Ageing as a price of cooperation and complexity

Self-organization of complex systems causes the ageing of constituent networks

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Summary

The analysis of network topology and dynamics is increasingly used for the description of the structure, function and evolution of complex systems. Here we summarize key aspects of the evolvability and robustness of the hierarchical network-set of macromolecules, cells, organisms, and ecosystems. Listing the costs and benefits of cooperation as a necessary behaviour to build this network hierarchy, we outline the major hypothesis of the paper: the emergence of hierarchical complexity needs cooperation leading to the ageing of the constituent networks. Local cooperation in a stable environment may lead to over-optimization developing an 'always-old' network, which ages slowly, and dies in an apoptosis-like process. Global cooperation by exploring a rapidly changing environment may cause an occasional over-perturbation exhausting system-resources, causing rapid degradation, ageing and death of an otherwise 'forever-young' network in a necrosis-like process. Giving a number of examples we explain how local and global cooperation can both evoke and help successful ageing. Finally, we show how various forms of cooperation and consequent ageing emerge as key elements in all major steps of evolution from the formation of protocells to the establishment of the globalized, modern human society. Thus, ageing emerges as a price of complexity, which is going hand-in-hand with cooperation enhancing each other in a successful community.

Introduction: Evolvability, robustness and ageing of hierarchical networks

The network approach proved to be a highly efficient cognitive tool to describe various levels of the hierarchical organization of complex systems from macromolecular structures to the currently emerging world-wide social networks. The description of a complex system as a

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network needs the identification of separable subsets of the system as network elements, and a catalogue of their interactions as network contacts or links. In most of the cases network elements themselves can be perceived as networks. Thus, elements of social networks, human individuals are networks of organs and cells, cells are networks of proteins, and proteins are networks of amino acids to name only a few of the possible representations. Networks display a lot of rather general properties, such as

(a) ***small-worldness***, meaning the existence of short pathways between most network elements;
(b) the ***existence of hubs***, which have a much higher number of neighbours than the average of network elements;
(c) ***modular structure***, which organizes networks to various overlapping groups;
(d) the co-existence of ***strong and weak links***, where the link-strength is usually defined as the real, physical strength of the connection, or as the probability of interactions and
(e) the existence of a ***network skeleton***, which is the subset of most important pathways in the network.

Regretfully, the scope of the current paper does not allow us to give exact definitions and a detailed description of all these network properties, therefore, the reader is referred to a number of recent reviews for more details.[1–4]

In the previous list of network properties almost all general network features referred to a structural, topological description of networks. However, an even more important task is the characterization of network dynamics, which became a centrepiece of network studies in the last couple of years. Networks continuously accommodate novel members, lose their original elements, as well as build, erase and rearrange their links. Networks may even undergo a profound structural reorganization, called topological phase transition, when they experience a large change in the resources providing the energy to maintain their links, or suffer a large stress, i.e. an abrupt change in the number and magnitude of perturbations arriving from the network environment and disturbing their original structure.[1–7]

Structural changes of biological networks often respond to the novel stimulus of the environment, especially if the stimulus is repeatedly experienced. This adaptation of network structure to the environmental signals – which is similar to the training of artificial neural networks – can be perceived as a learning process. The plasticity of a network enabling these adaptive changes can be described as network evolvability. In its more restricted and original sense, the evolvability of a biological system is the capacity of the system to generate a heritable phenotypic variation.[2,8,9] Evolvability is by itself an inheritable property,[10,11] which shows that the plasticity of biological networks is a carefully regulated key feature of the accommodation to the magnitude, speed, rhythm and unexpectedness of environmental changes.

The evolvability of a network (i.e. in this, broader sense its potential to produce and accommodate innovations changing the properties of the complex system) is balanced by network robustness. Robustness and evolvability at the level of network structure display a rather antagonistic relationship. In stark contrast with structural robustness, robustness at the level of network function (i.e. at the level of the phenotype of the complex system encoded by the network, Box 1) does not hinder, but actually may even promote the evolvability of the network structure.[12] Recent studies show, that variable network structures displaying a robust phenotype can step-by-step access large amounts of structural variation avoiding the penalty of natural selection.[13,14] However, there is an intricate balance in biological systems to maintain their functional robustness, and to allow the chance of an evolutionary change by preserving their evolvability. The interplay of evolvability-mediated innovations and the buffering effects of functional robustness often lead to jumps between stability-islands causing a bi-stable or biphasic network behaviour, where two markedly different functional states of the network are much more populated than any of the myriads of the potential mixtures between them.[15]

Ageing in a very general, network sense can be regarded as the gradual phenotypic appearance of the consequences of the suboptimal conditions caused by the uneven distribution of available resources of the complex system between its sub-systems. From this approach it is clear that aging will not occur, if the system enjoys unlimited resources, or the system does not have distinct sub-systems. However, this is a rather unlikely scenario at its extreme form. A good compromise allowing a close-to-optimal function and maintenance of all sub-systems may be regarded as a form of successful ageing. Due to the (1) plethora of possible segregations of the various sub-systems; (2) the numerous solutions of resource-distribution between them, and (3) the improbably high number of possible scenarios how the uneven resource distribution will gradually affect sub-system functions – ageing has been increasingly perceived as one of the most complex phenomena of biological systems. According to the antagonistic pleiotropy theory of ageing, genes, which are preferable during early development, become detrimental at later stages. The disposable soma theory of ageing expresses this like the insufficiency of somatic maintenance allowing a high fertility. The “network theory of ageing” accommodated the balance between various types of damage and repair mechanisms in the special case of the ageing of cellular systems. Ageing is accompanied by a general increase in noise in parallel with a decrease in complexity – these signs show a general deterioration of network plasticity, which is caused and aggravated by the accumulation damage. The costly repair systems decline, which contributes further to the loss of network flexibility. If biological networks lose their adaptation potential, and their structural robustness becomes more and more rigid, instead of the plastic, functional robustness outlined before, the network-coded complex system displays the properties of an aged organism.[16–19]

In the following sections of the paper we will show that cooperation of the constituent networks is necessary to build up the hierarchical network structure from macromolecules to

the globalized social network. We will describe the costs of this beneficial cooperation in a number of examples. We then outline our central hypothesis that cooperation leads to an additional, previously unrecognized cost: ageing and finally we will show the generality of this statement by pinpointing potential ageing phenomena of the constituent networks at each major steps of the evolution[20] starting in the formation of the protocell, through the development of coordinated replication, transcription and protein synthesis, the establishment of the eukaryotic cell, sexual proliferation, differentiated multicellular organisms, animal social groups and finally, human societies.

