

Enforcement is central to the evolution of cooperation

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Cooperation occurs at all levels of life, from genomes, complex cells and multicellular organisms to societies and mutualisms between species. A major question for evolutionary biology is what these diverse systems have in common. Here, we review the full breadth of cooperative systems and find that they frequently rely on enforcement mechanisms that suppress selfish behaviour. We discuss many examples, including the suppression of transposable elements, uniparental inheritance of mitochondria and plastids, anti-cancer mechanisms, reciprocation and punishment in humans and other vertebrates, policing in eusocial insects and partner choice in mutualisms between species. To address a lack of accompanying theory, we develop a series of evolutionary models that show that the enforcement of cooperation is widely predicted. We argue that enforcement is an underappreciated, and often critical, ingredient for cooperation across all scales of biological organization.

The evolution of cooperation is central to all living systems. Evolutionary history can be defined by a series of major transitions (Box 1) in which replicating units came together, lost their independence and formed new levels of biological organization^{1–4}. As a consequence, life is organized in a hierarchy of cooperation: genes work together in genomes, genomes in cells, cells in multicellular organisms and multicellular organisms in eusocial groups (Fig. 1). The identification of these major transitions has underlined the key conceptual challenge for all cooperative systems: why does natural selection favour investment in cooperation rather than self-serving rebellion that would undermine a particular genome, organism, society or mutualism between species?

Despite this generality, discussions of social evolution commonly focus on just one or two systems. Much attention has been paid to the amazing biology of the eusocial insects — bees, ants, wasps and termites — and the evolution of worker sterility^{5–8}. Eusocial insects are an example of what Queller⁹ termed a fraternal cooperative system, multicellularity being another¹⁰, in which the interacting individuals are genetically related members of one species or type (Fig. 1, Box 1 and Supplementary Table 3). A key feature of fraternal systems, and fraternal major transitions, is the importance of kin selection, which explains cooperative traits via their benefits to family members that pass on the same genes^{3–6,11} (Supplementary Table 3).

There is a concern, however, that the focus on particular empirical systems might be limiting our understanding of cooperative evolution. In particular, in the field of social evolution, less attention has been paid to what Queller called egalitarian cooperative systems, which are those formed between unrelated individuals⁹. These include cooperation among genes in genomes, the origin of complex cells via endosymbiosis and between-species mutualisms (Fig. 1 and Supplementary Table 3). Between egalitarian individuals, there are no interactions with brothers, sisters or clonemates, rendering kin selection powerless¹². Consideration of these systems makes it clear that family life is not a general explanation for the major transitions in evolution, or for cooperative systems in general¹³.

A major open question, then, is what, if anything, unites the evolution of cooperative systems? Here, we review cooperative evolution across all levels of biological organization, which reveals a growing amount of evidence for the importance of enforcement. By enforcement, we mean an action that evolves, at least in part, to reduce selfish behaviour within a cooperative alliance (see Box 2 for the formal definition). These mechanisms have long been considered as a way to promote cooperation¹⁴, particularly in genomes^{15–17} and humans¹⁸, and early experiments revealed the power of enforcement in the birds¹⁹ and the bees²⁰. By now, the growing list of examples covers all biological scales, with recent reviews highlighting, for example, the importance of the silencing of transposable elements for genomes^{21,22}, conflict mediation in mitochondrial–nuclear interactions for eukaryotic cells^{23–25}, enforcement of cooperation in microbes²⁶, cancer suppression for multicellular evolution^{27,28}, punishment and policing for animal sociality^{29,30} and host control for mutualistic symbioses³¹.

Such diverse examples, however, have resulted in an equally diverse terminology³⁰, which can hinder comparison and synthesis. In this paper, we bring together these examples and use mathematical models to formalise the shared evolutionary processes at work across the full array of cooperative systems. It is only by considering all scales of cooperation side by side — from genomes through complex cells, multicellular organisms and societies, to mutualism between species — that the reach of enforcement becomes clear.

Genomes

The earliest alliances were molecular. One of the major theories of early life — the RNA world hypothesis — centres on cooperating RNA molecules or related polymers³². Similarly, the hyper-cycle concept provided insights on the theoretical conditions under which cooperative networks might have first evolved from replicators¹². The lack of empirical details on these ancient systems makes it challenging to infer how they evolved, although mathematical models allow for exploration of general principles² (Figs. 2 and 3).

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Box 1 | Glossary

Altruism. Following Hamilton^{5,6}, we define this as an action that evolves to increase the fitness of a recipient while decreasing the lifetime reproductive fitness of the actor, sometimes known as strong altruism⁷.

Cooperative system. An alliance, however transient, between entities (genes, cells or individuals) that arises via the evolution of cooperative phenotypes.

Cooperation. A social phenotype that has a positive fitness effect on another individual and that evolved, at least in part, because of this effect.

Direct benefit. A fitness benefit to a focal individual that increases the individual's lifetime personal reproduction.

Egalitarian alliance. A cooperative system made up of unrelated individuals or entities; for example, different loci in a genome.

Enforcement. An action that evolves, at least in part, to reduce selfish behaviour within a cooperative alliance.

Fraternal alliance. A cooperative system that is made up of genetically related entities of the same species; for example, cells in a multicellular organism.

Genetic relatedness. Genetic similarity at a locus for a social phenotype as compared to the population's average

allele frequency. This is often driven by kinship, which is the probability of this similarity based on recent common ancestry.

Indirect benefit. A fitness benefit to a focal individual's relatives. Actions that yield indirect benefits to individuals can be promoted by kin selection.

Individual. A stable, physically integrated collective.

Kin selection (as a process, rather than as a theoretical framework⁷). Natural selection involving nonzero genetic relatedness between individuals. Kin selection involving family groups is a major explanation for cooperation.

Major transition. A shift in the way information is transmitted across generations. This often involves the coming together of formerly independently reproducing entities to form a new level of individuality, where entities become irreversibly dependent upon one another.

Preadaptation. An adaptation that evolved for one function in an ancestor that later enables a different function in a descendent.

Selfishness. A phenotype that benefits an actor and harms a recipient. When selfish traits harm other members of a cooperative system, they also reduce cooperation.

What is clear is that replicating molecules eventually became stably connected in DNA-based genomes. This connection likely reduced evolutionary conflict as at that point all loci shared an interest in their cell's fitness, and the notion of being 'in the same boat' is often considered to be central to the evolution and maintenance of cooperation^{33,34}. However, this effect is much weaker in genomes than is often assumed. Even small amounts of horizontal gene transfer can favour selfish genetic elements that proliferate at the cost of the host genome^{35,36}. Sexual reproduction pushes this effect to the extreme. Because genetic elements can spread to new lineages by outcrossing each generation, they can proliferate despite reducing host fitness³⁷. The evolution and maintenance of cooperation within genomes therefore requires additional explanation.

