells and is placed in a random vacant site. (b) Symmetric self-renewal: a site in the compartment of stem cells is selected, and hence, the selected stem cell, in the red box, divides. One of the daughter cells inherits the mother's site. Since the selected site, in the yellow box, is vacant, the second daughter cell is placed in this site. (c) Symmetric commitment: a vacant site in the compartment of undifferentiated cells committed to differentiation, in the yellow box, is selected, and hence, the selected stem cell, in the red box, divides. Both daughter cell migrate to the compartment of undifferentiated cells committed to differentiation. One of the daughter cells is placed in the selected site, and the other daughter cell is placed in a random vacant site. (d) Symmetric differentiation a full site in the compartment of undifferentiated cells committed to differentiation, in the yellow box, is selected. The cell in the selected site divides, and both daughter cells leave the compartment of undifferentiated cells committed to differentiation, and begin to differentiate.

fate of the undifferentiated cells. Not all undifferentiated cells can self-renew. Research indicates that stem cells are located in a restricted region of the bone marrow niche (Wineman et al., 1996; Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998; Verfaillie, 1998; Koller et al., 1999; Yin and Li, 2006; Zhang and Li, 2008; Cheng et al., 2014). In this paper, this region is referred to as the compartment of stem cells, whereas the compartment of committed undifferentiated cells refers to the region of the bone marrow niche which contains undifferentiated cells that can not self-renew. However, it is still unknown whether this representation gives an accurate description of the bone marrow niche in vivo: As discussed in the introduction, it is not possible to reconstruct a niche experimentally, and hence, relatively little is known about the exact behaviour of most types of undifferentiated cells, including the haematopoietic stem cells (Wineman et al., 1996; Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998; Verfaillie, 1998; Koller et al., 1999; Yin and Li, 2006; Zhang and Li, 2008; Cheng et al., 2014; Fuchs et al., 2004; Nikolova et al., 2007; Simons and Clevers, 2011). On the other hand, research on *Drosophila germline stem cells* provides a clear-cut example of how the stem cell compartment promotes stem cell maintenance (Yamashita et al., 2003; Morrison and Kimble, 2006; Wong et al., 2005). Germline stem cells are unique because they are solely dedicated to reproduction and transmission of genetic information. Exciting progress has been made in understanding molecular mechanisms underlying interactions between stem cells and stem cell compartments through the use of genetic techniques in *Drosophila* germline stem cells. The knowledge gained from studying the *Drosophila* germline stem cells has provided an intellectual framework for defining the niche and molecular regulatory mechanisms for other adult stem cells. The results on *Drosophila* germline stem cells have previously been used to describe systems and construct models of other types of stem cells, including the haematopoietic stem cells (Lemischka, 1997; Cinquin, 2009; He et al., 2009; Xia et al., 2012; Sada and Tumbar, 2013). The outcome of a *Drosophila* germline stem cell division depends on the spindle orientation relative to the Hub cells in the stem cell compartment, and the results from the unequal distribution of intracellular regulators and extracellular (Hub-derived) signals between daughter cells during mitosis. The result is that when a *Drosophila* germline stem cell divides under normal conditions, one daughter remains in the stem cell compartment and retains stem cell identity, and the other daughter is left outside the stem cell compartment and commits to differentiation. Yamashita et al. (2003), Morrison and Kimble (2006), Wong et al. (2005). This is a classical example of asymmetric stem cell division. Even though *Drosophila* germline stem cells normally divide asymmetrically, they can be induced to self-renew symmetrically to regenerate an additional stem cell after an experimental manipulation in which one stem cell is removed from the stem cell compartment. Thus, the experiments on *Drosophila* germline stem cells indicate that the stem cell compartment can contain up to a certain number of cells, and that the stem cell compartment is full under normal conditions. When a stem cell divides, one of the daughters inherits the mother's place in the stem cell compartment and retains stem cell identity. The fate of the other daughter depends on whether there is a vacant place in the stem cell compartment or not. If there is a vacant place in the stem cell compartment, the latter daughter remains in the stem cell compartment and retains stem cell identity. If the stem cell compartment is full, it is placed outside, and loses its stem cell identity. Hence, research on *Drosophila* germline stem cells implies that the stem cells do not “know” that they must divide asymmetrically or symmetrically, as discussed in Section 1.1. On the contrary, the stem cells divide at random, and the availability of the stem cell compartment, and perhaps other regulatory factors, determines whether the division is symmetric or asymmetric. This indicates that there are, in general, no phenotypic differences between daughter cells resulting from a symmetric and asymmetric stem cell division, which means that a cell must be in the stem cell compartment to function as a stem cell: Once a cell is placed outside, it is no longer a stem cell.

Similar to the *Drosophila* germline stem cell compartment, the stem cell compartment in the haematopoietic bone marrow niche plays an important role in the regulation of haematopoietic stem cell organisation (Wineman et al., 1996; Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998; Verfaillie, 1998; Koller et al., 1999; Yin and Li, 2006; Zhang and Li, 2008; Cheng et al., 2014). Even though there are no in vivo experiments that reveal exactly how proliferation of the haematopoietic stem cells is regulated, it is known that self-renewal depends on local growth conditions, namely, on the direct contact between stem cells and stroma cells (Wineman et al., 1996; Verfaillie, 1998; Koller et al., 1999). The model presented in this paper assumes that the results obtained from the experiments on *Drosophila* germline stem cell compartment and the implications that follow from these results, also hold true for the bone marrow niche. The main idea is illustrated in Fig. 1 and explained more thoroughly in Section 2.

1.3. Haematopoietic cytokines and extracellular regulation

It is commonly accepted that all types of blood cells are generated by haematopoietic stem cells (Baum et al., 1992; Morrison and Weissman, 1994), and that these cells go through a number of divisions, obtaining various stages of differentiation, until the fully mature haematopoietic cells stop dividing. However, as discussed by Dingli et al. (2007) and Furusawa and Kaneko (2009), Furusawa and Kaneko (2012), there is no unambiguous determination of the number of stages connecting haematopoietic stem cells and fully mature cells, let alone how fast cells go through different stages of maturation and exactly how these processes are regulated (Donohue et al., 1958; Cronkite and Fliedner, 1964; Ogawa, 1993). *Haematopoietic cytokines* are extracellular signalling molecules that regulate the generation of haematopoietic cells (Aglietta et al., 1989; Layton et al., 1989; Metcalf, 2008; Fried, 2009). Each of these cytokines can regulate one specific lineage or multiple lineages. Individual haematopoietic cytokines have multiple actions mediated by receptors that can initiate various responses – differentiation, maturation, functional activation, survival and proliferation (Metcalf, 2008). Furthermore, for some cell types, such as haematopoietic stem cells and megakaryocyte progenitors, the simultaneous action of multiple cytokines are required for proliferative responses. One of the reasons why it is very challenging to establish the precise source of cytokines and predict their ultimate fate, is that the haematopoietic cytokines have many tissue sources, for instance lung, kidney, muscle, liver and membrane-displayed factors on local stromal cells (Aglietta et al., 1989; Metcalf, 2008). Several models have been proposed to investigate different feedback mechanisms (Roeder and Loeffler, 2002; Fuchs et al., 2004; Nikolova et al., 2007; Simons and Clevers, 2011; Wong et al., 2005; Cinquin, 2009; He et al., 2009; Xia et al., 2012; Sada and Tumbar, 2013; Furusawa and Kaneko, 2009, 2012; Donohue et al., 1958; Cronkite and Fliedner, 1964; Ogawa, 1993; Aglietta et al., 1989; Layton et al., 1989; Metcalf, 2008; Fried, 2009; Potten and Loeffler, 1990; Wodarz, 2008; Lander et al., 2009; Høyem et al., 2015; Larsen, 2016; Mangel et al., 2016; Rompolas et al., 2016). Results from theoretical work modelling the haematopoietic system (Wodarz, 2008) and crypt cells (Potten and Loeffler, 1990) imply that changes in stem cell number and their cyclic activity are associated with changes in the demand of the mature cell stages. Lander et al. (2009) explore how secreted negative feedback factors may be used to control the output of multistage cell lineages, as exemplified by the actions of GDF11 and activin in a self-renewing neural tissue, the mammalian olfactory epithelium. The results by Lander et al. indicate that two feedback loops are in general better than one. That is, good control (robustness, stability, low progenitor load, and fast regeneration from a variety of conditions) is found over an increasing fraction of the parameter space when feedback loops are

added. These results might also apply to the haematopoietic system. Similar to the models presented in Dingli et al. (2007) and Høyem et al. (2015), we model differentiation as a multi-step process where cell replication and differentiation are coupled with cells moving through successive stages – compartments – of maturation in a series of steps from the haematopoietic stem cells all the way down to the fully differentiated haematopoietic cells.

1.4. Models for haematopoiesis in female Safari cats

The experiments on female Safari cats (Abkowitz et al., 1988, 1990, 1993) have inspired several mathematical models (Guttorp et al., 1990; Newton et al., 1995; Abkowitz et al., 1996; Golinelli et al., 2006; Fong et al., 2009). In 1990, Guttorp et al. proposed a state-space Markov model for haematopoiesis in Safari cats (Guttorp et al., 1990). It is assumed that in each cat there is a large pool of haematopoietic stem cells, and that a proportion p of these stem cells express d G6PD. The proportion p may vary between cats, but remains constant within each cat. The authors suppose that most haematopoietic stem cells are not involved in the production of mature blood cells, but are members of a primary pool of slowly self-replicating cells. A relatively small number of haematopoietic stem cells produce mature blood cells through asymmetric division and differentiation, and are referred to as *active stem cells*. It is assumed that the number of active stem cells is constant, N, and that the active stem cells do not have the ability to self-renew symmetrically. Consequently, when an active stem cell dies, a member of the primary pool of slowly self-replicating stem cells must become an active stem cell, in order to keep the number of active stem cells constant. Since N is much smaller than the total number of haematopoietic stem cells, the number of active stem cells that express d G6PD can be between 0 and N, even though the proportion of haematopoietic stem cell expressing d G6PD is constant. Indeed, the probability that i of the active stem cells express d G6PD is given by the probability mass function of the binomial distribution:

$$P_i = \binom{N}{i} p^i (1-p)^{N-i}.$$

Moreover, suppose that there are i active stem cells expressing d G6PD. When an active stem cell dies, the number of active stem cells expressing d G6PD can either increase by one, decrease by one or remain constant. The conditional probabilities for these three events are

$$P(i+1, i) = \left(1 - \frac{i}{N}\right)p,\ P(i-1, i) = \frac{i}{N}(1-p),\ P(i, i) = 1 - \left(1 - \frac{i}{N}\right)p - \frac{i}{N}(1-p),$$

respectively. Although the model proposed by Guttorp et al. can explain some of the results from the experiments on female Safari cats (Abkowitz et al., 1988, 1990, 1993), for instance that the proportion of cells expressing d G6PD remained relatively constant under normal conditions, the model cannot explain the results that indicate that the proportion of cells expressing d G6PD can change during regeneration. The reason for this is that Guttorp et. al. assume that stem cell self-replication is a deterministic process such that the proportion of d G6PD remains constant. The models presented in Abkowitz et al. (1996), Golinelli et al. (2006), Fong et al. (2009) and the model presented in this paper assume that self-replication is a stochastic process.