Cooperation: costs, benefits and the emergence of hierarchical complexity

Cooperation is a joint action for a common goal, which requires information exchange and strategy adjustment of the participating partners. Development of cooperation between the constituent networks is a key step in many major transitions in evolution.[20] Game theory describes a number of biologically relevant examples, where cooperation provides a smaller income for all participating agents than the opposing behaviour, cheating, or defection. However, in a large subset of these games, called social dilemmas, the limited private income of cooperators, i.e. the cost of cooperation is paralleled with the maximization of overall income for the whole cooperating community. How can selfish replicators forgo a part of their own replication potential, and use their resources to help each other? Various examples of the evolution of cooperation show that at least five major mechanisms: (1) kin-selection; (2) direct reciprocity; (3) indirect reciprocity; (4) network reciprocity and (5) group selection; help the survival of cooperation, if the selfish replicators play repeated games restricted to their neighbours in a network context.[21–24]

Let us illustrate the costs of cooperation on the rather extreme examples of self-destructive cooperation. Here cooperation has an especially high price: the loss of reproduction or death of the cooperating individual upon offering a higher accessibility of public goods for the overall population. Obviously, the sacrifice of self-destructive cooperation can be typical only to a limited fragment of the whole population, since otherwise it would lead to a general extinction.[25] A number of examples of self-destructive cooperation from bacterial lysis to examples of human behaviour are listed in Box 2.[26–33] Interestingly, even general, network-related benefits of cooperative behaviour can be perceived as special forms of self-destructive cooperation. Cooperators must form cooperation-enriched islands to survive, and the existence of hubs may also overcome the temptation to defect[21–24,34,35]. If we consider the costs of maintaining a network contact, both the emergence of densely connected islands and – especially – hubs (i.e. network marvels, who have countless numbers of acquaintances) may also be perceived as a rather costly, and in part, even self-destructive act, since these individuals certainly have less energy and time for their own offspring.

Cooperation was necessary to build the growing layers of embedded network hierarchy including the emergence of eukaryotic cells, multicellularity and eusociality.[20] Recently Richard E. Michod[36] developed a plausible model to show that in case of a hierarchical

network, cooperation transposes fitness from the lower level (e.g. from the costs of the individual cells) to the higher level (e.g. to the benefits of the multicellular assembly) and in this way it may extend the 'fitness-window' offering novel means of survival. In these non-zero-sum games cooperation acts as a mediator of conflict reducing the selection inside the organism or group, and increasing the selection between organisms. A similar effect has been mentioned before as group-selection, which is the 5th major mechanism of game theory promoting cooperation.[24] The recent work of Efferson et al.[37] demonstrates the same transposure of fitness from the individuals to their social group showing that cultural processes (e.g. the introduction of discriminating markers for group identification) can favour the evolution of previously unfavourable behavioural traits, like altruistic cooperation.

The importance of cooperation between the elements of growing layers of the hierarchical complexity of biological systems can be perceived as an effort of the self-organizing complex systems to become more autonomous by building in an increased amount of complexity and flexibility,[38] as well as to stabilize as large segment of their environment as possible. An example of this latter behaviour is the network-building property of horizontally transferred genes in the host's regulatory network.[39] However, the preservation of the evolvability of the system requires the preservation of non-cooperating, creative network elements as sources of innovation and network plasticity. These creative elements are exemplified by active centres of enzymes, molecular chaperones of cells, stem cells of complex organisms, or creative persons of social networks. Creative elements perform a random sampling of the whole network, and provide flexible links between variable network communities. The continuous jumps of creative elements exclude their prolonged cooperation with any of the constituent network groups.[34,40]

Two evolutionary strategies of survival: multiple cases of simultaneous ageing and cooperation

In the following two sections we outline our major hypothesis arguing that cooperation has another important cost besides those mentioned before: ageing. First, we summarize two major evolutionary strategies of survival, called small and big phenotypes at the level of various individuals,[41] or r/K-strategies at the level of ecosystems,[42] and show that in both strategy-pairs cooperative behaviour is associated with a greater predominance of ageing. Continuing the examples of simultaneous ageing and cooperation we show that anti-ageing homologues are significantly enriched among the 'non-cooperative/cheater genes' of the amoeba, *Dictyostelium discoideum*.[43] Finally, we summarize the properties of competitive, 'forever-young' networks, *versus* the cooperative, 'always-old' networks, and show that cooperation not only induces, but also helps a successful ageing process.

As we mentioned before, complex systems often show biphasic behaviour. Our first example of cooperation-related ageing is the small phenotype of the small and big phenotype-pair of humans and a wide range of other organisms summarized recently by Bateson et al.[41] In this dual strategy, small phenotypes with small size and slow metabolism become accommodated

to adverse circumstances, while big phenotypes may enjoy a larger size and a rapid metabolism due to more abundant resources. With a large individual variation, small phenotypes have a small number of offspring, cooperate in their nursing, have a long life expectancy, and in the meantime display numerous signs of gradual ageing. Here ageing is predictable and long, cooperation is tight and local, and it is very often restricted to a small subset of individuals, e.g. the family. On the contrary, individuals of the big phenotype have a larger number of offspring, are rather competitive instead of cooperation, and show less typical signs of gradual ageing. Here ageing is non-predictable and abrupt, cooperation is loose and mostly occurs in a highly global sense, meaning the exploration and integration of the whole large community and environment. As an example for the transition of global to local cooperation as well as of non-predictable/abrupt ageing to predictable/long ageing, caloric restriction has been shown to prolong life giving a chance of long and successful ageing. Caloric restriction also induces a reduction of competition-related fertility, and closely resembles the transition of the big small phenotype behaviour.[44,45] However, this transition is not immediate, but may require as many as three generations for completion.[41]

