

2. Matthews, J. V. Jr & Oviden, L. E. Late Tertiary plant macrofossils from localities in Arctic/Subarctic North America: A review of the data. *Arctic* **43**, 364–392 (2000).
3. Elias, S. A. & Matthews, J. V. Jr Arctic North American seasonal temperatures from the latest Miocene to the early Pleistocene, based on mutual climatic range analysis of fossil beetle assemblages. *Can. J. Earth Sci.* **39**, 911–920 (2002).
4. Harington, C. R. *Life at a 3.5 Million Year Old Beaver Pond in the Canadian Arctic Islands and the Modern Scene* 11–13 (Meridian, Ottawa, 2001).
5. Hutchison, J. H. & Harington, C. R. A peculiar new fossil shrew (Lipotyphla, Soricidae) from the High Arctic of Canada. *Can. J. Earth Sci.* **39**, 439–443 (2002).
6. Zakrzewski, R. J. & Harington, C. R. Unusual Pliocene rodent from the Canadian Arctic Islands. *J. Vert. Paleol.* **21**, 116A–117A (2001).
7. Björk, P. R. The Carnivora of the Hagerman Local Fauna (Late Pliocene) of southwestern Idaho. *Am. Phil. Soc. Trans.* **60**, 1–54 (1970).
8. Lindsay, E. et al. Recognition of the Hemphillian/Blancan boundary in Nevada. *J. Vert. Paleol.* **22**, 429–442 (2002).
9. Petter, G. Origine, phylogénie et systématique des Blaireaux. *Mammalia* **35**, 567–597 (1971).
10. Hulbert, R. C. & Harington, C. R. An early Pliocene hipparionine horse from the Canadian Arctic. *Paleontology* **42**, 1017–1025 (1999).
11. Tedford, R. H. et al. Yushe Basin, China: Paleomagnetically calibrated mammalian biostratigraphic standard for the late Neogene of eastern Asia. *J. Vert. Paleol.* **11**, 519–526 (1991).
12. Dompierre, H. & Harington, C. R. Dental microwear analysis of an Early Pliocene deerlet and hipparionine from Strathcona Fjord, Ellesmere Island, Canada. *J. Vert. Paleol.* **17** (suppl. 3), 43A (1997).
13. Rybczynski, N. & Harington, C. R. Tarsal evidence for ecomorph reconstruction in fossil lagomorphs. *J. Vert. Paleol.* **17** (suppl. 3), 72A (1997).
14. Shi, N. The late Cenozoic stratigraphy, chronology, palynology and environmental development in the Yushe Basin, North China. *Striae* **36**, 1–90 (1994).
15. Dowsett, H. J. et al. *Middle Pliocene Paleoenvironmental Reconstruction: PRISM2* 99–535 (US Geol. Surv. Rep., Washington DC, 1999).
16. Sher, A. V. Olyorian land mammal age of northeastern Siberia. *Palaontographica Italica* **74**, 77–112 (1986).
17. Stach, J. *Arctomeles pliocenica* new genus and species from Weze. *Acta Geol. Polonica* **2**, 129–157 (1951).
18. Viret, J. Monographie paléontologique de la faune de vertébrés des Sables de Montpellier, III, Carnivora, Fissipedia. *Trav. Lab. Geol. Fac. Sci. Lyon Mem.* **2**, 7–26 (1934).
19. Teilhard de Chardin, P. & Leroy, P. Les Mustélidés de Chine. *Inst. Geobiol. Pékin* **12**, 1–56 (1945).
20. Roshchin, A. D. A new genus of fossil mammal of the family Mustelidae from the Priudensk of Odessa. *Trudy Odeskogo Gosud. Univ.* **60**, 97–106 (1949).

Acknowledgements C.R.H. is grateful to J. G. Fyles, who discovered the Beaver Pond site in 1961 and the first vertebrate remains in 1988, for introducing him to the site in 1992, and to J. S. Tener and C. C. Kennedy for field assistance. The Canadian Museum of Nature and the Polar Continental Shelf Project provided support for field work. R.H.T. thanks E. Heck for graphics and A. Lora for manuscript preparation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to R.H.T. (tedford@amnh.org).

