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Enforcement is central to the evolution of cooperation

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Supplementary Materials for:

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Definition of enforcement (summary of Box 1)

We define enforcement as an action that evolves, at least in part, to reduce selfish behaviour within a cooperative alliance. Individuals can use enforcement in two key ways: either to change the strength of interaction with different partners (partner choice; Table 1; Box 2), or to manipulate the amount that their partners display selfish behaviours rather than cooperative ones (partner manipulation; Table 1; Box 2). We distinguish between the selfishness of group members, z , and the selfishness experienced by a focal individual, y . In the models below, these are sometimes equivalent but, where they are not, the key measure of selfishness is that experienced by an enforcer y (plotted in Figure 3), which captures any effects of enforcement from the enforcer's perspective (the key to the evolution of enforcement, see next section).

Consider a focal actor who experiences a level of selfishness, y , from others that is a function of that actor's investment in enforcement, a , such that $y = y(a)$. Here, $a = 0$ represents no enforcement and $a > 0$ represents enforcement. If investing in enforcement (increasing a) reduces the selfishness experienced by the focal actor (decreases $y(a)$) then the focal actor is enforcing cooperation. Specifically, in the absence of enforcement ($a = 0$), define the focal actor's strength of interaction with individual i of n partners as $\beta_i = \beta_i(0)$ and individual i 's level of selfishness as $z_i = z_i(0)$. (The β_i function quantifies how much an individual's selfishness matters to the focal actor's fitness; for example, when the focal individual interacts with all of its n partners equally, we would have $\beta_i = \frac{1}{n}$.) When the actor invests in enforcement ($a > 0$), these change to $\beta_i(a)$ and $z_i(a)$. Formally, the focal actor's experienced selfishness can be written without enforcement as

$$y(0) = \sum_i^n \beta_i(0)z_i(0) \quad (\text{S1})$$

and with enforcement as

$$y(a) = \sum_i^n \beta_i(a)z_i(a). \quad (\text{S2})$$

We are interested in the evolution of selfish traits and their suppression by enforcement. A trait z is selfish if, in the absence of enforcement, a marginal increase in the trait increases its fitness w (i.e., $dw/dz > 0$) but decreases the fitness of other individuals w' (i.e., $dw'/dz < 0$). Meanwhile, a trait a causes the enforcement of cooperation if a marginal increase the trait value decreases experienced selfishness (i.e. $dy/da < 0$). The evolution of enforcement can occur because of its effects on selfishness, or due to other unrelated effects (byproduct enforcement; Box S1). We are most interested in the former cases where the focal trait a evolves, at least in part, *because* of its effects on selfishness within a cooperative alliance, which implies that $dw/dy < 0$ and $dy/da < 0$, and by transitive logic $dw/da > 0$. Finally, we assume in the models below that enforcement carries a cost, C . For simplicity, we phrase the above in terms of the direct benefits to the enforcer, but enforcement can also evolve due to indirect fitness benefits, as we discuss next.

Direct and indirect benefits of enforcement

The evolution of enforcement, like cooperation, rests upon there being either direct benefits to an enforcer (via improved lifetime reproduction), or indirect fitness benefits via improved reproduction of the enforcer's relatives (Box 2). Examples of direct benefits include a plant that limits the access of nectar robbing insects, but not pollinators, using complex floral morphology¹ (Model 6, below). Another example is reciprocal altruism in humans, which uses cooperation to make other reciprocators cooperate back². Direct benefits can also come via effects on the whole social group³. For example, the evolution of a silencing allele that reduces the transposition rate of transposable elements will benefit the suppressor because it increases genome-level fitness⁴. Here, the benefit from enforcement is direct because it falls back on the allele that silences the elements. An example of indirect benefits driving enforcement is the evolution of distinct queen cells in corbiculate bees, which allows workers to control which larvae become queens and thereby prevents caste conflict⁵⁻⁷. This requires relatedness within the colony, such that there is an indirect fitness benefit to the non-reproductive workers from improving colony productivity.

With relatedness, enforcement can also occur via an allele that reduces the expression of selfishness in carriers (self-enforcement)⁸. For example, there is a tight pleiotropic link between proliferation and apoptosis in the regulatory networks of mammalian cells, which means many potentially cancerous cells simply enter programmed cell death^{9,10}. For this to be favoured by natural selection, clonemates have to benefit from the suicide of a pre-cancerous cell (Model 3). This then satisfies the condition that an individual carrying an enforcement allele (a) will experience reduced selfishness, i.e. $dy/da < 0$ because group members that did not undergo apoptosis will also tend to carry the allele, which allows $dw/da > 0$ to be satisfied (above).

In the models of enforcement that follow, we analyse examples of enforcement involving both direct and indirect benefits to the enforcer. To account for indirect benefits of enforcement, experienced selfishness $y(a)$ could be defined separately for each class of relatives and then prorated by genetic relatedness as appropriate. The models analysing indirect benefits of enforcement below (Models 1 and 4) simply assume that each relative in a cooperative group experiences the same level of selfishness, but this can be extended to models where different relatives may each have a separate experienced level of selfishness.

