**Supplemental Information: "Spatial population expansion promotes the evolution of cooperation in an experimental Prisoner's Dilemma"**

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**Supplemental Inventory**

**Heuristic description of model and derivation of analytical results:**

**Mechanism of selection for cooperation during range expansion**

The fate of cooperators during a range expansion is determined by two phases, which we will take in turn. In Phase I, stochasticity at the frontier due to serial genetic bottlenecking causes local fixation of genotypes and loss of genetic diversity. Despite being selected against within subpopulations, cooperators can nonetheless fix at the frontier if stochastic effects overwhelm purifying selection. Once genotypes fix at the frontier, the expanding wave of individuals (the "density wave") will almost always travel faster than the "allele frequency wave" (Figure S1). To see this, note that in a

population growing logistically with diffusion of individuals via dispersal into

neighboring habitat sites, the speed of such a wave in steady-state has a known solution,

$$v_d = 2\sqrt{D\overline{W}} \qquad (1a)$$

as does the speed of the traveling allele-frequency wave [1]

$$v_f = 2\sqrt{D\left(W_i - W_j\right)} \qquad (1b)$$
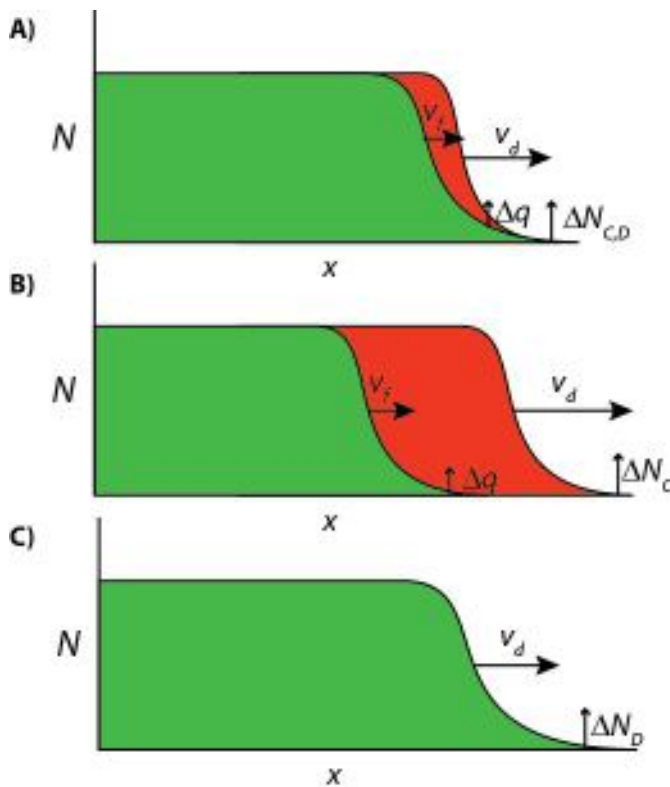


**Figure S1| Spatial expansion in one-dimension.** Population size, *N*, as a function of spatial coordinate, *x*, in a spatially expanding population. In the Prisoner's Dilemma, a mixed cooperator (red) and defector (green) front (**A**) will eventually resolve into a wave fixed for either cooperators (**B**) or defectors (**C**). Because cooperators lose in direct competition to defectors within each subpopulation (i.e., at each site *x*), outcome (B) requires that the stochastic effects of sampling at the leading edge overcome selection. However, once fixed, cooperators (or any other genotype) will outrun defectors, which advance in the trailing allele frequency wave. If defectors fix at the front (C), there is no trailing allele frequency wave because cooperators cannot invade defector subpopulations.