Volunteering leads to rock–paper–scissors dynamics in a public goods game

Dirk Semmann, Hans-Jürgen Krambeck & Manfred Milinski

Department of Evolutionary Ecology, Max Planck Institute of Limnology, 24306 Plön, Germany

Collective efforts are a trademark of both insect and human societies¹. They are achieved through relatedness in the former² and unknown mechanisms in the latter. The problem of achieving cooperation among non-kin has been described as the ‘tragedy of the commons’, prophesying the inescapable collapse of many human enterprises^{3,4}. In public goods experiments, initial cooperation usually drops quickly to almost zero⁵. It can be maintained by the opportunity to punish defectors⁶ or the need to maintain good reputation⁷. Both schemes require that defectors are identified. Theorists propose that a simple but effective mechanism operates under full anonymity. With optional participation in the public goods game, ‘loners’ (players

who do not join the group), defectors and cooperators will coexist through rock–paper–scissors dynamics^{8,9}. Here we show experimentally that volunteering generates these dynamics in public goods games and that manipulating initial conditions can produce each predicted direction. If, by manipulating displayed decisions, it is pretended that defectors have the highest frequency, loners soon become most frequent, as do cooperators after loners and defectors after cooperators. On average, cooperation is perpetuated at a substantial level.

Clean air to sustain the global climate and clean public toilets are examples of public resources that everybody is free to overuse. The social dilemma of public goods situations is that although a group of cooperators is always better off than a group of defectors, defectors exploit cooperators in groups. Since the late 1970s, economists, social scientists and evolutionary biologists have used the public goods game as a model to study the problem of maintaining cooperation in a group of unrelated individuals^{10–14}. For example, six players are asked to contribute money to a public pool; the money in the pool is, for example, multiplied by 3.6 and then equally distributed among the players irrespective of whether they contributed. The optimum outcome for the group is achieved if everybody cooperates; however, because each euro paid into the pool yields only a return of 60 cents for the contributor—that is, a net deficit of €0.40—no matter how the other players decide, the selfish decision is never to contribute to the pool. Studies have identified punishment^{6,15–17}, which is also combined with fairness¹⁸, and reputation through interaction with other social behaviour⁷ as mechanisms that can effectively maintain cooperation in public goods experiments.

In one model^{8,9}, a large population with three types of player, cooperators, defectors and loners, is considered. From time to time, sample groups of N players are randomly chosen and asked to participate in a single public goods game. Players either can refuse to participate, and will then receive a small fixed payoff, or can join the public goods game. In the latter case, they either defect or cooperate. Their strategies are specified beforehand and do not depend on the composition of the group. A continuing oscillation of the three strategies is predicted because each strategy, when most frequent, can be beaten by one of the others. Defectors can exploit a large group of cooperators, whereas loners have the highest profit when defectors are frequent. When loners are most frequent the public group size is reduced, which invites cooperation because the game is no longer a dilemma in small groups^{19–21}. For example, if the group consists of only three players, each euro paid into the public pool yields a return of €1.20 for the contributor, that is, a net gain of €0.20.

It is not just the fact that volunteering is possible that induces cooperation, but rather that volunteering reduces public goods groups to small sizes for which the individual cost-to-benefit ratio becomes more favourable. In addition, even though defectors are still better off than are cooperators in each group, cooperators do better when averaged over small groups according to Simpson’s paradox²². For example, a group of three players can consist of either three cooperators, two cooperators and one defector, one cooperator and two defectors, or three defectors; cooperators receive on average a net gain of €1.8, defectors only €0.8. Circumstantial evidence for the ‘small group advantage’ is potentially provided by fish that leave their shoal and take a risk to inspect a predator from a short distance: very often minnows, *Phoxinus phoxinus*, inspect a pike, *Esox lucius*, in small groups²³. Thus, after loners, cooperators will be most frequent for a while before defectors will take over again^{8,9}. Hence, volunteering relaxes the social dilemma: instead of defectors winning the world, coexistence among cooperators, defectors and loners is expected²⁴.

