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Robustness and evolvability of cooperation

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Abstract

Robustness and evolvability are indirectly selected properties of biological systems that still play a significant role in determining evolutionary trajectories. Understanding such second order evolution is even more challenging when considering traits related to cooperation, as the evolution of cooperation itself is governed by indirect selection. To examine the robustness and evolvability of cooperation, we used an agent-based model of digital evolution, Aevol. In Aevol individuals capable of cooperating via costly public good secretion evolve for thousands of generations in a classical tragedy of the commons scenario. We varied the cost of secreting the public good molecule between and within individual experiments and constructed and evaluated millions of mutants to quantify the organisms' position in the fitness landscape. Populations initially evolved at different regimes selecting against secretion, and then continued the evolution at a reasonably low cost of secretion. The populations that experienced a very strong selection against cooperation evolved less secretion than the ones that initially experienced a less drastic selection against cooperation via a high secretion cost. The mutational analysis revealed a correlation between the number of mutants with increased secretion and the secretion level across all costs of secretion. We also evolved several clones of each population to highlight a strong effect of history in general on cooperation. Our work shows that the history of cooperative interactions has an effect on evolutionary dynamics, a result likely to be relevant in any cooperative systems that are frequently experiencing changes in cost and benefit of cooperation.

Introduction

The interplay between robustness and evolvability is one of the central questions in evolutionary biology (Wagner, 2005; Lenski et al., 2006). While mutation robustness should be beneficial, due to avoiding deleterious mutations and maintaining the organism's phenotype, without the ability to adapt to a novel environment the organism may perish in a changing world. Both selection for robustness and for evolvability are indirect, making these properties potentially difficult to investigate experimentally. Past research has found evidence that evolvability (Bedau and Packard, 2003; Earl and Deem, 2004; Wagner and Altenberg, 1996; Woods et al., 2003), as well as robustness can be selected for (Altenberg, 2005; Wilke et al., 2001; Misevic et al., 2006; Azevedo et al., 2006) under a range of circumstances. However, in most of these studies the traits that evolved different robustness and evolvability had direct fitness benefit and were thus under direct selection. We extend this work by studying aspects of evolvability and robustness of an indirectly selected trait, specifically cooperation via public good secretion.

Cooperation among individuals is frequently present in natural world and yet it remains a fascinating evolutionary enigma. When helping others comes at a direct personal cost, natural selection predicts that individuals who do not cooperate would be favored over cooperating ones. A number of theories exist to explain the diversity and abundance of stable cooperation systems in nature, primarily relying on inclusive fitness, kin and group selection arguments (Axelrod, 1984; Sober and Wilson, 1998; Lenski et al., 2006; Nowak, 2006; Lehmann and Keller, 2006; Lehmann et al., 2007). Public good secretion in microbes has been a particularly successful model system for the study of the evolution of cooperation, allowing for great insight into the forces that shape its emergence and persistence (West et al., 2007; Racey et al., 2010).

The majority of both theoretical and experimental work on robustness and evolvability has been done under either fixed environmental conditions or traits that have direct fitness effects. Here we study cooperation, a trait under indirect selection, during evolution in variable environment, where the fitness cost of cooperation changes. To investigate the effect of evolutionary history in general, and changing costs of cooperation in particular, on the evolution of cooperation, we use a digital evolution platform, Aevol. As in bacteria, the public good in Aevol is a molecule that is secreted into the environment at a cost and can then benefit both the producer and all its neighbors, acting as an agent of cooperation. After establishing the parameter range allowing for the appearance of secretion, we performed experiments investigating whether strong selection against secretion will lead to genotypes residing in regions of the fitness landscape far away from cooperation. In other words, we wanted to test the hypothesis of strong selection against se-

cretion not only causing a direct pressure against secretion genes, but also an indirect pressure on the genome structure that will modify the generic architecture and make secretion genes less likely to appear via mutations. In nature, cooperative phenotype may have to repeatedly evolve after being outcompeted by "cheaters", organisms benefiting from the cooperation without contributing to it. Depending on phenotype frequencies and ecological interactions between different types of individuals present, the cost to benefit ratio of cooperation would change. Understanding these history effects is necessary for understanding the long-term evolution of cooperation and may also be relevant to treatment of bacterial infections whose pathogenicity depends on cooperation among individuals, such as *Pseudomonas aeruginosa* (Griffin et al., 2004).

