

S1 Text. Supplementary Information

Pleiotropy, cooperation and the social evolution of genetic architecture

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This files includes

- Supplementary analyses
- Supplementary tables S1-S2
- Supplementary figures S1-S17
- Supplementary references

1. Supplementary analyses

In this section, we first explore how sensitive our result that pleiotropy does not stabilise cooperation is to our model assumptions. Specifically, we explore different mutational pathways for pleiotropy. Second, using individual-based simulations, we explicitly model the pleiotropic link between cooperation and the private trait, by assuming that this link is controlled by a third independent locus.

1.1 Different mutational pathways

In the main text, we showed that pleiotropy does not stabilise cooperation unless the genetic architecture cannot evolve: the link between cooperation and an essential private trait cannot be broken. Here, we test how robust this result is with respect to changes in the mutational pathways, or mutational accessibility, allowed in our simulations. In the model presented in the main text, pleiotropy could be gained or lost due to a mutation in individuals who bear both the private and cooperation traits, and only one mutation in pleiotropic cooperators could lead to the individual expressing neither traits (Fig 3). Here, we explore 3 different scenarios:

In scenario (i), the only difference with our baseline model (main text) is that pleiotropy cannot be lost (no mutational accessibility), so the only mutation in pleiotropic cooperators, which occurs with probability μ , leads to the loss of the pleiotropic regulator, and hence, the individual's death, because the private trait is no longer expressed. This is an extreme scenario because pleiotropy becomes an attractor, as no other genotypes can be generated from pleiotropic cooperators. Therefore, pleiotropy entirely invades the population, but only when Hamilton's rule is satisfied (S7g-l Fig).

In scenario (ii), we allow mutations on both the cooperation and private trait in pleiotropic cooperators in addition to mutations on the pleiotropic regulator, so that with probability μ , a mutation occurs and leads (randomly) to either (a) cheats, (b) cooperative private non-producers, (c) non-pleiotropic cooperators, or (d) uncooperative private non-producers. This case can be viewed as the least constrained scenario, because all mutations are allowed, and pleiotropy can be lost. As before, pleiotropy does not stabilise cooperation in this case (S7j-l Fig). Instead, cooperation promotes pleiotropy, as shown by greater proportions of pleiotropic cooperators relative to other cooperative genotypes when cooperation is strongly favoured, especially with a high mutation rate (S17b and d Fig). We also found that more pleiotropic cooperators within patches result in lower proportions of cheaters at the end of the growth phase, confirming our cheat-load hypothesis (S17e Fig).

In scenario (iii), we prevent pleiotropy from being lost (mutation II in Fig. 3 of the main text), but we also allow mutations on the cooperation trait, so that cheats can be generated. Hence, in pleiotropic cooperators, a mutation occurs with probability μ , and leads (randomly) to either (a) uncooperative private non-producers (death), or (b) cheats. When Hamilton's rule is satisfied, this mutational pathway results in pleiotropy being an attractor as in scenario (i). This is because pleiotropic cooperators generate either unviable individuals or cheats who are favoured locally, but

counter-selected globally. As a consequence, when Hamilton's rule is satisfied, the population is mostly composed of pleiotropic cooperators (S7d-f Fig).

1.2 An explicit model of pleiotropy

In our baseline model presented in the main text, we have not explicitly modelled how the pleiotropic link could be formed between the cooperation and private traits. However, the details of how this link could form might influence our conclusion on how pleiotropy stabilises cooperation, or whether cooperation promotes pleiotropy. Here, we test whether these results are robust to changes in the way the pleiotropy link is formed.

We explicitly model the pleiotropic link between cooperation and a private trait. We introduce two regulators, *regA* and *regB*, which control the expression of the private and cooperation traits, respectively, when these traits are present (i.e. allele *P* and *C*, respectively; S8 Fig). As a result, the expression of a trait requires both the expressing allele and its regulator to be present. Each regulator can either be present or not present (0/1). In addition, we assume that the private regulator *regA* can become a universal regulator, i.e. with pleiotropic effects. That is, the regulator can activate both the private and cooperation traits, at the same time. During reproduction, each regulator undergoes mutations independently with probability $\mu_R = 0.001$. With these assumptions, the total number of genotypes is $3 \times 2 \times 2 \times 2 = 24$. This scenario reflects a situation where both regulators are independent from each other. Hence, we chose to let them be affected by mutations independently, such that a mutation on *regA* does not influence *regB*. So, *regB* can remain active (albeit redundant) even if *regA* becomes pleiotropic. We assume that only the private regulator *regA* can become universal for simplicity, otherwise the number of genotypes becomes considerably larger.

All 24 genotypes are presented in S2 Table. As in our baseline model, we assume that the private trait is essential, so that not expressing it leads to the individual's death. As a result, only 8 genotypes are viable, 5 uncooperative and 3 cooperative ones. Among the 3 cooperative genotypes, 2 are pleiotropic. Hence, we take a proportion of pleiotropic cooperators above 2/3 as indication that pleiotropy has an advantage over non-pleiotropic cooperators. Neutral simulations show that the proportion of pleiotropic cooperators relative to all 3 cooperative genotypes never exceeded 0.7 (S11 Fig), confirming that mutations do not generate more pleiotropic cooperators than expected.

S9-10 Figs show the results of this explicit model. As in our baseline model (main text), pleiotropy does not stabilise cooperation (S9a and c Fig). In addition, we also found that pleiotropic cooperators were more common relative to non-pleiotropic cooperators when cooperation is strongly favoured (top-right of S9b and S9d Fig). As before, pleiotropy is favoured in this model because it reduces the local cheat-load, as shown by lower proportions of cheats generated during a single growth phase, when the number of pleiotropic cooperators increases (S10 Fig). However, this effect is smaller than in our baseline model, as shown by small differences in the proportion of cheats with increasing number of pleiotropic cooperators (S10 Fig).

2. Supplementary Tables and Figures

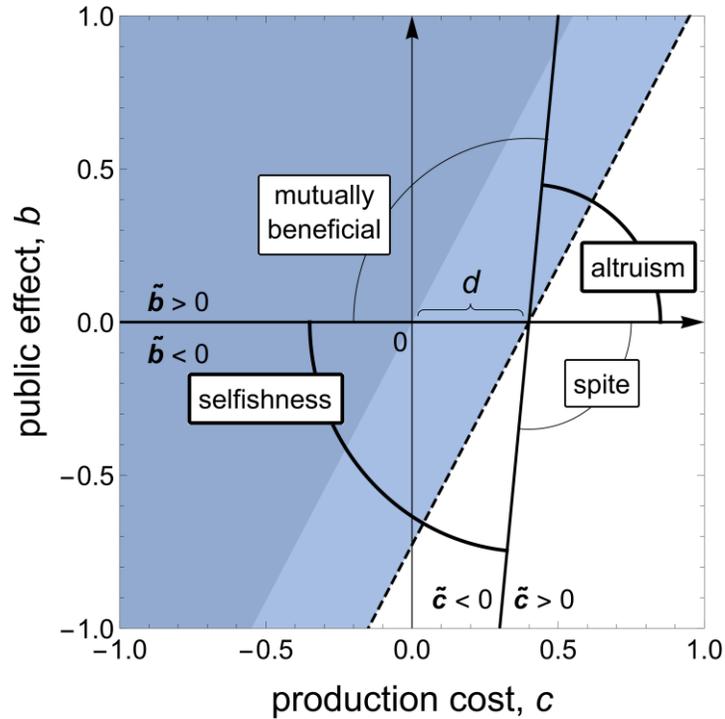
Table S1. Previous claims about the general importance of pleiotropy in stabilising cooperation.

Reference	Importance of pleiotropy for stabilising cooperation
Foster et al., 2004 (p. 695)	Pleiotropy is placed among other mechanisms such as (high relatedness) kin selection
Dandekar et al., 2012 (p. 266)	Pleiotropy is placed alongside single cell/bottle-neck dispersal (kin selection) mechanisms
Bruger & Waters, 2015 (p. 3)	‘metabolic constraint’ (private traits linked to cooperation) is one of the five mechanisms to maintain cooperation
Frénoy et al., 2015 (abstract)	“Our results [...] uncover an important genetic mechanism for the evolution and maintenance of cooperation.”
Wang et al., 2015 (p. 2189)	Pleiotropy (co-regulation) is placed alongside kin selection and policing.
Mitri & Foster, 2016 (p. 488)	“Pleiotropy [...] is thought to be a key mechanism for stabilizing cooperation against cheater mutants”
Majerczyk et al., 2016 (p. 1-2)	Different forms of pleiotropy (metabolic constraint and coregulation) are given as two of the three ways that have been shown to stabilise cooperative quorum sensing.
Özkaya et al., 2017 (p. 3)	Pleiotropy is given as one of the main ways to prevent cheating, alongside factors such as spatial structure (kin selection) and policing.
Asfahl & Shuster, 2017 (p. 99)	Pleiotropy is an ‘evolutionary force’ in addition to kin selection
Chisholm et al., 2018 (p. 47)	“Genetic interaction [...] may play a key role in the maintenance of altruistic traits.”