Some selfish genetic elements may fail to spread due to constraints on their function, or they may be self-limiting³⁸. For example, a meiotic drive gene that manipulates the meiotic process to increase its chance of transmission beyond the usual 50% can spread in diploids by killing gametes that do not carry it at meiosis, but if the driver spreads to fixation, the killing will stop³⁸. However, like being in the same boat, it is clear that such effects are again insufficient to protect cooperation within genomes. This is most evident with transposable elements, which are particularly interesting because of their suggested similarity to the replicators that formed the first genomes²¹. Transposons are found across all domains of life, harm their hosts³⁹ and make up the bulk of many eukaryotic genomes, including around half of the human genome.

While the shared genomic interest that comes with being in the same boat is not expected to remove genomic conflict, it can provide the basis for the evolution of enforcement. To extend the analogy, while being in the same boat does not protect you from conflict, it does provide a boat for you to protect (formalised with equation (S6) in the Supplementary Information). Empirical observations support these predictions. The P transposable element in *Drosophila* was first detected in *Drosophila melanogaster* in the 1970s⁴⁰ because it causes hybrid dysgenesis in crosses between males carrying the

element and females lacking it. Strikingly, American populations of *D. melanogaster* evolved silencing of the P element via a small RNA pathway in less than 40 years, consistent with an on-going co-evolutionary arms race between the host and the P element^{41,42}. The P element has recently entered the genome of *D. simulans* from *D. melanogaster*, in which it induces both morphological abnormalities and sterility, but several strains already appear to have the ability to silence the transposition⁴³.

Small-RNA-mediated silencing is also thought to limit transposon replication in many other species⁴⁴, including plants⁴⁵. The plant *Arabidopsis thaliana* seems to have lost the ability to effectively control long terminal repeat retrotransposons such that the transposable-element-derived sequence in *A. thaliana* is now larger than the entire genome of related species⁴⁶ (Fig. 1). In addition to transposable elements, there are many other forms of genomic conflict^{36,47}. As discussed, some meiotic drivers can be self-limiting but, for many others, there is evidence that the evolution of suppressors is central to maintaining organismal function⁴⁸. These conflicts are also easy to miss because selfish genetic elements and their suppressors commonly co-occur in populations, and it often takes crosses between divergent populations to see the elements' effects²¹.

Considerable effort is currently underway to engineer selfish genetic elements — so-called gene drives — to introduce desirable traits into natural populations, such as reduced carriage of malaria or dengue by mosquitos⁴⁹. By design, these elements are intended to invade and fix in a population. It is telling then that a key concern surrounding these approaches comes from theoretical⁵⁰ and empirical work⁵¹ demonstrating the ease with which genomic suppressor alleles can arise and spread. The best hope is in strategies where suppressor mutations are somehow constrained or where the presence of gene drives has little effect on host fitness; that is, cases where the 'selfish' elements actually cost less than enforcement. When the elements compromise organismal function, the prediction is clear: suppressor alleles will rapidly inhibit their spread and effects⁵⁰.

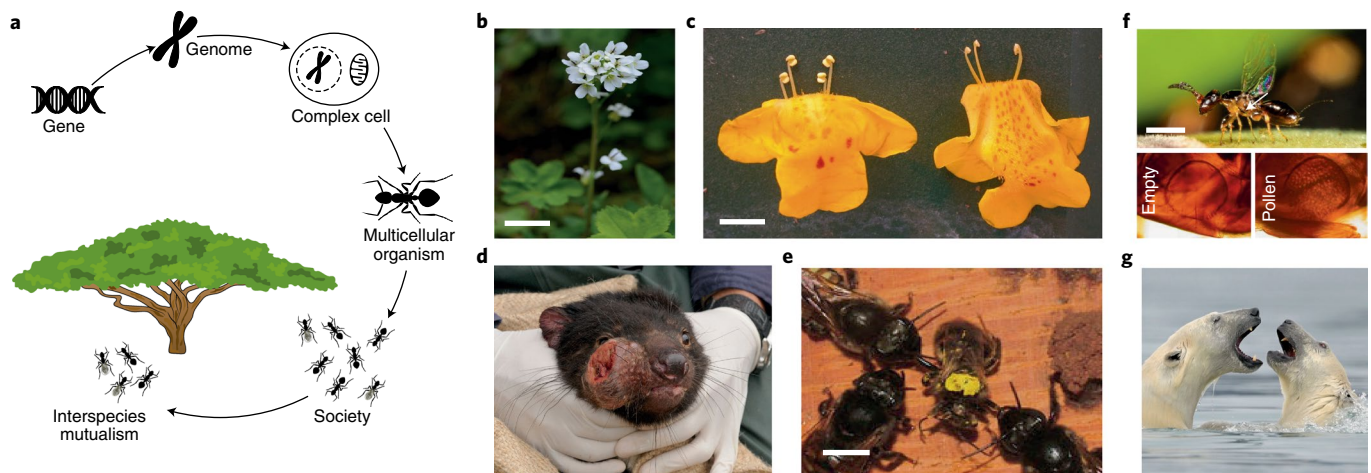


Fig. 1 | The importance of enforcement is revealed by its absence at all levels of biological organization. **a**, Life has a nested organization that rests upon cooperation at successive levels¹³. Here and in our discussion, we consider all cooperative systems, not just those that are considered a major transition in evolution. For example, many mutualisms between species are not a major transition. **b**, Genomes. *Arabis alpina* appears to have a reduced ability to silence and remove retrotransposons. Transposons contribute more DNA to its genome than occurs in the entire genome of some relatives⁴⁶. Scale bar, 5 cm. **c**, Complex cells. Cytoplasmic male sterility that is driven by mitochondria is revealed in *Mimulus guttatus* × *Mimulus nasutus* F₂ hybrids. Male fertile flowers with pollen-producing anthers (left) and male sterile flowers with deformed, sterile anthers (right)⁵². Scale bar, 1.5 cm. **d**, Multicellularity. Uncontrolled growth of the transmissible facial cancer tumour in the Tasmanian devil, which is associated with a loss of major histocompatibility complex diversity and tissue rejection^{85,86}. **e**, Sociality. Unlike many other eusocial species, caste fate is not forced upon larvae in several species of stingless bees. As a result, far more larvae develop into queens than the colony needs, which are then killed by workers upon emergence¹¹². Scale bar, 4.5 mm. **f**, Mutualism. Some fig wasps do not carry pollen in their pollen pocket (insets), but figs can abort fruits containing developing fig wasps if the wasps do not pollinate. In mutualisms in which the figs abort less often, the wasps less often carry pollen¹¹⁸. Scale bar, 1 mm. **g**, No cooperative system. Many reproductive conflicts occur with little prospect of enforcement, simply because there is no collective interest to protect, such as competition between males over a mate. Credit: Jon Ågren (**b**); Andrea Case (**c**); Dave Watts/Alamy Stock Photo (**d**); Tom Wenseleers (**e**); Christian Ziegler and Charlotte Jandér (**f**); Nature Picture Library/Alamy Stock Photo (**g**)

Complex cells

The evolution of the eukaryotic cell is an example of an egalitarian alliance and transition (Fig. 1 and Supplementary Table 3). The interaction between mitochondria, plastids and nuclear genes has been crucial to eukaryotic metabolism for over a billion years⁵² and is arguably the most intimate and successful of all symbiotic relationships⁵³. The long co-evolutionary association between mitochondrial and nuclear genes is clearly demonstrated by the large-scale transfer of genes from cytoplasmic to nuclear DNA⁵³. The mitochondrial genomes of most animal species now only carry a dozen or so genes, and in *Arabidopsis thaliana*, close to one in five nuclear genes are of chloroplast origin⁵⁴.

