The influence of the composition of tradeoffs on the generation of differentiated cells

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We study the emergence of cell differentiation under the assumption of the existence of a given number of tradeoffs between genes encoding different functions. In the model the viability of colonies is determined by the capability of their lower level units to perform different functions, which is implicitly determined by external chemical stimuli. Due to the existence of tradeoffs it can be evolutionarily advantageous to evolve the division of labor whereby the cells can suppress their contributions to some of the activities through the activation of regulatory genes, which in its turn inflicts a cost in terms of fitness. Our simulation results show that cell differentiation is more likely as the number of tradeoffs is increased but the outcome also depends on their strength. We observe the existence of critical values for the minimum number of tradeoffs and their strength beyond that maximum cell differentiation can be attained. Remarkably, we observe the occurrence of a maximum tradeoff strength beyond that the population is no longer viable imposing an upper tolerable level of constraint at the system. This tolerance is reduced as the number of tradeoffs grows.

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I. INTRODUCTION

The evolution of tradeoffs is an issue of intense debate in the current literature as tradeoffs have played a central role in shaping life histories and ecological/evolutionary dynamics in nature [1]. The tradeoff theory is key for our progress in evolutionary understanding. Traits are often linked in ways that prevent simultaneous optimization of all of them, as they reflect biophysical compromises [2-3]. Tradeoffs are referred to as the cost paid in terms of fitness when a beneficial change in one trait is linked to a detrimental change in another [4]. With the massive available data from experiments, especially in microbial populations, this problem has been more effectively addressed [5]. Today it is also known that shapes and magnitudes of tradeoff relationships are strongly influenced by the environment [6].

The presence of tradeoffs is one of the main conditions that are fulfilled by most biological systems for the appearance of the division of labor [7], being its emergence concomitantly favored by other factors [7-9]. One of these factors favoring the emergence of division of labor upon the existence of tradeoffs is developmental plasticity [8]. Developmental plasticity refers to the genotype’s ability to change its developmental processes and phenotypic outcomes in response to environmental changes, and influences the trait expression. Today it is well established that developmental plasticity is critical to the promotion of evolutionary innovation [10]. Indeed, plastic responses to environmental variation plays a key role for species to develop [11-12].

The main goal of the present study is to address the emergence of labor division through the differentiation of cells initiating from undifferentiated units. A key feature of the modelling is the use of the well-grounded acquaintance that formerly diversity in multicellular organisms stems from changes in the regulatory interactions that drive gene expression [13], and so many of the requirements for multicellularity evolved in unicellular ancestors [14]. Sophisticated sensing mechanisms and signal transduction systems in Eukariotic cells allow accurate dynamic outcomes in response to changing environment conditions [15].

Recent theoretical contributions address the evolution of multicellularity and the further specialization of cell types, mainly focusing on the differentiation between somatic and germinative functions [8-15]. Here the division of labor and subsequent differentiation is addressed to the competition among distinct somatic functions in a population which comprises genetically identical cells. Conditions upon the tradeoffs that allow cellular specialization to evolve are investigated. Cellular differentiation can evolve as an outcome of the selective advantage brought by the division of labor between the cells. The process is entirely grounded on regulation mechanisms, as it influences the viability of the aggregates which are formed. We assume the existence of tradeoffs among a set of somatic functions. An illustrative instance is provided by the cyanobacteria whose cells can specialize either in the carbon or nitrogen fixation processing [16-17]. The underlying concept is that there is a set of essential biological functions which contributes to the viability of the organism. These functions are subjected to biophysical constraints that impose tradeoffs between the different functions. These relations can lead to specialization of cells, as it becomes advantageous to develop compartmentalized function in order to reduce the cost brought about by the tradeoffs. The biophysical constraints are not explicitly considered, as it is not our aim to propose