Models of Enforcement

We develop a series of models to study the evolution of enforcement. Our goal is to identify the broad conditions that favour, or disfavour, the evolution of enforcement and thereby assess its generality as an explanation for cooperation. In order to preserve key biological details, we do not seek to reduce the problem to a single general model. Instead, we develop one or more dedicated models at each level of biological cooperation (Figures 1 and 2; Table S1). The examples also cover the different mechanisms of enforcement (partner choice versus manipulation; Table 1) as well as enforcement that benefits the enforcer (direct fitness) and cases that benefit relatives

Table S1 Summary of the models

<i>Model</i>	<i>Level^a</i>	<i>Mechanism^b</i>	<i>Benefit</i>	<i>Relatedness^c</i>
Replicators in a protocell	Genome	manipulation	Direct/Indirect	yes
Endosymbionts of isogamous cells	Complex cell	manipulation	Direct	no
Early multicellularity and cancer	Organism	choice	Indirect	yes
Reproductive conflict in animals	Society	manipulation	Direct/Indirect	yes
Host-symbiont mutualism	Mutualism	choice	Direct	no
Plant-pollinator mutualism	Mutualism	manipulation	Direct	no

a) Level of biological organisation (Figure 1). b) Mechanism of enforcement (Box 1, Table 1). c) Relatedness between enforcer and enforceses is possible in the model.

(indirect fitness). The evolution of enforcement has the potential to drive the evolution of novel selfish strategies that escape a particular form of enforcement^{11,12}, followed by the evolution of new more-effective enforcement, and so on¹³. While we do not consider this process explicitly, our models describe the conditions for selfishness and enforcement for any one round of this process. Finally, though the study of enforcement has often focused on derived systems, particularly in the social insects¹⁴⁻¹⁷, our examples often reflect an early stage in the evolution of a given social system. We do this to challenge the notion that enforcement is only important in the maintenance, and not the origin, of cooperative systems.

In the models that follow, we notate individual investment in enforcement and selfishness as a and z , respectively; group mean investment in these traits as A and Z ; population mean investment in these traits as \bar{a} and \bar{z} ; and the cost of enforcement as C .

Model 1: Replicators in a protocell (and review of Frank's policing model)

We first consider a model of a simple group of replicating molecules in an early cell (Figure 2a)¹⁸. In the absence of investment into selfishness (z), we assume that replicators are transmitted fairly each cell division. However, replicators can attract a catalytic molecule (equivalent to a retrotransposase in modern systems) with affinity z to afford them an additional duplication per cell division at the cost of other replicators. Additionally, we assume that a replicator can spend a fraction a of its time bound to the catalytic molecule without undergoing replication, which limits the potential for other replicators to replicate selfishly, at cost Ca to itself; such that when $A = 1$, no selfish replication can occur. This simple scenario can be captured with a modification of Frank's classic model of mutual policing¹⁹. We therefore first review Frank's model before we modify and apply it to the case of replicators.

Mutual policing is a type of enforcement by manipulation in which the enforcers are themselves also the targets of enforcement. Therefore, a focal individual expressing selfishness z and

enforcement a belongs to a larger group expressing average selfishness Z and average enforcement A . From Frank¹⁹, an individual's fitness is then

$$w = (-Ca + A + (1 - A)\frac{z}{Z})(1 - (1 - A)Z), \quad (\text{S3})$$

where C is the individual marginal cost of enforcement, accounting for the $-Ca$ term. A proportion A of all social interactions are policed. The fruits of a policed interaction are divided fairly among all members of the group, while the fruits of an unpoliced interaction accrue to each individual proportionally to their relative selfishness, z/Z . This accounts for the $A + (1-A)z/Z$ term. Finally, the group as a whole experiences a loss of cooperation-derived fitness due to any unpoliced selfishness, which accounts for the $1 - (1 - A)Z$ term.

Frank found that the equilibrium level of selfishness in the absence of enforcement was $z^* = 1 - R$, where R is the average group relatedness. Since there is no enforcement, we also have the equilibrium experienced selfishness $y^* = z^*$. If we allow enforcement to evolve, we have enforcement at equilibrium $a^* = 1$ and experienced selfishness at equilibrium $y^* = 0$ if $C < 1 - R$, and $a^* = 0$ and $y^* = z^* = 1 - R$ if $C > 1 - R$. That is, full policing evolves if the cost of enforcement is less than the equilibrium selfishness prior to enforcement $z^* = 1 - R$, while if the cost of enforcement is less than this cost of selfishness, enforcement does not evolve.

A number of modifications have been made to Frank's basic model²⁰. For example, one key assumption in the original model is that the cost of enforcement is independent of an individual's investment selfishness per se. This assumption is likely to be violated in our replicator system, where an individual's investment in enforcement by blocking the catalytic molecule will trade off with its replication proper. We can capture this tradeoff with

$$w = (A + (1 - A)\frac{z(1-Ca)}{Z(1-Ca)})(1 - (1 - A)Z), \quad (\text{S4})$$

where $A + (1 - A)\frac{z(1-Ca)}{Z(1-Ca)}$ captures the individual benefits of competition to replicators and $(1 - (1 - A)Z)$ captures the shared fitness interest ("being in the same boat", see main text) of the replicators, which is harmed by competition Z but improved by investment into enforcement A . In the absence of enforcement ($a = A = 0$), this is equivalent to Frank's model, meaning that equilibrium selfishness in the absence of enforcement is $z^* = 1 - R$. As for Frank's model then, without enforcement the model predicts the evolution of selfishness that will reduce cooperation and compromise the function of the protocell. This is also consistent with the prediction that early replicator systems would be susceptible to the evolution of selfish genetic elements that invest in biasing personal replication over replication of other sequences and the cell^{18,21}.

We can then ask whether enforcement, a , will increase from zero. This will occur when

$$\frac{\partial w}{\partial a} \Big|_{a=A=0, z=Z=1-R} + R \frac{\partial w}{\partial A} \Big|_{a=A=0, z=Z=1-R} > 0, \quad (\text{S5})$$

which eventually simplifies to

$$C < 1.$$

Moreover, we find that when $C = 1$, any nonzero enforcement ($a > 0$) makes enforcement easier to evolve, meaning that any unilateral expression of enforcement can select for group enforcement. As the system evolves, we find that $a^* = 1$ and $y^* = 0$ for all $C \leq 1$, meaning that policing always becomes absolute and reduces experienced selfishness to zero under these conditions. That is, for our scenario – where the cost of policing is in terms of an individual’s ability to selfishly replicate – policing is easier to evolve than under Frank’s original model (Figure 3a vs 3b). Evolution then is cost dependent but so long as policing is equally or less costly per unit investment than replication, it is predicted that enforcement removes all conflict (Figure 3b).