As for the evolution of genomes, the close association of organellar and nuclear genomes reduces the scope for conflict but is insufficient to remove important differences of evolutionary interest^{55,25}. The potential for conflict was probably at its strongest in early eukaryotes in which the fusion of outbred gametes would bring together genetically different endosymbionts (Fig. 2b and Supplementary Information)²⁴. The strength of this potential conflict has led several authors to argue again for the importance of enforcement, specifically that the uniparental inheritance of organelles evolved to limit the spread of selfish endosymbionts^{1,14,55–57}. While other factors may have contributed⁵⁸, our own modelling further suggests that isogametic fusion both strongly favoured the evolution of selfish organelles that bias their own transmission and the subsequent evolution of uniparental inheritance as a mechanism of enforcement (Figs. 2b and 3c and Supplementary Model 2). Uniparental inheritance appears to be the norm in modern isogamous species²⁵, making it challenging to test such predictions⁵⁹. However, the unicellular green alga *Chlamydomonas reinhardtii* is informative because it is isogamous and possesses both mitochondria and chloroplasts. Interestingly, uniparental inheritance differs

between mitochondria and plastids in the timing and mechanism of elimination, and in which mating type passes on which endosymbiont (the minus type passes on mitochondria, while the plus type passes on chloroplasts)⁵⁹. This may indicate two distinct evolutionary conflicts whereby selfishness, and later on, suppression, evolved for each endosymbiont independently.

The evolution of uniparental inheritance did not end conflict within complex cells²⁵. Uniparental inheritance contrasts with the biparental inheritance of autosomal nuclear genes, in that the evolutionary fate of mitochondria and chloroplast genes in anisogamous species depends only on their performance in females⁶⁰. As a consequence, there is a long-documented conflict driven by mitochondrial genes over sex determination and sex ratio, and several mitochondrial genes in plants are known to cause male sterility⁶¹. Enforcement is again important for resolving this conflict via the evolution of nuclear alleles that suppress selfish mitochondrial phenotypes and restore organismal functioning. As for selfish genetic elements (see above), however, the magnitude of co-evolutionary arms races between the organelles and the nucleus is only revealed by crossing diverged populations. For example, cytoplasmic male sterility is rarely detected in natural populations of the monkey flower *Mimulus guttatus*, but does occur in crosses with closely related *M. nasutus*^{62,63} (Fig. 1).

In contrast to the prevalence of mito-nuclear conflicts in plants, the mitochondria of animals do not appear to cause major conflicts, possibly due to the greatly reduced size of the genome (but see discussions of Mother's Curse⁶⁴). However, nuclear enforcement is required to suppress other endosymbiotic bacteria, particularly from the genus *Wolbachia*, that are carried by many insects. While they are intracellular and are transmitted vertically like mitochondria and chloroplasts, *Wolbachia* are typically classified as parasites because of the reproductive problems they cause in their attempts

Box 2 | Formal definition of enforcement

We define enforcement as an action that evolves, at least in part, to reduce selfish behaviour within a cooperative alliance. Selfish behaviours are those that benefit an actor and reduce its cooperation with one or more recipients in the alliance (Box 1). We more explicitly define selfishness (z) in a series of models in the Supplementary Information; z always corresponds to a specific behaviour that benefits an actor while reducing the benefits they provide to others in the context of a specific cooperative venture.

Enforcement can be unilateral or a joint effort, and a cooperative alliance can be fleeting, as between a plant and pollinator that may interact only once, or long-lasting, as among genes in a genome that are partners for (at least) the lifetime of the organism. Here, we formalize our definition of enforcement by considering individuals that can invest in enforcement, a , in two key ways (Table 1): either by changing the strength of interaction with different partners (partner choice), or by manipulating the amount that their partners display selfish versus cooperative behaviours (partner manipulation). An example of partner choice reducing selfishness is a bobtail squid host that prevents colonization by a bacterial strain that invests in its own growth rather than light production¹²⁴. An example of partner manipulation reducing selfishness is the workers of the queenless ant *Dinoponera quadricaps*, which physically restrain a member of the colony that is challenging the reproductive female, thereby restoring its cooperative behavior¹¹¹.

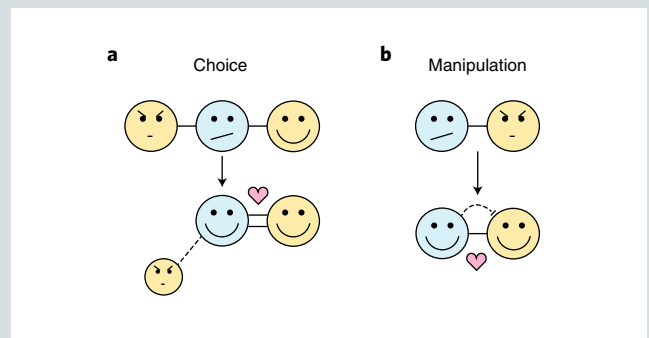
To be explicit about how enforcement can occur, we distinguish between the selfishness of group members, z , and the selfishness experienced by a focal individual, y . In the models in the Supplementary Information, these are sometimes equivalent but, where they are not, the key measure of selfishness is that experienced by an enforcer, y (plotted in Fig. 3), which captures any benefits of enforcement for the enforcer (for example, Supplementary Model 1). Consider a focal actor who experiences a level of selfishness, y , from others, which is a function of that actor's investment in enforcement, a , such that $y = y(a)$. If investing in enforcement (increasing a) reduces the selfishness experienced by the actor (decreases $y(a)$), then the focal actor is enforcing cooperation. Specifically, in the absence of enforcement ($a = 0$), the focal actor's strength of interaction with individual i of n partners is defined 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 $z_i(a)$ (see the figure in this Box). Formally, the focal actor's experienced level of selfishness can be written without enforcement as

$$y(0) = \sum_{i=1}^n \beta_i(0) z_i(0) \quad (1)$$

and with enforcement as

$$y(a) = \sum_{i=1}^n \beta_i'(a) z_i(a) \quad (2)$$

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 possessor's fitness, w (that is, $dw/dz > 0$), but decreases the fitness of other individuals, w' ($dw'/dz < 0$). Meanwhile, a trait a causes the enforcement of cooperation if a marginal increase in the trait value decreases experienced selfishness ($dy/da < 0$). The evolution of enforcement can occur because of its effects on selfishness within a cooperative alliance, or due to other effects unrelated to these effects (Supplementary Box 1). However, our discussion is focused on the former cases in which the focal trait a evolves, at least in part, because of its effects on selfishness, which implies that $dw/dy < 0$ and $dy/da < 0$, and $dw/da > 0$. For simplicity, we have phrased the above in terms of direct benefits to the enforcer, but enforcement also includes cases in which an actor reduces selfish behaviour within a cooperative alliance due to benefits to its relatives (indirect benefits; Box 3 and Supplementary Information).