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The tradeoffs are represented by a matrix $T = \{T_{ij}\}$ that measures the strength of the ascendency of the somatic function $j$ on the function $i$. The main gene $Y_i$ encodes for function $i$, whereas the regulatory genes $y_{ik}$ regulates the expression of gene $Y_i$ in a cell undertaking activity $k$. As we know, a cell is a complex self-regulated system that responds in different ways to different set of chemical signalling mechanisms [13]. Here we assume that there is temporal segregation of incompatible activities of cells which are induced by these external chemical signals. The activation of regulatory genes brings a cost, estimated as $c(y_{ik})$. It is assumed that $c(y_{ik})$ is a decreasing function of $y_{ik}$. As the system evolves incompatible cellular processes tend to suppress the expression of genes encoding other functions [15], thus contributing to the formation of aggregates with permanently specialized cellular functions.

The contribution of a cell subject to chemical signal $k$ to the overall somatic function $i$ ($f_{ik}$) of the colony is then calculated as

$$f_{ik} = [(1 - y_{ik})Y_i^{T_{ii}}c(y_{ik})\prod_{j\neq i}[1 - (1 - y_{jk})Y_j^{T_{ij}}]^{T_{ij}}c(y_{jk})].$$

We see that the direct effects of the major genes $Y_i$ increase the corresponding fitness components, whereas there are indirect negative effects of the other genes $Y_j$, $i \neq j$, on the major gene, and thereby Eq. (1) captures the essence of the tradeoff relationships. The case $T_{ij} = 0$ reflects the non-existence of tradeoff between the corresponding pair of genes. As the mechanisms of gene suppression and developmental plasticity embody a cost in fitness terms, it is incorporated into the estimation of $f_{ik}$ through the cost function $c(y_{ik})$, which is a decreasing function of the regulation effect $y_{jk}$. This means that the stronger suppression is more costly it becomes [8, 20]. Without this regulation, Eq. (1) reduces to its simplest form

$$f_i = Y_i^{T_{ii}}\prod_{j\neq i}[1 - Y_j^{T_{ij}}].$$

The cost function $c(y)$ is given by a Gaussian function $c(y) = \exp(-\frac{1}{2}\frac{y^2}{\sigma^2})$. The full expression (Eq. (1)) can be compactly written as

$$f_{ik} = \prod_j [(1 - \delta_{ij} - (1 - y_{jk})Y_j^{T_{ij}}]^{T_{ij}}c(y_{jk}).$$

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The paper is organized as follows. In Section II the model is described. Section III presents the simulation results, and finally Section IV presents our concluding remarks.

II. THE MODEL

The population consists of asexual haploid cells. The model assumes clonal development from a unicellular spore/zygote, thus giving rise to multicellular organisms (colonies) as cells undergo binary fission [8, 14]. Under clonal development genetic variation among cell lineages is relatively low within an organism and basically stems from somatic mutations. During clonal development the cell passes through binary fission processes until the colony at this expansion stage reaches size $S$. After reaching size $S$ each colony goes through an unicellular stage (propagate formation) in case it survives viability selection. Each cell can give rise to new daughter colonies with a given probability that depends on its fertility $f$. The viability

FIG. 1: Panel a: Average number of colonies versus the number of trade-offs $t$ for four biological functions (the remaining parameters are $s = 2$, $S = 16$, $\mu = 10^{-5}$ and $K = 50000$). Panel b: Average viability of a colony versus the number of trade-offs $t$ for four biological functions (the remaining parameters are $s = 2$, $S = 16$, $\mu = 10^{-5}$ and $K = 50000$). Each point is an average over 1000 independent configurations.
FIG. 2: Panel a: Average number of colonies versus the strength of the trade-off $s$ for three biological functions and one (dark blue), two (red), three (green) and six (orange) trade-offs (the remaining parameters are $S = 16, \mu = 10^{-5}$ and $K = 50000$). Panel b: Extinction probability versus the strength of the trade-off $s$ for three biological functions and one (dark blue), two (red), three (green) and six (orange) trade-offs (the remaining parameters are $S = 16, \mu = 10^{-5}$ and $K = 50000$). Each point is an average over 1000 independent configurations.