Note that, in this case, the evolution of enforcement requires a group benefit to succeed, which is exemplified by the shared fitness term $(1 - (1 - A)Z)$ of equation (S4). Unless enforcement improves group function, it does not evolve. Specifically, if we remove this effect from the fitness function above, yielding the alternative form

$$w = (A + (1 - A) \frac{z(1-ca)}{z(1-cA)})(1 - Z), \quad (\text{S6})$$

the condition for natural selection to favour an increase in enforcement becomes $R > 1$, which can not be fulfilled. This formalizes the intuition that while “being in the same boat” is insufficient to prevent conflict, it can create a shared collective interest that allows highly-effective enforcement to evolve (see main text).

Relation to definitions of selfishness and enforcement — In the model above, affinity for the catalytic molecule, z , meets the definition of selfishness. That is because, in the absence of enforcement, $w = \frac{z}{Z}(1 - Z)$, and hence an increase in z improves the fitness of a focal replicator ($\frac{dw}{dz} = \frac{1-Z}{z}$, and so $\frac{dw}{dz} > 0$ when $0 < Z < 1$), while an increase in Z decreases the expected fitness of any replicator ($\frac{dw}{dZ} = -\frac{z}{Z^2}$, and so $\frac{dw}{dZ} < 0$), which fulfils the conditions for selfishness.

Interference with the catalytic molecule, a , meets the definition of enforcement because it reduces experienced selfishness and hence restores fitness. Specifically, experienced selfishness is $y = (1 - A)Z$, so $\frac{dy}{dA} < 0$ when $Z > 0$. Moreover, $w = (A + (1 - A) \frac{z(1-ca)}{z(1-cA)})(1 - y)$, and so $\frac{dw}{dy} < 0$. Together, these fulfil the conditions for enforcement.

Model 2: Endosymbionts of isogamous cells

We next consider a well-mixed population of single-celled eukaryotic diploid organisms that carry endosymbiotic bacteria, such as early mitochondria or chloroplasts. Sexual reproduction was present in the last universal common ancestor of eukaryotes²². We therefore assume the host organisms occasionally undergo meiosis to form haploid gametes of two mating types (m_1 and

m_2), where gametes are assigned a mating type at random (Figure 2b). As for the protocell, we first consider the potential for endosymbiont selfishness to evolve, before secondly asking whether enforcement will evolve to suppress any selfishness that arises.

The scope for selfish symbionts is as follows. When two gametes fuse, the endosymbionts carried by one of the two gametes stand to gain a twofold transmission advantage if they can eliminate the endosymbionts of the other gamete, e.g. via one of the toxin-immunity protein pairs that are widespread in bacteria²³. To analyse this case, we use the logic of classical game theory before performing simulations to support our conclusions. Suppose that the population-level frequency of endosymbionts engaging in selfish behaviour is \bar{z} ; endosymbionts engaging in killing (killer type) can spread if the fitness cost of endosymbiont warfare, c_{war} , is less than $1/2$, since costs greater than $1/2$ cancel the twofold transmission benefit of endosymbiont warfare. This cost may capture both the effects of coping with a half-complement of endosymbionts as well as the cost of synthesizing toxins for endosymbiont warfare.

When gametes carrying killer and passive endosymbionts fuse, the killer symbionts are retained and the resulting zygote has fitness $1 - c_{\text{war}}$. When killer symbionts mix, both sets are killed and the zygote has negligible fitness. Despite the high cost of conflict when killers meet, natural selection will promote the killer phenotype so long as $\bar{z} < 1$, since passive endosymbionts always stand to gain from adopting the killer phenotype. This prediction occurs because both phenotypes are killed upon encountering a killer; but as long as there are passives left in the population, a killer stands to gain from its strategy. A simple game theory model then predicts that there is the potential for strong conflict between endosymbionts that will be very costly for cellular function (Figure 3b), which is consistent with the predictions of more extensive population genetic analysis²⁴.

We next consider the potential for enforcement to affect the outcome of this conflict. Specifically, we consider the evolution of a nuclear modifier (a_+) that degrades the DNA of its endosymbionts shortly before or shortly after gametic fusion, conditional on the gamete's mating type. This is consistent with the known biology of isogamous unicellular species, such as *Chlamydomonas reinhardtii* where with an hour of mating, the DNA in chloroplasts of one mating type is selectively degraded before the chloroplasts lacking DNA fuse with those of the other mating type²⁵. Without loss of generality, we assume the uniparental phenotype is only expressed in m_1 gametes. This phenotype prevents endosymbiont conflict at cost C to the resulting zygote. Degrading endosymbiont DNA in this way may carry a relatively low cost as it occurs without destroying the organelles themselves. This suggests that $C \leq c_{\text{war}}$, i.e. a cell that sacrifices one set of endosymbionts in an energy-efficient manner will do so with lower cost to the cell than a killer endosymbiont strain using toxins to kill a competing passive strain. In the worst-case scenario, an m_1 gamete can sacrifice its endosymbionts completely, which ought to be no costlier for the cell than it is for killer endosymbionts to destroy opposing endosymbionts, i.e. at most $C \approx c_{\text{war}}$. If true, enforcement results in the zygote having an expected fitness of $1 - C$ rather than 0, and so enforcement should always invade in a population approaching complete endosymbiont selfishness ($\bar{z} \rightarrow 1$).