Two modes of enforcement. **a**, Partner choice and related behaviours increase interactions with cooperators (changes β_i in equation (1)). **b**, Partner manipulation changes the behaviour of others to make them more cooperative (changes z_i in equation (1)). See Table 1 for examples.

to bias investment into female offspring⁶⁵. For example, *Wolbachia* kill male eggs in a range of insect species, including the Great Eggfly butterfly (*Hypolimnas bolina*). Killing of this species can be so effective that the sex ratio is driven down to a small percentage of males⁶⁶. However, as for the P transposable element, male killing can be suppressed in a few generations by the spread of a nuclear allele that restores male viability⁶⁷.

Cell groups and multi-cellularity

There is extensive cooperation within cellular groups. The evolution of cooperation by microorganisms is now known to be extremely common and central for both their functioning and their effects on human health⁶⁸. For example, many bacteria secrete a suite of extracellular factors that provide benefits for neighbouring cells,

including enzymes that digest nutrients and antibiotics, and molecules that scavenge rare resources like iron⁶⁹. The evolution of cooperation within microbial groups, perhaps more than in any other system, appears to rest upon genetic relatedness and the ability of binary fission to generate a large clonal patch that can share in cooperative benefits⁷⁰. This alignment of interests reduces the need for enforcement. But it does not remove it. Enforcement is thought to be important for cooperation in the social amoeba *Dictyostelium discoideum*²⁶. Moreover, the rapid proliferation of many microbes creates ample opportunity for the de novo evolution of cells that lack cooperative traits via mutation, often known as 'cheaters'⁷¹ (Fig. 2). This may select for genetic mechanisms to counter the effects of such mutations and lead cells to enforce their own cooperation ('self-enforcement'; Table 1 and Supplementary Model 3).