Table S2. All possible genotypes in our explicit model where the pleiotropic link takes the form of a universal regulator (i.e., regulating simultaneously the private and cooperation trait).

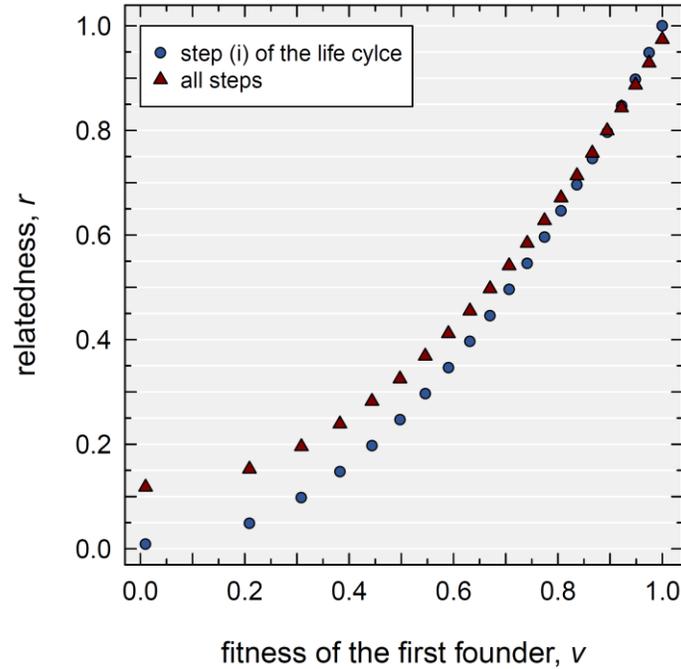
Regulator A (private)	Regulator B (cooperation)	Private loci	Cooperation loci	Phenotype	Genotype number
0	0	0	0	unviable	g1
0	0	0	1	unviable	g2
0	0	1	0	unviable	g3
0	0	1	1	unviable	g4
0	1	0	0	unviable	g5
0	1	0	1	unviable	g6
0	1	1	0	unviable	g7
0	1	1	1	unviable	g8
1	0	0	0	unviable	g9
1	0	0	1	unviable	g10
1	0	1	0	Cheat	g11
1	0	1	1	Cheat	g12
1	1	0	0	unviable	g13
1	1	0	1	unviable	g14
1	1	1	0	Cheat	g15
1	1	1	1	Cooperator	g16
2	0	0	0	unviable	g17
2	0	0	1	unviable	g18
2	0	1	0	Pleiotropic cheat	g19
2	0	1	1	Pleiotropic cooperator	g20
2	1	0	0	unviable	g21
2	1	0	1	unviable	g22
2	1	1	0	Pleiotropic cheat	g23
2	1	1	1	Pleiotropic cooperator	g24

0: absent; 1: present; 2: universal/pleiotropic regulator



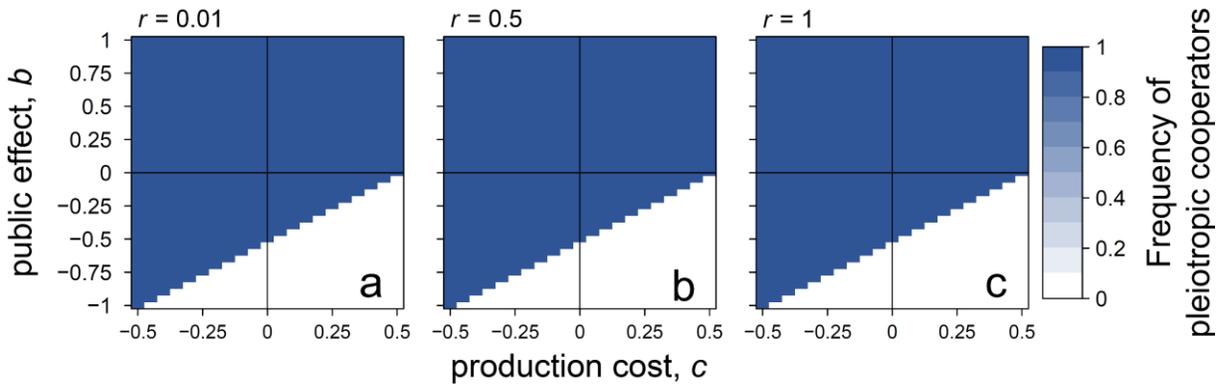
S1 Fig. Pleiotropy can help stabilise all forms of social trait.

We consider all possible forms of social interaction by considering a trait that has a fecundity effect $-c$ for the individual performing it, and a fecundity effect b that is shared amongst all the members of the group. If $b > 0$ then the trait is helpful, providing a benefit to both self and others (public good), whereas if $b < 0$ then the trait is harming, and costly to both self and others. If $c > 0$, then the trait has some fecundity cost to perform, but if $c < 0$, then performing the trait provides some fecundity benefit. The area to the left of the dashed line shows where the trait will be favoured when the trait also has some pleiotropic private benefit. The dark coloured area is when the trait would be favoured without the pleiotropic private trait. The light coloured area represents the extent to which pleiotropy can help stabilise social traits. We denote the lifetime fitness cost and benefits by $\tilde{c} = c - b/N - d$ and $\tilde{b} = (N - 1) b/N$, respectively. We follow Rousset (2014) by dividing the figure with the lines $\tilde{c} = 0$ ($c = b/N + d$) and $\tilde{b} = 0$ ($b = 0$), into the four classes of social behaviours - mutually beneficial, altruism, spite and selfishness. This classification holds for when the trait has some pleiotropic private benefit. Parameters: $N = 10$, $r = 0.5$, $d = 0.4$.



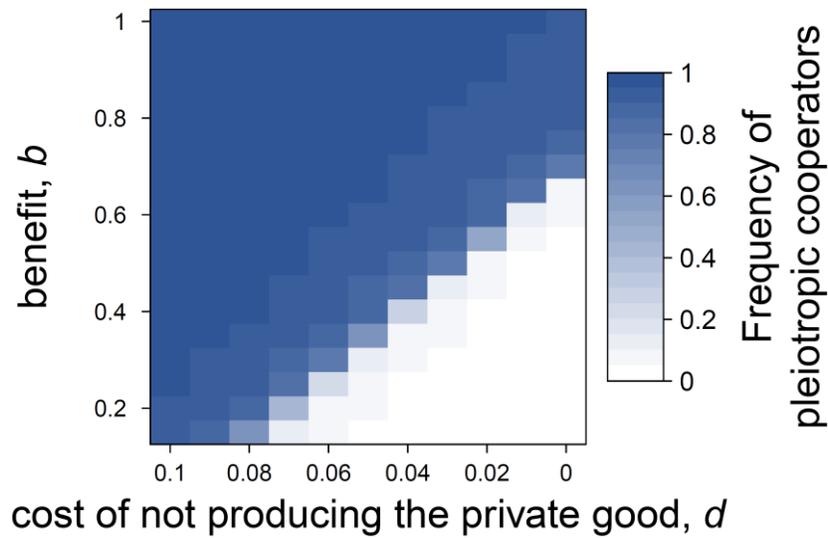
S2 Fig. Relatedness in neutral runs.

Shown is the average relatedness of 16 replicates during the last 20,000 generations, as a function of the fitness v of the first founder. Relatedness increases between step (i) and step $k = 10$ of the life-cycle, especially at low initial relatedness. At higher values of initial r , relatedness is mostly eroded by mutations. This increase in relatedness during the growth phase is explained as follows. Consider, for example, a neutral model where the number of cooperators and cheats doubles in each patch. Hence, the within-patch proportion of cooperators remains unchanged. However, the frequency of cooperators among the $N - 1$ partners experienced by a focal cooperator increases, and that of a focal cheat decreases. For example, if at generation t , a patch contains 2 cooperators and 1 cheat. Then, a focal cooperator has 1 cooperator and 1 cheat in its group (0.5:0.5), whereas a focal cheat has 2 cooperators and 0 cheat in its group (1:0). At generation $t+1$, population size doubles. Hence, a focal cooperator now has 3 cooperators and 2 cheats in its group (0.6:0.4), whereas a focal cheat now has 4 cooperators and 1 cheat in its group (0.8:0.2). The frequency of cooperators among the social partners of a focal cooperator increases, and that of a focal cheat decreases. Therefore, relatedness increases as population size increases, as long as there remains some non-homogeneous groups (in homogeneous groups, the frequency of cooperators among social partners no longer changes). Parameters: $c = 0.1, g = 0.5, \mu = 0.001$.