To verify this prediction, we simulate mating in a very large population of gametes with non-overlapping generations, which come in eight types, corresponding to mating type / endosymbiont type. We track the frequencies of the eight types: $m_1/\text{passive}/a_-$, $m_1/\text{passive}/a_+$, $m_1/\text{killer}/a_-$, $m_1/\text{killer}/a_+$, $m_2/\text{passive}/a_-$, $m_2/\text{passive}/a_+$, $m_2/\text{killer}/a_-$, and $m_2/\text{killer}/a_+$. When two gametes fuse, the fitness of the resulting zygote can be read from the following table:

		No enforcement by m_1 gamete	
		m_1 gamete	
m_2 gamete		passive	killer
		passive	1
killer	$1 - c_{\text{war}}$	0	

→

		Enforcement by m_1 gamete	
		m_1 gamete	
m_2 gamete		passive	killer
		passive	$1 - C$
killer	$1 - C$	$1 - C$	

After all gametes fuse into zygotes, they undergo viability selection (zygotes survive with probability proportional to their fitness w) and then undergo meiosis again, creating new gametes. Zygotes with both killer and passive endosymbionts pass on only the killers, while passive-passive zygotes pass on only passive endosymbionts. Gametes are randomly assigned a mating type, and mutation causes gametes to change their phenotype from killer to passive during meiosis with probability $\mu = 0.001$.

As predicted from game theory logic, we find that selfishness (the killer phenotype) sweeps to near-fixation in this scenario for any $c_{\text{war}} < 1/2$, and that when we allow enforcement (a_+ phenotype) to evolve, it also sweeps to fixation and causes realised selfishness (the frequency of gametic pairings resulting in endosymbiont warfare) to drop to zero for any cost of enforcement $C < 1$ (Figure 3c).

Relation to definitions of selfishness and enforcement — The killer gamete phenotype meets the definition of selfishness when $c_{\text{war}} < 1/2$ because the killer phenotype benefits gametes: in the absence of enforcement, a gamete's expected fitness is $w = (1 - z)(1 - \bar{z}) + 2z(1 - \bar{z})(1 - c_{\text{war}})$, so $\frac{dw}{dz} = -(1 - \bar{z}) + 2(1 - \bar{z})(1 - c_{\text{war}})$, which is positive when $c_{\text{war}} < 1/2$; and $\frac{dw}{d\bar{z}} = -(1 - z) - 2z(1 - c_{\text{war}})$, which is always negative. Accordingly, when $c_{\text{war}} < 1/2$, expressing the killer phenotype benefits a focal gamete ($\frac{dw}{dz} > 0$) and harms other gametes ($\frac{dw}{d\bar{z}} < 0$), fulfilling the condition for selfishness.

The a_+ nuclear modifier meets the definition of enforcement. With enforcement, an m_1 gamete's expected fitness is $w = (1 - a)[(1 - z)(1 - \bar{z}) + 2z(1 - \bar{z})(1 - c_{\text{war}})] + a(1 - C)$, which can be rewritten $w = (1 - a - y)[(1 - z) + 2z(1 - c_{\text{war}})] + a(1 - C)$, where $y = (1 - a)\bar{z}$ is the gamete's experienced selfishness, and the term in square brackets is the gamete's expected fitness in the absence of any enforcement or selfishness by others. Note that experienced selfishness y reduces this fitness component when the focal gamete is not an enforcer ($a = 0$), but has no impact upon fitness when the focal gamete is an enforcer ($a = 1$, which also makes $y = 0$). Since $\frac{dy}{da} < 0$ when $\bar{z} > 0$, enforcement decreases experienced selfishness.

Model 3: Early multicellularity and cancer

Here we consider the evolution of cancer suppression in a simple multicellular animal (Figure 2c). In some cases, like facial tumour in the Tasmanian devil, a cancer that arises within one individual can be transmitted to others²⁶. However, the majority of cancers are not transmitted and, as such, are short term evolutionary processes in the sense that they begin and end within the life of a multicellular organism. Nevertheless, cancer formally corresponds to the *de novo* evolution of a selfish phenotype within an organism that – within the timescale of one host generation – spreads for its own benefit but at a cost at the level of the organism²⁷⁻³¹. Moreover, phenotypes that suppress cancer and restore multicellular function correspond to the definition of enforcement against selfish phenotypes²⁷⁻³¹ (above).

Our model assumes that individuals start development as a single, non-cancerous cell, but that there is a per-cell-division risk of mutating to a cancer cell. For simplicity, we focus on those cancers in an organism that are ultimately fatal as these are the ones that are expected to have the greatest impact on multicellular function. To derive an organism of n cells from a single cell requires $n - 1 \approx n$ divisions. If the per-cell-division risk of cancer is μ , then the lifetime risk of cancer for an n -celled animal is

$$z = 1 - (1 - \mu)^{n-1} \approx 1 - \exp(-\mu n). \quad (\text{S8})$$

For simplicity, the amount of selfishness z in the system is the per-individual risk of getting cancer, which differs from our other models in that z is not expressed in terms of selfishness enacted at the lower level of biological organization, which would be per-cell selfishness. However, the two are equivalent in the sense that reducing z will also reduce the selfishness experienced per cell y . For fatal cancers, an individual's expected fitness is

$$w = 1 - z = \exp(-\mu n). \quad (\text{S9})$$

We then consider the potential evolution of self-enforcement⁸ whereby a fraction $A = a$ of cancerous cells undergo apoptosis, which is consistent with the well-known link between cell proliferation and apoptosis in animals⁸⁻¹⁰. Then the effective rate of cancer mutation is $(1 - A)\mu$, experienced selfishness is $1 - \exp(-\mu(1 - A)n)$, and fitness is $w = (1 - CA)\exp(-\mu(1 - A)n)$, where C is the cost of enforcement.