To estimate the viability of a given colony, first the average contribution of the cells to the fitness components of the group is computed [15], i.e.,

$$f_i = \frac{1}{N_c} \sum_k f_{ik}$$

where $N_c$ stands for the number of cells. The viability is then calculated as the geometric mean of the $f_i$-values, i.e., all functions are considered to be essential. Therefore the viability is expressed as

$$v = \sqrt[N_f]{\prod_i f_i}$$

and $N_f$ corresponds to the number of distinct biological functions. In Ref. [8] the viability of the colony is completely determined by a single somatic function. From the measurement of the viability it ensues that the likelihood a given organism survives to reproduction age equals [21]

$$1 + (S - 1) \frac{N}{Kv}$$

The above equation is a modified version of the Beverton-Holt stock-recruitment model which assumes that the per capita number of offspring is inversely proportional to a linearly increasing function of the number of mature colonies [21]. In Eq. (6) $N$ corresponds to the number of colonies and $K$ denotes the maximum carrying capacity of the population. As aforementioned, $S$ is the size of colony just before the unicellular stage takes place.

A. Summary of the parameters of the model

$s$ (tradeoff strength): In the simplest case the tradeoff strength is uniform, $s$, over all the tradeoffs. Therefore, under the assumption of an uniform tradeoff strength $T_{ij}$ is either equal to zero (if there is no tradeoff between a given pair of genes) or $s$. The assumption of an uniform tradeoff strength will be released later. In such situation, the strength $s$ is not a constant but rather taken from a given probability distribution.

$f$ (fertility): After surviving viability selection each cell of the colony can give rise to a newly formed colony with probability $f$, the fertility of the cell.

$\mu$ (mutation probability): During cell division there exists an uniform probability of mutation per gene, $\mu$. If a mutation takes place in a given gene $j$, $Y_j$ (in case it is a major gene) or $y_{jk}$ (in case it is a regulatory gene) changes to a randomly chosen value from an uniform distribution $[0, 1]$.

$K$ (maximum carrying capacity): The maximum carrying capacity, $K$, corresponds to the population size upon maximum fertility, $f = 1$ (all cells can successfully establish a new colony) and maximum viability, $v = 1$.

$t$ (number of tradeoffs): If the number of biological activities is $N_f$ there are up to $N_f(N_f - 1)$ tradeoffs (that corresponds to the number of degrees of freedom of the $T_{ij}$ matrix), so $t \leq N_f(N_f - 1)$.

III. RESULTS

As aforementioned, the strength of tradeoffs between a pair of genes is better described through the tradeoff matrix $T_{ij}$

$$T = \begin{bmatrix} T_{11} & T_{12} & \cdots & T_{1M} \\ T_{21} & T_{22} & \cdots & T_{2M} \\ \vdots & \vdots & \ddots & \vdots \\ T_{M1} & T_{M2} & \cdots & T_{MM} \end{bmatrix}$$
where the non-diagonal elements are randomly ascribed. The number of non-null off-diagonal elements is \( t \), and the strength of the tradeoff between a given pair of genes is either assumed to be constant \( T_{ij} = s \) or taken from a given probability distribution. Simulation results for the two cases are presented separately.

### A. Constant tradeoff strength

Simulations were run for different number of somatic functions. Unless stated otherwise it is assumed that number of cell before the unicellular stage is \( S = 16 \), mutation probability \( \mu = 10^{-5} \), fertility \( f = 0.5 \), tradeoff strength \( s = 2 \) and carrying capacity \( K = 50,000 \).