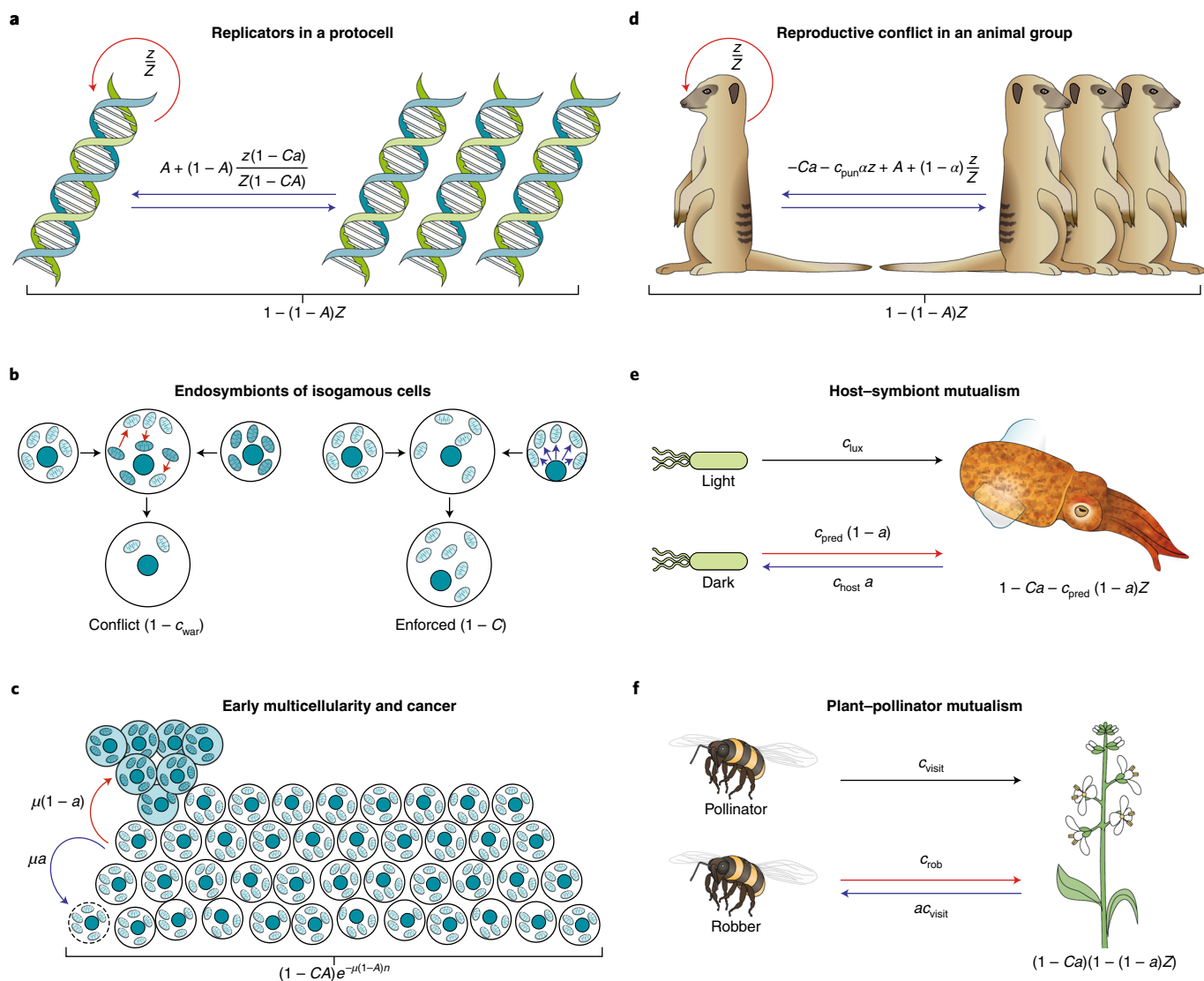


Fig. 2 | Models of enforcement across the levels of biological organization. Selfishness is indicated with red, and enforcement with blue. Throughout, z is individual selfishness, Z is group mean selfishness, a is individual enforcement, A is group mean enforcement and C is the cost of enforcement. **a**, Replicators and early genome evolution. A set of replicators in a protocell that can either just replicate (red arrow) or replicate more slowly to invest in the mutual inhibition of others' replication (blue arrows), which improves collective fitness (bottom). **b**, Endosymbionts and the evolution of complex cells. Endosymbiont conflict with cost c_{war} (red, left) can be reduced by nuclear modifiers that enforce uniparental inheritance of symbionts (blue, right). **c**, Multicellular evolution and cancer. Cells mutate at rate μ to become cancerous (red arrow) at a cost to the group of n cells (bottom), but a cell can invest in self-enforcement (blue arrow) that suppresses the impact of cancerous mutations, for example, by undergoing apoptosis. **d**, Animal groups. An individual can either just invest in reproduction (red arrow) or reproduce less and invest in punishment, which incurs cost c_{pun} to other individuals who reproduce a lot (blue arrows), which improves group fitness (bottom). **e**, Host-symbiont mutualism. A symbiont can either invest in cooperation at cost c_{lux} (black arrow), or in reproduction (red arrow) and face an enforcement cost c_{host} from the host (blue arrow), which functions to avoid the cost c_{pred} of predation owing to a lack of cooperation by symbionts. **f**, Plant-pollinator mutualism. A pollinator can either pollinate at cost c_{visit} (black arrow) or attempt to rob the flower at cost c_{rob} (red arrow), which can fail if the plant protects the flower and forces the insect to visit it normally (bottom, blue arrow). See the Supplementary Information for full model descriptions and Supplementary Table 1 for variables and parameters. Credit: Lena London, used under creative commons license (Meerkats in **d**)

Key mechanisms include the evolution of pleiotropy that links selfish phenotypes to personal cost, and redundancy that limits the phenotypic penetrance of mutations^{72,73} (Supplementary Box 1).

The importance of enforcement in cellular groups, however, is clearest within multicellular organisms. The transition to multicellular life from independently living cells has occurred many times throughout the history of life⁷⁴. Origins range from 3.5 billion years ago in cyanobacteria to 200 million years ago in the volvocine algae⁷⁵. Across these transitions, kinship has played a key role,

with obligate multicellularity only evolving in taxa in which group formations occur clonally and relatedness is maximal between cells⁷⁶. However, mutations pose a major threat to many multicellular systems, particularly animals as cancer, the disease associated with cells dividing in an unregulated fashion, is prevalent in these organisms^{28,77}.

Animal cells have evolved a wide range of mechanisms that inhibit the rise and spread of cancer^{27,28,77,78}. This includes strong pleiotropic links from cell proliferation and DNA damage to

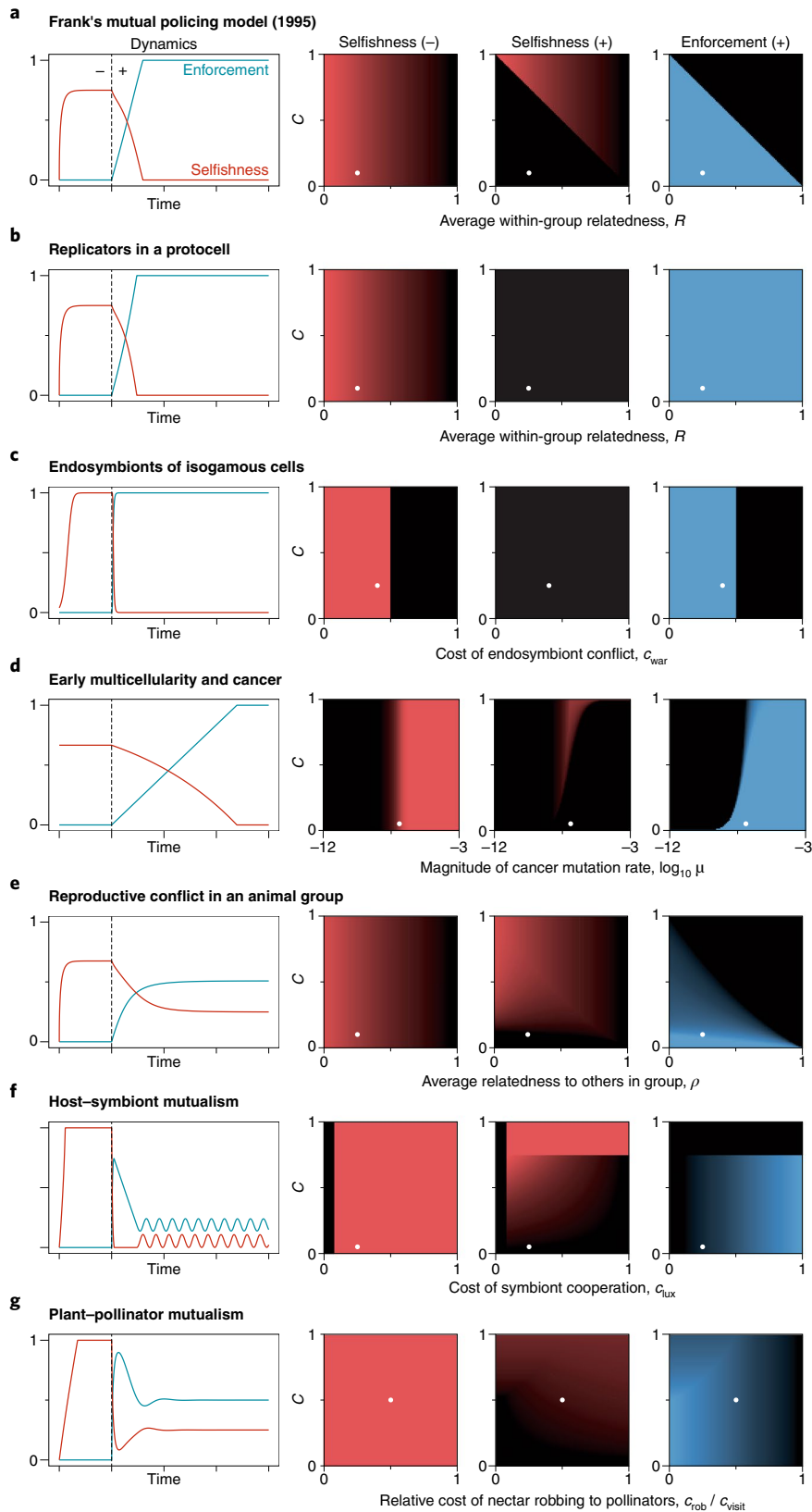


Fig. 3 | The evolution of enforcement is predicted to reduce selfishness and promote cooperation across all levels of biological organization.

a-g. Models of enforcement. The first column shows example dynamics of selfishness (red) and enforcement (blue); first without the possibility of enforcement and then when it is able to evolve (right of dashed line). Columns two to four show parameter sweeps of evolved selfishness (red intensity) without enforcement (column 2); evolved selfishness (red intensity) when enforcement can evolve (column 3); and evolved enforcement (blue intensity) (column 4). In the parameter sweeps, the y axis is always the cost of enforcement C , while the x axes sweep a second key parameter from each model. White dots show the parameters used in the example dynamics plots. See the Supplementary Information for full model descriptions and Supplementary Table 1 for descriptions of variables and parameters.