In 1995, Newton et al. (1995) used a simple stochastic model, similar to the model presented by Guttorp et al. (1990), to quantify the relationship between observed proportions of progenitors expressing d G6PD and unobserved haematopoietic stem cell populations.

Abkowitz et al. stimulated haematopoiesis by assuming that all stem cell decisions, that is, replication, apoptosis and initiation of differentiation, are determined by chance (Abkowitz et al., 1996). The paper was published in 1996. They show that stochastic stem cell behaviour can result in a wide spectrum of discrete outcomes observed in vivo (Abkowitz et al., 1988, 1990, 1993), and that clonal dominance can occur by chance. More precisely, each haematopoietic stem cell is randomly selected for replication, apoptosis (cell death) and differentiation at constant rates λ, α and μ, respectively. Furthermore, the probability that a stem cell is selected for replication is much higher than the probability that a stem cell is selected for apoptosis or differentiation, i.e.

$$\lambda > \alpha + \mu.$$

This means that the number of stem cells increases when the haematopoietic system regenerates after injury. When the number of stem cells reaches a certain limit, the stem cells ignore the signals that tell them to reproduce. This means that each stem cell must keep track of the total number of stem cells. In our paper, an alternative strategy is investigated, where the rates of replication and differentiation depend on the number of stem cells and undifferentiated committed cells. That is, when cells need to be replaced, the rate of symmetric stem cell division increases, whereas under normal conditions, the stem cells divide mostly asymmetrically.

In 2006, Golinelli et al. published a paper (Golinelli et al., 2006) that describe a stochastic process used to model early haematopoiesis in continuous time. The haematopoietic stem cells follow a simple linear birth-death process where each stem cell can either self-renew symmetrically or differentiate into a progenitor cell at constant rates λ and ν, respectively. Similar to the model presented in Abkowitz et al. (1988), the rates satisfy

$$\lambda > \nu,$$

so the stem cells can regenerate after injury. Moreover, if the stem cell compartment is full and a stem cell self-renews symmetrically, then a random stem cell dies.

Fong et al. (2009) performed Bayesian statistical inference on extensions of the model proposed by Golinelli et al. (2006), in order to determine if haematopoietic stem cell decisions are linked to cell divisions or occur independently. This paper was published in 2009. Their results show that haematopoietic stem cells must divide symmetrically in order to maintain haematopoiesis. They also demonstrate that a model that adds asymmetric division events provides a better fit to the competitive transplantation data. The conclusions drawn by Fong et al. correspond well with the results of this paper. However, unlike the model investigated by Fong et al., stemness is not treated as an explicit cellular property in this paper, but as the result of a dynamic process of regulation and self-organisation similar to the models presented by Loeffler and Roeder (2002), Roeder and Loeffler (2002).

2. Model of haematopoiesis with self-organisation

In this section, we present a compartmental model of the haematopoietic system with self-organisation. The model can reproduce several of the results from the experiments with female Safari cats (Abkowitz et al., 1988, 1990, 1993). At the root of the model are the stem cells, located in the SC-compartment. It is assumed that the committed cells go through K stages of differentiation. A committed cell at stage i is denoted DC^i and is located in the DC^i-compartment for $0 \leqslant i \leqslant K$. The dynamics of the compartments of undifferentiated cells are described in Section 2.1, whereas in Section 2.4, the differentiated cells are also included.

The results from the experiments on *Drosophila* germline stem cells (Yamashita et al., 2003; Morrison and Kimble, 2006; Wong et al., 2005) and female Safari cats (Abkowitz et al., 1988, 1990, 1993) which can be reproduced by our model, are discussed in Section 3, and the biological processes that the model are based on are examined in Section 4, whereas in this section we mainly focus on describing the model.

2.1. Compartments of undifferentiated cells

As discussed in Section 1, undifferentiated haematopoietic cells are, in general, located in the bone marrow. The model presented in this paper subdivides these cells into two groups: the undifferentiated cells located in the SC-compartment and the undifferentiated cells located in the DC^0-compartment. It is assumed that these two groups of cells are phenotypically identical. However, the cells located in the former compartment are stem cells because they self-renew and produce differentiated cells, whereas the cells in the DC^0-compartment are committed to differentiation and cannot self-renew, and hence, they are not stem cells. The compartments of undifferentiated cells regulate symmetric and asymmetric stem cell division. The main idea is that under steady-state the stem cells divide mostly asymmetrically, whereas when cells need to be replaced due to tissue damage, the stem cells start to divide symmetrically. Both compartments contain M sites. Each of the $2M$ sites can either contain exactly one cell or no cell, denoted *full* sites and *vacant* sites, respectively. Thus, $2M$ represents the carrying capacity of the bone marrow niche. Under steady-state there are approximately M cells in both compartments, and the stem cells typically divide asymmetrically – one daughter cell inherits the mother's site and the other daughter is placed in a vacant site in the DC^0-compartment. The DC^0s migrate to the DC^1-compartment when they divide and obtain the first stage of differentiation.

It is known that the number of undifferentiated cells can increase markedly when they are regenerated after injury to the bone marrow (Abkowitz et al., 1990, 1993; Morrison et al., 1997; Reya et al., 2001; Yamashita et al., 2003; Morrison and Kimble, 2006; McKenzie et al., 2006; Dingli et al., 2007). This type of injury is modelled by decreasing the number of cells in the SC-compartment and DC^0-compartment well below M. The stem cells start to divide symmetrically after injury to the compartments of undifferentiated cells. It is symmetric self-renewal if one daughter cell inherits the mother's site while the other daughter is placed in a vacant site in the SC-compartment, and symmetric commitment if both daughter cells are placed in vacant sites in the DC^0-compartment.

2.2. Markov process

The population dynamics in the compartments of undifferentiated cells, described in Section 2.1, are implemented by the following Markov process: At each elementary event, a random site and a random stem cell are selected. If a site in the SC-compartment is selected, the selected stem cell divides. One of the daughter cells inherits the mother's site. If the selected site is full, then the second daughter cell migrates to the DC^0-compartment, and is placed in a random vacant site, i.e. the division is asymmetric (see Fig. 1 (a)). If the selected site is vacant, the second daughter is placed in this site, resulting in symmetric self-renewal (see Fig. 1 (b)). On the other hand, suppose that a random site in the DC^0-compartment is selected. If the selected site is vacant, the selected stem cell commits symmetrically to differentiation, and both daughter cells are placed in random vacant sites in the DC^0-compartment (see Fig. 1 (c)). If the selected site is full, this cell leaves the DC^0-compartment (see Fig. 1 (d)). For boundary conditions, it is assumed that when all the sites in the SC-compartment are vacant, a cell from another SC-compartment migrates to the empty SC-compartment, so that symmetric division is possible. Moreover, it is assumed that when all the sites in the DC^0-compartment are full, then any cell that enters the DC^0-compartment undergoes apoptosis, i.e. programmed cell death. Thus, given that there are I stem cells and J DC^0s, we obtain the following transition probabilities:

$$P_{I,J}(I, J-1) = \frac{1}{2}\frac{J}{M}, \tag{1}$$

$$P_{I,J}(I, J+1) = \frac{1}{2}\frac{I}{M}, \tag{2}$$

$$P_{I,J}(I+1, J) = \frac{1}{2}\left(1 - \frac{I}{M}\right), \tag{3}$$

$$P_{I,J}(I-1, J+2) = \frac{1}{2}\left(1 - \frac{J}{M}\right). \tag{4}$$

That is, the conditional probability that a cell leaves the DC^0-compartment is given in (1), a stem cell divides asymmetrically is given in (2), a stem cell self-renews symmetrically is given in (3), and a stem cell commits symmetrically to differentiation in given in (4). Let $X(\Gamma)$ and $Y(\Gamma)$ be the expected number of cells in the SC-compartment and DC^0-compartment, respectively, at elementary event Γ. It follows from Eqs. (1)–(4) that

$$\begin{aligned} X(\Gamma+1) &= X(\Gamma) + \frac{1}{2M}((M - X(\Gamma)) - (M - Y(\Gamma))) \\ &= X(\Gamma) + \frac{1}{2M}(Y(\Gamma) - X(\Gamma)), \end{aligned} \tag{5}$$

$$\begin{aligned} Y(\Gamma+1) &= Y(\Gamma) + \frac{1}{2M}(X(\Gamma) + 2(M - Y(\Gamma)) - Y(\Gamma)) \\ &= X(\Gamma) + \frac{1}{2M}(X(\Gamma) + 2M - 3Y(\Gamma)), \end{aligned} \tag{6}$$

for $0 < X(\Gamma)$ and $Y(\Gamma) \leqslant M - 2$. When the SC-compartment is empty, the number of stem cells increases by two after symmetric self-renewal. Moreover, when there is only one vacant site in the DC^0-compartment, one of the daughters undergoes apoptosis when a stem cell commits symmetrically to differentiation, whereas if there are no vacant sites, both daughters undergo apoptosis. For simplicity, these boundary conditions are neglected in the following approximation of the mean functions: First, the system of linear difference equations given in (5)–(6) has exactly one equilibrium solution, namely

$$(X^*, Y^*) = (M, M),$$

which means that all sites in both compartments are full. The corresponding transition matrix is

$$\frac{1}{2M}\begin{bmatrix} -1 & 1 \\ 1 & -3 \end{bmatrix}$$

and the eigenvalues are

$$\lambda_1 = -2 + \sqrt{2},\ \lambda_2 = -2 - \sqrt{2}.$$

An eigenvector corresponding to λ_i is

$$\mathbf{v}_i = \begin{bmatrix} 1 \\ 1 - \lambda_i \end{bmatrix},$$

for $i \in \{1, 2\}$. One time step is defined as 2M elementary events. It follows that the expected number of stem cells and DC^0s at time step t are approximately

$$X(t) = M + c_1 \exp\left(\frac{\lambda_1}{2M}t\right) + c_2 \exp\left(\frac{\lambda_2}{2M}t\right), \tag{7}$$

$$Y(t) = M + c_1(1+\lambda_1)\exp\left(\frac{\lambda_1}{2M}t\right) + c_2(1+\lambda_2)\exp\left(\frac{\lambda_2}{2M}t\right), \tag{8}$$

respectively, where

$$\begin{bmatrix} c_1 \\ c_2 \end{bmatrix} = \frac{1}{(\lambda_2 - \lambda_1)M}\begin{bmatrix} 1+\lambda_2 & -1 \\ -(1+\lambda_1) & 1 \end{bmatrix}\left(\begin{bmatrix} M \\ M \end{bmatrix} - \begin{bmatrix} X(0) \\ Y(0) \end{bmatrix}\right)$$

and $X(0)$ and $Y(0)$ are the initial number of stem cells and DC^0s, respectively. It follows from (7) and (8) that it is expected that the system converges towards the steady state where both compartments are (approximately) full. However, given that the process runs long enough, stochastic realisation will lead to extinction of one of the phenotypes with probability one. As illustrated in the next subsection, for small populations, one phenotype gets extinct after a relative short time period, whereas for sufficiently large populations, both compart-

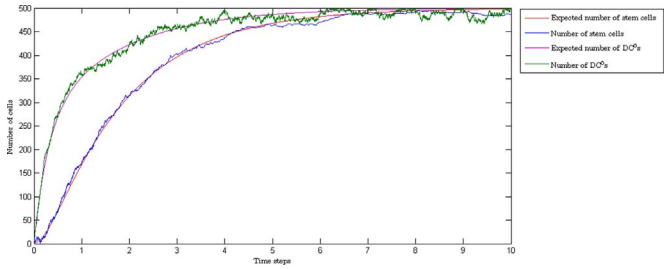

Fig. 2. Regeneration of the undifferentiated cells The compartments of undifferentiated cells are regenerated, starting with a single stem cell, with compartment size $M = 500$. The red and the purple smooth curves show the expected numbers of stem cells and DC^0s, respectively, and the jagged blue and green curves are simulations of stem cells and DC^0s, respectively.

ments of undifferentiated cells remain approximately full under normal conditions for any time interval corresponding to the lifetime of a mammal.

