DOI: http://dx.doi.org/10.7551/978-0-262-32621-6-ch004

Constrained genetic architecture promotes cooperation

Antoine Frénoy[1], François Taddei[1], and Dusan Misevic[1*]

[1]Center for Research and Interdisciplinarity, INSERM U1001, Université Paris Descartes, Sorbonne Paris Cité, France
[*]corresponding author: dule@alife.org

Abstract

Cooperation in nature often has direct costs but only indirect benefits. Kin and group selection theories comprehensively address its evolutionary origins, but our knowledge of the precise genetic mechanisms that prevent cheater invasion and maintain cooperation is incomplete. Here we review our published work on cooperation in Aevol, an agent-based, *in silico* genomic platform used to evolve and study populations of digital organisms that compete, reproduce, and cooperate by secreting a public good. Motivated by the observation of phenotypically identical individuals who had radically different evolutionary fates, we recorded and compared gene locations, effectively performing bio-inspired genomics analyses of our digital organisms. We found that the association between metabolic and secretion genes (promoter sharing, overlap via frame shift or sense-antisense encoding) was characteristic for populations with robust, stable cooperation. Such architecture arose *de novo* during the evolution of cooperation, but only when producing the public good was costly. Effectively, cooperation evolved to be protected and maintained through constrained, entangled genetic architecture. Beyond confirming the importance of second-order selection, we uncover a novel genetic mechanism for the evolution and maintenance of cooperation. Our results suggest a method to limit the evolutionary potential of synthetically engineered organisms, in order to reduce the change or loss of synthetic gene circuits, a major issue in synthetic biology.

Background and Introduction

The evolution of cooperation in microbial populations is a fascinating, rich and controversial evolutionary problem (West et al. 2006). Evolutionary explanations of cooperation are constantly being improved and refined by a host of mathematical tools such as game theory and meta-population models (Lehmann and Keller 2006). However, these methods typically do not distinguish between genotypes and phenotypes and are unable to investigate the structure of genomes that encode the cooperative traits. Microbial studies have already hinted at potential mechanisms by which genetic architecture can affect cooperation, including gene co-regulation and pleiotropy (Foster et al. 2004; Dandekar et al. 2012). Here we review our published work (Frénoy et al. 2013) that examines two specific types of genomic architecture: (1) operon sharing, when secretion and metabolism genes are on the same operon and share a promoter and a terminator, and (2) overlap, the base-pair sharing between metabolic and secretion genes possible when genes are in different reading frames or on different DNA strands. Well described in viruses, overlaps in bacteria are existent but rare. Similar to operons, they are thought to be caused by strong maximum genome size constraints or selection for co-regulation (Normark et al. 1983). However, overlaps have not themselves been studied as an evolutionary constraint, which we do here, in the context of cooperation.

Methods

In our work we used the *in silico* experimental evolution system Aevol (Batut et al. 2013), heavily inspired by microbial genetics. The implementation of an Aevol digital organism allows for continuous metabolic and cooperating phenotypes. For example, instead of the classical binary cheat/cooperate behavior, there is an infinite number of possible cooperation phenotypes. Individuals live on a grid and when they evolve cooperation, it is via a secreted public good molecule that diffuses and degrades (Misevic et al. 2012). The public good is costly to secrete but may benefit any neighboring organisms. Rules for transcription, translation and protein synthesis govern the complex mapping of a double-stranded binary string (genotype) to phenotype to fitness. Phenotypically similar or even identical individuals can have different genotypes, thus also having different evolvability, robustness, and evolutionary fate (Frénoy et al. 2012). All these properties of Aevol enable the two sets of evolutionary experiments we performed, in which genetic architecture constraints relating to cooperation were both observed and quantified. We first analyzed the dynamics of cooperation loss due to increased cost of secretion (*cheater invasion experiments*), and then evolved cooperation from non-secreting ancestors, under different costs (*de novo evolution of genetic links experiments*). To quantify genetic architecture, we identified instances of all operon sharing and overlap between genes. For each of the evolved cooperators we analyzed the architecture of all its secretion genes and classified them in different categories based on presence or absence of gene overlap and operon sharing. Additionally, we constructed millions of random mutants and measured the effect of mutations on both fitness and secretion to confirm the phenotypic effect of the constrained genetic architecture.

Results and Discussion

All experimental results supported the hypothesis of cooperation maintenance via constrained genetic architecture.

(1) *Cheater invasion experiments*. Using a bank of 50 evolved cooperators, we seeded new populations that continued evolving under a high secretion cost. Although the starting individuals had comparable level of secretion, under the new cost regime, the populations greatly varied in the resistance to cheater invasion. In replicate experiments, some populations consistently preserved low levels of secretion, while others always lost all secretion genes. The abundance of promoter sharing or overlapping between metabolic and secretion genes was a good predictor of cooperation maintenance. Mutational analysis confirmed that the constrained genetic architecture resulted in a greater proportion of cooperation-destroying mutations also having a direct negative fitness effect, than non-constrained architecture. Secretion-decreasing mutations were evolutionary dead-ends, resulting in evolvability suppression, a possible spontaneously-evolving mechanism for maintenance of far-sighted traits (Altenberg 2005). Interestingly, the mutation analysis was a good predictor of short-term evolution, while genetic architecture was better at explaining long-term evolutionary fate of cooperation.