$D$ is the diffusion constant, $\overline{W}$ is the mean Malthusian fitness of a subpopulation (the maximal rate of increase), and $W_k$ is the fitness of genotype $k$. In the Prisoner's Dilemma game we can write the fitness of cooperators and defectors as,

$$W_C = W_0\left(1 + bp_C - c\right) \qquad (2a)$$

$$W_D = W_0\left(1 + bp_C\right) \qquad (2b)$$

where $W_0$ is the baseline reproductive rate, $b$ is the fitness benefit donated by cooperators, $c$ is the loss in fitness from cooperating and $p_C$ is the frequency of cooperators in a subpopulation (we assume that subpopulations are, by definition, well mixed in terms of social interactions, so that every individual has an equal chance of interaction with any other individual, and that choice of social partners is indiscriminate). From this, we have,

$$v_d = 2\sqrt{DW_0\left(1 + (b-c)\,p_C\right)} \qquad (3a)$$

$$v_f = 2\sqrt{(\pm)DW_0 c} \qquad (3b)$$

where the $c$ is positive if cooperators are fixed at the front, and negative if defectors are fixed at the front. This means that defectors can deterministically invade subpopulations fixed for cooperators (Figure S1B), but cooperators cannot invade defectors (Figure S1C). So, we have 2 scenarios corresponding to Figures S1B,C:

*1) Cooperators stochastically fix at the frontier (Figure S1B):*

    a) Density wave of cooperators proceeds at speed:

$$v_d = 2\sqrt{DW_0\left(1 + b - c\right)} \qquad (4a)$$

    b) And is trailed by a lagging frequency wave of cheaters at speed:

$$v_f = 2\sqrt{DW_0 c} \qquad (4b)$$

c) Cooperation is stable at the front, and will contintue to gain ground and

increase in global frequency if:

$$\frac{1+b}{2} > c \qquad (4c)$$

*2) Defectors stochastically and/or deterministically fix at the frontier (Figure S1B):*

a) Density wave of defectors proceeds at speed:

$$v_d = 2\sqrt{DW_0} \qquad (5)$$

b) There cannot be a lagging frequency wave of cooperators, because its speed is

not a real number.

c) Cooperation is eliminated.

Because of this, cooperators can only increase in frequency in a one-dimensional range

expansion if they stochastically fix at the front. Thus, there is no positive force of

selection promoting cooperation in the PD in one dimension.

Importantly, this analysis has thus far been restricted to a single spatial dimension.

In reality, most range expansions will proceed along two spatial dimensions. Two-

dimensional range expansions can undergo Phase II: competition among neighboring

subpopulations at the frontier favoring genotypes with high productivity (Figure S2).

Note that within subpopulations, selection favors genotypes with the highest *relative*

fitness, which in the case of spite and selfishness (see below) actually causes a reduction

in total reproductive output as these traits sweep to fixation. This is known as the

"Tragedy of the Commons". However, at the frontier, expansion speed is determined by

*absolute* fitness, generating a force of selection promoting genotypes that increase

productivity. In the Prisoner's Dilemma, equations S4a,b tell us that subpopulations fixed

for cooperators will travel faster than those fixed for defectors if,

$$b > c \qquad\qquad (6)$$

Together, Phase I (genetic demixing) and Phase II ("survival of the fastest") interact to

create a force promoting high productivity strategies such as cooperation (Figure S2).

However, the specific parameter regimes in which this force can overcome selection

within groups favoring defectors, which is necessary for Phase I, are not immediately

clear. A more detailed theoretical analysis of the interactions between density and allele

frequency waves in two dimensions will be required to provide specific conditions

necessary for cooperation to evolve in expanding populations, taking into account both