2.3. Numerical simulations

Fig. 2 shows the regeneration of the population of undifferentiated cells, starting with a single stem cell. The red and purple smooth curves illustrate the approximation of the expected number of stem cells and DC^0s, given in Eqs. (7) and (8), respectively, whereas the jagged curves are simulations of the population dynamics described in Section 2.2. The figure illustrates that when the compartment size is sufficiently large, the simulations fit the expected numbers of undifferentiated cells well: The number of cells in both compartments grow steadily until the compartments are approximately full. The DC^0s grow significantly faster than the stem cells. Under stable, normal conditions, the number of cells in both compartments remain close to M.

The approximations of the expected number of undifferentiated cells, given in Eqs. (7) and (8), indicate that under steady-state, both compartments remain approximately full. In general, the simulations become more similar to the expected functions as the number of sites increases. In Fig. 3, different compartment sizes are tested. Figs. 3 (a)–(b) and (c)–(d), with compartment size M=10 and M=20, respectively, illustrate that the model works poorly with relatively small compartment sizes. In Figs. 3 (a)–(b), the number of stem cells is zero 24 times during 10^4 time steps. When the compartment size is M=20, as illustrated in Figs. 3 (c)–(d), extinction of stem cells has not been observed during simulations when both compartments were initially full. However, the number of cells in both compartments vary too much to be a realistic representation of the bone marrow niche. Figs. 3 (e)–(f) show that when $M = 50$, the number of cells in each compartment remains relatively close to 50. Moreover, several results from experiments by Abkowitz et al. (1988, 1990, 1993) can be reproduced by the model when $M = 50$. This corresponds well with the model by Abkowitz et al. (1996) – 50 is the minimum size of the stem cell compartment in their model. However, in the remaining examples, the compartment sizes are larger than M=50. Figs. 3 (g)–(h), (i)–(j) and (k)–(l), where the compartment sizes are 100, 500 and 1000, respectively, illustrate that the number of undifferentiated cells varies less as the compartment size increases.

In all remaining examples, we use compartment size $M = 500$, which makes it easy to compare the results obtained in the different examples. Moreover, we want to compare our results with the results obtained from the previous models (Guttorp et al., 1990; Newton et al., 1995; Abkowitz et al., 1996; Golinelli et al., 2006; Fong et al., 2009) based on the experiments on Safari cats, and in particular, with the results obtained by Abkowitz et al. (1996). In the model by Abkowitz et al., all undifferentiated cells are stem cells, and in their numerical simulations, the stem cell compartment can contain up to 750 undifferentiated cells, whereas there can be up to 500 stem cells and 1000 undifferentiated cells in the continuing examples of this paper.

In the remaining examples, the value $\mu - s$ will be referred to as the lower limit for *normal population level*, where μ is the estimated mean number of cells in a given compartment and s is the estimated standard deviation. When the mean numbers of cells in all compartments are approximately the same as the estimated mean, and, at the same time, the standard deviations are approximately equal to the estimated standard deviations, the system is said be in *stable, normal state*.

When the cells are subdivided into two neutral phenotypes, such as cells expressing G G6PD and d G6PD for the Safari cats, the percentage of cells that express one type is expected to remain constant. Indeed, Figs. 4 (a)–(b) show a numerical example where the percentage of d G6PD-positive cells varies relatively little during stable, normal conditions. Fig. 4 (b) displays the percentage of self-renewal divisions that are symmetric and illustrates that under stable, normal conditions, the stem cells generally divide asymmetrically. Indeed, on average, 2.34% of the self-renewals are symmetric. On the other hand, Figs. 4 (c)–(d) show a numerical example where the percentage of cells expressing d G6PD varies extensively during regeneration. Initially, 5% of the sites in both compartments are full. After 4 time steps, the DC^0s reach the normal population level, whereas the stem cells reach the normal population level at time step $t = 5.7$. The percentage of self-renewals that are symmetric during regeneration is shown in Fig. 4 (d), and illustrates that when a large proportion of the sites are vacant, the stem cells divide symmetrically at a high rate, and as the number of cells in both compartment gradually increase, the rate of symmetric division steadily decreases. Fig. 5 shows twelve numerical examples of regeneration, where the initial conditions are the same as in the example illustrated in Figs. 4 (c)–(d). The curves in each of these examples are unique, which corresponds well with the experimental and theoretical work by Abkowitz et al. (1988), Abkowitz et al. (1990), Abkowitz et al. (1993), Abkowitz et al. (1996). Moreover, the time the cell population uses to reach normal population levels also varies – in Fig. 5 (d), the stem cells reach normal population level at time step $t = 7.5$, whereas in Fig. 5 (f), normal population level is reached after 5 time steps.

As illustrated in Figs. 5 and 6, the system has not, in general, gained stable, normal state when it reaches normal population level after regeneration – the DC^0s reach normal level before the stem cells, and this causes an intermediate time interval with relatively high variance in the cell number. For instance, consider Figs. 6 (a)–(b): In the time interval 6.5–50, where both compartments have reached normal level, the mean percentage of cells expressing the d G6PD phenotype is 62% and the standard deviation is 1.9%. On the contrary, the standard deviation is 0.8% in the numerical example plotted in Figs. 6 (c)–(d), where the system is in stable, normal state with mean percentage of cells expressing the d G6PD phenotype equal to 62%. Since symmetric stem cell division causes variation in the cell number, it is reasonable to expect that the stem cells self-renew symmetrically at a higher rate in the intermediate time interval with high variance than under stable, normal conditions, and, indeed, it follows from Fig. 6 (b) that at time step $t = 6$ and $t = 7$, the percentage of symmetric self-renewal is above 9% and 5%, respectively, which is rarely observed under stable, normal state. Moreover, the mean percentage of symmetric self-renewal is 2.7% during the time interval 6.5–50 in Fig. 6 (b), whereas the estimated mean during stable, normal state is 2.3%. The intermediate time interval with high variance has more apparent effect on the population dynamics when compartments of differentiated cells are included, and is investigated more thoroughly in Section 2.6.

In our simulations of regeneration, illustrated in Figs. 5 and 6, the average time the population of cells uses to reach normal population level is 6.2 time steps. There are no in vivo data for the undifferentiated cells in the bone marrow niche. However, experiments on Safari cats showed that bone marrow BFU-E and CFU-GM, as well as progenitor cell-cycle kinetics, returned to baseline values a hundred weeks after transplantation, on average (Abkowitz et al., 1988, 1990, 1993). Moreover, the pattern of clonal contribution to haematopoiesis in each

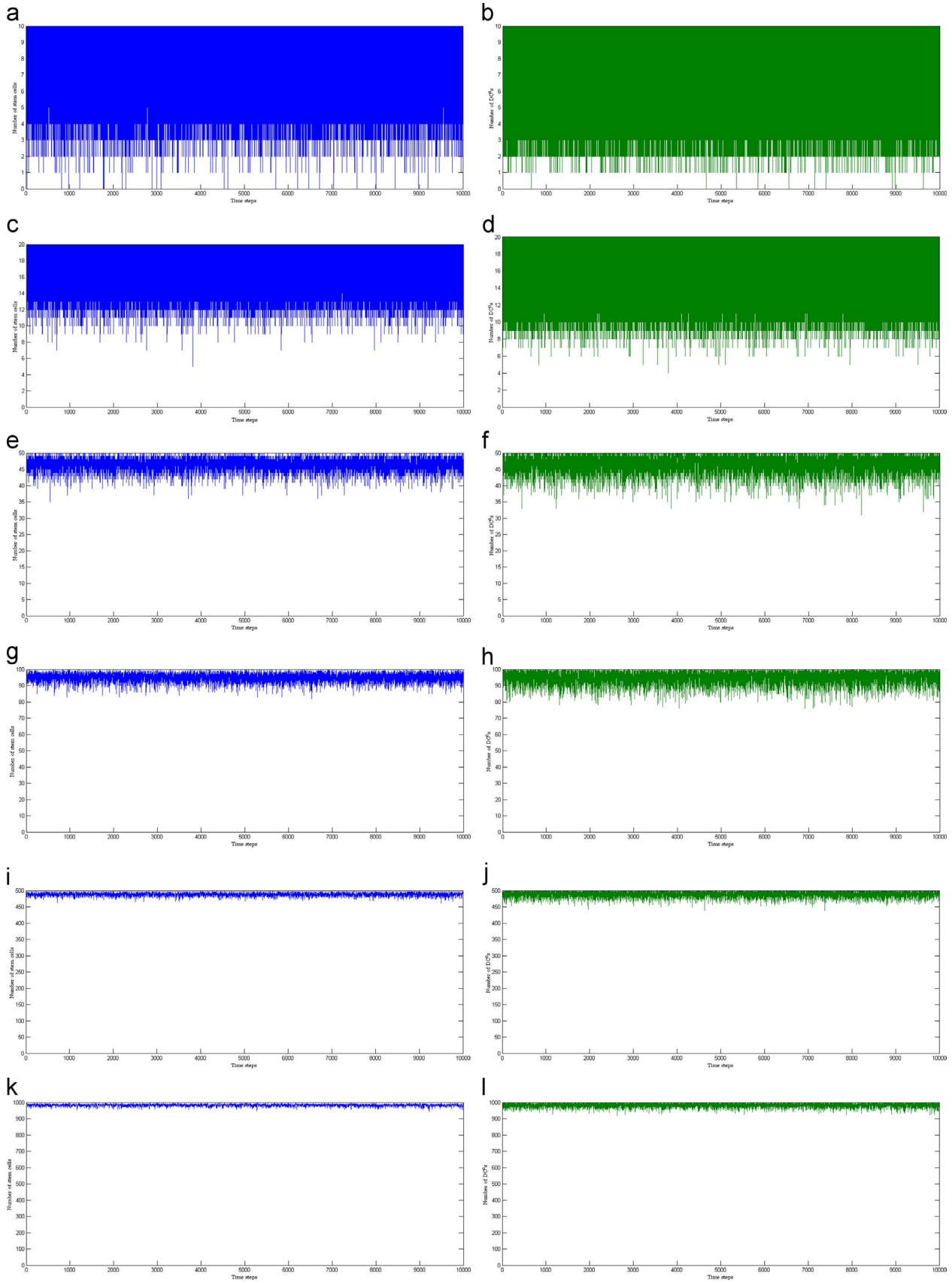

Fig. 3. Different compartment sizes Initially, all sites contain one cell. Different compartment sizes are tested, and it is verified that the simulations become increasingly more similar to the expected functions as the number of sites increases. (a) and (b) display the stem cells and DC^0, respectively, for $M = 10$. The system is highly unstable. (c) and (d) display the stem cells and DC^0, respectively, for $M = 20$. The system is unstable. (e) and (f) display the stem cells and DC^0, respectively, for $M = 50$. The system is quite stable. (g) and (h) display the stem cells and DC^0, respectively, for $M = 100$. The system is stable. (i) and (j) display the stem cells and DC^0, respectively, for $M = 500$. The system is stable. (k) and (l) display the stem cells and DC^0, respectively, for $M = 1000$. The system is stable.

