

Consider therefore the evolution of a rising CO₂-brine fluid on the idealized pseudobinary phase diagram shown in Fig. 2, which shows the single-phase/two-phase boundary as a function of temperature (and thus effectively depth). For rising H₂O-rich fluids (case A in Fig. 2) the very small non-wetting CO₂ bubbles exsolved in the fluid will simply rise through the porous network until trapping in the manner described above, either against a pore throat or against a previously trapped bubble. The eventual lengthening of bubbles thus produced to dimensions much longer than a grain size will reduce the threshold pressure gradient to below the actual driving pressure gradient, and the bubbles will move again. This coalescence of bubbles will prevent the dispersal required by the trapping mechanism described above.

On the other hand, if CO₂-rich fluids are rising (case B in Fig. 2), water will be exsolved as the minority constituent; because it is the wetting phase, it will adhere to mineral surfaces in thin lenses. When water accumulation increases enough at pore throats, the phenomenon of choke-off³¹ will block pore throats with water; rising CO₂ will be compartmentalized. Continued percolation of fluid through those parts of the network still open will in turn seal those parts. Thus, rising water-saturated CO₂ can eventually generate an immobile two-phase mixture.

Such sealing cannot be permanent below the brittle-ductile transition. Over long timescales at greater depth, ductile deformation of the rock matrix around the pore space will occur. Because the gas pressure is higher than the liquid pressure (by $\sim 10^{-3}$ bar for a bubble of 10^{-4} m radius), the rock will retreat from rock-gas interfaces and advance on rock-liquid interfaces, eventually leading to the isolation of the gas bubble inside the liquid away from rock surfaces, permitting easy liquid leakage around at least some parts of the bubble. For calculating deep-crustal water residence times, CO₂ can be regarded as part of the deformable matrix through which the water must flow; these times are geologically short⁶. Thus although CO₂ can remain trapped at greater depths, water cannot, consistent with current petrological views of the lower crust¹⁴. As sealing cannot occur below the brittle-ductile transition, it would probably be most pronounced at those depths where it first becomes possible for rising fluids, namely just above the brittle-ductile transition. At this depth, the fluid will pond from below against the sealing level.

Can such a process generate horizontally extended reservoirs, given the horizontal variability of rock properties and fluid

inputs? Probably yes, because the proposed trapping mechanism is self-healing. If by horizontal spreading, ponding fluid below the sealing level reaches an unsealed region, and leaks upward at that point, the water-exsolution process described above can generate a new seal. The final state of the reservoir zone might well resemble an inverted version of puddles on uneven ground after a rainfall. In exhumed rocks, these would presumably show as originally subhorizontal fractures (for faults and shear zones, if exhumation was associated with tectonic stress) with associated local retrogression. □