A similar dual strategy can also be observed at one level higher in the network hierarchy, in the ecosystems. The well-known r/K selection theory of ecology describes the emergence of two types of individuals: (1) r-strategists produce a lot of offspring, each of which has a relatively low probability of surviving to adulthood, and occupies every available niches, while (2) K-strategists live in crowded niches investing a lot to their relatively few offspring, which have a high probability to survive to adulthood. K-strategists often enjoy long life expectancies and populate relatively stable and predictable environments.[42] K-strategists tightly cooperate in the local sense restricting their cooperation to a small group, e.g. their family. On the contrary, r-strategists loosely cooperate in a global sense meaning the exploration and integration of the whole community and environment. Bacteria and yeast are typical r-strategists, where tight, local cooperation is usually not prevalent, and the small life-expectancy does not allow the accumulation of massive age-related damage. Moreover, bacterial and yeast r-strategists often ‘shed off’ damaged proteins and other cellular components by asymmetric cell division, which segregates most of the damaged material to mother-cells, producing a ‘fresh’ daughter-cell as an offspring.[46,47] Thus, many r-strategist organisms die non-predictably and abruptly, and do not age in the predictable and long sense of the phenomenon. On the contrary, K-strategists, while enjoying a long life and cooperating tightly and locally to protect and raise their offspring, accumulate all the damage to the extent r-strategists never reach. Similarly to the transitions of the big and small phenotypes mentioned above, the ratio of r- and K-strategies may also change according to the environment. Moreover, many organisms may also display an intermediate state. As an example of this, mice and other rodents can mostly be categorized as r-strategists, while showing a lot of aspects of cooperation and ageing.

An interesting example of the r/K-strategy change is the change in the soil microbial community after meadow mowing. Mown-meadow allows the growths of globally, loosely

cooperating r-strategists, due to the weakened competition of the grass for the available resources. On the contrary, un-mown-meadow has a predominance of locally, tightly cooperating K-strategists due to the intensified competition with the grass surrounding for resources.[48] The mown un-mown; r- K-strategy transition resembles well to the transition of caloric restriction – observed now at the community level.

We summarize the major properties of the two dualities of small and big individuals as well as of r- and K-strategists[41,42] in Table 1. The globally cooperative strategy may appear as a big phenotype at the level of the individual, and as an r-strategist at the level of the whole ecosystem. Conversely, the locally cooperative strategy usually appears as a small phenotype at the level of the individual, and as a K-strategist at the level of the ecosystem. Global cooperation is loose, and it is accompanied with local competition due to the speedy proliferation and network expansion of big/r-strategists. On the contrary local cooperation is tight, due to the slow, restricted, sparing life of small/K-strategists. The local character of tight cooperation is also in agreement with the preference of cooperating, local islands as described before.[21,24,34]

As a summary of this comparison, globally, loosely cooperating, locally competing big individuals, or r-strategists remain fast growing, expanding 'forever young' organisms, and achieve their success in this way. On the contrary, locally, tightly cooperating small individuals, or K-strategists behave as slow, wise, farseeing, 'always-old' organisms, and achieve their success in this way.

As we noted before, most of the times none of the above strategies exists in their pure form. In agreement with the general picture outlined before, the transition between the global locally cooperative strategies resembles to the previously mentioned topological phase transition of networks occurring at the reduction of resources, i.e. in times of prolonged stress.[5–7] As an extension of this analogy, in simple game models environmental stress promoted local cooperation even under circumstances, when cooperative behaviour was too costly and was avoided in normal conditions.[49] Thus the occurrence and ratio of global or local cooperative strategies depends on the environment. This is especially typical to r- and K-strategists, which describe this behaviour in context of the environment.

To provide a second example showing the correlation of ageing with cooperative behaviour we analyzed the recently described *Dictyostelium discoideum* model, where a rich genetic background of asocial, defecting, cheater (or cooperating, loser) mutants was uncovered allowing the hosting amoebas to produce more (or less) than their fair shares of spores in mixed colonies.[43] We have searched for the homologues of these genes and determined, if these homologous genes had been demonstrated to play any role in the ageing process. The results are summarized in Table 2. We found six cheater genes, the synaptogenic Unc-10,[50] the stem cell- and NMDA receptor-related Nfyc/NARG2,[51,52] a member of the *old-1/old-2*

tyrosine kinase family,[53] the Ca-calmodulin activated kinase kinase 2 (CAMKK2),[54] the ubiquitin-conjugating enzyme 2 and the mitochondrial fission-related chondrocyte protein with a poly-proline region (CHPPR),[55] which all participate in various processes hindering the ageing process. Notably, the best documented case of the six 'anti-ageing' genes, the Unc-10 homologue, was among the 6 strongest, 'conditional' cheaters, which produced more spores under the experimental conditions of the test applied in the original paper.[43] Six out of the tested 31 'cheater' and 6 'loser' genes may not seem to be a large hit-rate. However, we would like to note that in the unlisted 31 cases the lack of identified homologues (11 cases) or the lack of available biological information was rather predominant. Moreover, we did not find a single example, where the contrary of the expectations was ever described. We believe that this uneven occurrence of data gives a rather strong support to the note that the genetic background of the non-cooperating phenotype is helpful to reduce the deleterious consequences of ageing. This assumption is further substantiated by the fact that screening of the residual 161 genes examined in ref. 43 resulted in 6 additional genes related to ageing or longevity listed in the legend of Table 2. When identifying these genes we took all possibilities into account including their participation in neurodegenerative diseases, which is only indirectly related to the ageing process. In spite of this rather generous sampling the difference between the likelihood to find a 6-gene cohort in the 31, or 161 gene-samples is highly significant ($p < 0.005$ using the chi-square probe), which shows that ageing-related genes are significantly enriched among the cheater genes of *Dictyostelium discoideum*. In summary, these data provide an additional piece of corroborative evidence that ageing is a price for cooperation.

As a third and more general example, on Figure 1 we illustrate the typical network structure of both a competitive and a cooperative network. We highlight the main structural features of the two systems in the followings.

Globally, loosely cooperative, locally competitive systems (Figure 1A)

- have a looser network structure with a large number of predominantly weak links
- are less integrated locally, but more integrated globally
- have large overlaps of their modules, and
- have a suppressed importance of their network skeleton.

On the contrary, locally, tightly cooperative systems (Figure 1B)

- have a tight local structure with a small number of predominantly strong links
- are more integrated locally, but less integrated globally
- have small overlaps of their modules, and
- have a key importance of their network skeleton (for details, see Suppl. Table 1 of ref. 34).