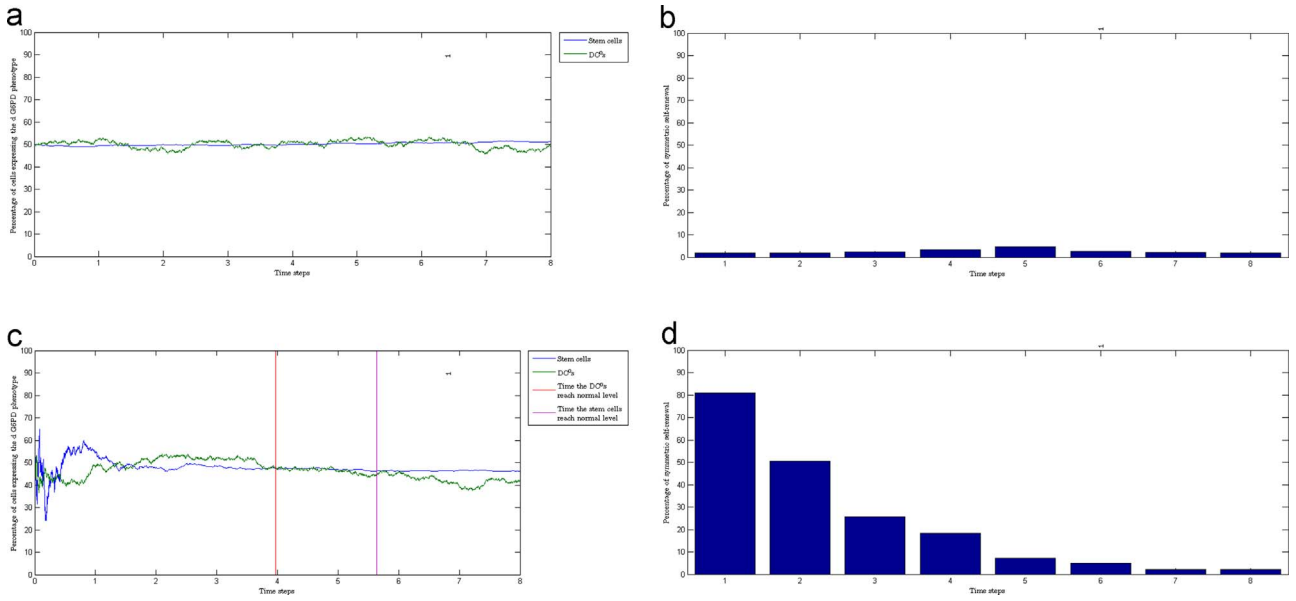

Fig. 4. Stable, normal conditions versus regeneration This figure illustrates that when the system is in stable, normal conditions, the percentage of cells expressing d G6PD is approximately constant and the stem cells typically divide asymmetrically, whereas when the system regenerates, the percentage of cells expressing d G6PD varies extensively, and the rate of symmetric division is relatively high. In both simulations, the compartment size is $M = 500$. (a) displays the percentage of stem cells and DC^0s expressing d G6PD when the system is in stable, normal state. (b) displays the percentage of self-renewals that is symmetric when the system is in stable, normal state. (c) displays the percentage of stem cells and DC^0s expressing d G6PD when the system regenerates and, initially, 20 (d) displays the percentage of self-renewals that are symmetric when the system regenerates.

cat was unique, and, in some cats, significant variation in the percentage of cells expressing d G6PD and D G6PD was observed for years after the number of cells reached normal population levels, whereas in other cats, the percentage remained approximately constant. The uniqueness and variation observed in vivo are, to some extension, captured by our model: In our simulations, the minimum number of time steps until normal population is reached, is five, and the maximum number of time steps is fifty percent greater, and, as discussed above, the system has not, in general, gained normal state when it reaches normal population level after regeneration – the system enters an intermediate time interval with high variance. On the contrary, for the model of haematopoiesis in Safari cats by Abkowitz et al. (1996), the time the system uses to regenerate varies little – less than five percent, and once the system reaches normal population size, it behaves exactly as under normal conditions.

2.4. Multi-compartmental model

In this subsection, the differentiated cells are also included in the model. That is, it is assumed that the committed cells go through K stages of differentiation, and that a cell at stage i in the differentiation process, denoted DC^i, is located in the DC^i-compartment for $0 \leqslant i \leqslant K$. All the cells in these compartments are *committed* to differentiation. However, the DC^0s are still undifferentiated whereas the DC^js, for $0 < j$, are actual differentiated cells. Moreover, when a cell in the DC^j-compartment divides, for $0 \leqslant j < K$, both daughter cells migrate to the DC^{j+1}-compartment. The cells in the DC^K-compartment are fully differentiated and stop dividing. The DC^i-compartment contains 2^iM sites. The sites in the compartments of differentiated cells are not just concrete, physical locations, but more abstract, representing the sum of signals in the environment of the cells. Similar to the compartments of undifferentiated cells, the sites in the compartments of differentiated cells are called vacant when they contain no cell, and unlike the compartments of undifferentiated cells, the full sites in a compartment of differentiated cells can contain more than one cell if all the other sites in this compartment are full. Under stable, normal conditions, there are approximately 2^iM cells in the DC^i-compartment for $0 \leqslant i \leqslant K$, and the cells commit symmetrically to differentiation at the same, constant rate. On the other hand, when the number of cells in the DC^{i+1}-compartment is significantly less than under normal conditions, the rate of symmetric commitment in the DC^i-compartment increases.

2.5. Extended markov process

The population dynamics of the multi-compartmental model are implemented by the following Markov process: At each elementary event, a random site is selected. Each site in the $K + 2$ compartments has the same probability of being selected. If a site in a compartment of undifferentiated cells is selected, the elementary event is as described in Section 2.2, whereas if a site in the DC^i-compartment is selected, for $1 \leqslant i \leqslant K$, and the site is full, then, for $i < K$, a DC^i divides symmetrically and both daughter cells migrate to the DC^{i+1}-compartment, i.e. symmetric commitment, while for $i = K$, a cell in this compartment dies. On the other hand, if the selected site is vacant, then a random cell from the DC^{i-1}-compartment commits symmetrically to differentiation. For boundary conditions, it is assumed that if a vacant site in the DC^{i+1}-compartment is selected and the DC^i-compartment is empty, then the process finds the highest integer j, where $0 \leqslant j < i$, such that the DC^j-compartment is not empty, and a random DC^j commits symmetrically to differentiation. If all DC^j-compartments are empty for $j < i$, then a random stem cell commits symmetrically to differentiation.

Given that there are I and J^i full sites in the SC-compartment and DC^i-compartment, respectively, for $0 \leqslant i \leqslant K$ and $0 < J^i \leqslant 2^iM$ for $0 < i$, we obtain the following transition probabilities:

$$P_{J^K}(J^K - 1) = \frac{J^K}{2^{K+1}M}, \tag{9}$$

$$P_{J^i,J^{i+1}}(J^i - 1, J^{i+1} + 2) = \frac{2^{i+1}M + J^i - J^{i+1}}{2^{K+1}M}, \tag{10}$$

$$P_{I,J^0}(I, J^0 + 1) = \frac{I}{2^{K+1}M}, \tag{11}$$

$$P_{I,J^0}(I + 1, J^0) = \frac{M - I}{2^{K+1}M}, \tag{12}$$

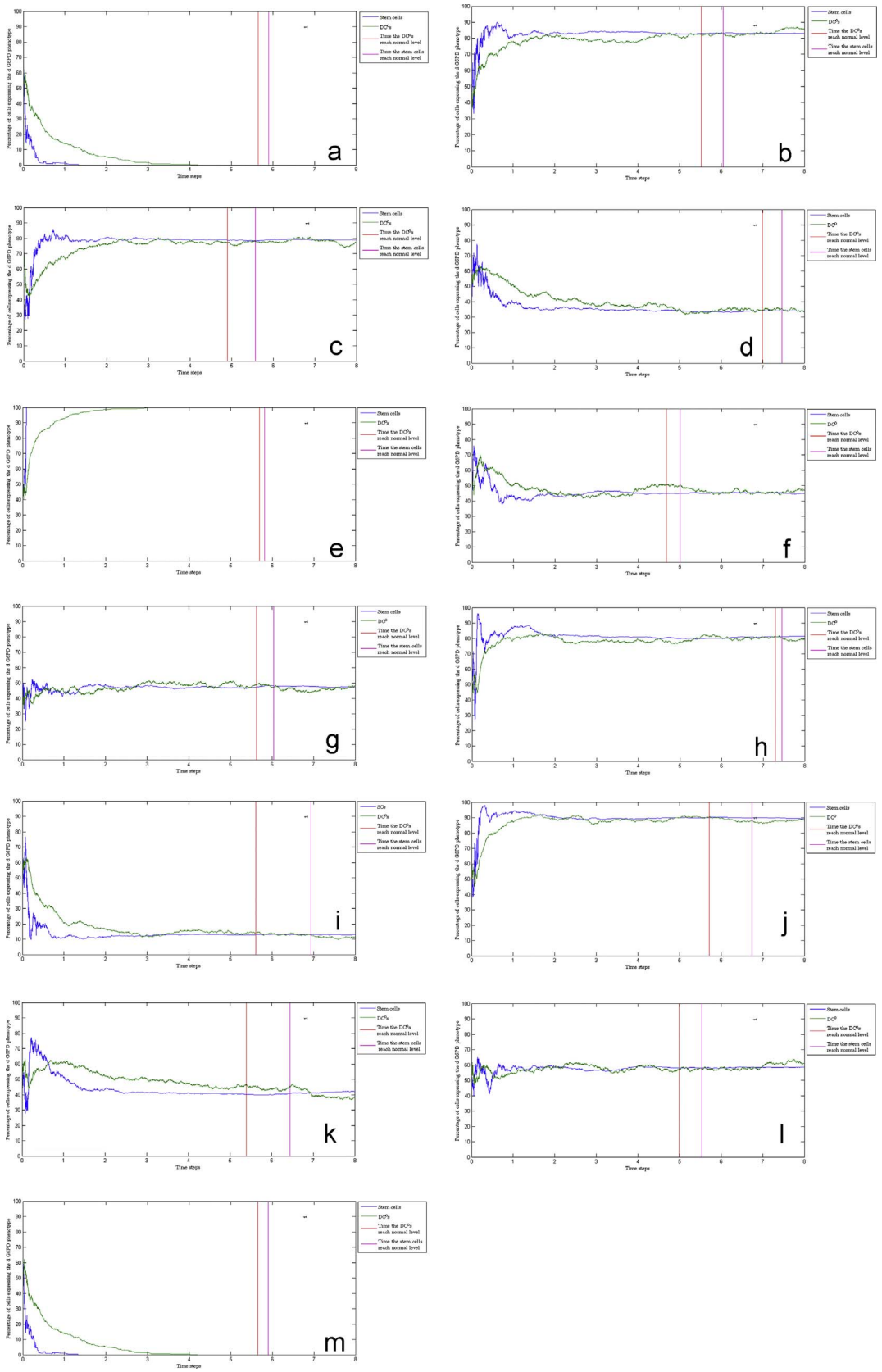

Fig. 5. Unique traits of regeneration This figure displays twelve simulations of regeneration where, initially, 20% of the sites are full, and illustrates that every regeneration is unique. In all simulations, the compartment size is $M = 500$.