Methods

Description of the model system

In this study we use the Aevol platform (Knibbe et al., 2008; Parsons, 2011), an individual-based, genetic algorithm-inspired model aimed at studying the evolutionary processes. It is especially well suited for examining the indirect selection pressures on the genome structure due to microbial-inspired, complex genotype-phenotype map (Parsons et al., 2010). The genomic layer of Aevol is inspired by bacterial genomic, but should be general enough for our needs. Aevol is an open-source project and is freely available at `www.aevol.fr/download`. In all our experiments we used the default parameters unless otherwise noted.

The genome of Aevol individuals is encoded by a double-stranded string of zeros and ones. The phenotype is a collection of traits that are represented by a 2D curve, each point on the curve specifying performance level for an abstract biological process, a metabolic trait. A single protein is obtained by transcription and translation of the binary genome strings, through a mathematical transformation. To be expressed, protein sequence must be found between start and stop codons, that in turn must be between a promoter and terminator sequences, and be preceded by a Shine-Dalgarno sequence. A protein can affect a number of different processes simultaneously, to a different degree, depending on its expression level. There is no explicit genetic regulation in this version of Aevol, but there are functional interactions (combining the effect of two proteins contributing to the same trait). The transcription efficiency, and thus the protein expression level can be affected by mutations in the promoter region. Such genotype-to-phenotype map is directly inspired by the complexities of bacterial genomics and allows us to study not only the evolutionary dynamics of phenotypic traits, but also the evolution of the genetic architecture supporting these traits, including the genome length, percentage of coding/non-coding DNA, number of genes, and number of operons (Knibbe et al., 2007b,a; Parsons, 2011; Parsons et al., 2010).

The fitness of an Aevol digital organism is a decreasing function of the gap between the curve representing its phenotype and a target curve representing the "perfect phenotype" for the chosen environment. This target phenotype is a combination of several gaussians, chosen by the researcher and fixed during the experiments. There may be many ways to encode the same protein and thus many genotypes may map to the same phenotype. Moreover, different phenotypes may have the same fitness. In our system, selection acts on the phenotypic variation created by random mutations of organisms' genome. We distinguish between two types of mutations: small mutations (single base substitutions, insertion or deletion of up to 6 neighboring bases) and large mutations (duplication, deletion, inversion, or translocation of a section of the genome whose size and location are chosen at random). The mutation rates we used are $5 * 10^{-5}$ per nucleotide per generation for small mutations, and $5 * 10^{-6}$ for large mutations. Given the typical genome size of 10^4 bases, for each individual we expect about one small mutation per generation and one large mutation every 5 generations. The stochastic nature of our model is derived from the random choice of mutations at each generation, combined with the probabilistic selection which we describe below. By modifying the random number seed, we can perform multiple experiments with the same set of parameters and analyze the statistical significance of our results.

In order to study robustness and evolvability of cooperation we extended the Aevol system to include the possibility of secreting and consuming a public good, a diffusible, degradable molecule that is produced at a cost but confers a benefit to each individual absorbing it (West et al., 2007; Racey et al., 2010). Based on the studies of public good dynamics in Aevol and other systems (Brown and Taddei, 2007; Misevic et al., 2012), we set the degradation rate to 10% per generation (the amount of the public good molecule that degrades each generation) and diffusion rate to 5% (the percentage of the public good that diffuses into each of the neighboring cells in the classical 3x3 Moore neighborhood). Under this scenario, 54% of the initially present public good remains in the grid cell after each generation.

To allow for the encoding of the public good production, we modified the genotype-phenotype map as follows: half of the phenotypic traits remain related to the "classical" metabolic phenotype and their levels have a direct effect on fitness, while the other half specifies the secretion-related phenotype. The metabolic fitness component is inversely proportionate to the gap between the metabolic part of the phenotype and the target phenotype. The gap between the secretion part of the phenotype and the secretion target phenotype is inversely proportionate to the amount of public good secreted by an individual. The total fitness of an organism is the combination of its metabolic fitness, the cost

it pays for secreting the public good and the benefit it gets from any public good present in its local environment. To be precise, $W = Wmet * (1 + B * (PG - C * S))$, where W is the total fitness, $Wmet$ is the metabolic fitness, PG is the amount of public good present in the local environment, S is the amount of the public good secreted by the individual, B is the contribution of cooperation to fitness (set to 0.5 in all our experiments), and C is the cost of secretion that we will vary in some of our experiments. As an individual does not directly benefit from the public good it secretes, but only from the public good secreted by its ancestors and neighbors, the selection for cooperation is indirect.