Table 1 | Mechanisms of enforcement

Mechanism	Illustrative examples
Partner choice^a and related mechanisms Increase interactions with cooperators (changes β_i in Box 2, equation (1))	Humans choose to preferentially interact with cooperators ^{98,102} Choice in mutualisms ^{12,96,97} ; for example, cleaner and client fish, plants and pollinators Increased resources to beneficial mutualists, including mammalian microbiota, legume sanctions of rhizobia, fruit abscission by figs ^{31,118,120} Harming non-cooperative mutualists, including immune suppression of harmful microbiota ³¹ , bobtail-squid enzymes thought to harm non-light-producing bacteria ¹²⁴ Self-enforcement that removes non-cooperators ²⁹ ; for example, apoptosis of proliferative cells ^b in mammalian development ⁷⁹
Partner manipulation Change behaviour of individuals (changes z_i in Box 2, equation (1))	Genomic suppressors of conflict; for example, silencing of transposable elements ^{41,42} Limiting competition between partners ¹⁴ ; for example, uniparental inheritance of endosymbionts and organelles ^{55,56} and termites enforcing high relatedness in their fungus ¹²² Reciprocal altruism and indirect reciprocity promotes cooperation in other reciprocators ^{99,101} Self-constraint via genetic redundancy ⁷³ ; for example, mammalian cell proliferation being robust to mutations in genes that affect the cell cycle ²⁷ Change behaviour via causing harm; for example, punishment in vertebrate societies, some worker policing ^b in insects ^{19,29,90,92,103,108,111} Compartmentation to limit resource access; for example, specialized queen cells ^b in honeybees ^{109,112} , epithelial barrier in microbiome ³¹ , floral morphology that directs nectar only to pollinators ¹³⁵ (Supplementary Model 6)

^aPartner choice is a form of enforcement that increases the likelihood that a partner is a cooperator, thereby reducing selfishness in an alliance. However, it does not guarantee that cooperators preferentially associate, as cooperation and choice can be unlinked traits (for example, a selfish host will benefit from selecting cooperative symbionts)¹⁵. ^bRelatives of the enforcer receive direct benefits of enforcement, not the enforcer itself (Box 3).

apoptosis, which cause many precancerous cells to undergo programmed cell death^{29,73,79} (Box 3, Figs. 2 and 3 and Supplementary Model 3). Such self-enforcement may explain why elephants, despite their massive body size, experience a lower rate of mortality due to cancer than humans. Compared to a single copy in humans, there are multiple copies of the tumour-suppressor gene *TP53* in elephants, and their cells undergo apoptosis at a much higher rate than human cells in response to DNA damage^{80,81}. Another important response seen in precancerous cells is differentiation. For example, many mutations that promote tumour formation in mammalian blood stem cells also promote differentiation into a non-proliferative cell type. This helps to ensure that mutant lineages are replaced by healthy cells in the dividing stem cell population rather than progressing toward tumour formation⁷⁷. There is even evidence that clumps of cancerous cells can be physically pushed out of proliferative tissues via a process known as apical extrusion⁸². Finally, the immune system can act as an enforcement system that targets tumour cells for destruction^{83,84}. The transmissible facial tumour in the Tasmanian devil appears to spread because low major histocompatibility complex diversity in the devils limits foreign tissue rejection⁸⁵ (Fig. 1). The tumors are a threat to the species but the devils seem to be evolving resistance, thereby restoring multicellular integrity to a most charismatic of examples⁸⁶.

Interestingly, plants and fungi appear to suffer fewer harmful cancers than animals for reasons that are currently unknown. Features like the cell wall, lack of cell motility and the modularity of plants may limit the spread of malignant tumors^{28,87}. Recent work, however, has found that cell proliferation and cell death are again positively linked in plants via regulators such as retinoblastoma-related protein 1 (ref. ⁸⁸), raising the possibility that self-enforcement mechanisms convergently evolved in the plant and animal lineages after each became multicellular.

Societies and eusociality

The field of social evolution has long revolved around the study of animal societies. This includes cooperative breeding in vertebrates, particularly birds and mammals, where there is again a clear role for genetic relatedness. Either high average relatedness or mechanisms of kin discrimination (that allow more related individuals to interact) are seen across cooperative breeders⁸⁹. However, there is also

evidence that enforcement is central to cooperation^{29,90}, although a convincing demonstration can require challenging manipulative experiments⁹¹. For example, superb fairywren (*Malurus cyaneus*) helpers that usually assist in the care of breeders' offspring are punished by breeding males if they are experimentally prevented from helping¹⁹, and if a subordinate banded mongoose (*Mungos mungo*) is made to reproduce without the dominants, its offspring are killed⁹².

The importance of enforcement in vertebrates arguably reaches its zenith in humans, where egalitarian cooperation between non-relatives is commonplace¹⁸. Understanding cooperation in humans is challenging owing to the complex interaction of genetics, individual learning and culture⁹³. Nevertheless, it is clear that human cooperation is enforced in many ways⁹⁴. This includes partner choice, whereby individuals form partnerships and strong bonds with cooperative individuals^{95–98} (Table 1). And within particular relationships, cooperation often rests upon reciprocation whereby cooperation is provided in return for receiving cooperation^{99,100} or is directed to individuals with a reputation for being cooperative¹⁰¹. There is also punishment, through which transgressions are met with harm from other individuals^{102,103}. Punishment is particularly favoured by powerful individuals¹⁰⁴ or when meting out punishment itself improves an individual's reputation¹⁰⁵.

The social insects have been central to the insight that kin selection can be key to the evolution of cooperation^{5–7}. Specifically, a morphologically distinct worker caste appears to have only evolved in species that are monogamous, which ensures high relatedness between the workers (the queen's daughters) and the queen's other offspring^{106,107}. While relatedness is important in the origin of eusociality, its role in the derived social insects is less clear¹⁰⁸. In derived species, multiply-mated queens are common, and sibling relatedness is much reduced as a result. The canonical example is the honeybee, *Apis mellifera*; queens of this species mate tens of times. It is striking then that honeybees have one of the most conflict-free societies described. To achieve this, honeybees and other derived species employ a diverse set of enforcement mechanisms that limit the potential for conflict^{108,109}.