Figure 1a shows that the number of colonies is a decreasing function of the number of tradeoffs. As the number of tradeoffs is augmented more specialization is required in order to keep the colony functional. At the individual level, specialization brings a cost in terms of fitness, though it provides a benefit at the group level. This condition is better understood if one looks at the dependence of the mean viability on the number of tradeoffs \( t \) (see Fig. 1b). As can be noticed the viability is a monotonic decreasing function of the number of trade-offs. If there are no tradeoffs the mean viability goes to one, meaning that all traits can be maximised simultaneously as there are no constraints. As tradeoffs are added specialization requires the suppression of the expression of more genes entailing a greater cost in terms of fitness.

In the following, Figure 2a, explores the effect of the tradeoff strength on the evolution of the system. In the plot the number of colonies is shown as a function of the tradeoff strength, \( s \), for several values of the number of tradeoffs \( t \). As a general scenario, it is verified that the augmentation of the strength of the interaction between the genes produces detrimental effect on the viability, and thereby reducing the number of colonies at the stationary state. From the plot it is possible to remark another interesting pattern. As the tradeoff strength is enlarged up to a certain value of \( s \) the number of colonies shrinks, beyond that point the number of colonies remains roughly constant. Subsequently, there exists a second critical value of the tradeoff strength at which the population is no longer viable and then the population goes extinct (number of colonies goes to zero). This is also corroborated from Fig. 2b that exhibits the probability of population extinction versus \( s \). One notices the occurrence of a clear transition from a regime in which the population always persists (probability of extinction equals zero) to a regime where the population is always doomed to extinction and is no longer viable (probability of extinction equals one). As the number of tradeoffs increases the transition region becomes sharper. There are two important features worth mentioning. First, the critical tradeoff strength at which the population is no longer viable decreases with the number of tradeoffs, i.e., the transition region is shifted towards lower \( s \), and thereby the tolerance to the strength of the tradeoff decreases with the number of tradeoffs. Second, the range of the tradeoff strength at which the number of colonies remains constant is also shortened with the number of tradeoffs.

As a next step, now we will see how the evolution of the population subject to tradeoffs can ultimately drive its constituents to the process of differentiation. As a criterion for establishment of differentiation among the cells we propose as a metric the distance \( d \) between the response to two stimuli \( i \) and \( j \), which is calculated as

\[
d_{ij} = \sqrt{\frac{\sum_{k=1}^{n_f} (y_{ki} - y_{kj})^2}{N_f}},
\]

where \( N_f \) denotes the number of biological functions. If the distance \( d \) is higher than \( d_c \) (\( d_{ij} > d_c \)) one considers that there is a differentiated response to those stimuli (i.e., cells subject to different stimuli differentiate into different types). Since \( y_{ij} \in [0,1] \), \( d_{ij} \) also lies in the range between 0 and 1. For our purposes the threshold \( d_c \) is set at \( d_c = 0.2 \), which demonstrated to provide a good criterion for determining the differentiation among the cells.

The number of differentiated types is then the number of different responses to the different stimuli, that is, the number of different phenotypes found in the cells of the same organism. The number of differentiated cells (cell types) are presented when the number of functions is equal to three (see Figure 3). Therefore, in this case the maximum number of distinct cell types that can be reached is also equal to three. Figure 3a shows the ultimate number of cell types versus the tradeoff strength \( s \) and distinct values of the number of tradeoffs \( t \). It is pretty clear that the existence of tradeoffs can strongly favor the emergence of cell differentiation. This can be achieved by either increasing the number of tradeoffs, i.e., the number of non-null elements of the tradeoff matrix \( T_{ij} \), or increasing the strength of those tradeoffs. As the number of functions rises it ensues that the minimum number of tradeoffs needed to attain maximum differentiation is also augmented. It also follows that the lesser the number of tradeoffs the larger the tradeoff strength must be in order to produce maximum differentiation. In Figure 3a the tradeoff strength is now held at \( s = 2 \) and the number of tradeoffs is varied. In that case the number of biological functions is four. In agreement with the previous results, as number of tradeoffs \( t \) grows cell differentiation is facilitated. We see that maximum differentiation is only achieved at \( t = 10 \).