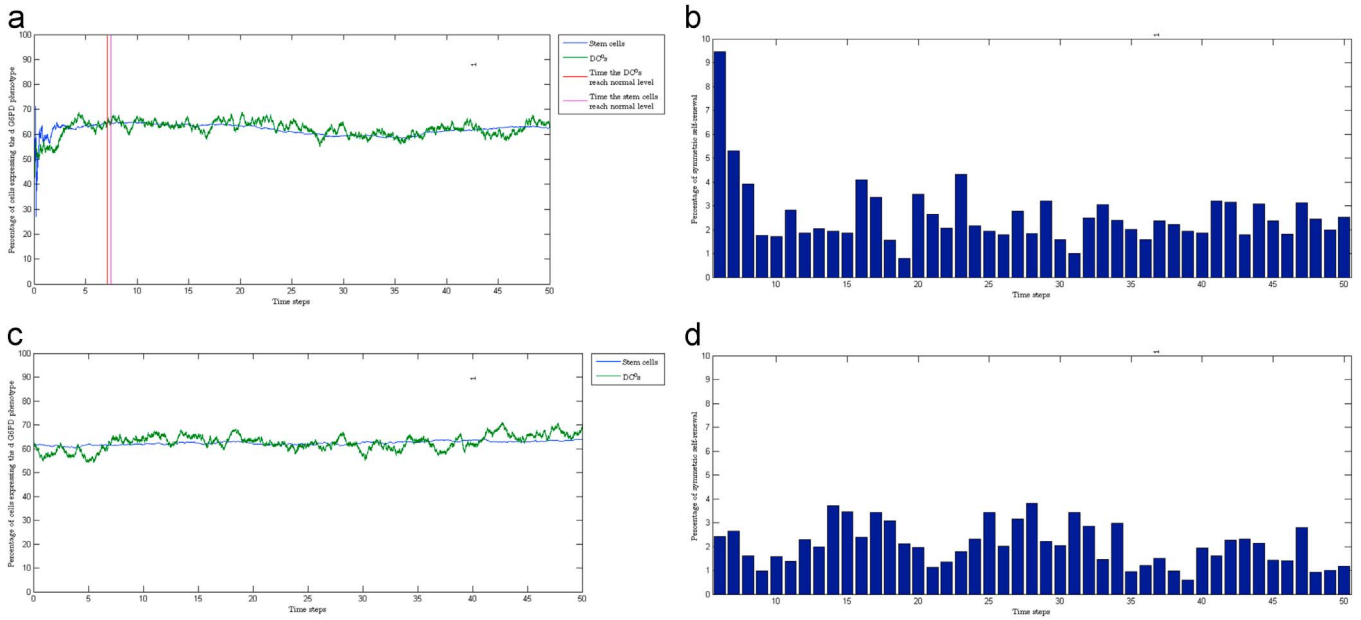

Fig. 6. Stable, normal conditions versus intermediate time interval with high variance This figure illustrates that when the system reaches normal population levels, the stem cells continue to divide symmetrically at a slightly higher rate than under stable, normal conditions, and consequently, the percentage of cells expressing d G6PD might vary more in the intermediate time interval with high variance than under stable, normal conditions. The compartment size is $M = 500$. (a) The percentage of stem cells and DC^{0}s expressing d G6PD under regeneration and the intermediate time interval with high variance. (b) The percentage of self-renewals that are symmetric in the intermediate time interval with high variance. (c) The percentage of stem cells and DC^{0}s expressing d G6PD under stable, normal conditions. (d) The percentage of self-renewals that are symmetric under stable, normal conditions.

$$P_{I,J^0}(I-1,\, J^0+2) = \frac{M-J^0}{2^{K+1}M}. \tag{13}$$

That is, the conditional probability that a DC^K is selected to die is given in (9), a DC^i commits symmetrically to differentiation is given in (10), a stem cell divides asymmetrically is given in (11), a stem cell self-renews symmetrically is given in (12), and a stem cell commits symmetrically to differentiation is given in (13). Let $X(\Gamma)$ and $Y^i(\Gamma)$ be the expected number of cells in the SC-compartment and DC^i-compartment, respectively, at elementary event Γ. It follows from Eqs. (9)–(13), given that $0 < X(\Gamma)$, $Y^0(\Gamma) \leqslant M-2$ and $Y^j \leqslant 2^jM$, for $0 < j$, we have that

$$X(\Gamma+1) = X(\Gamma) + \frac{1}{2^{K+1}M}(Y^0(\Gamma) - X(\Gamma)), \tag{14}$$

$$Y^0(\Gamma+1) = Y^0(\Gamma) + \frac{1}{2^{K+1}M}(X(\Gamma) + Y^1(\Gamma) - 3Y^0(\Gamma)), \tag{15}$$

$$Y^j(\Gamma+1) = Y^j(\Gamma) + \frac{1}{2^{K+1}M}(2Y^{j-1}(\Gamma) + Y^{j+1}(\Gamma) - 3Y^j(\Gamma)), \tag{16}$$

$$Y^K(\Gamma+1) = Y^K(\Gamma) + \frac{1}{2^{K+1}M}(2Y^{2-1}(\Gamma) + 2^KM - 3Y^j(\Gamma)), \tag{17}$$

where $0 < j < K$. Because of the boundary conditions when the compartments of differentiated cells are empty, it is not possible to derive a simple approximation of the mean function, as it was for the model of undifferentiated cells illustrated in Fig. 2. Hence, we simply inspect the stability of the system of linear difference equations given in (14)–(17). The system has exactly one equilibrium solution, namely

$$(X^*,\, Y^{0*}, \ldots, Y^{i*}, \ldots Y^{K*}) = (M,\, M, \ldots, 2^jM, \ldots, 2^KM).$$

The corresponding transition matrix is:

$$\begin{bmatrix} -1 & 1 & 0 & 0 & 0 & \cdots & 0 \\ 1 & -3 & 1 & 0 & 0 & \cdots & 0 \\ 0 & 2 & -3 & 1 & 0 & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & 2 & -3 & 1 & 0 \\ 0 & \cdots & 0 & 0 & 2 & -3 & 1 \\ 0 & \cdots & 0 & 0 & 0 & 2 & -3 \end{bmatrix}, \tag{18}$$

It follows from the work by Kulkarni et al. (1999) that the corresponding eigenvalues are negative (see Appendix A). Hence, if all sites are initially vacant or contain exactly one cell, it is expected that the number of cells increases until approximately all sites are full.

2.6. Numerical simulations

Since there are M sites in the SC-compartment and 2^iM sites in the DC^i-compartment, for $0 \leqslant i \leqslant K$, it follows that the total number of sites in the multi-compartmental model is

$$M\left(1 + \sum_{i=0}^{K} 2^i\right) = M\left(1 + \frac{1-2^{K+1}}{1-2}\right) = 2^{K+1}M.$$

In the numerical examples in this subsection, one time step consists of $2^{K+1}M$ elementary events. Since each site has the same probability of being selected at any elementary event, it follows that, on average, each site is selected once during a time step.

Fig. 7 shows the multi-compartmental model in stable, normal state. In Figs. 7 (a)–(b), the ratio

$$\frac{\text{number of cells in compartment}}{\text{number of sites in the compartment}}$$

is plotted for cells of all stages in the multi-compartmental model. The figures verify that under stable, normal state, all sites contain approximately one cell. Since the number of cells in the compartments of undifferentiated cells cannot exceed M, the corresponding ratios remain under one. On the other hand, the sites in the compartments of differentiated cells may contain more than one cell. Consequently, the corresponding ratios fluctuate over one. Fig. 7 (c) shows the percentage of self-renewal divisions that are symmetric. The estimated mean is 2.46%. This verifies that during normal conditions the stem cells divide mainly asymmetrically. Consequently, the number of stem cells fluctuate less than the number of DC^{0}s and the number of differentiated cells with compartments sizes that are relatively small. Indeed, Fig. 7 (d) displays the intervals $(\mu - s, \mu + s)$ for all compartments, where μ is the estimated mean in a given compartment and s is the estimated standard deviation. It can be verified that the estimated coefficient of variation, s/μ, is significantly larger for the DC^i-compart-

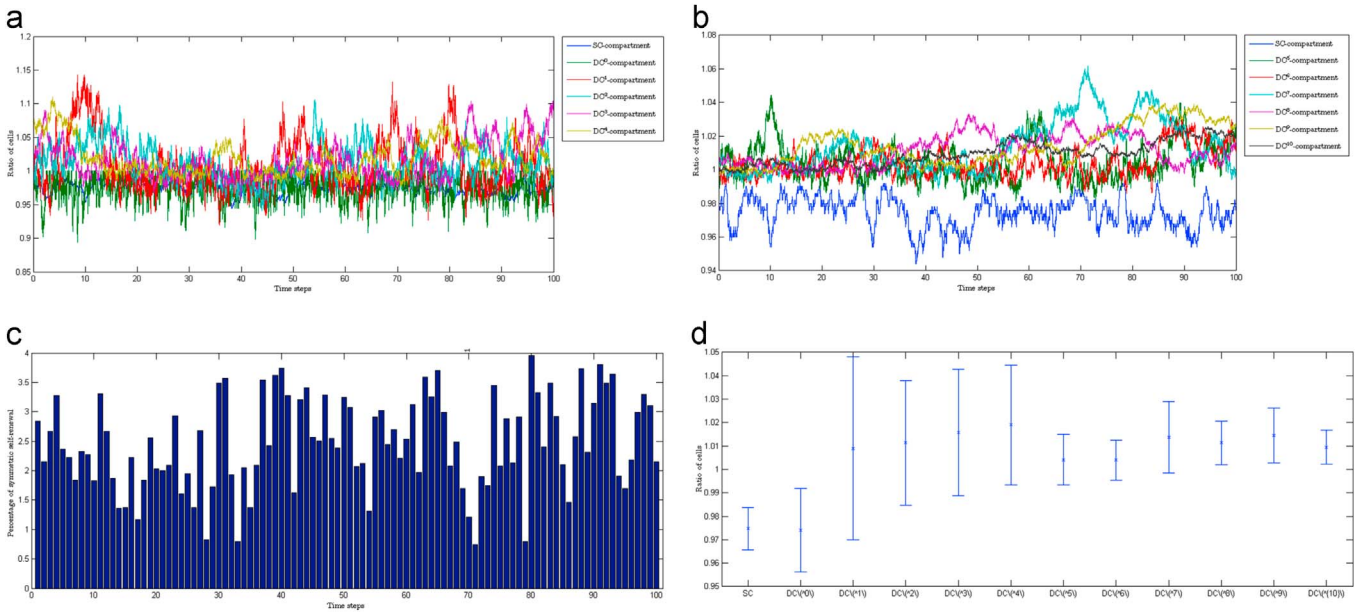

Fig. 7. The multi-compartmental model under stable, normal conditions This figure illustrates the multi-compartmental model under stable, normal conditions. The compartment size is $M = 500$. The ratio (number of cells in compartment)/(number of sites in compartment) is plotted for all compartments. (a) The ratio of cells in the SC-compartment and DC^i-compartment, for $0 \leqslant i \leqslant 4$. (b) The ratio of cells in the SC-compartment and DC^i-compartment, for $4 \leqslant i \leqslant 10$. (c) The percentage of self-renewal divisions that are symmetric. (d) The intervals $(\mu - s, \mu + s)$ for all compartments, where μ is the estimated mean in a given compartment and s is the estimated standard deviation.

ment than for the SC-compartment for $i \leqslant 4$, whereas for $4 < i$, the DC^i-compartment has smaller or approximately the same estimated coefficient of variation as the SC-compartment. We will use the values $\mu - s$, given in Fig. 7 (d), as lower limits for normal population levels in the examples where the multi-compartmental model is regenerated.