Spatial structure is thought to have a major impact on the evolution of public-good secretion: cooperation is likely to be favored by kin selection when related individuals are spatially close to each other (West et al., 2007; Nowak and May, 1992; Hauert and Doebeli, 2004). In order to enable the potential evolution of cooperation, our individuals evolve in a square toroidal grid with 1024 positions (32x32). Each position is inhabited by a single individual and there are no empty positions. The selection is done on a purely local basis: to compute a new generation, for each grid position we synchronously compete the nine individuals in its neighborhood. The higher the fitness of an individual is, the higher is the probability it will reproduce. All mutations happen during the reproduction step, after which the fitness of the new individual is recomputed, based on the changed levels of the available public good and mutations that occurred. To avoid the drastic decrease of the selection pressure as organisms approach the target phenotype, we use rank based, rather than fitness based selection in the neighborhoods. Additionally, the rank contributes exponentially to the probability of being selected for reproduction, in line with previous work on genetic algorithms in general and Aevol in particular (Bickle and Thiele, 1994; Knibbe et al., 2007b). We choose the exponential rank selection parameters that give the individual with the highest fitness in the neighborhood a 31.3% probability of reproducing in the central cell of that neighborhood, while that probability is 1.8% for the individual with the lowest fitness. We determined these selection probabilities by testing a range of parameters and choosing ones that result in evolution of the highest level of secretion over time (data not shown).

Experimental design

Secretion cost and the evolution of cooperation. The ratio between the cost paid by the individual that produces the public good and the benefit received from its consumption is a crucial parameter affecting the evolution of cooperation (Hamilton, 1964; Nowak, 2006; West et al., 2007). In order to quantify the dynamics of cooperation in Aevol under different cost-benefit ratios, we performed 50 experiments for each of the 7 different levels of secretion cost, $C = 0.01$, 0.05, 0.1, 0.2, 0.3, 1 and 2. Each experiment lasted $30,000$ generations and we recorded the average amount of the public good secreted by the individuals over time. We used the results from these experiments to inform our parameter choices in remainder of the study.

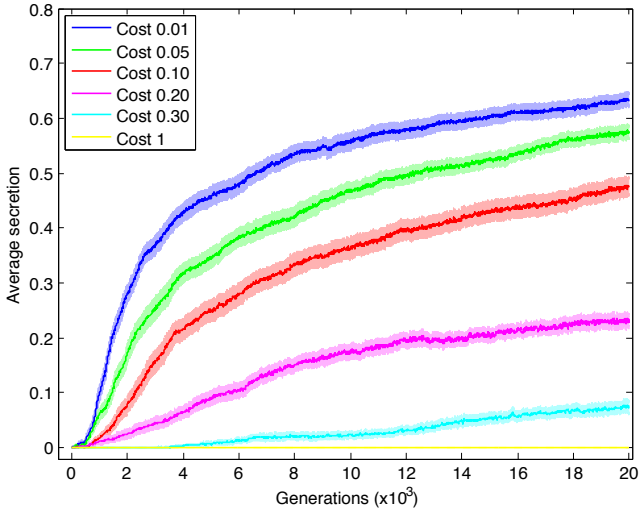

Figure 1: Effect of secretion cost on the evolution of cooperation. Each line represents an average of 50 replicate experiments conducted at the same secretion cost. The shaded area is one standard error of the mean. Results for cost = 2 are indistinguishable from cost = 1 and are thus not shown.

Historical cost of secretion and the evolution of cooperation. To quantify the strength of the historical effects, as well as robustness and evolvability of cooperation in Aevol, we performed a series of experiments in which populations evolved for $10,000$ generations at one of the three regimes with different cost of secretion, specifically $C = 0.8$, $C = 0.5$, $C = 0.35$. We also tested an additional regime, $NoSec$, where the biological processes that were assigned to the secretion part of the phenotype are associated with metabolism instead and their optimal expression level is set to zero. The cost parameters we chose should completely inhibit the evolution of cooperation, or allow for it only at extremely low levels. After $10,000$ generations the cost of secretion is set to $C = 0.25$ for all treatments, and the secretion target phenotype in $NoSec$ treatment becomes the same as in the three other treatments. Specifically, the values y for all processes in the target phenotype with $x \in (0, 1)$ are described by four Gaussian functions of the form $y = He^{-(x-M)^2/2W^2}$, where $(H, M, W) = \{(0.35, 0.3, 0.04), (0.5, 0.2, 0.02), (0.5, 0.7, 0.02), (0.35, 0.8, 0.04)\}$. All processes with x-values less that 0.5 are associated with metabolism while the others are associated with secretion. During all these experiments we recorded the average amount of secreted compound.