One such mechanism is worker policing, whereby the low relatedness between workers leads them to prevent each other's reproduction and allow the queen alone to reproduce^{110,111}. Some forms of worker policing may evolve as a by-product of worker-worker

Box 3 | Why does enforcement evolve? Kin selection, direct benefits and by-products

Why do cooperative behaviours evolve? A typical answer identifies benefits to an actor that are either direct — which improves personal reproduction — or indirect, which improves the reproduction of relatives (kin selection)^{5,6}. Here, we have argued that a process is missing from many explanations: enforcement. However, like cooperation itself, enforcement is also a behaviour whose evolution needs to be explained^{14,103}. We model diverse examples in the Supplementary Information, which illustrate that enforcement — again like cooperation — evolves due to either direct or indirect fitness benefits to an actor¹⁰³. For example, a host can benefit directly from enforcing cooperation in a symbiont (Fig. 2e), while some forms of cancer repression require self-sacrifice by cells that is only possible with kin selection (Fig. 2c). In this sense, therefore, kin selection and enforcement are not independent explanations for cooperation.

However, the typical kin selection explanation for cooperation does not include enforcement — for example, in the major transitions⁴ — and in this sense, kin selection and enforcement evolution are alternative ways of explaining cooperation whose importance can be experimentally tested across biological systems. And, here, enforcement clearly has the greater reach as it is important in both egalitarian and fraternal interactions, which

is why we are arguing it is central to cooperation. In a similar vein, our models also identify several cases in which natural selection favours little cooperation through either kin selection or direct benefits, but highly effective enforcement is favoured through these processes (compare evolved selfishness without (–) and with (+) enforcement in Fig. 3). For example, in Supplementary Model 1, the shared interest of being in the same cell is insufficient to generate much cooperation between replicators. However, it is sufficient to drive enforcement that then allows high levels of cooperation to evolve (Fig. 3b; equation (S4) versus equation (S6) in the Supplementary Information). Such examples emphasize the importance of including enforcement in the theories of social evolution.

Our definition of enforcement is focused on mechanisms whereby the direct or indirect benefits of enforcement come, at least in part, from improving cooperation (Box 2). However, mechanisms that limit selfishness can evolve independently of their effects on cooperation as a by-product of natural selection on other traits^{4,91}. We discuss such by-product enforcement in more detail in Supplementary Box 1, along with the potential for species-level selection to enrich for such mechanisms.

competition, which is not an evolved enforcement mechanism in a strict sense (see discussion in Supplementary Box 1). Nevertheless, the effect is promotion of cooperation and colony efficiency¹¹⁰. A second route to enforcement in the social insects is the differential feeding of larvae destined to become new queens as opposed to new workers. In the honeybee (*Apis* spp.), this is achieved by raising new queens in special cells under tight worker control¹⁰⁹. Control over caste fate is thereby taken away from larvae. Strikingly, this control is lacking in some stingless bees; in these species, larvae are free to determine their own caste. The result is that around a fifth of female larvae emerge as new queens, only to be immediately executed by the workers¹¹² (Fig. 1). In other stingless bees, workers have evolved to make larger cells for queens that limit or remove this conflict by again taking control over caste fate away from the larvae¹¹². This suggests that queen-worker dimorphism — the defining feature of derived eusociality and a major transition in societies⁸ — can evolve as a mechanism of enforcement.

Interspecific mutualism

The evolution of cooperation between species (mutualism) occurs in a diverse range of systems, from plants and pollinators, through client and cleaner fish, to the vast diversity of microbial symbioses of plants and animals revealed by DNA sequencing¹¹³. Like the evolution of genomes and eukaryotic cells, interactions between species represent egalitarian alliances of individuals among which Hamilton's relatedness never occurs (except within members of each species)¹². Coinheritance of partners is a feature of some — including endosymbionts (above), lichens and the symbionts of leafcutter ants (*Atta* spp. and *Acromyrmex* spp.) and termites — but is far from general. Indeed, horizontal transmission of symbionts is so common that it was recently called a 'paradox' on the basis of the assumption that vertical transmission is the best way for a host to manage conflicts¹¹⁴.

This conclusion, however, overlooks a large body of theoretical and empirical work showing the importance of enforcement in mutualisms^{12,115}. As in humans (see above), mutualistic species engage in partner choice to interact preferentially with more cooperative partners^{12,96} (Table 1). Bumblebees (*Bombus* spp.) pollinate plants and receive nectar in return; if too little nectar is

provided, a bee may leave a plant, remember the encounter and not return¹¹⁶. Many ants tend and protect aphids on plants, receiving sugary honeydew in return. If too little honeydew is provided by the aphids, the ants may leave or even eat the aphids¹¹⁷. Figs (*Ficus* spp.) can abort fruits containing developing fig wasp larvae if their mothers did not pollinate the fig when depositing eggs. The fig species with the most effective sanctions (that abort most readily) have more cooperative wasps that pollinate the figs more often¹¹⁸ (Fig. 1f). Finally, cleaner fish remove parasites from much larger client fish at specific sites known as cleaner 'stations'. If a cleaner also bites and takes host tissue, their client will be less likely to return to that station¹¹⁹.

Comparable mechanisms are used by hosts to choose and promote cooperative microbial symbionts. For example, legumes preferentially provide nutrients to root nodules that contain nitrogen-fixing bacteria¹²⁰ (Supplementary Box 1). Leafcutter ants farm vertically transmitted fungi in large gardens, which digest the leaf material for the ants. Workers actively tend the gardens and remove other strains and species of fungi¹²¹, which ensures that the beneficial fungi dominate. Mammalian hosts mount an inflammation response against bacteria that breach the gut epithelial barrier³¹. Mutualists also directly manipulate their partner's behaviour to make it more favourable (Table 1). For example, floral morphology often appears adapted to reward pollinators rather than robbers that collect nectar without pollination (Fig. 2f and Supplementary Model 6), and some termites ensure that only a single genotype of fungus is transmitted between generations. This, like uniparental inheritance of symbionts (Fig. 2b), is hypothesised to have evolved to limit conflicts between fungal strains¹²².

The bobtail squid provides a particularly striking example of enforcement³¹. This type of squid has multiple mechanisms that help select the bacterium *Vibrio fischeri* over other species of bacteria to enter its light organ and, once there, promote the luminescence that helps the squid hunt and hide^{123–125}. The squid–*Vibrio* system is striking because enforcement appears to eliminate nearly all selfish behaviour in symbionts, despite them being horizontally acquired by each generation of squid (Fig. 2e). This suggests that enforcement alone can create alliances that function as a single evolutionary individual; coinheritance is not a requirement.