Phase I and II; this is an interesting topic for further work.
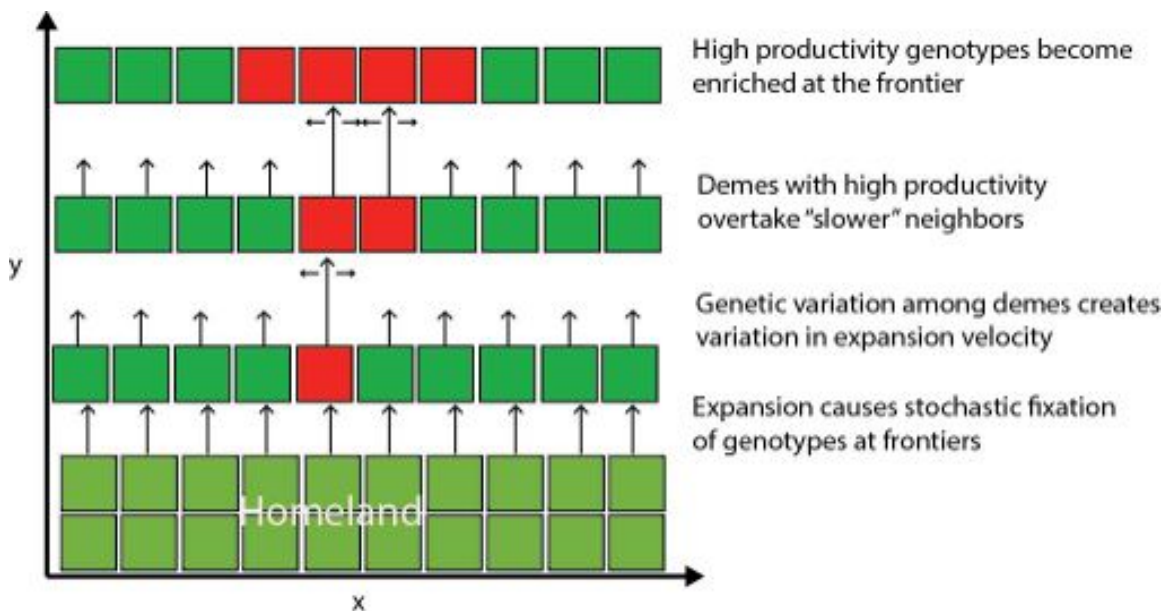


**Figure S2| Spatial expansion in two-dimensions allows selection for cooperation.**
Populations expanding in two spatial dimensions, with each site at coordinate (*x,y*)
representing a subpopulation connected to nearest neighbors by dispersal according to
Kimura's stepping stone model [2]. A mixed homeland with rare cooperators will
eventually demix upon expansion into subpopulations fixed for either cooperators (red)
or defectors (green). Because subpopulations with cooperators expand faster than
subpopulations of defectors, cooperators become enriched at the frontier by overtaking
neighboring defector sectors.

**Extension to other social behaviors**

Spatial population expansions may influence the evolution of other social behaviors as well. Consider spite and selfishness, strategies that reduce mean population fitness, but may spread nonetheless. Spite occurs when an individual reduces its own personal fitness to harm others ($b < 0$, $c > 0$) [3, 4]. Despite counterselection within subpopulations (due to fitness cost, $c$), spite can nonetheless stochastically fix at the frontier of an expanding population. Once this happens, equation 4c tells us that spitefull genotypes will increase in frequency in one-dimensional populations as long as $c < (1 - b)/2$, which can be satisfied over a wide range of parameter space.

Selfish individuals increase their direct fitness while reducing the fitness of neighbors ($b, c < 0$). Selfish genotypes are more likely to fix at frontiers than non-selfish genotypes because of positive selection (direct benefit of magnitude, $c$), and will also establish an allele-frequency wave that will chase non-selfish genotypes that stochastically fix at the frontier.

Two-dimensional spatial expansions, however, select against both spite and selfishness. Lower productivity of spiteful and selfish subpopulations makes them vulnerable to being overtaken by neighboring subpopulations of non-spiteful or non-selfish strategies. A full exploration of this effect awaits further study.


**Experimental Procedures**

**Strains:** Strains were haploid (MATa) prototrophs with deletions of *mal11* and *mal12* genes, constructed from W303 background with *ADH*1 promoter-driven expression of the fluorescent markers ymCherry (cooperators) and ymCitrine (*suc2Δ* defectors).

**Experimental setup:** Overnight cultures grown in YPD were washed twice with sterile water, resuspended to a density of $2\text{x}10^8$ cells/mL as measured by Coulter Counter, and mixed in appropriate ratios quantified by FACS. *Stationary (non-expanding) competitions* were conducted in round-bottom 96-well culture plates with 128 uL of liquid media: YEP (1% yeast extract, 2% peptone) plus 2% filter sterilized sucrose and the appropriate concentration of cycloheximide, both added after autoclaving. Cycloheximide stocks were diluted in ethanol, filter sterilized and stored at -20 C until use. Wells were inoculated with 1uL of initial culture, and 1uL was passaged from each well into fresh media every 24 hours. Plates were incubated at 30 C on an orbital plate shaker at 1000 rpm. Strain frequencies were measured using FACS at days 0, 1, 2, and 6, with 3 replicates of each condition. *Range expansion competitions* were conducted on 7 mL agar media (same recipe as above plus 2% agar) in 6-well culture plates. The two center wells were left empty to avoid plate effects. 1 uL of initial culture was spotted onto the center of each well and plates were incubated at 30 C, with 3 replicates of each condition. For frequency analysis, 3 replicates from each condition were chosen at random and harvested at the appropriate time point by repeatedly pipetting 2 mL PBS until colony was completely detached from the agar and well mixed, then the culture was diluted appropriately for FACS analysis. *Growth rates* were conducted as with the range expansions, but conducted in individual petri plates on 12 mL of agar media.