We tested these predictions with 280 first-semester biology students in 20 groups of 14 students that played the optional public goods game for 57 consecutive rounds. The students observed the

introduction and the complete game on a public screen. They were told that they had a starting account of €10 and would make their decisions anonymously. In each round, six players were randomly selected from the 'population' of 14 players to decide first whether to join the public goods group and, thereafter, if they chose to join whether to contribute to the public pool. In the first seven rounds, we manipulated the displayed decisions in such a way that defector, cooperator or loner was pretended to be the most frequent strategy of the population. This manipulation was necessary to test the three possible predictions of the model experimentally. Without this manipulation our results would be only descriptive. In the eighth round, we expected that being a loner (after staged defector), defector (after staged cooperator) or cooperator (after staged loner) would be the most frequent strategy according to the players' real decisions. Thereafter, the game proceeded with unmanipulated display to test whether oscillations of the three strategies would occur and, if so, whether they would occur predominantly in the predicted sequence over 50 rounds.

After the manipulated start in the first seven rounds, we found that the predicted strategy was the most frequent strategy in round eight after all three starting schemes ($P < 0.004$, $n = 20$ groups, sign test, two-tailed; Fig. 1a–c). We use each group of 14 players as a statistical unit. In the following 50 rounds, we determined for each group of 14 players the number of cases where two conditions were met: one strategy was most frequent and one of the other strategies was most frequent in the following round. We compared all cases where the predicted strategy became most frequent with all cases where an unpredicted strategy became most frequent. In this way, we identified switches of the most frequent strategy between rounds and checked whether their direction was as predicted. The predicted

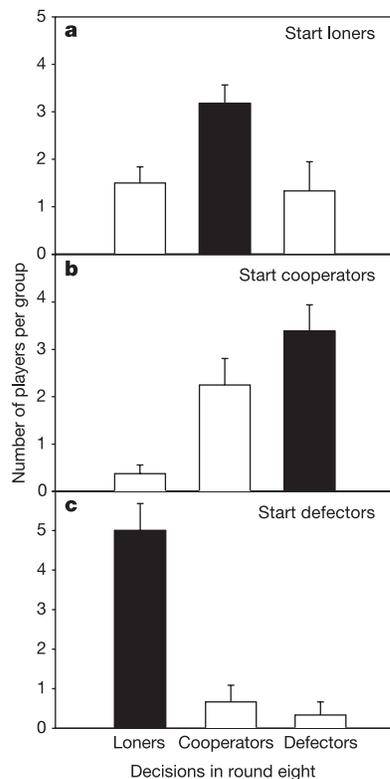


Figure 1 Decisions in round eight after the staged standstill with one single strategy that was most frequent in the first seven rounds. The bars of the strategy predicted to be most frequent are shown in black. The average frequency of chosen strategies per group of 14 players is shown (mean ± s.e.m.). **a**, Start loners: $n = 6$ groups of 14 players with a simulated prevalence of loners in rounds 1–7. **b**, Start cooperators: $n = 8$ groups of 14 players with a simulated prevalence of cooperators in rounds 1–7. **c**, Start defectors: $n = 6$ groups of 14 players with a simulated prevalence of defectors in rounds 1–7.

strategy became most frequent significantly more often than did the alternative strategy ($P < 0.001$, $n = 20$; paired t -test, $t = 6.588$, two-tailed; Fig. 2).

Although the above analysis provided a formal proof of the predicted oscillations, we made an example of these oscillations visible in Fig. 3. We synchronized the 20 groups in the 50 'not-manipulated' rounds by selecting similar starting points in each group, because the cycles were not expected to have the same duration in each group. For example, if we were to select the round from each group that had the highest proportion of loners, we would expect that cooperators would be most frequent next, followed by defectors in all 20 groups. The same procedure was used to find such starting points for cooperators and for defectors.

From several maxima of a strategy, we defined the first as the starting point. Thereafter, we averaged all 20 starting point rounds and each of the following 9 rounds over all groups, for loners (Fig. 3a), cooperators (Fig. 3b) and defectors (Fig. 3c) as starting points. The oscillations can be observed in all three cases, although the groups became increasingly asynchronous during the ten rounds. As the model predicts, after loners have the highest frequency, cooperators become most frequent, thereafter defectors, and then loners again (Fig. 3a). After a prevalence of cooperators, defectors become most frequent, followed by loners, and then cooperators again (Fig. 3b). Figure 3c shows that the prevalence of defectors is followed, as predicted, by an increase of loners that is closely trailed by increasing numbers of cooperators, followed again by defectors, and thereafter by loners.