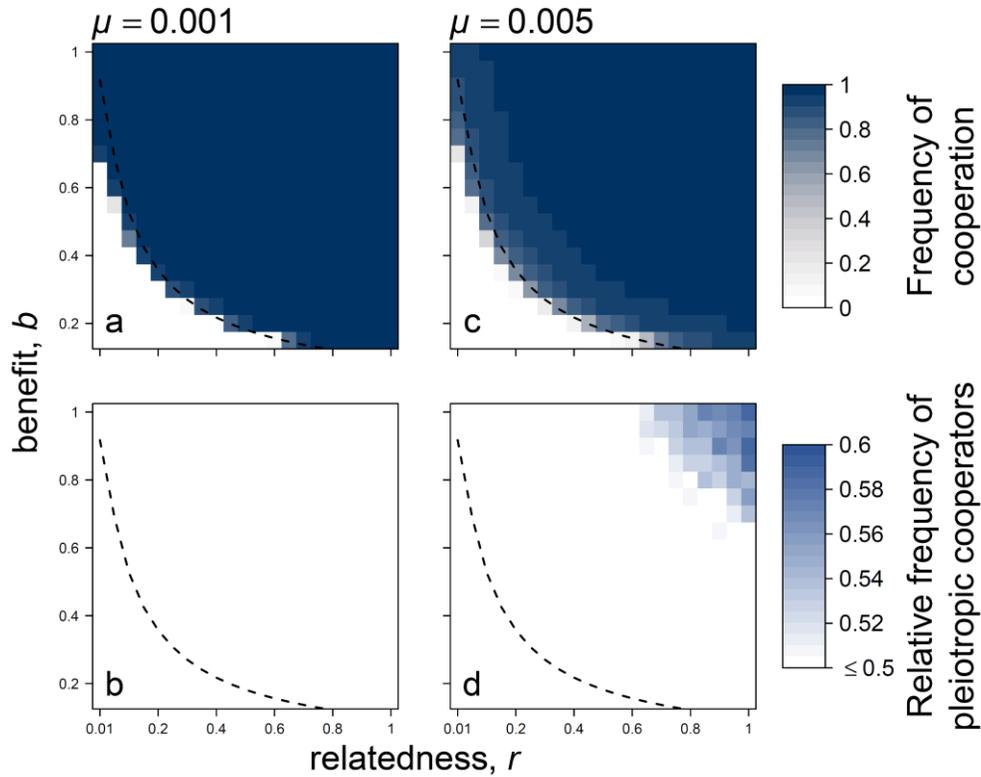
S3 Fig. Pleiotropy promotes cooperation and anything else.

Pleiotropic cooperators compete with non-cooperative private non-producers. Shown is the average pleiotropy as a function of the production cost c , and the public good benefit, b . Pleiotropy prevails even when producing public goods is harmful to both the actor and its partners, i.e., $b \leq 0$, as long as expressing both the private and social traits leads to a fitness that is greater or equal to one. Otherwise, the population goes extinct (white area). Parameters: $c = 0.1, g = 0.5, \mu = 0.001$.



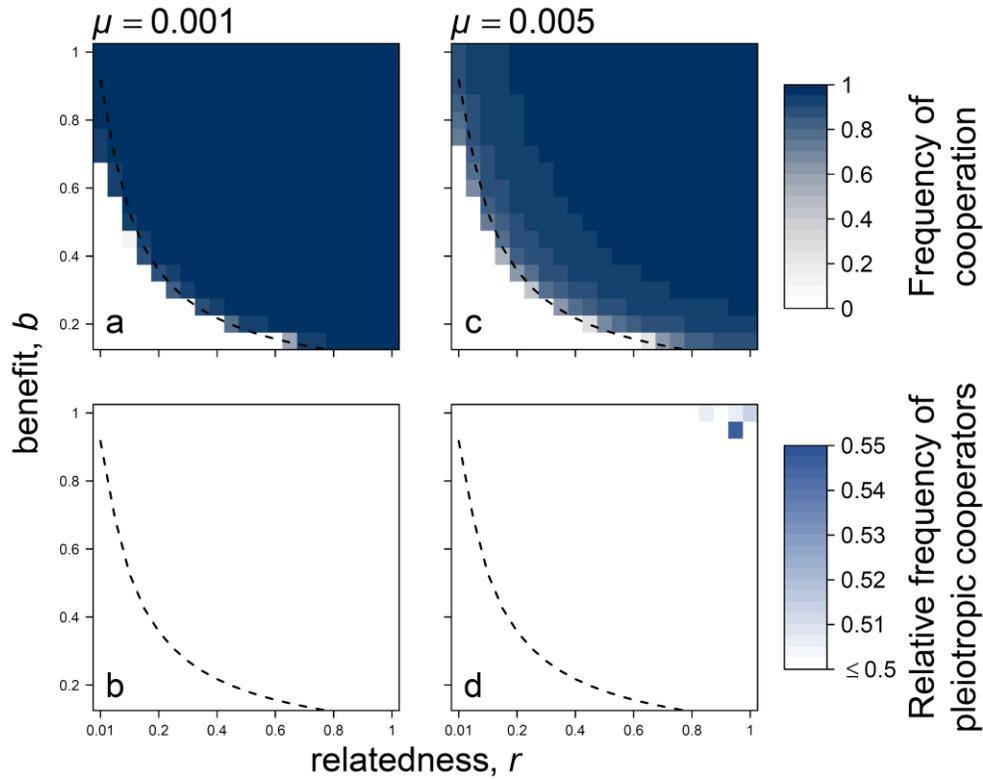
S4 Fig. Pleiotropy and the cost of not producing the private good.

Pleiotropic cooperators compete with non-cooperative private non-producers. Pleiotropy prevails as long as expressing both the private and cooperation trait leads to a sufficiently better growth rate. Otherwise, non-cooperative private non-producers prevail (white area). In these runs, relatedness, $r = 0.01$, which represents the most difficult condition for non-pleiotropic cooperation to evolve in our simulation. The baseline growth rate of pleiotropic cooperators $g = 0.5$. Parameters: $c = 0.1, \mu = 0.001$.