**Image analysis:** Image analysis was performed with Matlab. Colony radii were determined from circle fits to the colony boundary, detected using edge detection or thresholding on the brightfield image of the colony. Sector boundaries were identified by

edge detection in the fluorescent images. Each sector was assigned a color by comparing its average intensity to the average intensity of its neighboring sectors.

**Simulations:** Simulation were conducted on an *n*x*m* square lattice. Each site of the lattice contained a subpopulation of size $N_T$ undergoing logistic population growth with genotype-independent carrying capacity, *K*, non-overalapping generations, and growth rate of genotype *i* at local frequency $p_i$ following: $N_i' = (p_i + \Delta p_i)(N_T + \Delta N_T)$, where

$$\Delta N_T = W_0 (1 + (b-c)p_C) N_T (1 - N_T/K), \quad \Delta p_i = p_i(1-p_i)(W_i - W_j), \text{ and } (W_C - W_D) = -c.$$

Stochasticity was introduced by first computing $N_i'$ as a real number, then using the non-integer part as the binomial probability of success over $N_i$ trials. The life cycle was as follows: cooperators produce a fitness benefit *b*, which is shared equally by all $N_i$ group-mates, at a personal fitness cost *c*, individuals then reproduce based on their fitness, die, and finally disperse. The number of migrants of each type was a binomial random draw with success probability equal to *m*. Simulations were initiated with a "homeland" population with each site at carrying capacity and a binomial random number of cooperators with mean frequency equal to $p_0$. In linear expansions, the homeland consisted of 10 rows of sites filling the bottom of a cylindrical lattice (boundaries were periodic only in the direction perpendicular to the direction of expansion). Radial expansions were initiated as a square block of demes in the center of a lattice. Scripts were written in Matlab with plots and analysis performed in Matlab and Mathematica.

**Additional Supplemental Figures**
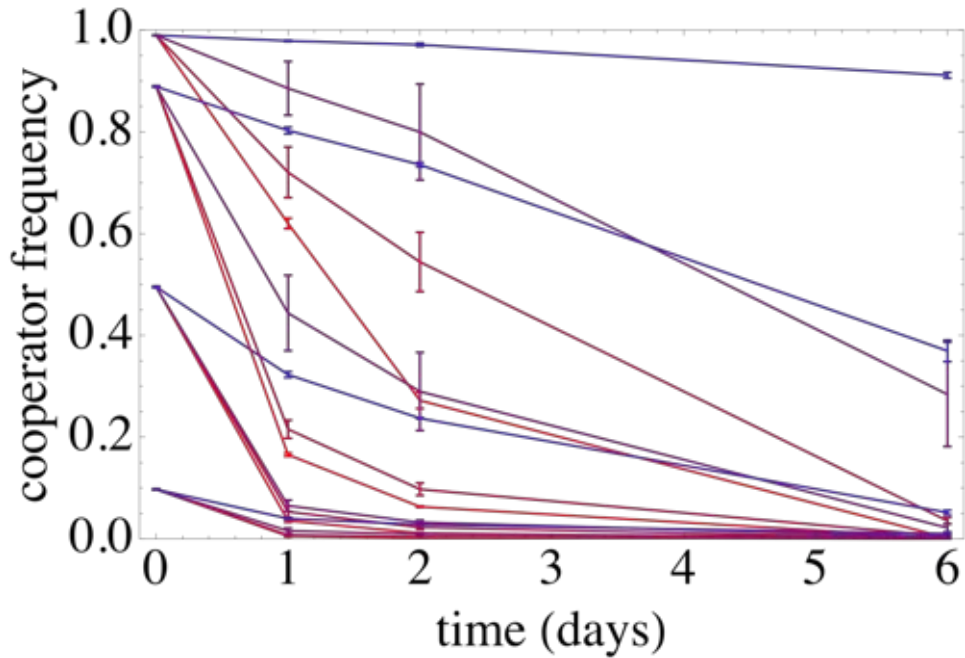
**Figure S3| Selection against cooperators in a stationary environment.** Competition between cooperator and defector strains in shaken liquid culture for a range of imposed costs (cycloheximide concentration varied from 50 (blue) to 150nM (red): 50 nM, 75 nM, 100 nM, 150nM). Strain frequencies measured by FACS. Any potential equilibrium between cooperators and defectors is below 0.01, making our system function as a Prisoner's Dilemma for all conditions considered: increase in cooperator frequency in our range expansions cannot be due to rare advantage.