Fig. 8 shows the regeneration of the whole system, starting with a single stem cell, and verifies that the number of cells converges towards the steady-state where all compartments are approximately full. It follows from Fig. 8 (a) that the DC^{i+1} s grow, in general, faster towards the normal population level than the DC^is, for $0 \leqslant i < 10$, and that the stem cells typically grow slowest. Fig. 8 (b), which displays the percentage of self-renewal divisions that are symmetric, verifies that during regeneration, the rate of symmetric self-renewal increases. I.e. in the beginning of the regeneration, the percentage of symmetric self-renewal is close to 100%, and it decreases steadily down to approximately 2.5%. All the differentiated cells have reached normal population levels at time step $t = 19$. However, the stem cells continue to self-renew symmetrically at a higher rate than what is observed under stable, normal state. This illustrates the phenomenon, denoted intermediate time interval with high variance, which occurs in all of our numerical trials: When the cells reach normal population level, the stem cells continue to self-renew symmetrically at a relative high rate for some period of time, before the rate stabilises at normal level, and the whole system enters stable, normal state. The time-laps from the moment the cells reach normal population level to the system reaches stable, normal state, varies both in length and in how much it affects the population dynamics of the multi-compartmental model. In particular, when the cells are subdivided into two neutral phenotypes, such as G G6PD-positive and d G6PD-positive cells for the Safari cat, the percentage of cells that expresses each type might change radically during the intermediate time interval with high variance. When the system is in stable, normal state, the percentage of each phenotype remains approximately constant. This is illustrated in Figs. 9 and 10. The blue and green curves plotted in Fig. 9 are, respectively, the ratio of full sites in the SC-compartment and the ratio of stem cells expressing d G6PD when the multi-compartmental model regenerates. The initial conditions are that 70% of the sites in all compartments are vacant and that 40% of the cells in the SC-compartment express d G6PD. It follows from Fig. 9 (a) that the ratio of d G6PD-positive stem cells fluctuates most intensely during the first ten time steps. At time step $t = 22$, when all the compartments of committed cells have reached their normal population level, 26.12% of the stem cells express d G6PD. The stem cells reach their normal population level at time step $t = 26$, followed by a relatively long period with high fluctuation in the population size. When the system stabilises at stable, normal state at time step $t = 60$, the percentage of stem cells that express d G6PD is on average 14.69%. However, in other numerical trials the percentage of d G6PD-positive cells does not change significantly after the committed cells reach normal population level. For instance, in the example displayed in Fig. 10, where the multi-compartmental model is regenerated, starting

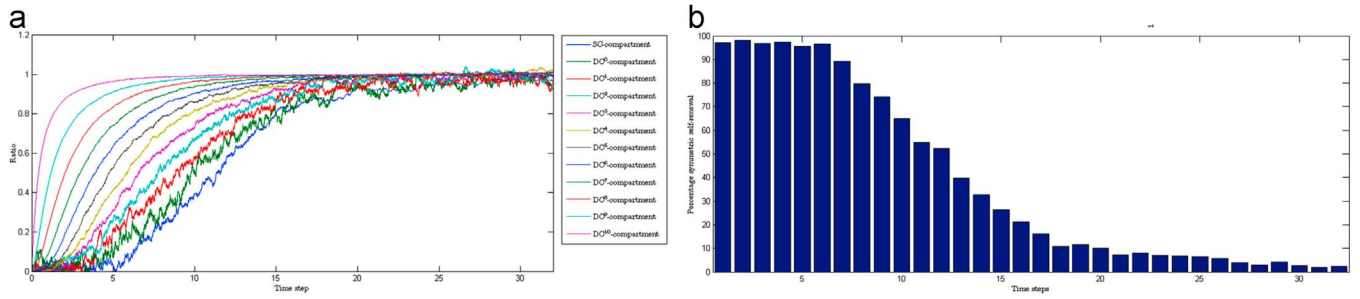

Fig. 8. Regeneration of the multi-compartmental model The whole system regenerates, starting with a single stem cell. The compartment size is $M = 500$. (a) displays the ratio (number of cells in compartment)/(number of sites in compartment) for all compartments. (b) displays the percentage of self-renewals that are symmetric.

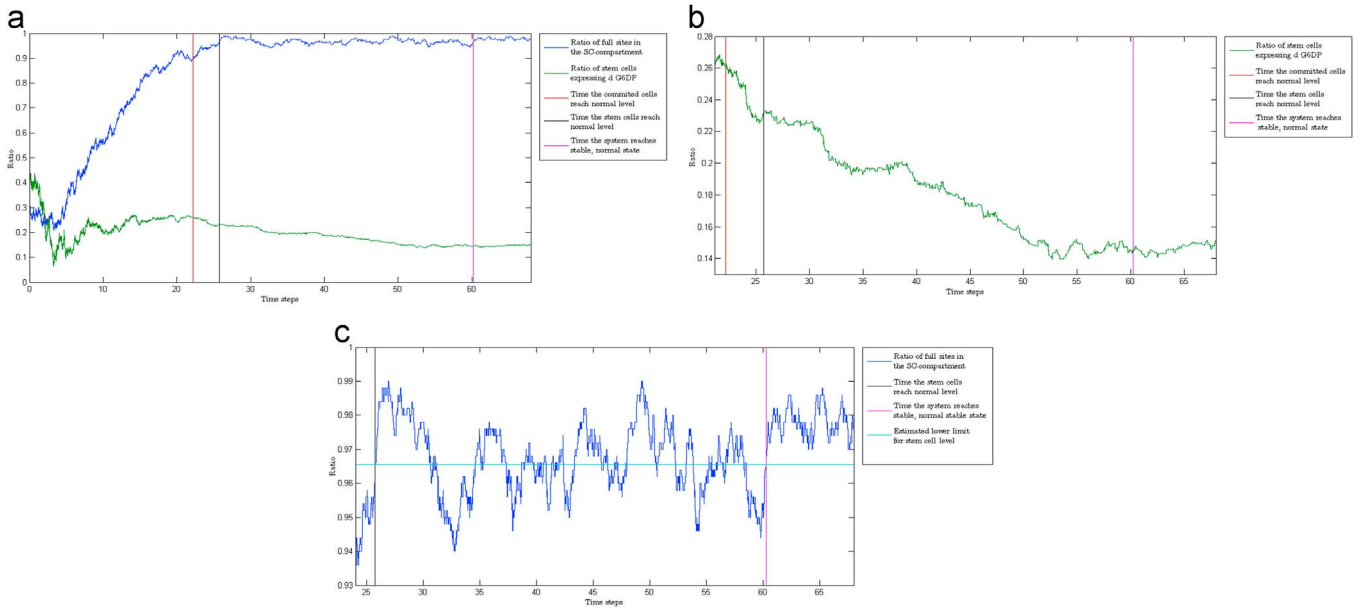

Fig. 9. The intermediate time interval with high variance This figure illustrates that the intermediate time interval with high variance can cause a significant fluctuation in the number of stem cells and the percentage of stem cells expressing d G6PD. Initially, 70% of the sites in all compartments are vacant and 40% of the stem cells express d G6PD. (a) displays the ratios (number of stem cell)/(number of sites) and (number of stem cell expressing d G6PD)/(number of stem cells) as blue and green curves, respectively, during regeneration, the intermediate time interval with high variance, and when the system reaches stable, normal conditions. (b) displays the ratio of stem cells expressing d G6PD during the intermediate time interval with high variance and when the system reaches stable, normal state. (c) displays the ratio of full sites in the SC-compartment during the intermediate time interval with high variance and when the system reaches stable, normal state. The horizontal, turquoise line is the estimated lower limit for stem cell level. During the intermediate time interval, the ratio of full sites in the SC-compartment is frequently below this limit, whereas under stable, normal conditions, the ratio is in general above this limit.

with 80% vacant sites in all compartments and 50% of the cells expressing d G6PD, the stem cells expressing d G6PD get extinct at time step $t = 3$, and eventually all the cells express G G6PD. As illustrated by Fig. 10 (b), the percentage of mature cells expressing d G6PD does not follow the fluctuation of d G6PD-positive stem cells during regeneration. In particular, the d G6PD-positive stem cells get extinct at time step $t = 3$ in the example illustrated in Fig. 10, though there are still mature cells expressing d G6PD at time step 13. However, at time step $t = 14$, all cells in the system are G G6PD-positive. Under stable, normal state, all the compartments have approximately the same percentage of d G6PD-positive cells as the SC-compartment. Hence, it is possible to estimate the percentage of d G6PD-positive stem cells by measuring the percentage of mature cells expressing d G6PD, under stable normal conditions.

In all examples each site is, on average, selected once during a time step. However, in 2.3, it is only the two compartments of undifferentiated cells that regenerate, whereas in this subsection, both the compartments of undifferentiated cells and the compartments of differentiated cells regenerate, and this is the reason why the average time the population of cells uses to reach normal population level is 6.2 time steps in the former subsection, whereas in this subsection, the average number of time steps is 27.6. As illustrated by Figs. 5, 6, 9 and 10, the intermediate time interval with high variance has more apparent effect on the population dynamics when compartments of differentiated cells are included. However, as discussed in Sections 4 and 5, none of our simulations could reproduce all the results from the experiments on Safari cats (Abkowitz et al., 1988, 1990, 1993).

3. Results

The compartmental model of haematopoiesis presented in this paper is inspired by the results from the experiments on the *Drosophila* germline stem cell compartment (Yamashita et al., 2003; Morrison and Kimble, 2006; Wong et al., 2005). As discussed in Section 1.2, these results support the following conjectures:

Conjectures.