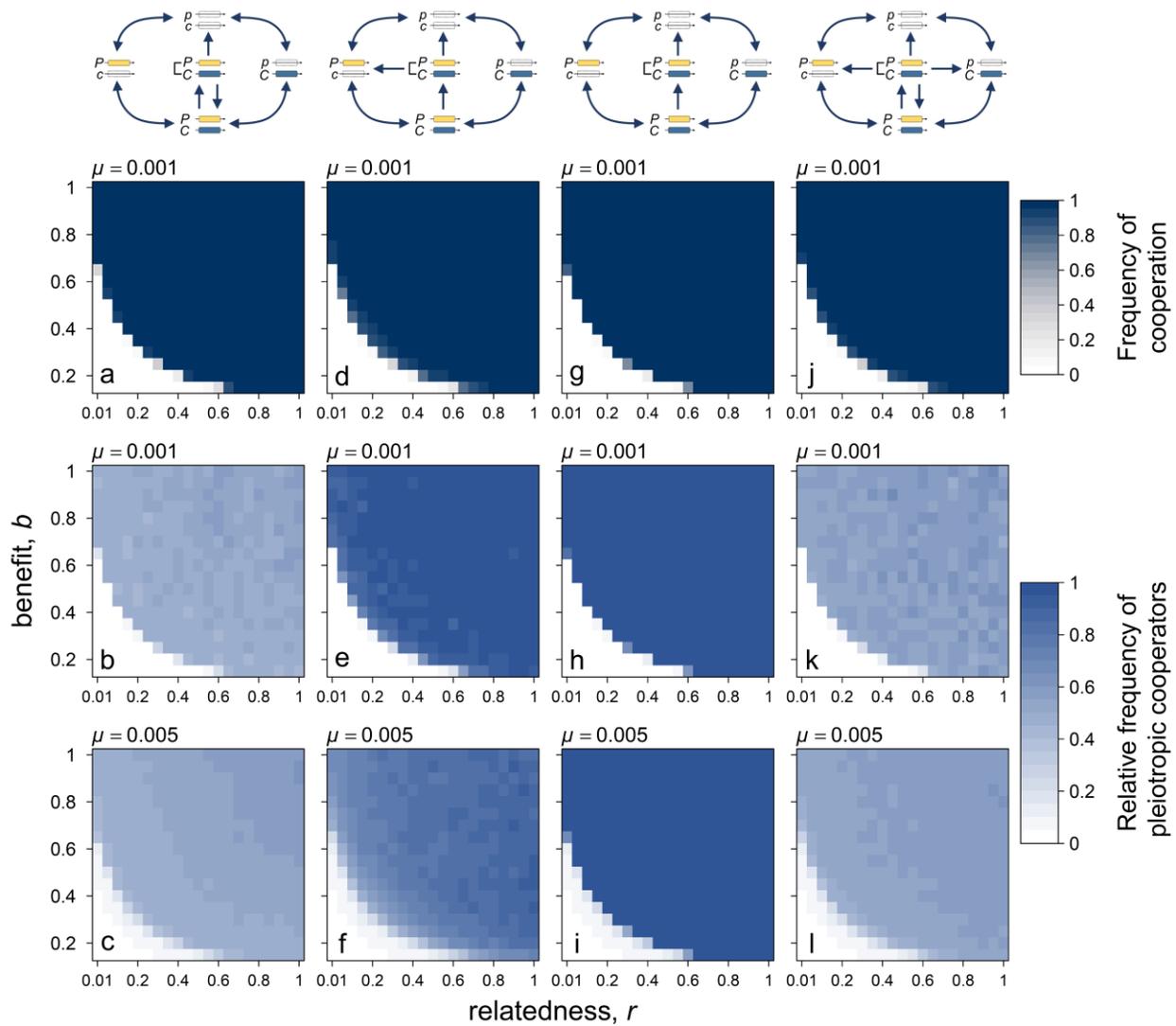
S5 Fig. Pleiotropy and a mutational bias of 10.

Losing a function (i.e., cooperation, private trait, and pleiotropy) is 10 times more likely than gaining one. For example, if a mutation occurs in a non-pleiotropic cooperator, it has 10 times more chances to lead to a loss of either cooperation or private production, than a gain of the pleiotropic link. As before, cooperation only evolves when Hamilton's rule is satisfied. However, pleiotropy is only favoured under high relatedness and large cooperation benefits, and a high mutation rate. Panels (a) and (c) show the frequency of cooperation, and panels (b) and (d) show their respective proportion of pleiotropic cooperators. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that migration occurs every generation (i.e., $k = 1$ in Eq.(3) in the main text). In all panels, all genotypes and mutations I and II in Fig. 3 of the main text are allowed. Parameters: $c = 0.1, g = 0.5, k = 10$.



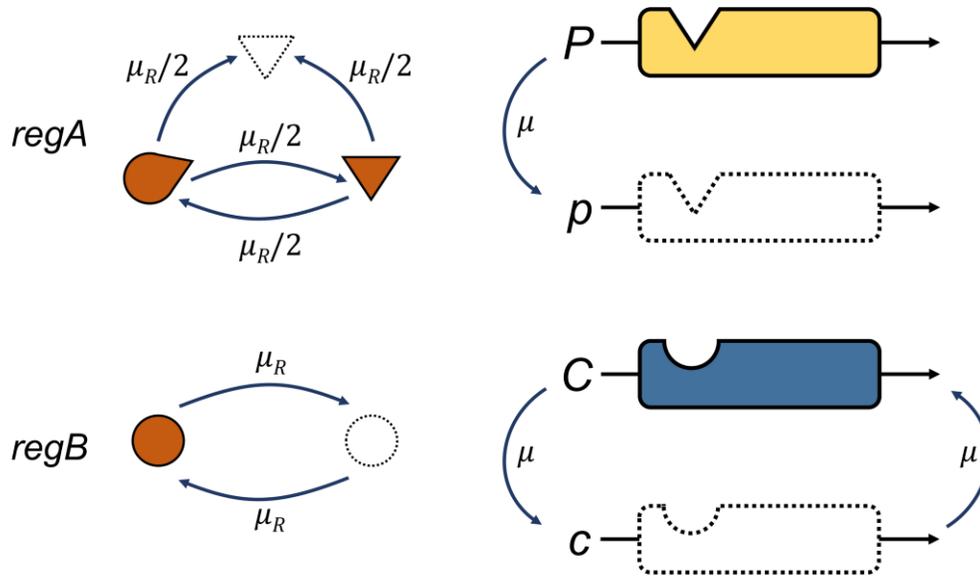
S6 Fig. Pleiotropy and a mutational bias of 100.

Losing a function (i.e., cooperation, private trait, and pleiotropy) is 100 times more likely than gaining one. For example, if a mutation occurs in a non-pleiotropic cooperator, it has 100 times more chances to lead to a loss of either cooperation or private production, than a gain of the pleiotropic link. As before, cooperation only evolves when Hamilton's rule is satisfied. However, pleiotropy is only favoured with relatedness and cooperation benefits close to 1, and a high mutation rate. Panels (a) and (c) show the frequency of cooperation, and panels (b) and (d) show their respective proportion of pleiotropic cooperators. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that migration occurs every generation (i.e., $k = 1$ in Eq.(3) in the main text). In all panels, all genotypes and mutations I and II in Fig. 3 of the main text are allowed. Parameters: $c = 0.1, g = 0.5, k = 10$.



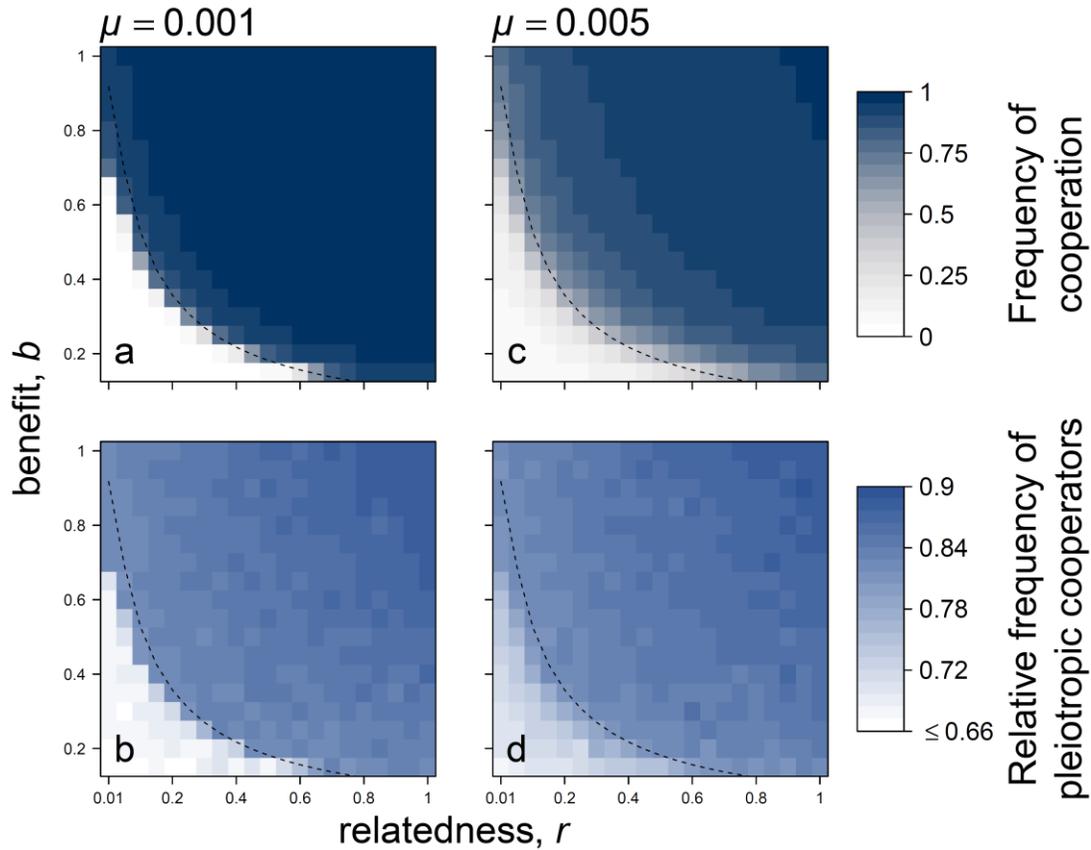
S7 Fig. Cooperation and pleiotropy frequencies in different scenarios.