The consequences of the oscillation of the strategies should be an always-recurring rise of each of the three strategies and thus a fairly cooperative outcome of the game after initial perturbations. In the last 30 rounds (21–50), the frequencies of the three strategies seemed on average rather stable (rounds 21–35: $32.22 \pm 1.0\%$ loners, $30.11 \pm 0.9\%$ cooperators, and $37.67 \pm 1.0\%$ defectors; rounds 36–50: $32.39 \pm 1.4\%$ loners, $29.06 \pm 1.3\%$ cooperators, and $38.56 \pm 1.3\%$ defectors). According to the model, we would expect that there would be at least 42% loners and that 58% would choose to join the public goods group⁹. Only $33 \pm 2.5\%$ (mean ± s.e.m.) chose the loner option, which is significantly less than expected ($P = 0.003$, $n = 20$; Wilcoxon signed rank test, $Z = 2.95$, two-tailed). Of the players joining the public goods group, 38% would be expected to cooperate and 62% to defect, respectively⁹. We found, as expected, more defectors ($56.51 \pm 1.7\%$) than cooperators ($43.48 \pm 1.7\%$, $P = 0.004$, $n = 20$; Wilcoxon signed rank test, $Z = 2.91$, two-tailed). Although these numbers are close to the expected ones, the percentage of cooperators was significantly higher than predicted ($P = 0.011$, $n = 20$; Wilcoxon signed rank test, $Z = 2.54$, two-tailed).

In the long run (averaging over many cycles), the net payoff of both defectors and cooperators should be same as the payoff of loners—that is, €1.25. We found that defectors earned slightly but significantly more than expected, $1.46 \pm €0.04$ ($P < 0.001$, $n = 20$;

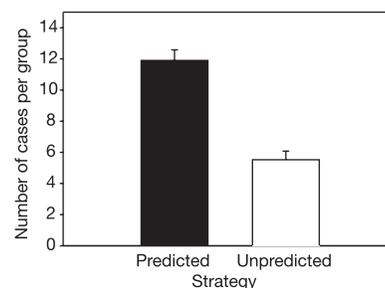


Figure 2 The predicted prevalence switch occurred more frequently than the unpredicted prevalence switch during the 50 rounds that followed the seven manipulated start rounds. Columns show mean ± s.e.m. per group of 14 players.

Wilcoxon signed rank test, $Z = 3.36$, two-tailed). Cooperators had a payoff that did not significantly differ from the expected one, $1.32 \pm \text{€}0.09$ ($P = 0.43$, $n = 20$; Wilcoxon signed rank test, $Z = 0.78$, two-tailed). Defectors probably profited because they were less frequent than expected at the equilibrium.

We found that volunteering (the option to choose between joining the public goods group and taking the loner strategy) indeed protected cooperation in the public goods game by inducing small group sizes. On average, there was a rather stable frequency of cooperators that was higher than what is usually found in public goods games after several rounds^{5,17}. As predicted by the model^{8,9}, the dynamics of the games showed oscillations of the rock–paper–scissors succession of cooperators, defectors and loners, even though our players were less averse to risk than expected: only about a third chose the loner option.

Volunteering is a mechanism that potentially sustains cooperation in various species. Like some large predatory animals, ancestral humans also acted as groups when hunting large prey such as mammoths, but went out solitarily for small prey such as antelopes²⁵. Thus, volunteering was possible and might have supported cooperation in addition to potential relatedness by reducing the public (hunting) group size. Obviously, we are not free to decide whether we stop sharing the global climate with others, but there are many other human social dilemmas in which volunteering is possible.