Natural selection will promote a small increase in enforcement A if

$$dw/dA > 0 \quad (\text{S10})$$

$$(-C)(\exp(-\mu(1 - A)n) + (1 - CA)(\exp(-\mu(1 - A)n)n\mu > 0 \quad (\text{S11})$$

$$n\mu > \frac{C}{1 - CA}. \quad (\text{S12})$$

This last line can be interpreted as follows: Natural selection will increase enforcement if the per-individual rate of cancer mutations ($n\mu$) exceeds the marginal relative cost of enforcement ($\frac{c}{1-cA}$).

As expected then, the evolution of self-enforcement in cancerous cells will depend on the size of the organism, because large organisms will, all else being equal, experience more cancerous mutations^{31,32}. However, an organism does not need to be very large to benefit from enforcement. From inequality S9, we can see that self-enforcement becomes important once n gets of the order $1/\mu$. Eukaryotes exhibit about 3×10^{-3} coding-sequence mutations per genome per cell division³³. However, the proportion of mutations that lead to a cancer phenotype in early organisms is unknown. Nevertheless, by comparison with modern single-celled organisms, many routes to increasing cell division rate within an organism are expected, such as a loss-of-function mutations in pathways that generate cooperative secretions³⁴. To give an example, if we assume that one in a thousand nonsense mutations led to cancer phenotypes in early multicellular organisms, and a given point mutation causes a nonsense mutation with approximate probability³⁵ 0.038, then the per-cell-division rate of cancer mutations is $\mu = 3 \times 10^{-3} \cdot 1 \times 10^{-3} \cdot 0.038 = 1.1 \times 10^{-7}$, and our model predicts that enforcement will be important for organisms of on the order of millions of cells or above (c.f. 10^{13} cells in the human body³⁶). But whatever the exact ancestral mutation rate, whenever cancer is prevalent enough to damage multicellular function, the prediction is that self-enforcement will rapidly evolve that restores cooperation (Figure 3d).

Relation to definitions of selfishness and enforcement — Cancer meets the definition of selfishness because, at the scale of selection between cells in a developing individual, the fitness of an individual cell is positively correlated with the cancer phenotype (the unchecked proliferation and growth of cancer cells are what make them cancerous by definition). Although we do not model this process explicitly here, the increase in frequency of mutant cells during cancer evolution is a form of natural selection within individuals²⁷⁻³¹.

Apoptosis A meets the definition of enforcement because fitness can be written $w = (1 - CA)(1 - y)$, where experienced selfishness y is $1 - \exp(-\mu(1 - A)n)$. Hence, we have both $\frac{dw}{dy} < 0$ (cancer is harmful to the multicellular organism) and $\frac{dy}{dA} = -\mu n \exp(-\mu(1 - A)n) < 0$ (enforcement decreases the effect of selfishness on the group).

Model 4: Reproductive conflict in an animal group

We next consider the evolution of enforcement in a non-eusocial animal group, akin to a cooperatively breeding vertebrate like meerkats (Figure 2d). Individuals can invest in aggression that gains them a greater share of group resources, and thereby reproduction, but at a cost to group productivity. They also have the possibility of investing in enforcement that punishes selfish individuals in the group. As for the case of replicators, we capture this scenario with a modification of Frank's mutual policing model because enforcers are again also subject themselves to enforcement²⁰. Specifically, we make two changes to Frank's model to better fit punishment in an animal group: 1) punishment incurs a direct cost for the selfish individual being policed; and 2) an

individual's investment in punishment only affects others in the group, rather than also affecting themselves (individuals do not self-police). Under these conditions, an individual's fitness is

$$w = (-Ca - c_{\text{pun}}\alpha z + A + (1 - \alpha)\frac{z}{z})(1 - (1 - A)Z), \quad (\text{S13})$$

where c_{pun} is the cost of being punished and α is the average level of enforcement among individuals in the group *other* than the focal individual.

We calculate group relatedness R from the parameters ρ (relatedness to other members other than oneself) and n (total group size). This average relatedness to the entire group then is

$$R = \frac{n-1}{n}\rho + \frac{1}{n} \quad (\text{S14})$$

As in the replicator model, equilibrium selfishness in the absence of enforcement is $z^* = 1 - R$ in this model. Enforcement of others will increase from zero when

$$\frac{\partial w}{\partial \alpha} \Big|_{a=\alpha=A=0, z=Z=1-R} + \rho \frac{\partial w}{\partial \alpha} \Big|_{a=\alpha=A=0, z=Z=1-R} + R \frac{\partial w}{\partial A} \Big|_{a=\alpha=A=0, z=Z=1-R} > 0, \quad (\text{S15})$$

which simplifies to

$$-C - (c_{\text{pun}}z + 1)\rho + \left(1 + \frac{z}{1-z}\right)R > 0. \quad (\text{S16})$$

The term $-C$ captures the individual's cost of enforcement; the term $-(c_{\text{pun}}z + 1)\rho$ captures the indirect-fitness cost of punishing relatives, $c_{\text{pun}}z\rho$, and the indirect cost of reducing relatives' selfishness, ρ . The term $\left(1 + \frac{z}{1-z}\right)R$ captures the benefit R of the group sharing group resources due to policing and the benefit $\frac{z}{1-z}R$ of reduced degradation of the environment due to less expressed selfishness. Note that when $c_{\text{pun}} = 0$ and $\rho = R$, this recovers Frank's condition for the evolution of altruism, $C < z$. In our model, natural selection for enforcement is higher in smaller groups because policing impacts upon fewer relatives in the group. However, increasing relatedness to the other group members ρ decreases the benefits of enforcement because of the harm caused by punishing relatives (Figure 3e). And, as for the other models, increases to the cost of punishment c_{pun} also reduce natural selection for enforcement. The result of the additional punishment cost c_{pun} is that enforcement is less favoured than in Frank's original model (Figure 3a vs 3e), and intermediate levels of enforcement ($0 < a < 1$) are stable. However, for a low cost C of enforcement, the result is again highly effective enforcement and little impact of selfishness on a group.