Mutational robustness. We analyzed the genetic architecture of all the individuals from each population at generation $10,000$ by performing large number of mutations and recording the overall fitness and the amount of the public good secreted by the mutants. Each organism was reproduced $10,000$ times with its offspring having the probability of acquiring mutations in the same way as during the reproduction in typical experiments, for a total of $10,240,000$ mutants analyzed from each population. We evaluated the frequency of beneficial, neutral and deleterious mutations as well as their magnitude.

History versus chance. To quantify the effect of history (versus chance) on the amount of secretion after generation $10,000$, we performed an experiment similar to the classic "adaptation, chance and history" studies (Travisano et al., 1995; Wagenaar and Adami, 2004). In these experiments, for each of our cost treatments, we selected 10 populations at random as the available computational power did not allow us to study all 100 populations per treatments. Each of these 10 populations was cloned 10 times when releasing the secretion cost (generation $10,000$), to obtain 10 groups of 10 replicates. We measured the average amount of secreted compound during $3,000$ additional generations for each of these populations. An analysis of the variance between the different groups compared to the variance within each group provides a measure of the influence of history and chance on the evolution of these populations. We do not specifically discuss the effects of adaptation here as they are apparent from the change in amount of cooperation in all treatments.

Results and discussion

Direct relationship between evolved secretion and its cost. The cost of secreting the public good had a direct and strong effect on the average amount of secreted public good molecule (Fig. 1). This is in accordance with our expectations, both in terms of the direct trade-off between cost and benefit of cooperation and in relation to classical results (West et al., 2007; Nowak, 2006). We used these experiments to establish a baseline cost of cooperation for which no population would evolve and maintain significant levels of secretion during at least the initial $10,000$ generations of evolution. In particular, we find that costs higher than 0.3 have this property and are thus suitable for use in the experiments from the second part of our study.

History affects future secretion levels. The phenotype of individuals that evolved for $10,000$ generations under high costs of secretion or $NoSec$ regime was generally identical: they did not secrete any public good molecules, as expected. However, once the selection pressure against secretion was released (at generation $10,000$), the faiths of different populations quickly diverged. By $10,000$ generations, mutations and evolution erased any statistical differences between the treatments so we used an earlier time point in our analysis. Rather than using just the final secretion which may be strongly affected by stochastic factors, we measured the amount of cooperation that evolved by averaging the amount secreted during the first $3,000$ generations after releasing secretion cost (Fig. 2), and used the Mann-Whitney nonparametric test to compare different treatments. We find a general trend of lower secretion in populations that underwent the $NoSec$ regime (strong direct selection against secretion) compared to the ones that experienced a high cost of secretion (less drastic selection against secretion) in their past (Mann-Whitney U test, $p = 0.010$). However we did not find any significant difference between the three secretion costs. This trend, although very noisy at our levels of replication, indicates that genotypes have preserved some information of their evolutionary history. The ones that evolved with strong direct pressure against secretion ($NoSec$ treatment) are more robust and less likely to change, while the ones that evolved with less strong pressure via secretion cost are more evolvable.

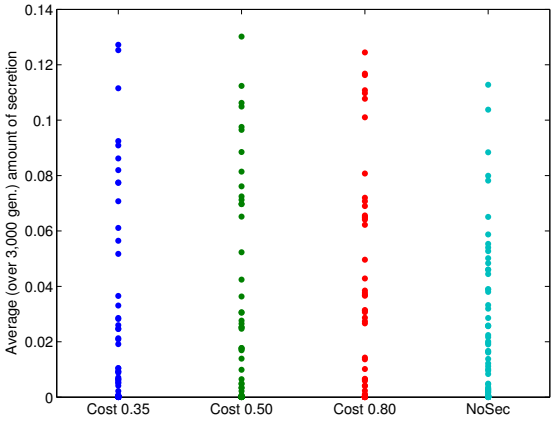

Figure 2: Average amount of secretion between generation $10,000$ and generation $13,000$, sorted by the cost regime in the first $10,000$ generations. Each point represents a single replicate population. There are 100 independent replicates for each treatment.