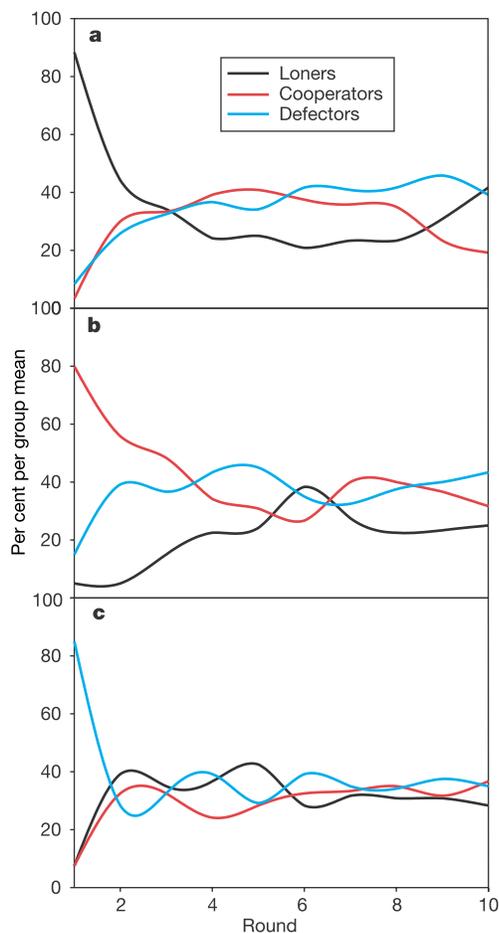


Figure 3 Average frequencies of the three strategies over a period of ten rounds after synchronizing the 20 groups as follows. The starting round of the ten rounds was defined for each group as the round when one of the strategies reached its maximum proportion throughout the game (rounds 8–57) for the first time. All 20 starting rounds and each of the following 9 rounds were averaged over all groups for loners (a), cooperators (b) and defectors (c). The sequence follows the predicted most frequent strategies according to rock–paper–scissors dynamics: that is, loners, cooperators, defectors, loners, and so on.

Volunteering does not produce overwhelming cooperation, but it might help to avoid the fate of mutual defection in many human collective enterprises and thus might pave the way for other mechanisms of cooperation to take over. For example, direct²⁶ or indirect reciprocity^{27–30} may be catalysed when the population happens to be in a cooperator period of the rock–paper–scissors dynamic and anonymity is relaxed after repeated interactions. Loners, although unsocial by definition, help cooperators to become most frequent and thus to escape the social dilemma. □

Methods

Subjects

A total of 280 human subjects of the universities of Bonn, Hamburg and Kiel played a public goods game with optional participation that lasted for 57 rounds. The students were completely anonymous, sat between partitions, saw the introduction to the game including one example round and the complete game on a large screen. They did not know the total number of rounds. They interacted by means of a computer program with silent ‘yes’ and ‘no’ switches.

Basics of the public goods game

For each round the computer program randomly selected 6 of the 14 students. Each student had played almost the same number of rounds at the end of the game. Because the expected cycles are predicted to become smoother with increasing population size⁹, we mimicked a larger population. The students were told that there was a pool of additional players in the form of strategies recorded from earlier sessions and that the program would sometimes choose ‘players’ from this pool.

A light at each person’s desk signalled who was to decide. Each of the six players had to decide first whether to play the loner strategy, thereby obtaining a fixed payoff (€1.25), or to join the public goods group with a second decision to make. The minimum public goods group size was two players. If only one player decided to play in the public goods group, he/she knew that he/she would automatically also become a loner. If the public goods group size was either two or larger, the players that had chosen to play in the public goods group had to decide whether they would contribute €1.25 or nothing to the public pool. At this point, they did not know how many subjects had decided to play in the public goods group. After all players of the public goods group made their final decision, the content of the pool was multiplied by 3.6 and divided evenly among the players that had joined the public goods group irrespective of their actual contribution. With an interest rate of 3.6, the model system has a fixed point, which refers to substantial proportions of cooperators, defectors and loners. The dynamic then predicts periodic cycles of all three strategies around these proportions; this requires an interest rate larger than 2.

Only at this point were the decisions of all players displayed simultaneously on the screen that all 14 subjects could see: that is, the numbers of loners and public good group players, their payoffs and their eventual costs (for example, one player was a loner and obtained €1.25 without cost, five had chosen to join the public goods group, of which three were defectors who received a payoff of €1.80 from the pool without costs and two were cooperators who also received €1.80 from the pool, but they had costs of €1.25 each). It never happened that one subject had to play loner because he/she had no money left.