Globally, loosely cooperative, locally competitive systems resemble to the stratus-type, or 'stringy-periphery' networks, while locally, tightly cooperative systems are similar to the cumulus-type, or 'multi-star' networks.[56,57]

Networks of globally cooperating, loose systems are good solutions in a rapidly changing environment posing a continuous challenge to the network with novel and novel stimuli providing a resource for restructuring and a chance for dynamic damage control, which delays ageing. Globally cooperative, loose systems have a predominance of competitive constituents. Having this hostile internal structure, globally cooperative, loose systems have to maintain their integrity with efficient and dynamic mediation of conflict between their competing parts. If this is not successful, globally cooperative, loose systems may lose control over their competing parts, and stochastically, all-of-a-sudden disintegrate. This process resembles to the necrosis of the cells.

On the contrary, networks of locally, tightly cooperating systems are good solutions in a constant environment lacking novel stimuli and lacking ample resources for continuous restructuring. This stability allows the development of optimal responses to regularly expected changes, and due to its rigidity suffers from the accumulation of damage causing the accelerated senescence of these, locally cooperating, tight systems. Locally cooperating, tight systems may delay ageing by the development of efficient repair mechanisms. The repair systems become less and less efficient as the locally cooperative system ages. Thus, locally cooperative, tight systems die in a gradual, well-planned manner. This process resembles to the apoptosis of the cells.

An ageing network makes the older nodes rather isolated, and allows only a limited local spread of information instead of global coupling. The limited availability of resources in an ageing network reduces the number of links, which diminishes modular overlaps, and gives and increased value of the remaining, tighter local structures and network skeleton.[18,58] These age-induced changes of network structure closely resemble the structure of locally cooperative, tight networks, which shows that the two processes, the emergence of continuous, unchanged local cooperation, and the age-induced adaptation go hand-in-hand, and enhance each other in network evolution.

Ageing as a price of both local and global cooperation

In the previous section we demonstrated the co-occurrence of cooperation and ageing in many examples, including the duality of locally cooperative/tight/ageing/gradually dying and globally cooperative/loose/locally competitive/stochastically dying evolutionary strategies; the enrichment of the anti-ageing gene homologues of the non-cooperating genes of *Dictyostelium discoideum*; and the description of the similarities of cooperative and aged network structures. Here, we extend our hypothesis showing that cooperation leads to ageing, both

(1) ***at the level of the complex network***, where the well-defined, non-overlapping modules of the network locally cooperate with each other; as well as

(2) ***at one level higher***, where the whole, overlapping, integrated network cooperates globally with similarly integrated networks.

The two types of ageing, the slow and predictable ageing versus the fast and stochastic ageing described below represent an extension of the network theory of ageing described before.[16–19]

(1) **Slow, predictable ageing gradually leading to death.** The balance of repair and damage can be disturbed ***by over-optimization***, where repair becomes predominant over damage, cooperation of network elements leads to their over-specialization. Here the elements form the rigid, locally integrated network of Figure 1B typical to 'always-old' organisms. Ageing here is slow, and rather predictable. If this process goes to the extreme, the system may run into an 'over-optimization catastrophe', which resembles to the robust apoptotic process of programmed cell death.

(2) **Fast, stochastic ageing causing a sudden death.** The balance of repair and damage can also be disturbed ***by over-perturbation***, where damage becomes predominant over repair, and the global cooperation of the whole, overlapping, integrated network (resembling to the 'always-young' network of Figure 1A) at one level higher exhausts network resources leading to increased damage and noise. Ageing here is fast and stochastic. If this process goes to the extreme, the system may run into an 'over-perturbation catastrophe', which resembles to the stochastic disintegration of cell necrosis.

We illustrate the two types of ageing related to the two levels of cooperation on Figure 2.

Over-optimization of a network may occur, when the environment is stable, and the network has a long, undisturbed time to adapt to a single set of environmental conditions. Under these circumstances networks become rigid and shed off a large segment of their original richness, which had helped them to adapt to the changing environment. A good example of this phenomenon is the reductive evolution of symbiotic organisms, which lose a large segment of their original genome, and form a tightly cooperating metabolic unit inside their host.[59] Another example of network over-optimization is cell differentiation, which leads to cellular senescence. Differentiated cells represent the cooperating small phenotype as opposed by the proliferating, non-cooperating big phenotype of the tumour and stem cell lineages, where cellular senescence never occurs.[60] Over-optimization certainly leads to the excessive loss of symmetry, which was shown to be related to the ageing process. Moreover, both the maintenance of symmetry and the repair of over-optimized cells are costly, which accelerate both the development and ageing of the differentiated, small-phenotype cells during limited resource availability. This connects the over-optimization scenario both to the antagonistic pleiotropy and the disposable soma theories of ageing.[61,62] Network closure, i.e. the development of tightly connected network subsets helps local cooperation, but, when becoming predominant, prevents adaptability and innovation even in social networks.[63] Thus, the over-optimization of network structure to local cooperation develops the rigid, locally integrated network of Figure 1B, which is typical to the 'always-old', slowly ageing organisms.

The over-perturbation of a network may occur, when network perturbation and concomitant damage becomes so extensive and continuous, which exhausts the available resources including the repair capacity. An interesting example of resource exhaustion may develop during the switch from fermentation to respiration in yeast at high population densities (Box 3.).[64–66] Here respiration may be regarded as a cooperative strategy, since it does not consume glucose and other energy resources in an inefficient, but fast way as fermentation does. However, the cooperative respiration may lead to an increased production of free radicals, which becomes especially true, when collection efforts of the sparsely available glucose lead to respiration-bursts increasing the level of perturbation further. The low energy resources compromise the repair mechanisms inducing the accumulation of oxidative damage and a consequent ageing.[16,44,67] As an extension of the fermentation/respiration duality, tumour cells are typical competitors: they grow rapidly, most of the times opt for fermentation and do not typically age.[65] We have to emphasize that the above example is true only in the case, when the equilibrium of increased oxidative damage and limited repair capacity becomes severely unbalanced. Moreover, recently more and more examples are published, which question the predominant role of oxidative stress in ageing.[67] This emerging controversy may reflect the dual role of oxidative stress as both a trigger of repair functions, and a continuous burden leading to an overload of the repair mechanisms.