**A)**

B)
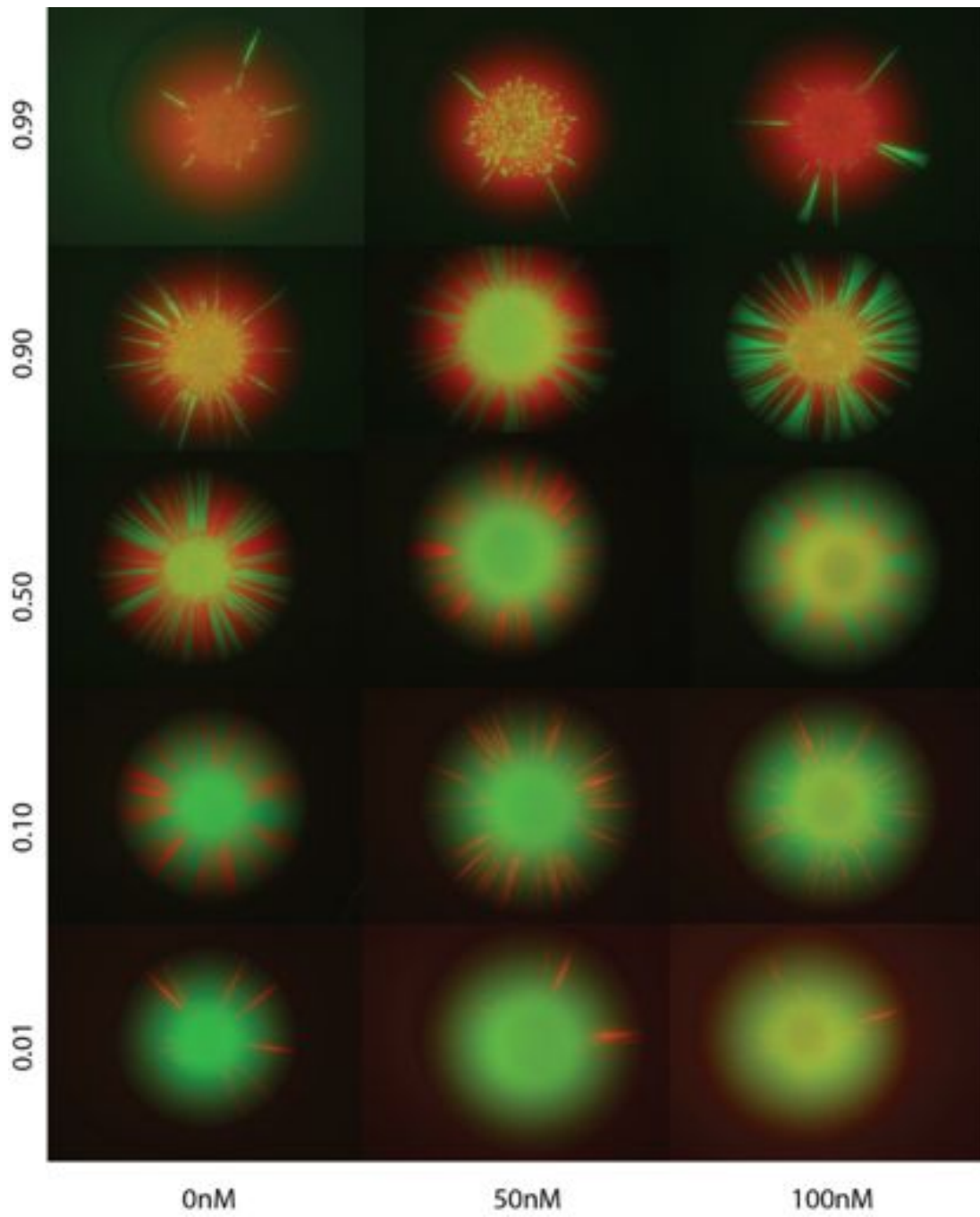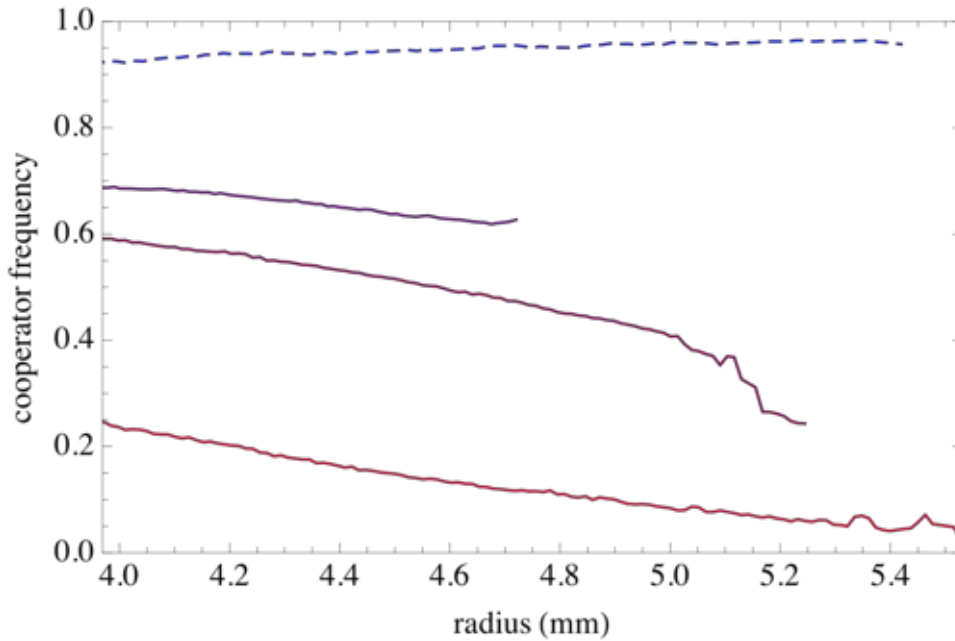
**Figure S4| Range expansions in glucose media controls. A)** Cooperator (red) and defector (green) strains competing in expanding colonies on glucose rich media (YEP + 2% glucose + 2% agar). The abundance of monosacharides and the absence of sucrose in the media render the coopertive phenotype, sucrose digestion, unnecessary. Thus, the benefit of cooperation is eliminated leaving only the cost of cooperation to distinguish strains. **B)** Image analysis of glucose controls for colonies with an initial frequency of cooperators of 0.90. Cycloheximide concentrations, from top to bottom: 0nM (dashed blue line), 75nM, 100nM and 150nM. Importantly, in these control experiments cooperators decline in frequency (from 90% in this case) when cycloheximide is applied, in contrast to the case when cooperation is beneficial in sucrose media (Figure 2E in main text). Note that the 0nM (dashed blue) line increases in frequency slightly from 90%, indicating a slight cost to cycloheximide resistance in the cheaters in the absence of cycloheximide.

**Supplemental References**

1. Fisher, R.A. (1937). The wave of advance of advantageous genes. Annals of Eugenics *7*, 355-369.
2. Kimura, M., and Weiss, G.H. (1964). Stepping Stone Model of Population Structure + Decrease of Genetic Correlation with Distance. Genetics *49*, 561-&.
3. Hamilton, W.D. (1970). Selfish and Spiteful Behaviour in an Evolutionary Model. Nature *228*, 1218-1220.
4. West, S.A., and Gardner, A. (2010). Altruism, spite, and greenbeards. Science *327*, 1341-1344.