Relation to definitions of selfishness and enforcement — The trait z meets the definition of selfishness. That is because, in the absence of enforcement, $w = \frac{z}{z}(1 - Z)$, and hence an increase in z improves the fitness of a focal animal ($\frac{dw}{dz} = \frac{1-Z}{z}$, and so $\frac{dw}{dz} > 0$ when $0 < Z < 1$),

while an increase in Z decreases the expected fitness of all group members ($\frac{dw}{dZ} = -\frac{z}{Z^2}$, and so $\frac{dw}{dZ} < 0$), which fulfils the conditions for selfishness.

The trait a meets the definition of enforcement because it reduces experienced selfishness and hence restores fitness. Specifically, experienced selfishness is $y = (1 - A)Z$, so $\frac{dy}{dA} < 0$ when $Z > 0$. Moreover, $w = (-Ca - c_{\text{pun}}\alpha z + A + (1 - \alpha)\frac{z}{Z})(1 - y)$, and so $\frac{dw}{dy} < 0$. Together, these fulfil the conditions for enforcement.

Model 5: Mutualism, partner choice in host-symbiont systems

Many host-symbiont systems have large amounts of horizontal transmission whereby symbionts can move between hosts, and often are picked up *de novo* each generation³⁷. Such transmission introduces a large potential for conflict, both between different symbionts and between the host and symbionts. The mammalian microbiome is one such system³⁸, but we motivate our model here on one of the best-studied examples of horizontally acquired symbionts, the mutualism between the bobtail squid *Euprymna scolopes* and luminescent *Vibrio fischeri* bacteria^{39,40} (Figure 2e). The bacteria are housed in a special light organ by the squid that provides them with nutrients. Light produced by the bacteria is thought to help the squid to limit its moonlight shadow at night, aiding in both predation and avoiding predation. However, dark mutants of *V. fischeri* are easily observable, raising the question of what maintains the energetically costly reaction.

We assume that a bacterial strain with selfishness z has a probability z of not luminescing. The expected level of selfishness (dark phenotype) is \bar{z} . If a cell is luminescent it pays cost c_{lux} . There is a potential cost for the dark phenotype if it leads to the squid getting eaten by a predator, which is fatal for both host and symbiont. With high rates of horizontal transfer and mixing of genotypes (low average relatedness among *V. fischeri* cells, R), there is the potential for luminescent strains to be outcompeted within the squid light organ. However, we also assume that the squid has the option of investing in an enforcement mechanism – such as enzymes that produce toxin compounds in the absence of the light reaction³⁹. This selectively harms dark strains of bacteria by subjecting them to a cost c_{host} .

The level of enforcement in the a focal squid is captured by a ; the average level of enforcement among all squids is \bar{a} . A squid pays the cost Ca to enforce. Moreover, a squid that experiences selfishness y pays the cost $c_{\text{pred}}y$, where c_{pred} is the probability that an unilluminated squid gets eaten. The squid's experienced selfishness is $y = (1 - a)Z$, where Z is the average selfishness of *V. fischeri* cells in the squid. Accordingly, the fitness of a focal squid is

$$w_{\text{squid}} = 1 - Ca - c_{\text{pred}}(1 - a)\bar{z}, \quad (\text{S17})$$

while the fitness of a focal *V. fischeri* cell is

$$w_{\text{Vibrio}} = 1 - c_{\text{lux}}(1 - z) - c_{\text{host}}z\bar{a} - c_{\text{pred}}(1 - \bar{a})Z. \quad (\text{S18})$$

Natural selection will promote an increase in *V. fischeri* selfishness if

$$c_{\text{lux}} - c_{\text{host}}\bar{a} - Rc_{\text{pred}}(1 - \bar{a}) > 0. \quad (\text{S19})$$

Above, the first term c_{lux} gives the benefit of selfishness owing to saving on investment in luminescence; the second term $-c_{\text{host}}\bar{a}$ gives the marginal risk of experiencing enforcement from the squid; and in the third term, $-Rc_{\text{pred}}(1 - \bar{a})$ is the marginal cost of the risk of being eaten.

In the absence of enforcement, *V. fischeri* selfishness will increase from zero if

$$c_{\text{lux}} > Rc_{\text{pred}}. \quad (\text{S20})$$

This formally captures the intuition that with high rates of horizontal transfer that leads to many strains associating with a host (low R), the cost of light production will lead to the loss of light producing strains. However, natural selection will promote an increase in squid enforcement if

$$c_{\text{pred}}\bar{z} - C > 0. \quad (\text{S21})$$

That is, increased enforcement by the squid is selected for if the benefit of avoiding predation exceeds the cost of increasing enforcement. If light production is important to squid survival, therefore, enforcement is expected to evolve, even if it is costly. The prediction then is that enforcement is strongly favoured in this system so long as the benefits of mutualism are high (Figure 3f).