The fermentation to respiration switch of yeast at high population densities was already an example of potential cooperation-induced exhaustion of system resources, where cooperation occurred not at the level of network elements (i.e. proteins of the yeast cell) but between entire networks represented by the yeast cells themselves. Intercellular cooperation may generally compromise intracellular repair mechanisms. As a rather general example there is an increasing overlap between signalling networks and repair functions as we proceed from unicellular organisms to humans. The key signalling elements of the Ras-family, the p53 protein, the poly-ADP-ribose-polymerase, and the critical node of insulin signalling, the Akt/PI-3-kinase complex all overlap with critical repair functions. Conversely, the molecular chaperone families, which provide a central mechanism of protein repair, have numerous connections to various signalling pathways.[68,69]

The repair function at even one level higher, at the level of organ-repair exemplified by wound-repair or bone-repair requires the action of the Hedgehog, Notch, TGF-beta, Wnt and growth factor signalling pathways of stem cells or other cell types specialized to the repair of various tissues. All these organ-repair signalling pathways have an increasing overlap with the inter-organ communication pathways of the (1) nuclear hormone receptors, (2) JAK-STAT cytokine signalling and (3) insulin as we go from simple multicellular organisms to humans. Here again, a significant suppression of organ-repair capacity may occur by the increase of inter-organ communication during embryo development.[69] The examples can be continued: several layers higher in complexity, much less attention could be paid for the ‘repair’ of brokers’ physical and mental health during the recent economic turmoil than at former, ‘business as usual’ situations. Indeed, during the selling and buying frenzy the

intensive cooperation of brokers and clients certainly exhausted their resources (not only financially, but also personally).

As we have seen from the above examples, cooperation may occur at all levels of complexity. When the organisms want to catch a lot from the multitude of surrounding resources, they may re-organize their hierarchical networks suppressing local cooperation, while expanding global exploration and successive global cooperation. However, during stress and resource-exhaustion the loose, global cooperation will be inhibited, and the remaining resources become re-channelled to maintain a tight, local cooperation. The former, resource-rich situation may lead to the suppression of repair leading to the sudden ageing of the 'forever-young' network in case of an over-perturbation, while the latter, resource-poor situation may cause the over-optimization of the network, inducing an aged, 'always-old' network structure. However, the ageing of the individual networks may not necessary cause the ageing of the network of networks at one level higher of the hierarchical complexity.

Network elements (representing constituent networks themselves) age differently and at different speed. As we described before, non-cooperating, creative elements may preserve the 'forever young' phenotype even in the middle of an 'always-old' network.[40] Creative entrepreneurs in the age of 50 preserve a risk-taking behaviour typical to the age of 25, which gives a good example of the slow ageing of these non-cooperators.[70] Recent data suggest that the senescence of creative elements, such as stem cells, is an especially important step in the ageing process of the whole complex system.[19] The emerging diversity of either differently over-optimized or differently damaged networks may actually trigger their cooperation. This shows that the age-induced development of diversity and the emergence of cooperation once again, go hand-in-hand, and enhance each other in a successful community.

Ageing as a price of complexity

In this section we extend our hypothesis outlined in the previous section proposing that cooperation has the additional cost of ageing in a large variety of organisms. In the extension we go beyond the multiple examples of the past section, and show that the above hypothesis can be generalized to all major steps of evolution.[20,71–76] All these major steps led to the development of a higher level of complexity, which required the cooperation of more and more complicated parts as detailed in Table 3. Simultaneously with the occurrence of cooperation, in all these evolutionary innovations novel types of cooperation-related constraints have been introduced again and again, which all led to various forms of over-optimization and unbalance between repair and accumulating damage, causing an ageing-like process at the respective level of complexity.

We summarize the most important appearances of evolution-related ageing-type processes in the following examples.

- During the assembly of the protocell, the development of macromolecular complexes and networks led to the appearance of 'hot spots', i.e. macromolecular segments accumulating

a high amount of local energy as well as collecting and amplifying the perturbations of the whole, integrated system.[40,71] The amplification of perturbations not only gave excellent chances for increased catalytic actions, but also concentrated the damage and required the emergence of repair mechanisms.

- The evolution of coordinated replication, transcription and protein synthesis induced increasing physical constraints as well as coordination problems of adjustable transport and speed. All of these led to an increase in the local molecular damage as well as in the synthesis of truncated or damaged RNA-s and proteins. The accumulation of such types of damages is a typical sign of the ageing process.
- The development of eukaryotes required the cooperative action of respiration as detailed in Box 3, which set free a continuous bombardment of free radicals, which significantly contribute to the damage-load leading to ageing.
- The development of sexual reproduction induced a variety of sexual conflicts, which have a well-documented contribution to the ageing process.[72–74]
- The appearance of multi-cellular, differentiated organisms not only required a sophisticated and often malfunctioning transport system of nutrients and oxygen, but also led to the damage-inducing amplification of the perturbations at one level higher in the cellular networks of the neurons, immune and muscle cells etc.
- The development of social networks invoked various types of psychosocial stress, which – if experienced in a chronic form – rapidly promote ageing.
- Finally, the current development of globalized communication and transportation networks caused an information-overload, and an acceleration of everyday life, which led to previously inexperienced types of civilization stresses, and caused a massive environmental pollution leading to the currently experienced climate change and the ageing of the global ecosystem.

This chain of events shows that (1) cooperation is a general feature of all major evolutionary innovations at higher and higher levels, and (2) all novel forms of this cooperation evoked novel types of accumulating damage, leading to an ageing-like phenomenon of the respective complex system. Thus, ageing emerges not only as a price of cooperation, but also as a price of the emerging complexity of the self-organizing matter.

Conclusions

In conclusion, in this paper we have outlined the hypothesis that cooperation generally leads to the ageing of cooperating units. First, we gave several examples, where ageing co-occurred with cooperation: (1) we showed that in two major evolutionary strategies of survival cooperative behaviour is associated with a greater predominance of ageing (Table 1.), (2) we listed the enriched anti-ageing homologues of ‘non-cooperative, cheater genes’ of the amoeba *Dictyostelium discoideum* (Table 2.) and (3) we described the resemblance of locally cooperative network structures to that of the aged, ‘always-old’ network (Figure 1).