Rounds of the public goods game

In the first seven rounds, the display was manipulated such that the players were led to believe that they were in a group that played a high percentage of only one strategy. In six groups loners appeared to be most frequent; there were eight groups with cooperators and six with defectors as the apparent most frequent strategy. The players could make decisions, however, which were not displayed. Instead, six predetermined decisions with corresponding payoffs and eventual costs were shown. Each of the three possible real decisions of a player (that is, loner, cooperator and defector) was included at least once to ensure that each player would find his actual decision on the screen and nobody would doubt that the displayed decisions were real. The maximum number of defectors or cooperators displayed on the screen was four per round; for example, four defectors, one cooperator, one loner. In the case of loners prevailing in the first seven rounds, it was possible to show up to 100% of loners in one round, because each player who decided to join the public goods group would believe that he/she was the only one with this decision and thus became a loner. To have some variation, we chose a percentage of loners that was slightly lower in the seven rounds. On average, there were 79% of loners in the staged loner groups, 61% of cooperators in the staged cooperator groups, and 64% of defectors in the staged defector groups. Starting with round eight, there was no manipulation of the display for 50 consecutive rounds. The students did not know the total number of rounds to be played.

Received 30 June; accepted 6 August 2003; doi:10.1038/nature01986.

1. Trivers, R. *Social Evolution* (Benjamin Cummings, Menlo Park, California, 1985).
2. Hamilton, W. D. Genetical evolution of social behaviour. *J. Theor. Biol.* **7**, 1–52 (1964).
3. Hardin, G. Tragedy of commons. *Science* **162**, 1243–1248 (1968).
4. Hardin, G. Extensions of ‘the tragedy of the commons’. *Science* **280**, 682–683 (1998).
5. Ledyard, J. O. in *Handbook of Experimental Economics* (eds Kagel, J. H. & Roth, A. E.) 111–194 (Princeton Univ. Press, Princeton, New Jersey, 1995).
6. Fehr, E. & Gächter, S. Altruistic punishment in humans. *Nature* **415**, 137–140 (2002).
7. Milinski, M., Semmann, D. & Krambeck, H. J. Reputation helps solve the ‘tragedy of the commons’. *Nature* **415**, 424–426 (2002).
8. Hauert, C., De Monte, S., Hofbauer, J. & Sigmund, K. Volunteering as red queen mechanism for cooperation in public goods games. *Science* **296**, 1129–1132 (2002).

9. Hauert, C., De Monte, S., Hofbauer, J. & Sigmund, K. Replicator dynamics for optional public good games. *J. Theor. Biol.* **218**, 187–194 (2002).

10. Sugden, R. *The Economics of Rights, Co-operation and Welfare* (Blackwell, Oxford, UK, 1986).

11. Ostrom, E. *Governing the Commons* (Cambridge Univ. Press, Cambridge, 1999).

12. Gintis, H. *Game Theory Evolving* (Princeton Univ. Press, Princeton, New Jersey, 2000).

13. Berkes, F., Feeny, D., McCay, B. J. & Acheson, J. M. The benefits of the commons. *Nature* **340**, 91–93 (1989).

14. Colman, A. M. *Game Theory and Its Applications in the Social and Biological Sciences* (Butterworth-Heinemann, Oxford, UK, 1995).

15. Boyd, R. & Richerson, P. J. Punishment allows the evolution of cooperation (or anything else) in sizable groups. *Ethol. Sociobiol.* **13**, 171–195 (1992).

16. Gintis, H. Strong reciprocity and human sociality. *J. Theor. Biol.* **206**, 169–179 (2000).

17. Fehr, E. & Gächter, S. Cooperation and punishment in public goods experiments. *Am. Econ. Rev.* **90**, 980–994 (2000).

18. Fehr, E. & Rockenbach, B. Detrimental effects of sanctions on human altruism. *Nature* **422**, 137–140 (2003).

19. Schelling, T. C. Hockey helmets, concealed weapons, and daylight saving—study of binary choices with externalities. *J. Confl. Resolut.* **17**, 381–428 (1973).