Each column represents a distinct scenario, whose corresponding genetic architecture is shown at the top. In (a-c) and (j-l), pleiotropy can revert to non-pleiotropic cooperation (mutation II. in Fig. 3 of the main text). In (d-f) and (j-l), mutations on the cooperation trait in pleiotropic individuals are possible, and generate cheats. In (j-l), mutations on the private trait in pleiotropic individuals are possible. Pleiotropy prevails only when Hamilton's Rule is satisfied when in competition with all non-pleiotropic genotypes (Fig. 3 in the main text). Whenever pleiotropy cannot revert to a two-regulator system (panels d-i), the population is entirely invaded by pleiotropic cooperators. Parameters: $c = 0.1$, $g = 0.5$.



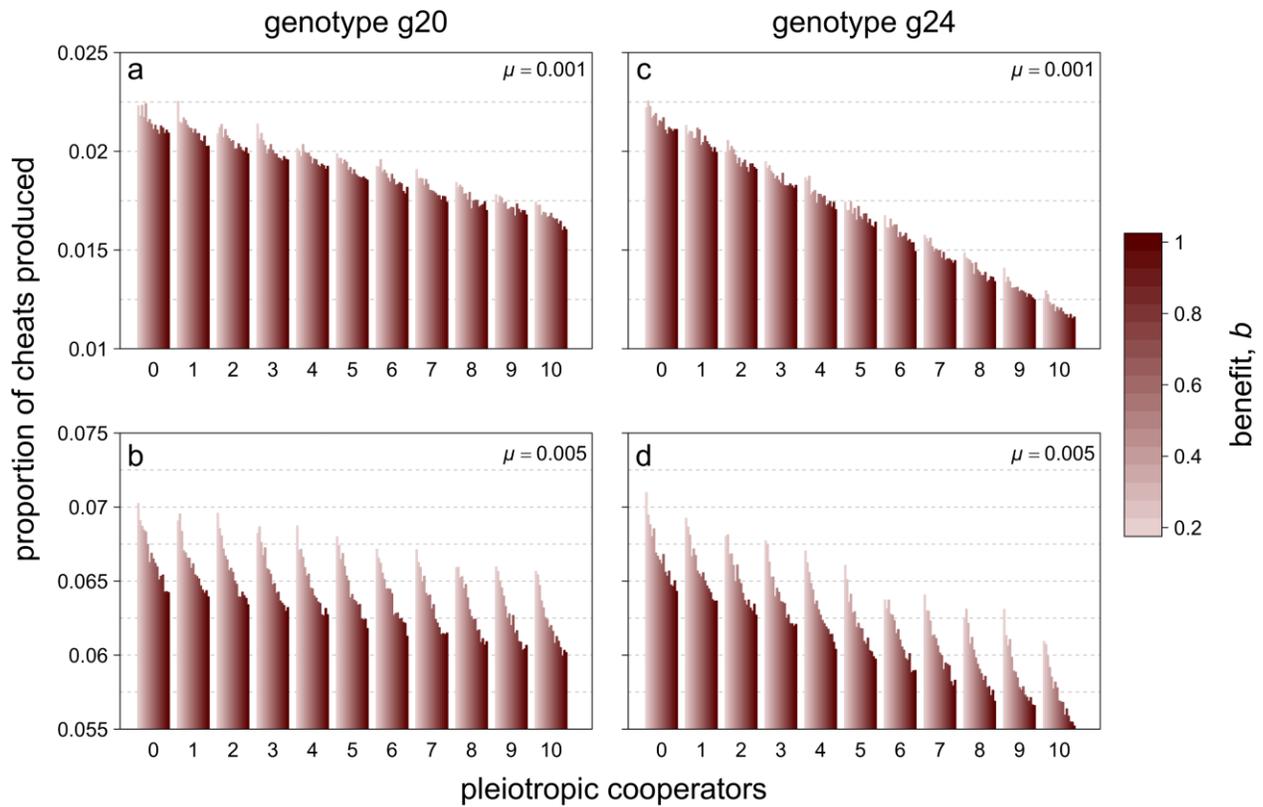
S8 Fig. Genetic elements in the explicit model.

We model more explicitly the pleiotropic link between the private and cooperation genes. Each gene is expressed only if both the expressing version of the allele (*C* or *P*) and the corresponding expressing version of its regulator (filled *regA* for the private trait and filled *regB* for the cooperation trait) are present. We assume that each regulator can be lost, and that the private regulator, *regA*, can become pleiotropic, by being able to regulate both genes at the same time (pointy orange circle). During reproduction, each regulator mutates independently with probability μ_R , and each gene mutates with probability μ . We assume that the private trait is essential, so in case the private regulator and/or private gene is lost through mutation, the individual dies. All the possible genotypes resulting from these elements and their corresponding phenotypes are listed in Table S2.



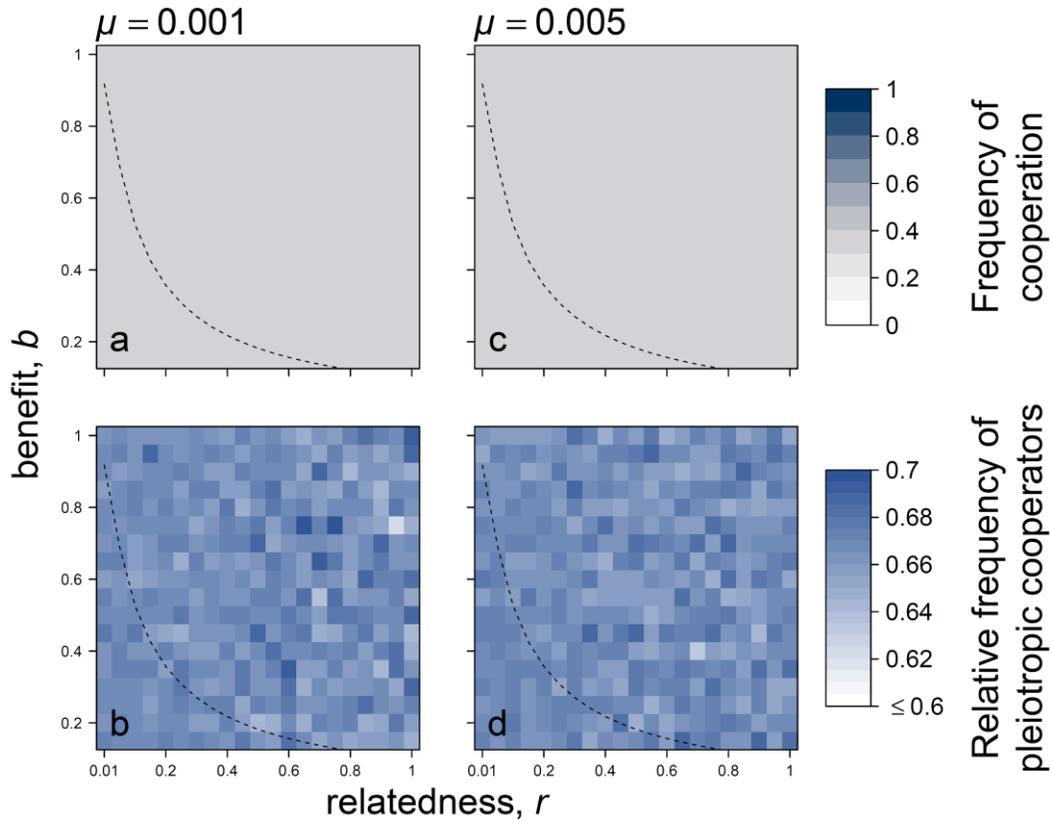
S9 Fig. Cooperation and pleiotropy when the pleiotropic link is explicitly modelled as a universal regulator.

Panels (a) and (c) show the frequency of cooperation, and panels (b) and (d) show their respective relative proportion of pleiotropic cooperators (i.e., frequency of genotype number $g_{20} + g_{24}$ over the sum of frequencies of the genotype number g_{16}, g_{20}, g_{24}). The dashed line represents the analytical prediction $r(N-1)b/N = c-b/N$, for when Hamilton's Rule is satisfied assuming that migration occurs every generation (i.e., $k = 1$). Parameters: $n_p = 500, c = 0.1, g = 0.5, k = 10, \mu_R = 0.001$.