Next, we outlined an extension of the “network theory of ageing” hypothesis showing that the equilibrium of repair and damage may become unbalanced by (1) local cooperation leading to

an over-repaired, over-optimized, ‘always-old’-type network structure ageing slowly, and dying in an predictable, apoptosis-like process. The equilibrium of repair and damage may also become unbalanced by (2) global cooperation over-perturbing the system, and exhausting its resources leading to inefficient repair, fast ageing and death in a stochastic, necrosis-like process (Figure 2). We gave the reductive evolution of symbionts and cell differentiation as examples of over-optimization-induced cellular senescence. We related over-optimization to the antagonistic pleiotropy and disposable soma theories of ageing. To illustrate the multitude of cooperation-related cases leading to resource exhaustion we listed (1) the production of age-promoting free radicals by the respiration-driven cooperative use of external glucose in yeast and differentiated cells (as opposed to fermenting tumours, Box 3), (2) the overlap of signalling pathways with cellular repair mechanisms and (3) the overlap of tissue repair-related and inter-organ communication-related signalling pathways all raising significant conflicts of interest in using the resources of the cells. We also gave examples for over-optimized structures and over-perturbing, resource-exhausting situations in social networks.

Finally, we extended the hypothesis and showed that the cooperation of more and more complex units was a necessary behaviour in all major steps of evolution, and it induced a novel, ageing-like phenomenon at each of these evolutionary innovations (Table 3). Thus, ageing emerges as a price of complexity, which is not only induced, but also helped by cooperation going hand-in-hand, and enhancing each other in a successful community.

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Box 1. Phenotypic, functional robustness of biological networks

Robustness of biological networks at the level of network function (i.e. at the level of the phenotype of the complex system described by the network) is helped by a number of mechanisms.

1. ***Strong links*** (meaning intensive, high probability, high affinity interactions) often form negative or positive feedbacks helping the biological system to return to the original state (attractor) or jump to another, respectively. This systems control enables the system to move between two stable states.
2. The contribution of ***weak links*** (meaning non-intensive, low probability, low affinity interactions) is more diffuse. Weak links provide (i) alternative, redundant, degenerate pathways; (ii) flexible connections disjoining network modules to block perturbations and re-assembling the modules in a slightly altered fashion, and (iii) additional, yet unknown mechanisms buffering the effects of the original perturbation further, and de-coupling physical perturbations from functional activities. We must note that weak links grossly outnumber strong links in cellular networks, which may often make the effects of their fail-safe mechanisms larger than those of negative or positive feedbacks.
3. Finally, robustness of cellular networks is also helped by an ***increased average robustness of their elements*** (e.g. proteins), which, most of the time are networks by themselves.[2,12]

Box 2. Examples of self-destructive cooperation

1. Bacterial virulence factors leading to destruction by the innate immune system
- *Salmonella typhimurium* Type III secretion systems and flagella enhancing gut inflammation and colonization[26]

2. Bacterial lysis
- *Streptococcus pneumoniae* pneumolysin helping lung colonization[27]
- *Clostridium difficile* TcdA enhancing gut inflammation and colonization[28]

3. Bacterial quorum sensing leading to reduction of virulence
- *Pseudomonas aeruginosa* LasR quorum sensing regulator enhancing survival in dense populations[29,30]

5. Yeast invertase enzyme secretion
- *Saccharomyces cerevisiae* Suc2 invertase secretion enhancing the availability of glucose[31]

6. Hara-kiri of neutrophil granulocytes
- neutrophils kill themselves by releasing extracellular structures of chromatin and granule proteins called neutrophil extracellular traps (NETs) to capture and kill invading bacteria[32]

7. Animal infertility
- workers of insect colonies enhancing offspring survival

8. Alarm calls
- several animals, e.g. marmots, risk self-sacrifice warning other members of their community on the appearance of a predator[33]

9. Soldiers' heroism

10. Altruism of nuns, monks and catholic priests

11. Blood donation

12. Charity donations

In many of the above examples the benefit is not evident at the level of the immediate community of the self-destructive cooperator, but becomes manifest at one or more levels higher in the hierarchical complexity of networks. As an additional example of these multi-layer effects, we may consider antibodies 'altruistic', when they sacrifice themselves co-degrading with the antigen for the health of the hosting organism living at least two levels higher in the hierarchical complexity.

Box 3. Respiration, cooperation and resource-exhaustion: their occurrence in yeasts, tumours, during the evolution of multicellular organisms and consequences in ageing

Experimental evidence of yeast cells[64] shows that respiratory ATP production yielding about 32 mol of ATP per mole of glucose can be regarded as a form of cooperation. On the contrary, fermentation (having a 16-times lower yield, but a much higher rate of ATP production) is a defective strategy, which uses up the available glucose in an extremely fast manner forcing the competitors to starve. In agreement with the features of competitive and cooperative strategies outlined in Table 1, at a low level of resources cooperation wins over asocial behaviour. However, at a high level of resources non-cooperating, fermenter yeast cells progressively outperform cooperating respirators. Similarly to this, tumour cells most often use fermentation instead of respiration, which is in agreement with their non-cooperating, invasion-prone behaviour.[65] Thus respiration emerges as a pre-requisite of the cooperation necessary to maintain a multicellular organism at high cellular density and low amount of resources. However, respiration (especially respiration-bursts) may lead to the release of free radicals, which promote the ageing of cooperating cells.[66] In agreement with this tumour cells opting for the competitive fermentation do not typically age. Thus the switch from fermentation to respiration is not only a key, cooperating step in the evolution of multicellular, complex systems, but in parallel with this respiration-driven free radicals may exhaust the repair capacity of the host causing an accelerated senescence of the constituent cells of the multicellular organism.