For the sake of completeness we also survey the dependence of the number of colonies on the size of group just before the unicellular state \( S \) and the carrying capacity \( K \). Both quantities influence the survivorship, as inferred from Eq. 4. For number of tradeoffs equal to three the number of colonies exhibits an abrupt growth with \( S \) in the regime of small \( S \) and then saturates for intermediate and large \( S \). However, if the number of tradeoffs is enlarged (\( t = 6 \) in the plot) we already observe an abrupt
FIG. 3: Panel a: Average number of cell types versus the strength of the trade-off $s$ for three biological functions and one (dark blue), two (red), three (green) and six (orange) trade-offs (the remaining parameters are $S = 16$, $\mu = 10^{-5}$ and $K = 50000$). Panel b: Average number of cell types versus the number of trade-offs $t$ for four biological functions (the remaining parameters are $s = 2$, $S = 16$, $\mu = 10^{-5}$ and $K = 50000$). Each point is an average over 1000 independent configurations.

FIG. 4: Panel a: Average number of colonies against its size just before the reproduction stage ($S$), for three biological functions and three trade-offs (the remaining parameters are $s = 2$, $\mu = 10^{-5}$ and $K = 50000$). Panel b: Average number of colonies versus the maximum carrying capacity of the system ($K$) for three biological functions and three (blue) and six (red) trade-offs (the remaining parameters are $s = 2$, $S = 16$ and $\mu = 10^{-5}$). The straight line is a linear fit of slope +1 as expected in the limit of large $K$ (please see Eq. (6)). Each point is an average over 1000 independent configurations.

drop of the number of colonies at intermediate, which owes to the extinction of the population. Indeed, the fall in the number of colonies is also found for $t = 3$ but this effect occurs at much larger $S$. This outcome also shows that the colony size $S$ can not be enlarged without bound as its augmentation reduces the probability of survival. This critical colony size $S$ depends on the number of trade-offs $t$. On the other hand an increased carrying capacity allows the population to hold a larger number of colonies, as expected (please see Fig. 3b). Nevertheless, one can remark that existence of minimum levels of carrying capacity $K$ in such way the population can be sustainable. The minimum value of $K$ required to sustain the population increases with the number of tradeoffs.

B. Variable tradeoff strength

Here we release the assumption of constant tradeoff strength. The tradeoff strength $s$ is a variable quantity drawn from an uniform distribution $s \in [s_{inf}, s_{sup}]$, that also varies across the different pairs of genes. Though $t$ still tunes the number of non-null off-diagonal elements. By changing $s_{inf}$ and $s_{sup}$ the mean value of $s$ and also its variance are modified. In order to compare with the outcomes of the previous section we changed $s_{inf}$ and $s_{sup}$ such that the mean value of $s$ is kept at two, i.e. $\langle s \rangle = 2$. And so here we explore the effect of increasing variance on the ultimate number of differentiated cells. From Figure 5 one can infer that as distribution becomes broader, and so also covering smaller values of $s$, the number of differentiated cells becomes considerably smaller in comparison to the case of constant trade-offs. At the extent the variance is reduced the number of differentiated cells readily approaches the outcome seen for constant strength, corroborating the finding that not only the existence of tradeoffs but also their magnitudes are essential for promoting cell differentiation.
We have investigated how cell differentiation can arise as a consequence of division of labor, which in its turn evolves due to developmental plasticity. The study is performed under different scenarios for the distribution of tradeoffs. Here we have assumed that fertility is constant thus restricting the analysis to the differentiation of cells concerning their somatic functions. In the beginning of the evolutionary process the cells are completely undifferentiated, which means that they undertake any function regardless of the chemical stimuli. As evolution proceeds they can suppress their contributions to some of the functions and mostly contribute to one or few tasks through the activation of regulatory genes that can suppress some of their activities when exposed to a given chemical stimulus. Although beneficial from the group perspective, the suppression mechanism produces a cost at the individual level. As we can tune the number of tradeoffs but also their strength it is possible to decouple these effects on the process of cell differentiation.