The model also predicts that there may be oscillations in the investment into cooperation and punishment. This mirrors an earlier model studying the coevolutionary dynamics of enforcement and cooperation in mutualisms⁴¹. Instabilities can occur if, once cooperation has evolved, the benefits of enforcement are reduced leaving only its cost ($\bar{z} = 0$, equation S21). The lack of natural selection for enforcement can then cause it to decline, but then cooperation also declines, which restores the benefit of, and natural selection for, enforcement, and so on. The magnitude and persistence of these dynamics in real systems remains unclear; frequent mutation to, or immigration of, less cooperative symbionts is predicted to maintain the benefits of enforcement and remove instabilities⁴¹. Both mutation and immigration of symbionts are expected to be important for bobtail squid which i) sample environmental bacteria each generation, many of which are not light producing and ii) carry bacteria that can become dark via a single mutation (e.g. loss of function mutation in *luxA*)^{39,40}. Nevertheless, the prediction of oscillations underlines again the potential for complex coevolutionary dynamics in cooperative systems under enforcement¹³.

Relation to definitions of selfishness and enforcement — Being dark is beneficial to a focal *V. fischeri* cell in the absence of enforcement when $c_{\text{lux}} > Rc_{\text{pred}}$ (Equation S20)—under these conditions, darkness increases the inclusive fitness of the cell. A lack of luminescence is also

harmful to the squid in the absence of enforcement because $\frac{dw_{\text{squid}}}{d\bar{z}} = -c_{\text{pred}} < 0$. Hence, the dark phenotype meets the definition of selfishness.

Selective harming of dark bacteria meets the definition of enforcement because a squid's experienced selfishness is $y = (1 - a)Z$, and hence $\frac{dy}{da} < 0$ when $Z > 0$.

Model 6: Mutualism, partner manipulation in plant-pollinator interactions

The biology of enforcement is particularly clear and diverse in mutualisms. We therefore finish with a second major example from mutualisms, which corresponds to partner manipulation, rather than partner choice (Box 1; Table 1). A well-known threat to the plant-pollinator mutualism is nectar robbers that cut through the side of a flower and remove nectar without collecting pollen¹ (Figure 2f).

We assume for simplicity that pollinators visit flowers at random, attempting to selfishly rob with probability z . By robbing, the nectar-robber has a fitness bonus, as it can visit more flowers in a shorter amount of time (getting more nectar per unit time), suggesting that robbing will invade if left unchecked.

However, cutting through a plant can be time consuming. It can take twenty minutes for small insect to reach nectar this way¹, suggesting that a plant can significantly inhibit robbing by thickening or toughening the flower corolla or sepals where a robber attempts its cut (or equivalently investing in the production of secondary compounds in these regions that are toxic to a robber). We assume that this investment comes at the cost of flower production. At $a = 0$ it invests fully in flowers and pollinators can always rob. At $a = 1$ it has flowers that are impossible to rob but so few flowers that the plant's reproductive fitness is negligible.

Upon attempting to rob nectar, an insect will try chewing through the plant, with probability of success $1 - a$. Each action taken by the insect incurs a cost in terms of time and energy expended. Assume that the cost of chewing through the plant is c_{rob} ; if successful, the pollinator moves on. If unsuccessful, the pollinator visits the flower legitimately at cost c_{visit} . Both costs can be expressed relative to the cost of travelling between plants, which we fix at 1. A pollinator's expected fitness can then be written as

$$w_{\text{pollinator}} = z/(1 + c_{\text{rob}} + \bar{a}c_{\text{visit}}) + (1 - z)/(1 + c_{\text{visit}}), \quad (\text{S22})$$

while a plant's expected fitness can be written as

$$w_{\text{plant}} = (1 - Ca)(1 - (1 - a)\bar{z}), \quad (\text{S23})$$

where the plant produces a number proportional to $1 - Ca$ flowers and $1 - (1 - a)\bar{z}$ is the probability that, given a visit, a flower is legitimately visited.

A pollinator should increase selfishness z if

$$1/(1 + c_{\text{rob}} + \bar{a}c_{\text{visit}}) - 1/(1 + c_{\text{visit}}) > 0, \quad (\text{S24})$$

which means

$$c_{\text{rob}} + \bar{a}c_{\text{visit}} < c_{\text{visit}},$$

that is, nectar robbing will invade when it costs less than a legitimate visit.

A plant will evolve to increase its enforcement a if

$$-C(1 - (1 - a)\bar{z}) + (1 - Ca)\bar{z} > 0, \quad (\text{S25})$$

that is, if the loss of fecundity from investing in enforcement (first term) is smaller than the gain in legitimate visits through enforcement (second term). More generally, we see that enforcement is again broadly predicted so long as it carries a relatively low cost ($C < 1$ in Figure 3g). However, as in the squid system, a benefit to enforcement – thickening the flower corolla or sepal – requires that there is some robbing ($\bar{z} > 0$, equation S25). As a result, oscillations also occur for some parameters (not shown) and the equilibrium level of enforcement often occurs at a point where there remains a significant level of robbing (Figure 3g). This is consistent with the observation that both robbers and legitimate pollinators often co-occur in pollination mutualisms¹.

Relation to definitions of selfishness and enforcement — Nectar robbing is beneficial to a focal pollinator in the absence of enforcement when $c_{\text{rob}} < c_{\text{visit}}$, by equation (S24), and hence under these conditions nectar robbing increases the fitness of the pollinator. Robbing is harmful to a plant in the absence of enforcement because then $w_{\text{plant}} = 1 - \bar{z}$, and so $\frac{dw_{\text{plant}}}{d\bar{z}} < 0$. Nectar robbing therefore meets the definition of selfishness.

A plant investing in reducing the efficacy of nectar robbing is enforcement because $w_{\text{plant}} = (1 - Ca)(1 - y)$, where experienced selfishness is $y = (1 - a)\bar{z}$, and hence $\frac{dy}{da} < 0$ when $\bar{z} > 0$.