Evolutionary theory of enforcement

There is empirical evidence for the importance of enforcement at all biological scales. However, our survey is necessarily limited by the number of examples in which enforcement has been looked for. Do these examples reflect a general tendency for enforcement to evolve across biological scales? This is where mathematical theory is valuable for its ability to reveal general predictions that are robust across many parameters and systems. However, there is currently no general theory of enforcement that covers all biological scales. There are several models predicting the importance of enforcement for animal groups and mutualisms^{14,103,126–129}. This includes the seminal models of reciprocal altruism and tit-for-tat, in which individuals only cooperate if a partner is also cooperating^{99,100} (although this strategy can only enforce cooperation when the partner is also a reciprocator). Also important is Frank's model of mutual policing, which was aimed to be applicable to a diverse set of social systems¹³⁰ (Fig. 3a). However, it was subsequently criticized for relying on assumptions that, it was argued, made the evolution of policing particularly likely¹¹. The general issue is that enforcement models have to be relatively complex if they are to capture both the evolution of cooperation and the evolution of enforcement as separate traits¹⁴. This can make them parameter heavy and may explain why we lack a general evolutionary model that captures enforcement across biological scales.

On this basis, we decided to build a suite of models that are each consistent with a specific biological example, where the examples span the scales of cooperation (Fig. 2 and Supplementary Information). With the models, we are able to mathematically define cooperation and enforcement in each case, enabling the formal comparison of very different examples. Modelling also allows one to explore ancestral scenarios that are hard to study empirically. Accordingly, we focussed on relatively simple systems — including early replicators, isogamous cells and simple multicellular organisms — to ask in particular whether enforcement is predicted at early stages of cooperative evolution (Fig. 2).

We first explored how each system functions without enforcement (Fig. 3 and Supplementary Information). Importantly, all models recapitulated the key prediction from social evolution that cooperative systems are susceptible to the evolution of selfish phenotypes that compromise higher level function^{1–4}. With this benchmark in place for each model, we then asked, across a range of parameters, whether enforcement will evolve and, if so, how this affects the evolution of cooperation. Despite being based on diverse examples across biological scales, all models identify conditions under which enforcement will evolve and increase cooperation (Fig. 3). As is typical for evolutionary models⁵, benefits and costs matter. In particular, high costs to enforcement can make it less likely to evolve, or less effective when it does evolve (for example, Fig. 3g). Nevertheless, enforcement is predicted in every system, and importantly, this occurs over a wide range of parameters, which is consistent with the general importance of enforcement independently of both biological details and scale.

Conclusions

We have considered cooperative systems ranging from genomes to interspecific mutualisms. Putting these diverse examples side-by-side reveals that enforcement plays a central role at every level of biological organization (Figs. 1 and 3). Of course, enforcement is not the only process that limits evolutionary conflicts in cooperative systems. Nor is enforcement always the most important mechanism in every example of cooperation. Some potential conflicts are constrained by pre-existing biology such that they are never expressed or are self-resolving¹¹⁰, and some conflicts have a weak effect on cooperation when they are expressed¹³¹. Furthermore, it is clear that family life plays a central role in fraternal systems, in which it enables the evolution of a stable reproductive division of

labor^{3,5,6}. Egalitarian interactions lack relatedness and the reproductive division of labour, but co-inheritance can nevertheless help to align evolutionary interests^{33,34,55}. Although they are much discussed, however, co-inheritance and relatedness only apply to specific systems, such as endosymbiosis or animal groups, respectively. By contrast, when costly selfish behaviour emerges, the evolution of enforcement that restores cooperation is possible (Fig. 3), and observed (Fig. 1), across all biological scales. It is in this sense that we find enforcement to be central to cooperative evolution (Box 3).

While generally important, the effects of enforcement vary, and it is arguably most critical in egalitarian interactions in which relatedness is lacking¹³⁰. The effect of enforcement also varies within each level of biological organization³, including among very similar species. Striking examples of this variation are the eusocial hymenoptera¹¹⁰ and the fig-fig wasp mutualism¹¹⁸. In both cases, the effectiveness of enforcement (rates of removal of worker eggs and senescence of unpollinated figs, respectively) has been experimentally measured across a set of related species, and, in both cases, more effective enforcement predicts higher cooperation (low worker ovary activation and few non-pollinating fig wasps). Such large datasets are hard to obtain, but are telling because they suggest that enforcement is a dominant shaper of cooperation over evolutionary time.

At a finer scale still, the effectiveness of enforcement varies within species. The honeybee is often considered a pinnacle of conflict resolution, and worker policing in this species is typically extremely effective. However, there are worker lineages that both lay eggs and escape policing, which can drive colony collapse and devastate apiaries^{132,133}. Failures of enforcement are also seen at other levels of organization, including the P element in *Drosophila*^{41,42}, killing of eggs containing males by *Wolbachia*⁶⁶ and transmissible facial tumours in Tasmanian devils⁸⁵. Like between-species comparisons, these examples underline the importance of enforcement; when it fails, there are major consequences for cooperation that can even threaten species with extinction⁸⁵ (Fig. 1). Within-species examples further emphasize how enforcement can drive complex evolutionary dynamics, where social evolution combines with coevolution to influence cooperation³⁸.

In spite of this complexity, such examples also show that enforcement is often highly effective. When new selfish elements are introduced to a population, the emergence of suppression is often rapid, again as seen with the P element⁴³ and killing of males by *Wolbachia*⁶⁷. Such rapidity can make enforcement difficult to detect. Moreover, once in place, the most effective enforcement will typically be the least tested or seen, which may lead its importance to be underestimated⁹¹ (Fig. 1). The best evidence of enforcement often comes from challenging manipulative experiments that simulate what would happen were individuals to rebel (for example, refs. 19,92,120). Similarly, the phenotypic consequences of selfish genetic elements are often seen in hybrids in which enforcement fails, but not in within-population crosses where it functions²¹.

There is now a large body of evidence that enforcement shapes cooperation across all levels of biology. This includes systems that have undergone a major transition, like some social insects, as well as those that have not, like many mutualisms. Both theory and data emphasize that biological details matter for how enforcement evolves. Looking forward, a key challenge is to identify general principles for the evolution of enforcement that are applicable across many systems despite the diversity and details of each system^{3–6,11}. It is already clear that the costs and benefits of enforcement will be important (Fig. 3 and Supplementary Information). While high costs can be prohibitive, a given cooperative system may explore diverse enforcement strategies over evolutionary time until a low-cost solution arises. Low costs to enforcement are further facilitated by power asymmetries^{3,38,104,110,134}. Enforcement can be enabled by a single powerful individual, such as a host who exercises control over

symbionts (Fig. 2e), or by majority rule, such as within an animal society^{108–110} (Fig. 2d). There is also a role for pre-adaptations: pre-existing features of the biology of a system can enable enforcement and promote its effectiveness (Supplementary Box 1). Systems in which enforcement is not possible will be less likely to see increases in cooperation over evolutionary time, or they may even see cooperation fall away. It is in those systems where enforcement does arise that we see the full extent, and wonders, of cooperative evolution.

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All authors contributed to the writing of the article. J.A.Å. and K.R.F. conceived the article, N.G.D. and K.R.F. developed the models and N.G.D. did the analysis.

Competing interests

The authors declare no competing interests.

Additional information

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