20. Dawes, R. M. Social dilemmas. *Annu. Rev. Psychol.* **31**, 169–193 (1980).

21. Boyd, R. & Richerson, P. J. The evolution of reciprocity in sizable groups. *J. Theor. Biol.* **132**, 337–356 (1988).

22. Sober, E. & Wilson, D. S. *Unto Other: The Evolution and Psychology of Unselfish Behavior* (Harvard Univ. Press, Cambridge, Massachusetts, 1999).

23. Magurran, A. E. & Pitcher, T. J. Provenance, shoal size and the sociobiology of predator-evasion behaviour in minnow shoals. *Proc. R. Soc. Lond. B* **229**, 439–465 (1987).

24. Michor, F. & Nowak, M. A. Evolution - the good, the bad and the lonely. *Nature* **419**, 677–679 (2002).

25. Ridley, M. *The Origins of Virtue* (Penguin, London, 1996).

26. Axelrod, R. & Hamilton, W. D. The evolution of cooperation. *Science* **211**, 1390–1396 (1981).

27. Nowak, M. A. & Sigmund, K. Evolution of indirect reciprocity by image scoring. *Nature* **393**, 573–577 (1998).

28. Wedekind, C. & Milinski, M. Cooperation through image scoring in humans. *Science* **288**, 850–852 (2000).

29. Bolton, G. E., Katok, E. & Ockenfels, A. What's in a reputation? Indirect reciprocity in an image scoring game. Working paper. (Penn State University, 2001).

30. Seinen, I. & Schram, A. *Social status and group norms: indirect reciprocity in a helping experiment. Discussion paper TI2001-003/1* (Tinbergen Institute, Amsterdam, 2001).

Acknowledgements We thank students at the universities of Bonn, Kiel and Hamburg for participation; T. Bakker, H. Brendelberger, E. Heinz, K.-P. Sauer and M. Zbinden for support; C. Hauert for calculating parameters; and the Max-Planck-Institute of Meteorology for hospitality.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to M.M. (milinski@mpil-ploen.mpg.de).

Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins

Colette Tournaire-Roux¹, Moira Sutka¹, H el ene Javot¹, Elisabeth Gout², Patricia Gerbeau^{1*}, Doan-Trung Luu¹, Richard Bligny² & Christophe Maurel¹

¹Biochimie et Physiologie Mol culaire des Plantes, Centre National de la Recherche Scientifique (Unit  Mixte de Recherche 5004), Institut National de la Recherche Agronomique, Universit  Montpellier 2 et Ecole Nationale d'Agromomie, Place Viala, F-34060 Montpellier cedex 1, France
²Physiologie Cellulaire V g tale, Commissariat   l' nergie Atomique, Rue des Martyrs, F-38054 Grenoble cedex 9, France

* Present address: Phytobiologie Cellulaire, Universit  de Bourgogne, BP 47 870, F-21078 Dijon cedex, France

Flooding of soils results in acute oxygen deprivation (anoxia) of plant roots during winter in temperate latitudes, or after irrigation¹, and is a major problem for agriculture. One early response of plants to anoxia and other environmental stresses is down-regulation of water uptake due to inhibition of the water permeability (hydraulic conductivity) of roots (L_p)^{2–5}. Root water uptake is mediated largely by water channel proteins

(aquaporins) of the plasma membrane intrinsic protein (PIP) subgroup^{6–8}. These aquaporins may mediate stress-induced inhibition of L_p ^{2,4,9} but the mechanisms involved are unknown. Here we delineate the whole-root and cell bases for inhibition of water uptake by anoxia and link them to cytosol acidosis. We also uncover a molecular mechanism for aquaporin gating by cytosolic pH. Because it is conserved in all PIPs, this mechanism provides a basis for explaining the inhibition of L_p by anoxia and possibly other stresses. More generally, our work opens new routes to explore pH-dependent cell signalling processes leading to regulation of water transport in plant tissues or in animal epithelia¹⁰.

The molecular bases of aquaporin gating in plants and animals by phosphorylation^{11–13} or other mechanisms^{14,15} remain elusive. Certain mammalian aquaporins are regulated by external pH^{14–16} when expressed in *Xenopus* oocytes. In plants, the water channel activity of purified plasma membrane vesicles can be blocked by protons¹⁷. We investigated the relevance of this process for regulation of water uptake by roots in *Arabidopsis*.