Table S2 Parameters

<i>Parameter</i>	<i>Actor</i>	<i>Parameter meaning</i>	<i>Range (point estimate)^a</i>	<i>Rationale of range (rationale of point estimate)</i>
<i>Replicators in a protocell</i>				
<i>C</i>	gene	cost of enforcement	0-1 (0.1)	full range (relatively low cost)
<i>R</i>	gene	group relatedness (including self)	0-1 (0.25)	full range (for comparison with animal group model)
<i>Endosymbionts of isogamous cells</i>				
<i>C</i>	zygote	cost of enforcement	0-1 (0.1)	full range (assumed lower than c_{war} , see model description)
c_{war}	zygote	cost of losing one set of mitochondria	0-1 (0.4)	full range (intermediate value)
<i>Early multicellularity and cancer</i>				
<i>C</i>	organism	cost of enforcement	0-1 (0.05)	full range (estimate)
<i>n</i>	organism	number of cells in primitive adult organism	(10^7)	estimate
μ	cell	per-division mutation-to-cancer rate	10^{-12} to 10^{-3} (1.1×10^{-7})	wide range (conservative estimate, see model description)
<i>Reproductive conflict in an animal group</i>				
<i>C</i>	animal	cost of enforcement	0-1 (0.1)	full range (relatively low cost)
c_{pun}	animal	cost of being punished	(1)	(exiled individuals cannot reproduce)
ρ	animal	group relatedness (not including self)	0-1 (0.25)	full range (half sibs, consistent with data ⁴²)
<i>n</i>	animal	group size	(10)	(small group)

<i>Mutualism: partner choice in host-symbiont systems</i>				
C	squid	cost of enforcement	0-1 (0.05)	full range (low cost)
C_{pred}	squid	risk of death for a wholly-dark squid	(0.75)	(assume light production is important)
C_{lux}	bacteria	cost of luminescence	0-1 (0.25)	full range (luminescence assumed costly)
C_{host}	bacteria	cost to <i>Vibrio</i> of host squid punishing cheating	(1)	(assume enforcement is effective)
R	bacteria	relatedness between bacterial cells in squid light organ	(0.1)	(6 crypts in squid light organ, 1-2 cells per crypt)
<i>Mutualism: partner manipulation in plant-pollinator interactions</i>				
C	plant	cost of enforcement	0-1 (0.5)	full range (assume that thickening corolla is relatively costly to future flower production)
C_{visit}	pollinator	cost of legitimately visiting a flower	(1)	(arbitrary)
C_{rob}	pollinator	cost of nectar robbing	$0 - C_{visit}$ (0.5)	full range for which $c_{rob} \leq c_{visit}$ (robbing is twice as efficient as legitimate visit)

^a Point estimates are used for the illustrative “Dynamics” plots in Fig. 3.

Table S3: Fraternal versus egalitarian alliances*

Feature	Egalitarian	Fraternal
Example of cooperative alliance forged	Different molecules in compartments; genes in chromosomes; nuclear genes and organelle in cells, mutualisms between species	Same molecules in compartments; cells in multicellular organisms; individuals in family group
Units	Unlike, non-fungible	Like, fungible
Reproductive division of labour	No	Possible
Initial advantage	Division of labour; combination of functions	Economies of scale; later division of labour

*Based on Queller's definitions^{43,44}. To encompass the full spectrum of cooperative systems, we do not only consider major transitions in evolution and instead discuss fraternal and egalitarian *alliances* that may or may not have undergone a major transition.

Box S1 Enforcement as an evolutionary byproduct

We have defined enforcement relatively narrowly as processes that evolve because they limit selfishness and promote cooperation (Definition of enforcement, above). However, similar mechanisms that limit selfishness can also evolve independently of their effects on cooperation^{27,45}. One potential example is worker policing via egg eating in the eusocial hymenoptera¹⁷. Here, workers are thought to remove other workers' eggs primarily because they are more related to the queen's eggs. By removing the incentive for workers to lay their own eggs, this behaviour leads to the evolution of increased worker cooperation¹⁷. The increase in cooperation only occurs after a delay, however, and is unlikely to contribute to natural selection for policing, which would be required for enforcement proper. This said, policing can immediately promote cooperation in cases where it directly modifies worker behavior⁴⁶, or when it interacts with another conflict (over sex allocation⁴⁷), which renders it enforcement *sensu stricto* according to our definition (above). There are many other potential examples of byproduct enforcement. For example, genetic recombination breaks down linkage groups that may act against the interest of the genome⁴⁸ but may have evolved for a different reason⁴⁹. Similarly, pleiotropy (Model 3, above) is a major feature of all genomes so there may be many cases where this limits the mutational target size that generates rebellious phenotypes as a byproduct³⁴.

There are two important caveats to this discussion of byproduct enforcement. First, byproduct enforcement may be improved upon by subsequent natural selection for cooperation. Take for example, the finding that legumes use root senescence to cut off non-cooperative (non-nitrogen fixing) bacteria⁵⁰. It has been argued that this may be a preadaptation because the senescence of low productivity roots is a general feature of plant biology that existed before root nodules⁵¹. This would be byproduct enforcement. However, the mechanism of root senescence was likely fine-tuned once root nodules evolved, thus rendering it enforcement proper. The second caveat is that species-level selection is predicted to enrich for enforcement mechanisms, byproduct or otherwise, if within-species conflict is linked to extinction risk⁵². Consistent with this, there is evidence that transposon abundance is associated with extinction in bacteria, and that termite species with lower societal conflict will outcompete species with higher levels⁵³. Species-level processes are often overlooked in the literature, probably due to long-standing nervousness associated with the misapplication of group-selection thinking⁵⁴. However, these processes may greatly enrich for cooperative systems with enforcement or byproduct enforcement⁵² (in the same way they are thought to enrich for sexual species⁵⁵). In this sense, then, byproduct enforcement is also likely to be selected due to its effects on cooperation.

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