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1. Fyfe, W. S., Price, N. J. & Thomson, A. B. *Fluids in the Earth's crust* (Developments in Geochemistry Vol. 1, Elsevier, New York, 1978).
2. Etheridge, M. A., Wall, V. J., Cox, S. F. & Vernon, R. H. *J. geophys. Res.* **89**, 4344-4358 (1984).
3. Sibson, R. H. *Geology* **15**, 701-704 (1987).
4. Rice, J. R. in *Fault Mechanics and Transport Properties of Rocks* (eds. Evans, B. & Wong, T.-F. 475-503 (Academic, New York, 1992).
5. Jones, A. G. *Geophys. J.* **89**, 7-18 (1987).
6. Bailey, R. C., *Geophys. Res. Lett.* **17**, 1129-1132 (1990).
7. Brace, W. F. *Int. J. Rock Mech. Miner. Sci. Geomech. Abstr.* **17**, 241-251 (1980).
8. Hyndman, R. D. & Shearer, P. M. *Geophys. J. Int.* **98**, 343-365 (1989).
9. Walder, J. & Nur, A. J. *Geophys. Res.* **89**, 11539-11548 (1984).
10. Marquis, G. & Hyndman, R. D. *Geophys. J. Int.* **110**, 91-105 (1992).
11. Thomson, A. B. *Nature* **358**, 295-302 (1992).
12. Sibson, R. H., *J. struct. Geol.* **11**, 873-877 (1989).
13. Fyfe, W. S. in *Saline Water and Gases in Crystalline Rocks* (eds Fritz, P. & Frape, S. K.) 1-3 (Spec. Pap. No. 33, Geol. Ass. Canada, Ottawa, 1987).
14. Yardley, B. W. D. *Nature* **323**, 111 (1986).
15. Thompson, A. B. & Connolly, J. A. D. *Tectonophysics* **182**, 47-55 (1990).
16. Secor, D. T. & Pollard, D. D. *Geophys. Res. Lett.* **2**, 510-513 (1975).
17. Yardley, B. W. D. & Bottrell, S. H. *Geophys. Res. Lett.* **16**, 199-202 (1988).
18. Frantz, J. D., Popp, R. K. & Hoering, T. C. *Chem. Geol.* **98**, 237-255 (1992).
19. Zhang, Y. G. & Frantz, J. D. *Chem. Geol.* **74**, 289-308 (1989).
20. Bowers, T. S. & Helgeson, H. C. *Geochim. cosmochim. Acta* **47**, 1247-1275 (1983).
21. Johnson, E. L. *Geology* **19**, 925-928 (1991).
22. Frape, S. K. & Fritz, P. in *Saline Water and Gases in Crystalline Rocks* (eds Fritz, P. & Frape, S. K.) 19-37 (Spec. Pap. No. 33, Geol. Ass. Canada, Ottawa, 1987).
23. Stegemeier, G. L. in *Improved Oil Recovery by Surfactant and Polymer Flooding* (eds Shah, D. O. & Schechter, R. S.) 55-91 (Academic, New York, 1977).
24. Dullien, F. A. L., Lai, F. S. Y. & MacDonald, I. F. J. *Colloid Interface Sci.* **109**, 201-218 (1986).
25. Katz, A. J. & Trugman, S. A. *J. Colloid Interface Sci.* **123**, 8-13 (1988).
26. Nassau, K. & Schonhorn, H. *Gems Gemol.* **15**, 354-360 (1978).
27. Watson, E. B. & Brenan, J. M. *Earth planet. Sci. Lett.* **85**, 497-515 (1987).
28. Adamson, A. W. *Physical Chemistry of Surfaces* 70 (Interscience, New York, 1960).
29. Rowlinson, J. S. & Widom, B. *Molecular Theory of Capillarity* (Clarendon, Oxford, 1982).
30. Brace, W. F., Walsh, J. B. & Frangos, W. T. *J. geophys. Res.* **73**, 2225-2236 (1968).
31. Mohanty, K. K., Davis, H. T. & Scriven L. E. in *Proc. Soc. Petrol. Engrs 55th A. Conf.* (Publ. No. 9406, Soc. Petrol. Engrs, Dallas, 1980).

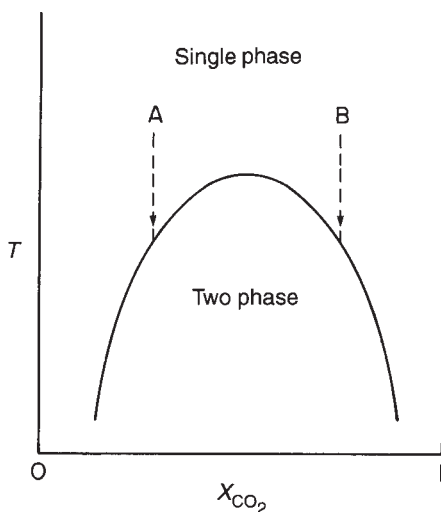


FIG. 2 Temperature defining the miscibility limit as a function of composition X_{CO_2} (mole fraction of CO₂) for an idealized pseudobinary phase diagram of the saline H₂O-CO₂ system. A and B are evolutionary paths for two possible fluid compositions described in the text.

Parental choice selects for ornamental plumage in American coot chicks

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ORNAMENTAL traits such as colourful bird plumage were the prime motivation for Darwin's theory of sexual selection¹. Other evolutionary mechanisms could also select for ornamental traits²⁻⁶, but such mechanisms have received far less attention, and empirical evidence for their existence is weak. Here we show that parental choice selects for the bizarre ornamental plumes of newly hatched American coot (*Fulica americana*) chicks. Experimental manipulations of chick plumage revealed that parent coots feed ornamented chicks preferentially over non-ornamented chicks, resulting in higher growth rates and greater survival for ornamented chicks.

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Moreover, we show that parental preference is relative, rather than absolute, an important element in the evolution of exaggerated traits^{7,8}. These observations provide the first empirical evidence that parental choice can select for ornamental traits in offspring.