Mutational robustness is strongly correlated with future secretion levels. Specifically, we suspect that the genotypes that evolved robustness against secretion were located in regions of the fitness landscape mutationally far away from genotypes that confer the secretion phenotype. To test for such genotypic memory, we performed a mutagenesis test (Fig. 3), as described in the methods. We found a strongly significant difference in the proportion of mutants with increase in secretion (weighted by the magnitude of these effects) between on one side the three high cost treat-

ments and on the other side the $NoSec$ treatment (Welch's t-test, $p = 0.0001$), but no significant difference when comparing the three different costs between them. We furthermore found a very strong within-treatment correlation between the proportion of mutants with increase in secretion (weighted by the magnitude of these effects) and the average amount of public good secreted during the first $3,000$ generations after regime change (Table 1). This correlation is still present if we pool all the data, with the coefficient of correlation of 0.37 and $p < 10^{-13}$, and suggests that history, encoded as genotypic memory, does strongly matter.

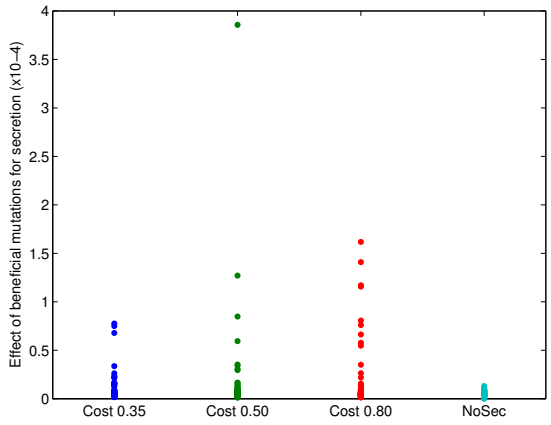

Figure 3: Beneficial mutations for secretion at generation $10,000$, depending on the regime during the first $10,000$ generations. Each point represents the average effect of $10,240,000$ mutations within single replicate population. There are 100 independent replicates for each treatment.

Cost of secretion	Correlation coefficient	p-value
0.35	0.6639	$< 10^{-13}$
0.5	0.3669	$< 10^{-3}$
0.8	0.4134	$< 10^{-4}$
NoSec	0.1790	0.07

Table 1: Correlation between the proportion of mutants with increase in secretion (weighted by the magnitude of these effects) at generation $10,000$ and the average amount secreted between generation $10,000$ and generation $13,000$ for each treatment.

ANOVA shows a strong effect of history versus chance. Following the experimental protocol described in the methods, we performed a one-way ANOVA to assess the influence of history (versus chance) on the evolution of secretion (Table 2). We found a significant influence of history for each of our three cost treatments, even if this history is not necessarily dependent on the cost of secretion, as we expected initially. As our previous experiments already found the $NoSec$ treatment to have different historical effect on robustness and evolvability of the cooperation phenotype, here we omitted it from the analysis and focused instead on the historical effect of the three cost treatments.

Cost of secretion	SShist/SStot	F statistic	p-value
0.35	0.63	16.9349	$< 10^{-15}$
0.5	0.44	7.7311	$< 10^{-7}$
0.8	0.57	13.3905	$< 10^{-13}$

Table 2: Influence of history versus chance on secretion. SShist is the sum of squares due to history, while SStot is the total sum of squares (history plus chance).

Conclusion

Using the Aevol digital system we performed a series of experiments to test the effect of evolutionary history on the robustness and evolvability of cooperation. Our results generally showed a weak effect of the strength of selection against secretion on the future evolution of secretion, and a strong effect of history in general. The data was extremely noisy and may require a much greater number of replicates than we could produce for this study. The difference in the mutational neighborhood occupied by populations that have evolved at different secretion costs was not significant; however, the difference between the three cost-driven regimes (indirect pressure against secretion due to moderately high cost) and the NoSec regime (strong direct pressure against secretion) was large. Moreover the accessibility of beneficial mutations for secretion did strongly correlate with the amount of secretion in our experiments, generally validating the mutational analysis approach. The analysis of several clones of each population highlighted a strong influence of history on the robustness and evolvability of cooperation, however the cost of cooperation does not seem to be the main factor creating this history. Much research remains to be done in terms of fully understanding these complex interactions.

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Contributions

AF conceived and designed the study, performed the experiments, analyzed the results, and wrote the paper. FT provided input on the study design, result analysis and interpre-

tation. DM wrote and edited the paper and contributed to data analysis and presentation.

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