(2) *De novo evolution of genetic links experiments*. In the second set of experiments, populations starting from non-cooperators evolved under different secretion cost regimes. Cooperators evolved to employ the constrained, protective encoding, and more so when the cooperation cost was high (Figure 1). This confirms that the entangled genetic architecture is subject to selection because of its protective effect on cooperation.

In the work reviewed here, we provide a new set of potential explanations for the evolution of operons and overlaps, both important building blocks of life. Operons and overlaps may help protect genes that are at risk of removal because of a short-term cost and in spite of the long-term benefit they may provide. In the case of cooperation, genetic architecture constraints may be highly relevant to a better understanding of the much-studied siderophore-mediated cooperation in *P. aeruginosa*, where cooperative as well as metabolic traits are under the control of the same quorum sensing mechanism (Dandekar et al. 2012). However, beyond explaining evolutionary outcomes in the field of cooperation, the mechanism we described here could be actively used to prevent mutations from removing the genes introduced into bio-engineering organisms, a major problem in the field of synthetic biology (Sleight et al. 2010; Yang et al. 2013; Renda et al. 2014). We have already designed tools that would allow manipulation of digital genomes in a way that would create or eliminate constrained architecture without changing the phenotype. In ongoing work, we are experimentally testing the effect of overlap on the maintenance of costly genes in synthetic *E. coli* strains, effectively connecting *in silico* and *in vitro* research to practical applications.

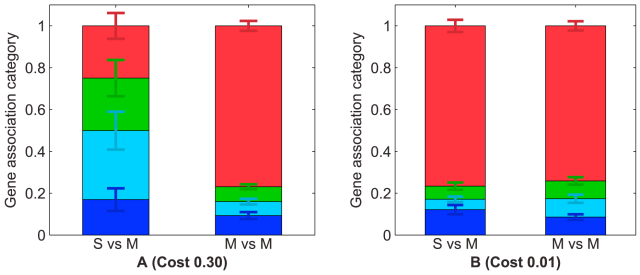

Figure 1. Genetic associations after *de novo* cooperation evolution at different secretion cost (from (Frénoy et al. 2013)). We quantified operon sharing and overlaps between secretion genes and metabolic genes (S vs M) and between metabolic genes and other metabolic genes (M vs M). Four classes of gene pairs were identified: sharing an operon without overlapping (dark blue), overlapping and sharing an operon (light blue), overlapping without sharing an operon (green), not sharing an operon nor overlapping (red). Error bars represent standard error of the mean.

References

Altenberg L. 2005. Evolvability suppression to stabilize far-sighted adaptations. Artificial Life 11:427-443.

Batut B, Parsons DP, Fischer S, Beslon G, and Knibbe C. 2013. In silico experimental evolution: a tool to test evolutionary scenarios. BMC Bioinformatics 14.

Dandekar AA, Chugani S, and Greenberg EP. 2012. Bacterial quorum sensing and metabolic incentives to cooperate. Science 338:264-266.

Foster KR, Shaulsky G, Strassmann JE, Queller DC, and Thompson CRL. 2004. Pleiotropy as a mechanism to stabilize cooperation. Nature 431:693-696.

Frénoy A, Taddei F, and Misevic D. 2012. Robustness and evolvability of cooperation. Pp. 53-58 *in* C Adami, DM Bryson, C Ofria, and RT Pennock, eds. Proceedings of Artificial Life 13. MIT Press.

Frénoy A, Taddei F, and Misevic D. 2013. Genetic architecture promotes the evolution and maintenance of cooperation. PLoS Computational Biology 9:e1003339.

Lehmann L and Keller L. 2006. The evolution of cooperation and altruism - a general framework and a classification of models. Journal of Evolutionary Biology 19:1365-1376.

Misevic D, Frénoy A, Parsons DP, and Taddei F. 2012. Effects of public good properties on the evolution of cooperation. Pp. 218-225 *in* C Adami, DM Bryson, C Ofria, and RT Pennock, eds. Proceedings of Artificial Life 13. MIT Press.

Normark S, Bergstrom S, Edlund T, Grundstrom T, Jaurin B, Lindberg FP, and Olsson O. 1983. Overlapping genes. Annual Review of Genetics 17:499-525.

Renda BA, Hammerling MJ, and Barrick JE. 2014. Engineering reduced evolutionary potential for synthetic biology. Molecular BioSystems.

Sleight SC, Bartley BA, Lieviant JA, and Sauro HM. 2010. Designing and engineering evolutionary robust genetic circuits. Journal of Biological Engineering 4:12-12.

West SA, Griffin AS, Gardner A, and Diggle SP. 2006. Social evolutionary theory for microorganism. Nature Reviews Microbiology 4:597-607.

Yang S, Sleight SC, and Sauro HM. 2013. Rationally designed bidirectional promoter improves the evolutionary stability of synthetic genetic circuits. Nucleic Acids Research 41.