I. The stem cell compartment promotes stem cell maintenance.
II. The stem cell compartment can contain up to a certain number of cells.
III. The stem cells self-renew at random.
IV. When a stem cell self-renews, one of the daughter cells inherits the mother's place in the stem cell compartment and retains stem cell identity, whereas the fate of the second daughter depends on the

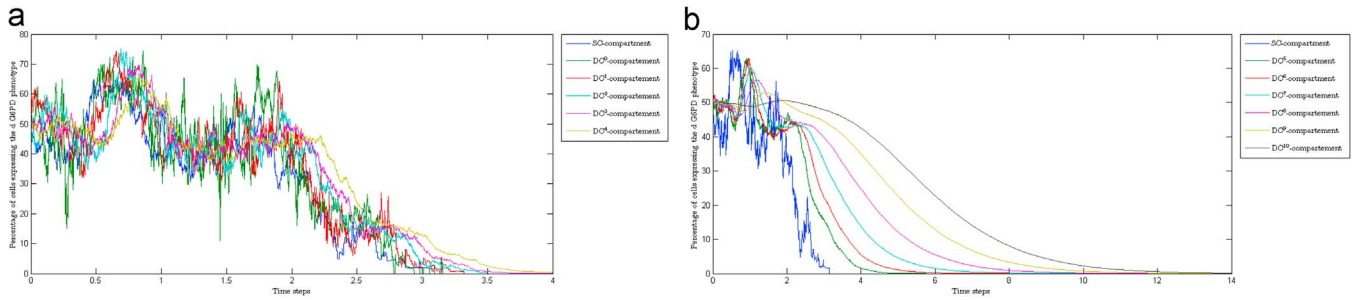

Fig. 10. The cells expressing d G6PD get extinct Initially, 80% of the sites in all compartments are vacant and 50% of the cells express d G6PD. At time step $t = 3$, the stem cells expressing d G6PD get extinct, and at time step $t = 14$, all the d G6PD-positive cells are extinct. The compartment size is $M = 500$. (a) displays the percentage of d G6PD-positive cells in the SC-compartment and DC^i- compartment, for $0 \leqslant i \leqslant 4$. (b) displays the percentage of d G6PD-positive cells in the SC-compartment and DC^i-compartment, for $5 \leqslant i \leqslant 10$.

availability of space in the stem cell compartment – it either slips into a random vacant place in the stem cell compartment and remains a stem cell (symmetric self-renewal), or the second daughter leaves the stem cell compartment and loses its stem cell identity (asymmetric self-renewal).

V. Under normal conditions, the stem cell compartment is approximately full, and the stem cells typically self-renew asymmetrically.

VI. When the stem cell compartment is not full, the rate of symmetric self-renewal generally increases, which leads to an expansion in the number of stem cells. The cells swift back to asymmetric self-renewal as the stem cell compartment reaches normal conditions.

As illustrated in Figs. 3 (a)–(d), if the number of sites in the SC-compartment is rather small, Conjectures IV–IV do not hold: The stem cells divide symmetrically at a relatively high rate when the SC-compartment is approximately full, causing a high variation in the number of cells, and more than not, the stem cell population continues to decrease when the number of cells in the SC-compartment is significantly less than the number of sites. In particular, when there are ten sites in the SC-compartment, the stem cell population frequently goes extinct. These results indicate that if the bone marrow niche can contain only a few active cells, then self-renewal is not a random process, but regulated deterministic. On the other hand, when there are fifty or more sites in the SC-compartment, all the conjectures hold (see Figs. 2, 3 (e)–(l), 4–10). This demonstrates that dynamic self-organisation of self-renewal and differentiation requires that the number of stem cells is sufficiently large.

The model can reproduce the following results from experiments with female Safari cats (Abkowitz et al., 1988, 1990, 1993):

Results from experiments.

I. The percentage of cells expressing d G6PD is approximately constant in healthy cats.

II. The pattern of clonal contribution to haematopoiesis is unique when the bone marrow regenerates. For instance, one of the G6PD phenotypes might get extinct during regeneration, but it is also possible that the percentage of each phenotype remains constant.

III. Significant variation in the percentage of cells expressing d G6PD might occur in a period after the cells have reached normal population level.

As shown in Figs. 4 (a)–(b), the percentage of cells expressing d G6PD varies relatively little under stable, normal conditions: The stem cells typically divide asymmetrically, and the daughter that inherits the mother's site has the same G6PD-phenotype as the mother. Figs. 5, 8–10 illustrate that each pattern during regeneration is unique: The rate of symmetric division increases, causing great fluctuation in the percentage of cells expressing d G6PD, and in some of the simulations, one of the G6PD phenotypes gets extinct. As demonstrated in Figs. 5, 6, 8–10, the system does not, in general, gain stable, normal condition when it reaches normal population levels after regeneration. Typically, the DC^{i+1} s grow faster towards normal population level than the DC^is, for $0 \leqslant i < K$, whereas the stem cells grow slowest. This causes an intermediate time interval where the number of cells varies more than under stable, normal condition. The time-laps from the moment the cells reach normal population level to the system reach stable, normal condition varies both in length and in how much it affects the population dynamics. In Fig. 9, the percentage of cells expressing d G6PD changes considerably during the intermediate interval with high variance, whereas in Figure 10, there is no significant change in the percentage after the cells reach normal population level. Hence, the model can reproduce Results I–III. As discussed in Section 1.4, several other models recreate Results I-II. However, to our knowledge, none of the previous models describing haematopoiesis in female Safari cats can explain Result III.

Results from simulations.

I. For sufficiently large population sizes, the percentage of each phenotype remained approximately constant under stable, normal conditions.

II. Each regeneration was unique, both with respect to the number of time steps until normal population was reached and with respect to the percentage of each phenotype.

III. The system did not, in general, gain stable, normal condition when it reached normal population levels after regeneration, and in some simulations, variation in the percentage of each phenotype occurred in a period after the system reached normal population level.

4. Discussion

The model of haematopoiesis presented in this paper, includes flexible and dynamically regulated self-organisation based on extracellular regulations and cell–cell and cell–environment interactions. The classical definition of stem cells – an undifferentiated cell capable of self-renewal, production of a large number of differentiated cells, regenerating tissue after injury and a flexibility in the use of these options – is fundamentally based on a functional perspective. As discussed by Loeffler and Roeder (2002), when the definition of stem cells was first introduced, the flexibility criterion attracted little attention. However, several experimental results indicate that flexibility is a fundamental property of the stem cells. For instance, a level of flexibility was found for lineage specifications within the haematopoietic system (Zhang et al., 1999): Zhang et al. managed to bias the degree of lineage commitment by several maneuvers that altered the growth environment. The present explanation of the fluctuations observed in lineage specification is based on a dynamic network of interacting transcription factors involving the PU-1 and GATA molecules. Cross and Enver introduced the concept of fluctuating levels of transcription factors within the haematopoietic system with threshold-dependent commitment (Cross and Enver, 1997). Moreover, several experiments indicate that stem cells specified for one type of tissue (e.g. haematopoiesis) can be manipulated in such a way that they can act as stem cells for another tissue (e.g. neuronal, myogenic) (Bjornson et al., 1999; Brazelton et al., 2000; Seale and Rudnicki, 2000; Goodell et al., 2001). The growth environment seems to be an important factor when tissue specification of stem cells are redirected. These results might support to our assumption that self-renewal is a property of undifferentiated cells located in the stem cell compartment, and that once a cell leaves the stem cell compartment, it loses the ability to self-renew. This implies that the cells located in the stem cell compartment and the compartment of undifferentiated cells committed to differentiation are phenotypically identical and cannot be distinguished in a laboratory.

Theoretical work also implies that flexibility is one of the most fundamental properties of the stem cells, because models without self-organisation must, in general, require that the cells somehow know how to behave under different circumstances (Loeffler and Roeder, 2002). For instance, as discussed in Section 1.4, the model presented by Abkowitz et al. in Abkowitz et al. (1996) has no self-organisation, and assumes that the stem cells ignore the signals that tell them to self-renew symmetrically when the number of stem cells reaches a certain limit. This means that each stem cell must keep track of the total number of stem cells, in order to make the right decision. Moreover, Loeffler and Roeder argue that a number of models include assumptions about symmetric and asymmetric stem cell division that in one way or another requires that the cells somehow explicitly “know” how to behave (Vogel et al., 1968; A comprehensive mathematical model, 1980; Loeffler and Grossmann, 1991; Loeffler et al., 1993, 1997). Loeffler and Roeder conclude that such concepts are too rigorous and potentially misleading, and hence, no implications about symmetric or asymmetric stem cell division are included in the definition of tissue

stem cells in Loeffler and Roeder (2002). The model presented in this paper includes symmetric and asymmetric stem cell division. Moreover, even though the stem cells have the flexibility to undergo self-renewal, produce mature cells by differentiation, and regenerate undifferentiated cells and differentiated cells when necessary, each cell in the system behaves completely random. This is implemented by subdividing the compartments into sites which represent physical space as well as signals and the environment (Wineman et al., 1996; Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998; Verfaillie, 1998; Koller et al., 1999; Yin and Li, 2006; Zhang and Li, 2008; Cheng et al., 2014; Fuchs et al., 2004; Nikolova et al., 2007; Simons and Clevers, 2011; Aglietta et al., 1989; Layton et al., 1989; Metcalf, 2008; Fried, 2009). Moreover, it is assumed that when a cell in the SC-compartment divides, both daughter cells remain undifferentiated, whereas when a cell in any of the DC-compartments divides, both daughter cells are more differentiated than the mother cell. This means that when a cell migrates from the SC-compartment to the DC^0-compartment, no change occurs in the phenotype. However, the cell is no longer a stem cell, because when the cell divides in the DC^0-compartment, none of the daughter cells remain undifferentiated, which means that the cell can no longer self-renew (Yamashita et al., 2003; Morrison and Kimble, 2006; Wong et al., 2005). Furthermore, it is assumed that the cells in the DC^0-compartment release signals that inhibit migration from the SC-compartment to the DC^0-compartment. If a cell in the SC-compartment does not receive these signals when it divides, then both daughter cells migrate to the DC^0-compartment, which means that the cell commits symmetrically to differentiation. This is implemented by selecting a random site in the compartments of undifferentiated cells and a random stem cell. The absence of signals that inhibit migration from the SC-compartment to the DC^0-compartment is represented by selecting a vacant site in the DC^0-compartment, in which case the selected stem cell commits symmetrically to differentiation. Hence, symmetric commitment is a random event, and the probability that this type of division occurs increases when the number of cells in the DC^0-compartment decreases. On the other hand, if a cell in the SC-compartment receives the signals that inhibit migration when it divides, then one of the daughter cells inherits the site of the mother, whereas the other daughter cell is placed by a random site in the SC-compartment. If this site is vacant, then the second daughter cell inhabits the site, which means that the division is symmetric self-renewal. On the contrary, if the site is occupied by another cell, then the second daughter cell migrates to the DC^0-compartment. That is, the division is asymmetric. Thus, both symmetric self-renewal and asymmetric division are random events, and the probability that each of these divisions occurs increases and decreases, respectively, when the number of cells in the SC-compartment decreases. The model presented in this paper also assumes that the cells in the DC^i-compartment are regulated by negative feedback from the cells in the DC^{i+1}-compartment, for $0 \leqslant i < K$ (Aglietta et al., 1989; Layton et al., 1989; Metcalf, 2008; Fried, 2009). More precisely, the cells in the DC^{i+1}-compartment release signals that inhibit symmetric commitment in the DC^i-compartment, such that under normal conditions, the cells in the latter compartment differentiate symmetrically at approximately constant rate. However, if the concentration of the signals that inhibit symmetric commitment to differentiation decreases, the rate of this type of division increases. This is implemented by selecting a random site in the compartments of differentiated cells. The absence of signals that inhibit symmetric commitment in the DC^i-compartment is represented by selecting a vacant site in the DC^{i+1}-compartment, in which case a random DC^i commits symmetrically to differentiation. On the other hand, if the selected site is full, a DC^{i+1} commits symmetrically to differentiation if $i < K - 1$ or dies if $i = K - 1$. Consequently, the K feedback loops from the DC^{i+1}-compartment to the DC^i-compartment, for $0 \leqslant i < K$, ensure that the system of cells regenerates the differentiated cells after injury, even though each cell in the system behaves completely random.