Importantly, we have noticed that the tradeoffs affect not only the outcome of the division of labor but also the viability of the population as a whole. At the same time, an increased number of tradeoffs and their strength contribute to the development of division of labor it also reduces the average viability of the population, and in extreme scenarios can even lead to the population extinction. The magnitude of the tradeoffs that can be tolerated by the population decreases with the number of tradeoffs. Although tradeoffs can strongly influence population’s viability it also enhances the likelihood of differentiation. We have observed that maximum differentiation, when the number of cell types equals the number of functions, is reached when the number of tradeoffs increases, while the strength of tradeoff required to attain this outcome is reduced.

Conclusions

FIG. 5: Average number of cell types as a function of the number of tradeoffs \( t \) for three biological functions. Here the tradeoff strength is variable and drawn from an uniform distribution. The blue points correspond to a constant tradeoff \( s = 2 \), the red points denote an uniform distribution with \( s \in \{1,\,3\} \), whereas the green points also denote an uniform distribution with \( s \in \{0.5,\,3.5\} \). The remaining parameters are \( S = 16, \mu = 10^{-5} \) and \( K = 50000 \). Each point is an average over 1000 independent configurations.

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References

[1] Y. Saeki, M. Tuda, and P. H. Crowley, Oikos 123, 786 (2014).
[2] I. Gudelj, R. Beardmore, S. Arkin, and R. MacLean, Journal of evolutionary biology 20, 1882 (2007).
[3] J. R. Meyer, I. Gudelj, and R. Beardmore, Nature communications 6 (2015).
[4] S. C. Stearns, Functional ecology 3, 259 (1989).
[5] R. C. MacLean, Heredity 100, 471 (2008).
[6] C. M. Jessup and B. J. Bohannan, Ecology Letters 11, 947 (2008).
[7] C. Rueffler, J. Hermisson, and G. P. Wagner, Proceedings of the National Academy of Sciences 109, E326 (2012).
[8] S. Gavrilets, PLoS Comput Biol 6, e1000805 (2010).
[9] G. Boza, A. Szilágyi, A. Kun, M. Santos, and E. Szathmáry, PLOS Comput Biol 10, e1003936 (2014).
[10] A. P. Moczek, S. Sultan, S. Foster, C. Ledón-Rettig, I. Dworkin, H. F. Nijhout, E. Abouheif, and D. W. Pfennig, Proceedings of the Royal Society of London B: Biological Sciences 278, 2705 (2011).
[11] R. D. Holt, Trends in Ecology & Evolution 5, 311 (1990).
[12] A. A. Hoffmann and C. M. Sgro, Nature 470, 479 (2011).
[13] E. de Nadal, G. Ammerer, and F. Posas, Nature Reviews Genetics 12, 833 (2011).
[14] R. K. Grosberg and R. R. Strathmann, Annual Review of Ecology, Evolution, and Systematics pp. 621–654 (2007).
[15] R. E. Michod, Proceedings of the National Academy of Sciences 104, 8613 (2007).
[16] J. A. Shapiro, Scientific American 258, 82 (1988).
[17] E. Flores and A. Herrero, Nature Reviews Microbiology 8, 39 (2010).
[18] I. Ispolatov, M. Ackermann, and M. Doebeli, Proceedings of the Royal Society of London B: Biological Sciences p. rspb20111999 (2011).
[19] L. Tam and D. L. Kirk, Development 112, 571 (1991).
[20] P. H. Van Tienderen, Evolution pp. 1317–1331 (1991).
[21] R. J. Beverson and S. J. Holt, On the dynamics of exploited fish populations, vol. 11 (Springer Science & Business Media, 2012).