S10 Fig. Proportion of cheats produced by mutation when the pleiotropic link is explicitly modelled as a universal regulator.

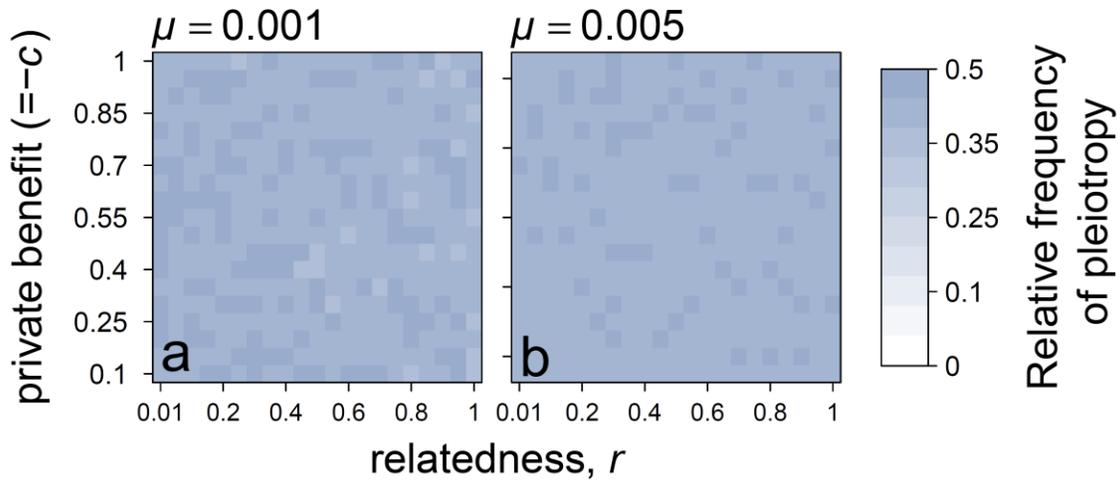
All panels show the proportion of cheats produced during a single growth phase, for different values of the cooperation benefit b and mutation rate μ . Each patch is started with 10 cooperators, and the x axis shows the number of those cooperators where cooperation was pleiotropically linked to an essential private trait, using pleiotropic cooperators with genotype number g20 (a-b) or g24 (c-d). Increased pleiotropy leads to a decreased accumulation of cheats. Each bar represents the average of 10^4 patches. Parameters: $c = 0.1$, $g = 0.5$, $k = 10$, $\mu_R = 0.001$.



S11 Fig. Cooperation and pleiotropy in neutral runs when the pleiotropic link is explicitly modelled as a universal regulator.

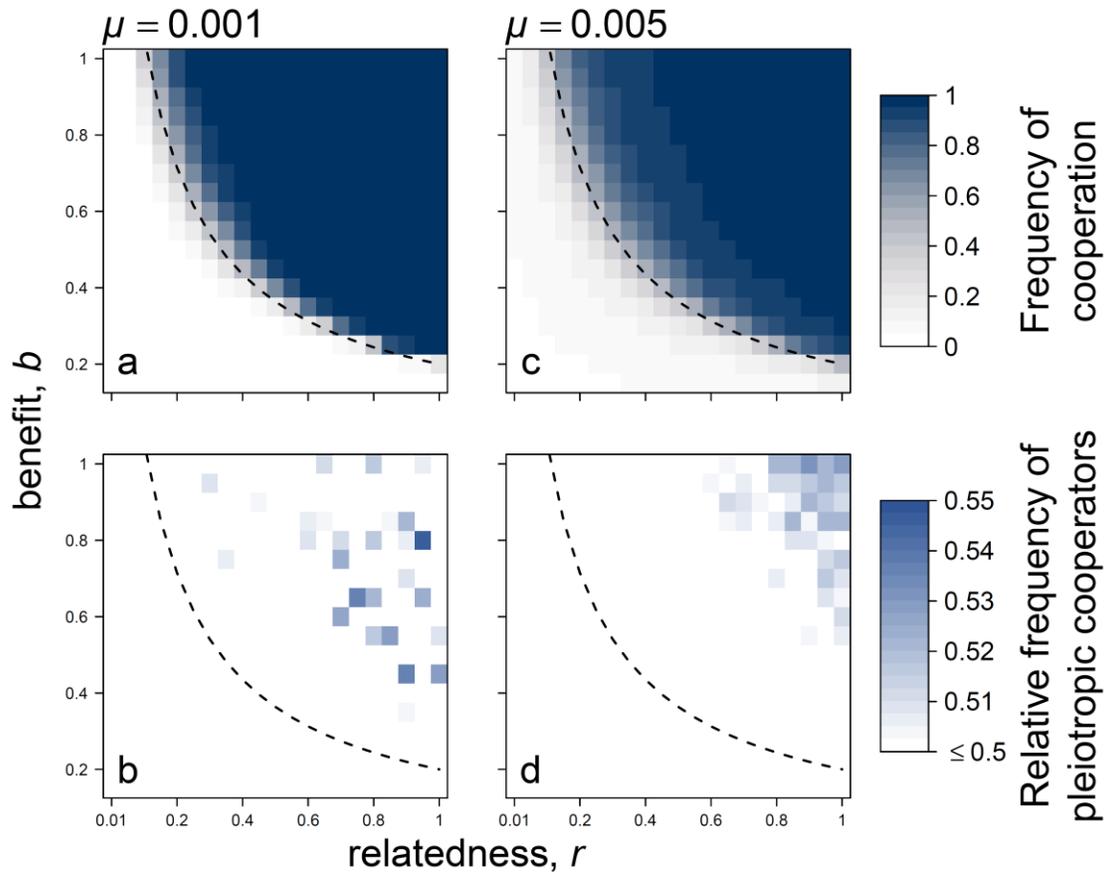
Panels (a) and (c) show the frequency of cooperation, which ranges between 0.36 and 0.38. This is because there are three cooperative genotypes among a total of 8 viable genotypes (Table S2), with $3/8 = 0.375$. In panels (b) and (d) show their respective relative proportion of pleiotropic cooperators (i.e., frequency of genotype number $g_{20} + g_{24}$ over the sum of frequencies of the genotype number g_{16}, g_{20}, g_{24}). The dashed line represents the analytical prediction $r(N-1)b/N = c-b/N$, for when Hamilton's Rule is satisfied assuming that migration occurs every generation (i.e., $k = 1$). All runs are neutral with respect to the cooperation trait, but not the essential private trait, i.e., all individuals who express the essential private trait have fitness $1 + g$, while the others die.

Parameters: $n_p = 500, g = 0.5, k = 10, \mu_R = 0.001$.



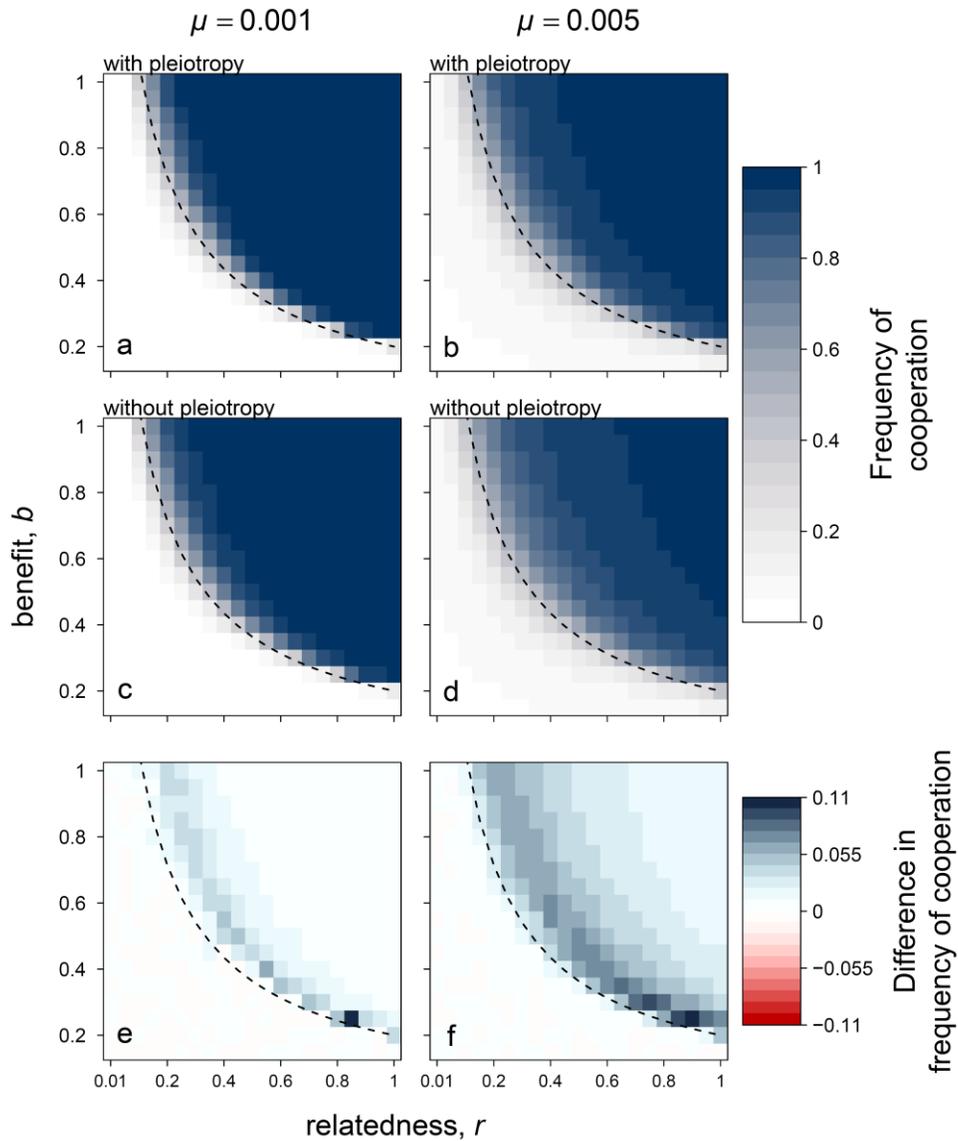
S12 Fig. Pleiotropy between two privately beneficial traits.