Table 1. General properties of the two major evolutionary strategies of survival: the association of cooperation and ageing

Property	**Global, loose cooperation** (big phenotype, r-strategist)	**Local, tight cooperation** (small phenotype, K-strategist)
Level of cooperation	low	high
Community-control	loose	tight
Fluctuation, noise, creativity	high	low
Ageing	atypical, fast	typical, long
Death	unpredictable	predictable
Efficiency of solving simple, goal-oriented tasks	low	high
Sustainability in a changing environment	high	low
Status and fate in a stable environment with a low intensity of input, resources	gradual, stochastic disintegration, fast ageing, and unpredictable death	optimal
Status and fate in an unstable environment with a high intensity of input, resources	optimal	accumulation of damage, long ageing, leading to a predictable death
Summary of strategy	**'forever young'**	**'always old'**

Table 2. Ageing/longevity-related homologues of asocial (non-cooperating, cheater, defecting) genes in the amoeba *Dictyostelium discoideum*

Asocial amoeba gene	**Level of asociality**	**Ageing-related homologous gene(s)**	**Relation to ageing/longevity**
DDB0219502	64.7%	Unc-10 (*C. elegans*)	causes an approx. 35% increase in longevity[50]
DDB0191265	63.2%	Nfyc (*C. elegans*); NARG2 (human)	involved in developmental processes in stem cells and neurons[51,52]
DDB0191503	62.3%	Src-2 (*C. elegans*)	member of longevity inducing *old-1/old-2* tyrosine kinase family[53]
DDB0220010	56.8%	CAMKK2 (human)	preservation of synaptic plasticity in ageing[54]
DDB0187308	56.0%	Ubiquitin conjugating enzyme E2	participation in proteasomal degradation of damaged proteins
DDB0169123	52.2%	CHPPR (human)	participation in antioxidation defence[55]

The Table summarizes those genes, which influenced the social behaviour of the amoeba *Dictyostelium discoideum*,[43] and had a homologous gene in the database of GenAge[77] or homologous ageing-related gene or protein in Pubmed. Gene homology has been established by the help of InParanoid[78], Wormbase[79] and Ensembl[80] databases. From the residual 161 *Dictyostelium discoideum* genes examined in ref. 43, we have found 6, which were related to either longevity, or accelerated ageing (in parentheses please find the PubMed ID of the respective papers, where appropriate): acad8 (PMID: 17387528); DDB0231250 chaperonin; irlB (PMID: 11846374); kynureine aminotransferase (PMID: 7650530); psmD1 proteasomal subunit and rpl10 (PMID: 17174052).

Table 3. Cooperation and ageing at major transitions of evolution

Evolutionary transition[20]	Types of cooperation*	Cooperation-related ageing	
		at one level lower in the hierarchy**	at the same level of hierarchy
primordial soup protocell	• macromolecular complexes • metabolic and signalling networks	• accumulated perturbations at hubs and other key points of macromolecular networks leading to increased local molecular damage[40,71]	• growth-induced tensions and subsequent division of the protocell
independent replicators chromosomes	• coordinated RNA/DNA replication	• increased physical constraints leading to a larger probability of RNA/DNA breaks and damage	• chromosomal aberrations and failures
RNA-world genetic code	• coordinated DNA transcription and protein synthesis	• regulation constraints (e.g. limited monomer availability): synthesis of damaged RNA and protein molecules	• transport- and speed-adjustment insufficiencies leading to propagating damage
prokaryotes eukaryotes	• mitochondrial and membrane networks • rearranged, elimination (proteasome)-centred protein-protein interaction network	• mitochondrial free radical production leading to oxidative damage[66]	• energy surplus causing an accelerated, error-prone synthesis of macromolecules and an elimination-centred macromolecule metabolism
asexual clones sexual populations	• mate-selection[72]	• fertility-induced excesses of metabolism and consequent acceleration of ageing	• sexual conflict-induced ageing[73,74]
protists differentiated multicellular organisms	• hormonal regulation • immune system • neuronal networks	• accumulated perturbations at hubs and other key points of cellular networks leading to increased local damage (muscle injuries, immunodeficiencies, neurodegeneration, etc.)	• insufficient mediation of conflict (civilization diseases, autoimmune diseases, etc.)
solitary individuals social networks	• group-related nursing of offspring • division of labour	• increased longevity (menopause)[2] allowing the development of ageing • stress of group-hierarchy establishment	• accelerated dispersal of homogenous network groups[75,76]
social networks human society	• communication and transportation networks	• information-overload, acceleration-and civilization-induced stress	• environmental pollution (climate-change)

	• globalization		

*In many cases the emerging cooperation helps the elimination of the damage caused by the already established cooperation at one level lower. Thus coordinated replication helps the development of cell division which is an emerging problem of cell growth, the energy surplus of mitochondria helps the repair of the accumulated damage of concerted transcription and translation, etc. The mediation of conflict in the use of resources is often helped by the complexity of cooperation at one level higher in the organization.