The model presented in this paper is very simplistic and has only two parameters, M and K – the number of sites in the SC-compartment and the number of compartments of differentiated cells, respectively. It is possible to add more parameters to the model, for instance letting the SC-compartment and DC^0-compartment have different number of sites, or selecting random cells to undergo apoptosis. However, the scope of this model is to link self-organisation with symmetric and asymmetric cell division, and these parameters do not lead to the revealing of new structures or any other relevant information. Hence, we choose to keep the model simple and comprehensible with two parameters only. If we want to extend the model such that it becomes more realistic and sophisticated, several aspects should be addressed. For one thing, the extended model should divide the committed haematopoietic cells into the erythroid lineage, the lymphoid lineage and the myeloid lineage. The first lineage is composed of red blood cells, the second of immune cells and the third includes granulocytes, megakaryocytes and macrophages (Morrison and Weissman, 1994; Verfaillie, 1998; Gehling et al., 2000). As discussed in Section 1.3, it is still unclear exactly how differentiation of haematopoietic cells is regulated. More than half a century ago, Waddington (1957) presented an epigenetic landscape to describe the differentiation of cells as the trajectories of balls rolling at random into branching valleys, each of which represents a developmental state. Based on Waddington's model, Furusawa and Kaneko (2009) propose a dynamical system model of cells with intracellular protein expression dynamics and interactions with each other. The model predicts that cells with irregular, or chaotic, oscillations in gene expression dynamics have the potential to differentiate into other cell types. During development, such complex oscillations are lost successively, leading to loss of pluripotency. Their results are consistent with the view that pluripotency is a statistical property defined at the cellular population level, correlating with intra-sample heterogeneity, and driven by the degree of signalling promiscuity in cells.

Another aspect that should be addressed in a more realistic and sophisticated version of the model, is that the cells in the SC-compartment are homogeneous with respect to functionality in the model presented in this paper. Nevertheless, phenotypic heterogeneity has been observed in haematopoietic stem cells with regards to various markers (e.g. CD34, CD38, c-kit, Sca 1) (Uchida et al., 1993; Lord, 1997). Moreover, experiments by Sato et al. indicate that both CD34-positive and CD34-negative cells can be effective stem cells and that the cells can even alter the CD34 property (Sato et al., 1999). As discussed by Huss (2000), CD34-negative stem cells are considered to be predominantly part of the quiescent stem cell pool of the haematopoietic system, and it is possible that haematopoietic stem cells alter the CD34 property from positive to negative when they go from active to quiescent state, and vice versa. Roeder and Loeffler propose a single-based stochastic model of haematopoietic stem cells that includes quiescence (Roeder and Loeffler, 2002). This model does not incorporate regulation of asymmetric and symmetric stem cell division. However, similar to the model presented in this paper, the model by Roeder and Loeffler introduces a perspective on stem cell organisation where stemness is not treated as an explicit cellular property but as the result of a dynamic process of self-organisation. That is, the model makes the novel concept of within-tissue plasticity operational – within a range of potential options, individual cells may reversibly change their actual set of properties, like going from active to quiescent state and vice versa, depending on the influence of the local growth environment. Stochastic switching between the growth environments introduces fluctuations that eventually generate heterogeneity.

5. Conclusion

In this paper, a simplistic model of haematopoiesis that links self-organisation with symmetric and asymmetric cell division is proposed. Each cell in the system behaves randomly and the daughter cells

resulting from symmetric and asymmetric stem cell divisions are, in general, phenotypically identical, and still, the haematopoietic system has the flexibility to self-renew, produce mature cells by differentiation, and regenerate undifferentiated cells and differentiated cells when necessary, due to self-organisation. Moreover, the compartments of committed cells are regulated by feedback loops, so that the system of cells regenerates the differentiated cells after injury. To our best knowledge, no previous model implements symmetric and asymmetric division as the result of self-organisation. Different models of self-organisation are discussed and compared by Osborne et al. in Osborn et al. (2016). The model of self-organisation proposed by Loeffler and Roeder (2002), Roeder and Loeffler (2002) and the potential impact of the stem cell niche on asymmetry of stem cell fate are discussed by Roeder and Lorenz in Roeder and Lorenz (2006). The authors state that since no implications about symmetric or asymmetric stem cell division are included in the definition of tissue stem cells in Loeffler and Roeder (2002) and Roeder and Loeffler (2002), this perspective of stem cell organisation does explicitly preclude asymmetric cell division. However, Roeder and Lorenz suggest that symmetric cell fates might be indirectly linked to self-organisation. On the contrary, our model implements symmetric and asymmetric division as the direct result of self-organisation.

The model can reproduce several of the results from experiments with female Safari cats (Abkowitz et al., 1988, 1990, 1993). Similar to previous models of haematopoiesis in female Safari cats (Guttorp et al., 1990; Newton et al., 1995; Abkowitz et al., 1996; Golinelli et al., 2006; Fong et al., 2009), the model presented in this paper can explain why the percentage of d G6PD-positive cells is approximately constant in healthy cats, whereas the pattern of clonal contributions to haematopoiesis is unique when the bone marrow regenerates. In addition, the model indicates that self-organisation of haematopoiesis might cause significant variation in the percentage of d G6PD-positive cells after the number of cells has reached normal population level. In general, the DC^{i+1} s reach normal population level before the DC^is, for $0 \leqslant i \leqslant K$, whereas the stem cells grow slowest, and this generates an intermediate time interval with relative high rate of symmetric stem cell division and corresponding high variance in the cell number. Eventually, the system self-regulates such that the rate of symmetric stem cell division decreases and the system enters stable, normal state.

Several of the results from experiments with female Safari cats (Abkowitz et al., 1988, 1990, 1993) cannot be reproduced by the model. For instance, for the first 10–12 weeks after transplantation, the percent of progenitors with d-G6PD was unchanged from that observed prior to transplantation in each cat (Abkowitz et al., 1990). This might indicate that when the number of stem cells is very small, self-renewal is strictly regulated. It may also be the case that a relative large number of quiescent stem cells are activated. However, after 10–12 weeks, the percents of progenitors with d-G6PD fluctuated widely, which indicates that self-renewal occurs more randomly. The model presented in this paper does not capture the difference between before and after 10–12 weeks because the model is very simplistic. For instance, it assumes that self-renewal always occurs at random, and quiescent stem cells are not included in the model. Moreover, Abkowitz et al. (1988) found that when the peripheral blood counts and the number of marrow progenitors detected in culture had reached normal level, the percentages of erythroid burst-forming cells and granulocyte/macrophage colony-forming cells in DNA synthesis increased. The main reason that the model presented in this paper does not capture this result, is that the committed haematopoietic cells are not divided into different lineages.

It is possible to extend the model such that it becomes more realistic and sophisticated by including quiescence for the stem cells and subdividing the committed cells into different lineages, similar to the models presented by Roeder and Loeffler (2002) and Furusawa and Kaneko (2009), respectively. As discussed in Section 4, these two models are based on similar assumptions as the model presented in this paper, namely that everything is totally random at single cell level. However, self-organisation of the system of cells ensures that self-renewal, production of mature cells, and regeneration of undifferentiated cells and differentiated cells are well orchestrated. The intermediate time interval with high variance has an apparent effect on the population dynamics when the differentiated cells are included. However, none of our simulations could reproduce all the results obtained by Abkowitz et al. (1988), Abkowitz et al. (1990), Abkowitz et al. (1993). By including quiescence for the stem cells and subdividing the committed cells into different lineages, the model would become more complex and richer, and it might capture a broader spectre of the results from experiment on female Safari cats.

Appendix A. Appendix

The transition matrix given in (18) might be the most the most natural representation of the system given in (14)–(17). However, the number of cells at elementary event Γ may be given by the vector

$$(Y^K(\Gamma), \ldots, Y^i(\Gamma), \ldots, Y^0(\Gamma), X(\Gamma)),$$

and in this case the corresponding transition matrix is

$$\begin{bmatrix} -3 & 2 & 0 & 0 & 0 & \cdots & 0 \\ 1 & -3 & 2 & 0 & 0 & \cdots & 0 \\ 0 & 1 & -3 & 2 & 0 & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & 1 & -3 & 2 & 0 \\ 0 & \cdots & 0 & 0 & 1 & -3 & 1 \\ 0 & \cdots & 0 & 0 & 0 & 1 & -1 \end{bmatrix}, \tag{A.1}$$

Clearly, the stability of the system given in Eqs. (14)–(17) does not depend on the representation of the transition matrix.

Kulkarni et al. (1999) present the general $n \times n$ tridiagonal Toeplitz matrix, denoted $T_n(a, b, c)$, in Section 2. By letting $a = -3$, $b = 1$ and $c = 2$, we obtain the following tridiagonal Toeplitz matrix:

$$T_n(-3, 2, 1) = \begin{bmatrix} -3 & 2 & 0 & \cdots & 0 \\ 1 & -3 & 2 & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 1 & -3 & 2 \\ 0 & \cdots & 0 & 1 & -3 \end{bmatrix}.$$

It from Theorem 2.2 given in Kulkarni et al. (1999), that the eigenvalues of $T_n(-3, 1, 2)$ are

$$\lambda_k = -3 - i2\sqrt{6}\cos(k\pi/(n+1))$$

for $k \in \{1, 2, \ldots, n\}$.

Kulkarni et al. study the eigenvalues of those tridiagonal matrices with upper left blocks which are Toeplitz matrices. If we let $a = -3$, $b = 1$, $c = 2$, $a_1 = -1$ and $b_1 = c_1 = 1$ in the matrix presented in the first example, where k=1, given in Section 4 of Kulkarni et al. (1999), the pseudo-Toeplitz matrix, denoted $T_n^1(-3, 1, 2)$, is the transition matrix given in (A.1). By examining the roots of the characteristic polynomial of $(1/\sqrt{6})T_n^1(-3, 1, 2)$ and using the substitution $-3/\sqrt{6} - \lambda = 2x$, Kulkarni et al. show that the roots must satisfy the equation

$$4(1/\sqrt{2} + x)U_n(x) - U_{n-1}(x) = 0,$$

where $U_n(x)$ denotes the nth degree Chebyshev polynomial of the second kind. By studying intersections of graphs in the xy-plane, Kulkarni et al. show that if $b_1c_1 > 0$ and $bc > 0$, then the $(n+1) \times (n+1)$-matrix $T_n^1(a, b, c)$ has $n+1$ real distinct eigenvalues. It can be verified by these graphs that for $a = -3$, $b = 1$, $c = 2$, $a_1 = -1$ and $b_1 = c_1 = 1$, all eigenvalues are negative.

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