As in our baseline scenario, the private trait is essential. However, the cooperation trait is replaced by a second privately beneficial trait (with $b = 0$ and $c < 0$). As a result, individuals expressing both private traits can either be pleiotropic or non-pleiotropic. In both panels, individuals with a pleiotropic link between two private traits are never more common than individuals without this pleiotropic link, as their proportion never exceeds 50%. Parameters: $b = 0$, $g = 0.5$, $k = 10$.



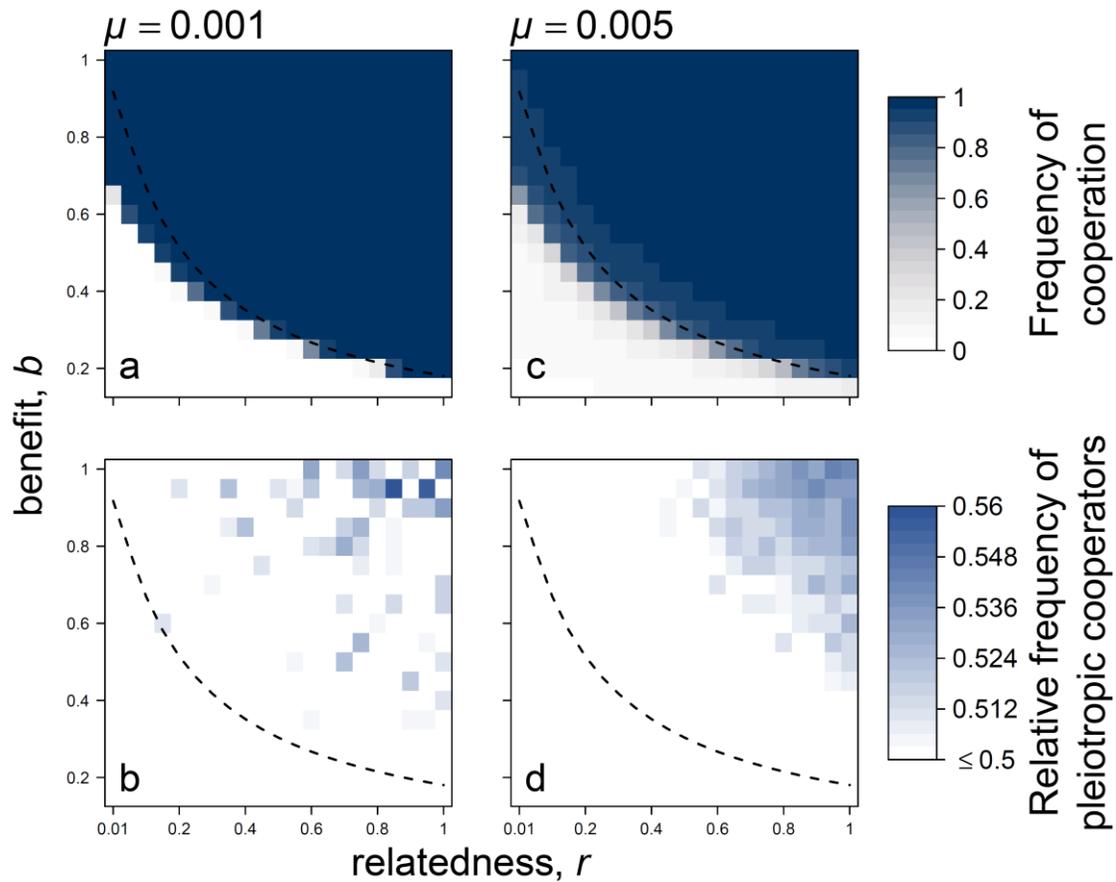
S13 Fig. Cooperation and pleiotropy with fluctuating benefit b .

The benefit of cooperation is alternating between 0 and the value shown on the y axis every 10 generations (i.e., every 1 growth phase). Cooperation is less likely to evolve under such fluctuating environment, and pleiotropy only evolves when Hamilton's rule is satisfied. Panels (a) and (c) show the frequency of cooperation, and panels (b) and (d) show their respective proportion of pleiotropic cooperators. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that the benefit is $b/2$ and that migration occurs every generation (i.e., $k = 1$; and substituting $b=b/2$ in Eq.(3) in the main text). In all panels, all genotypes and mutations I and II in Fig. 3 of the main text are allowed. Parameters: $c = 0.1, g = 0.5, k = 10$.



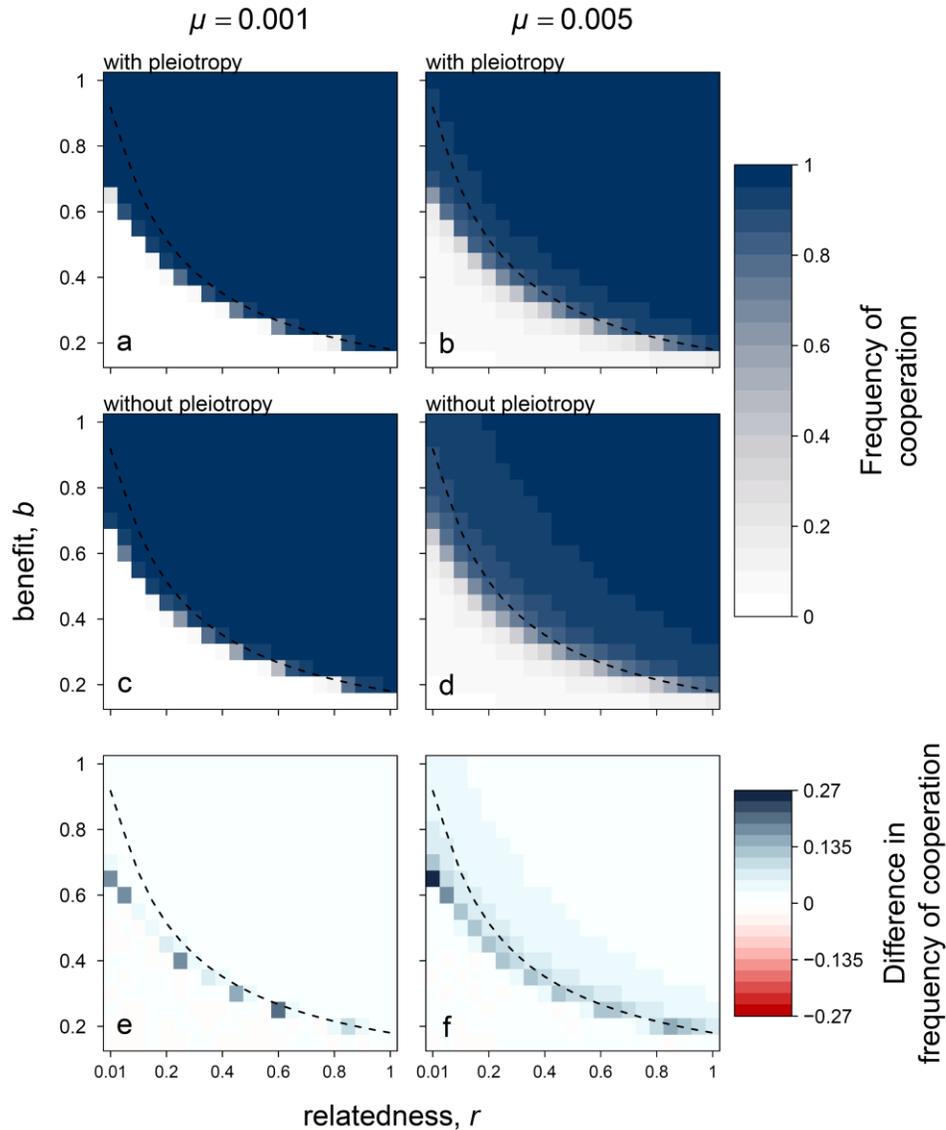
S14 Fig. Cooperation with and without pleiotropy and fluctuating benefit b .