**Ageing at this level does not necessarily lead to the ageing of the next, emerging level of complexity.

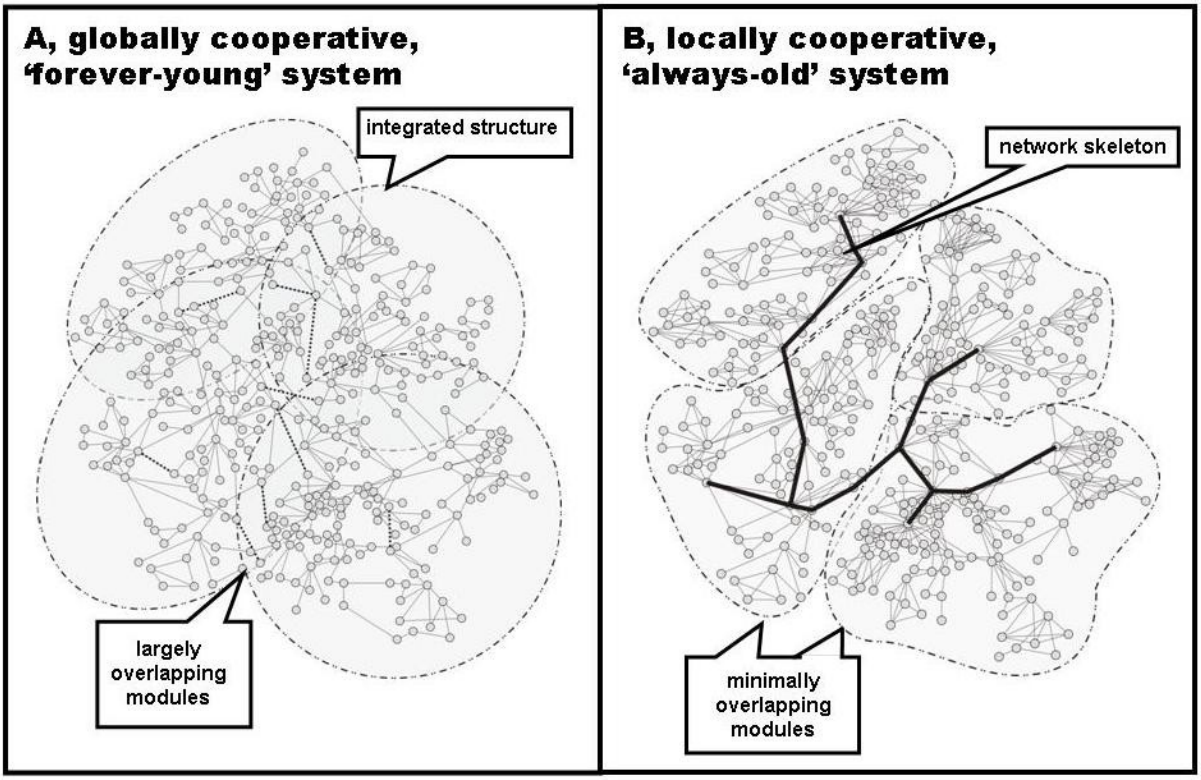

Figure 1. Typical network structure of globally, loosely cooperative, locally competitive, 'forever-young' and locally, tightly cooperative, 'always old' systems. **A:** Networks of globally cooperative, locally competitive systems have a looser structure with a large number of predominantly weak links, are less integrated locally but more integrated globally, have large overlaps of their modules, and have a suppressed importance of their network skeleton. This structure is typical to the exploratory, 'forever-young' systems. **B:** Networks of locally cooperative systems have a tight local structure with a small number of predominantly strong links, are more integrated locally but less integrated globally, have small overlaps of their modules, and have a key importance of their network skeleton. This structure is typical to the restrictive, 'always-old' systems.

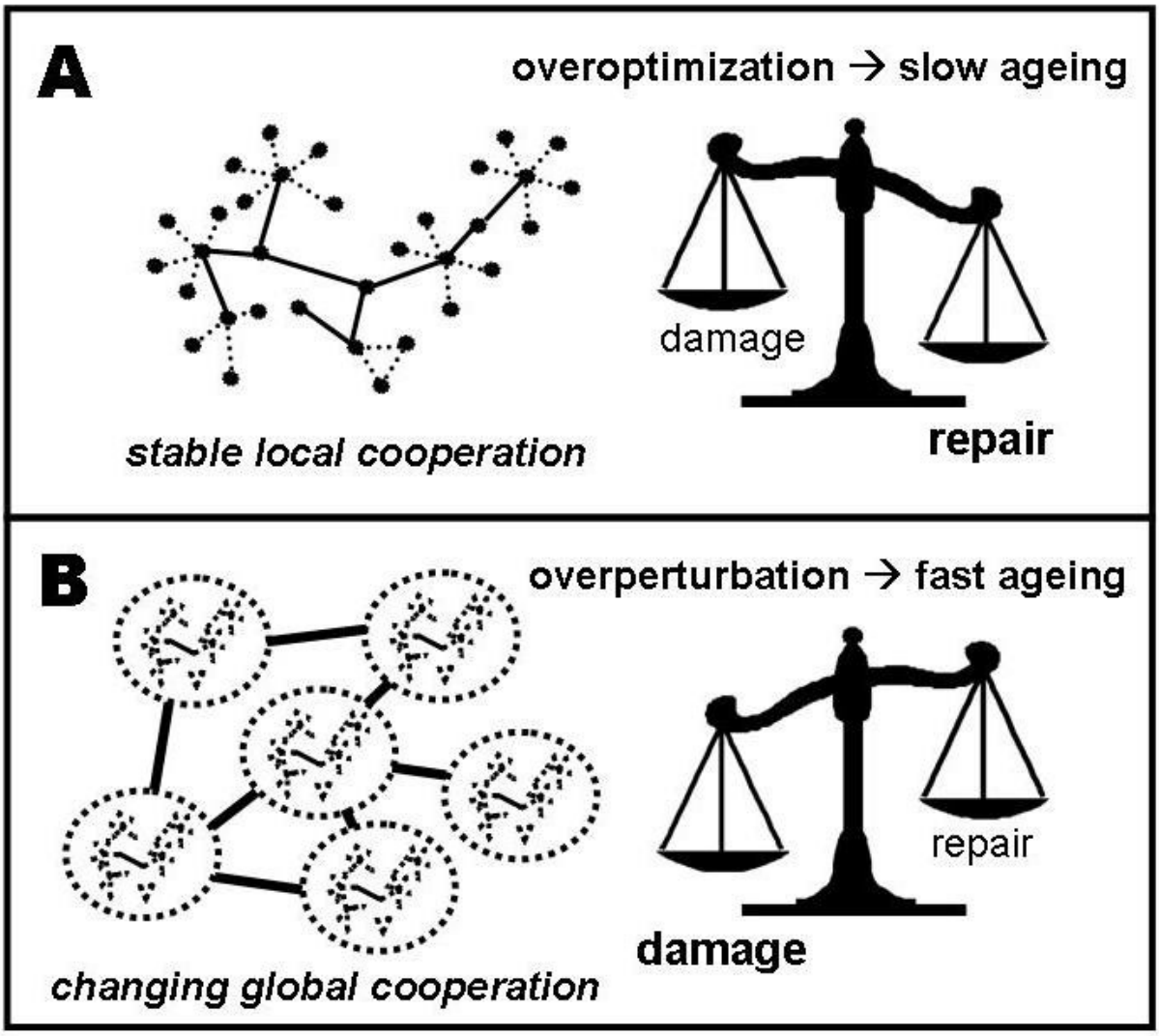

Figure 2. Ageing as a price of cooperation. We illustrate the plenitudes of factors causing an unbalance of repair- and damage-related processes. **A.** The equilibrium of repair and damage may be disturbed by over-optimization, where repair becomes predominant over damage. Here the local cooperation of network elements leads to their over-specialization. Network elements form a rigid, locally integrated network typical to aged organisms with a slow, predictable death. **B.** The equilibrium of repair and damage may also be disturbed by over-perturbation leading to resource-exhaustion, where damage becomes predominant over repair. Here the global cooperation of the whole network at one level higher exhausts network resources leading to the increased damage, noise, disintegration typical to unexpectedly, stochastically and rapidly dying organisms.