Roots detached from plants grown in hydroponics were inserted into a pressure chamber and bathed in a well-aerated standard root bathing solution (RBS) at pH 6.0. Applied pressure (P) induced a flow (J_v) of exuded sap, with a linear J_v to P relationship for P up to 0.5 MPa. The slope reported to root dry weight indicates a mean L_p of $61.5 \pm 6.7 \text{ ml g}^{-1} \text{ h}^{-1} \text{ MPa}^{-1}$ ($\pm \text{s.e.m.}$; $n = 4$) (Fig. 1a). Oxygen deprivation was induced in the same roots by N_2 bubbling for 30 min in RBS and resulted in a 42% reduction in L_p to $35.6 \pm 7.4 \text{ ml g}^{-1} \text{ h}^{-1} \text{ MPa}^{-1}$ ($\pm \text{s.e.m.}$) (Fig. 1a). Because the J_v curves cross the P axis at similar values close to the origin, measurements of J_v at a high (>0.3 MPa) constant P can be used to monitor relative changes in L_p . For instance, the anoxic treatment above induced a 46% reduction in J_v at 0.35 MPa. A similar reduction in J_v by $49.2 \pm 3.7\%$ ($\pm \text{s.e.m.}$, $n = 5$) was observed on another set of plants. Altogether, these results extend to *Arabidopsis* observations previously made in crop species^{3,5}. A fall in cytosolic pH is, besides fluctuations in cytosolic Ca^{2+} , one early cellular response that typically accompanies anoxia^{18–20}. This response was investigated in *Arabidopsis* roots using *in vivo* proton-decoupled ³¹P-nuclear magnetic resonance (NMR) spectroscopy. Spectra (see Supplementary Information 1) revealed three major peaks corresponding to phosphorylcholine (P-Cho), at 3.47 parts per million (p.p.m.), and to cytosolic and vacuolar pools of inorganic phosphate (Pi), at 2.45 and 0.55 p.p.m.—pH values of 7.7 and 5.9, respectively. Within the 10 min after the onset of oxygen deprivation, the peaks of cytosolic Pi and P-Cho shifted upfield to 1.94 p.p.m. and 3.38 p.p.m., respectively, indicating a decrease in cytosolic pH to 7.20–7.25 ($n = 3$).

Inhibitors of cytochrome pathway respiration can be used to mimic oxygen deprivation^{4,5}. We found that sodium azide (1 mM NaN_3) and potassium cyanide (0.5 mM KCN) induced a marked inhibition of J_v by $87 \pm 1\%$ (NaN_3 ; $n = 6$) and $81 \pm 1\%$ (KCN; $n = 7$), with half times ($t_{1/2}$) of 2.6 ± 0.2 min and 4.6 ± 0.1 min, respectively (Fig. 1b). Wash out of NaN_3 and KCN induced a significant reversal of J_v inhibition to $92 \pm 3\%$ ($n = 3$) and $76 \pm 3\%$ ($n = 4$) of initial values, respectively, over a similarly short period of time (NaN_3 , $t_{1/2} = 4.6 \pm 0.6$ min; KCN, $t_{1/2} = 3.2 \pm 0.7$ min; Fig. 1b). In parallel NMR experiments the chemical shift of P-Cho indicated that exposure of roots to 1 mM NaN_3 or 0.5 mM KCN resulted in a rapid drop in cytosolic pH to a steady-state value of 6.9–7.0 (NaN_3) or 7.0–7.25 (KCN; $n = 3$) after 10–20 min (Fig. 1c; see also Supplementary Information 1). Wash out of the inhibitors resulted in a complete recovery of initial cytosolic pH values (pH 7.7) in less than 5 min (Fig. 1c).

Loading of root cells with 20 mM propionic acid/potassium propionate (KProp), by substituting at constant pH (pH 6.0) the weak acid for 20 mM KCl in a standard RBS, induced a marked drop in cytosolic pH down to 6.5–6.6 ($n = 3$), with a secondary equilibration to steady-state values in the range of pH 6.7–6.8 (Fig. 2b). A