The benefit of cooperation is alternating between 0 and the value shown on the y axis every 10 generations (i.e., every growth phase). Cooperation is more likely to evolve if pleiotropy is allowed. In (a) and (b), pleiotropic cooperators are allowed (all genotypes and mutations I and II in Fig. 3 of the main text). In (c) and (d), pleiotropic cooperators are replaced by non-pleiotropic cooperators (this maintains a similar ratio of cooperative strategies to when pleiotropic cooperators are present). Panels (e) and (f) show the difference in cooperation frequency between (a) and (c), and (b) and (d), respectively. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that the benefit is $b/2$ and that migration occurs every generation (i.e., $k = 1$; and substituting $b=b/2$ in Eq.(3) in the main text). Parameters: $c = 0.1, g = 0.5, k = 10$.



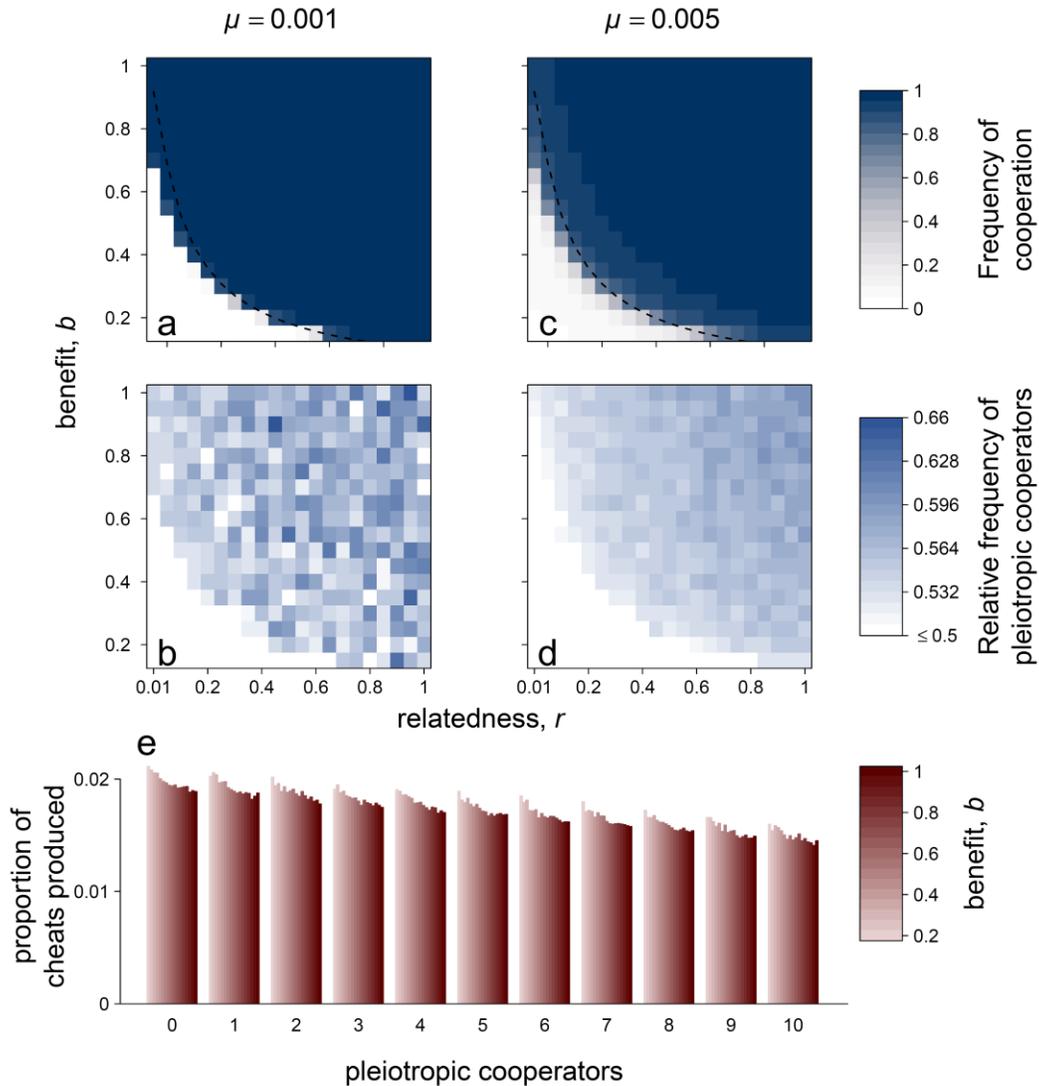
S15 Fig. Cooperation and pleiotropy with fluctuating population structure.

Population structure (relatedness r) is alternating between 0.01 and the value shown on the x axis every 10 generations (i.e., every 1 growth phase). Cooperation is less likely to evolve under such fluctuating environment, and pleiotropy only evolves when Hamilton's rule is satisfied. Panels (a) and (c) show the frequency of cooperation, and panels (b) and (d) show their respective proportion of pleiotropic cooperators. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that relatedness is $(r+0.01)/2$ and that migration occurs every generation (i.e., $k = 1$; and substituting $r=(r+0.01)/2$ in Eq.(3) in the main text). In all panels, all genotypes and mutations I and II in Fig. 3 of the main text are allowed. Parameters: $c = 0.1, g = 0.5, k = 10$.



S16 Fig. Cooperation with and without pleiotropy and fluctuating population structure.

Population structure (relatedness r) is alternating between 0.01 and the value shown on the x axis every 10 generations (i.e., every 1 growth phase). Cooperation is more likely to evolve if pleiotropy is allowed. In (a) and (b), pleiotropic cooperators are allowed (all genotypes and mutations I and II in Fig. 3 of the main text). In (c) and (d), pleiotropic cooperators are replaced by non-pleiotropic cooperators (this maintains a similar ratio of cooperative strategies to when pleiotropic cooperators are present). Panels (e) and (f) show the difference in cooperation frequency between (a) and (c), and (b) and (d), respectively. The dashed line represents the analytical prediction for when Hamilton's Rule is satisfied assuming that relatedness is $(r+0.01)/2$ and that migration occurs every generation (i.e., $k=1$; and substituting $r=(r+0.01)/2$ in Eq.(3) in the main text). Parameters: $c = 0.1, g = 0.5, k = 10$.



S17 Fig. Cooperation promotes pleiotropy under relaxed assumptions.

Panels a and c show the frequency of cooperation for different mutation rates μ , and panels b and d show their respective proportion of pleiotropy relative to all cooperative genotypes (i.e. pleiotropic cooperators, non-pleiotropic cooperators and cooperative private non-producers). The dashed lines represent the analytical prediction for when Hamilton's Rule is satisfied assuming that migration occurs every generation (i.e., $k = 1$; Eq.(3) in the main text). Panel e shows the proportion of cheats produced by mutation and growth, during a single growth phase (mutation rate $\mu = 0.005$), for different values of the cooperation benefit b . Each patch is started with 10 cooperators, and the X axis shows the number of those cooperators where cooperation was pleiotropically linked to an essential private trait. Increased pleiotropy leads to a decreased accumulation of cheats, but to a lesser extent than when mutations in pleiotropic individuals cannot generate cheats (compare panel c with Fig.4c). Each bar represents the average of 10^4 patches. Parameters: $c = 0.1$, $g = 0.5$, $k = 10$.

3. Supplementary references

Rousset F (2004) Genetic structure and selection in subdivided populations. Princeton University Press, Princeton.