American coots are monogamous rails (Rallidae) that breed throughout marshes in western North America. Both sexes share in territory defence, incubation, and feeding the precocial offspring for at least a month after hatching. American coot chicks are a striking exception to the general pattern of cryptic natal plumages in precocial chicks^{9,10}. Conspicuous orange, waxy-tipped filaments cover the front half of the body, brilliant red papillae surround the eyes and base of the bright red bill, and the top of the head is naked and brightly coloured (Fig. 1). The structure of the colourful plumes indicates that they are unlikely to play a role in thermoregulation (Fig. 1), and thus probably function strictly as signals.

The presence of conspicuous ornaments like colourful plumes and skin patches in newly hatched chicks is particularly astonishing because flightless chicks should be especially vulnerable to predators. Coot chicks frequently hide their heads when adults give alarm calls (personal observation), indicating that this colourful plumage does have associated costs. Although sexual selection would normally be invoked to explain the occurrence of potentially costly ornamental traits like conspicuous coloration^{8,11,12}, that mechanism cannot apply here because the colourful plumage is lost by the age of three weeks. However, a variety of social interactions other than sexual selection could potentially favour the evolution of exaggerated traits²⁻⁶. For example, West-Eberhard⁵ noted that parents are often in a position to exercise favouritism among their offspring through the allocation of critical resources like food. If parents preferentially feed offspring that bear the most extreme expression of a particular trait and if such feeding enhances offspring fitness, parental choice could result in the evolutionary elaboration of the offspring trait.

This idea, previously untested in any animal, may apply to the plumage of coot chicks. Parental feedings are essential for chick survival and between one third and one half of all chicks that hatch subsequently perish, mainly as a result of starvation¹³. Eggs hatch asynchronously and later hatched chicks suffer significantly higher mortality than early hatched chicks¹³. Aggres-

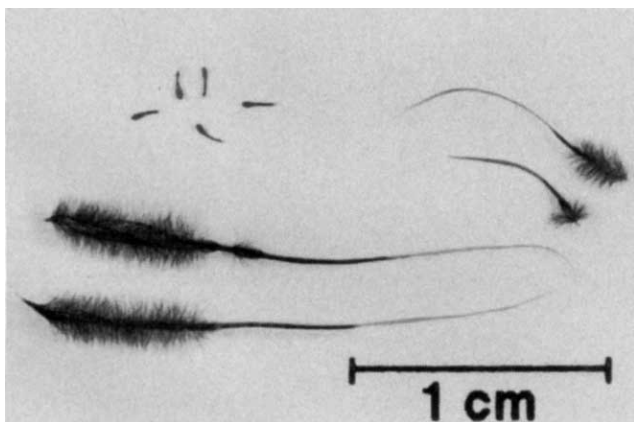


FIG. 1 Structure of the ornamental plumes from three regions of the body. The conspicuous coloration occurs on the modified distal ends of the throat feathers (upper right) and back feathers (bottom) while the black down typical of Rallid chicks is restricted to the basal portion. The reddish papillae-like feathers that surround the eyes and base of the bill (upper left) are highly modified and lack down completely. The structure of the colourful portions of these ornamental feathers implies a modification from a thermoregulatory function to a signalling function.

sion among chicks does not occur and parents exercise total control over the delivery of food among offspring (B.E.L., unpublished data). Beginning the week following hatching, brood division occurs whereby each parent specializes in feeding a subset of the brood. The youngest chicks attended by each parent are fed the most (B.E.L., unpublished). Thus, parents control the allocation of resources critical for chick survival and are in a position to exert choice. Chicks do not vocalize when begging but rather approach parents and display their plumage and, less frequently, the naked skin on the top of their head¹⁰. These displays suggest that parents assess chick plumage during feeding interactions and that parental choice may underlie the conspicuous plumage.

To test this hypothesis, we experimentally manipulated the plumage of chicks in a population of American coots in central British Columbia, Canada, during the breeding season of 1992. Our study population comprised 90 pairs of coots on three permanent wetlands (5–15 ha). A pilot study indicated that it was not feasible to dye chicks—the dye made chicks ill and removed waterproofing oils from their plumage. We therefore altered the appearance of chicks by trimming the orange tips off their body feathers. In each experimental brood, we trimmed half of the chicks in the brood, hereafter termed ‘black’ chicks, and left the remaining half of the brood with their orange plumes intact (‘orange’ chicks). On black chicks, the orange plumage was restricted to the throat area, a plumage coloration that closely resembles the natal plumage of most Rallids, including several congeners^{10,14}. Thus, our experimental manipulations produced an ancestral appearance rather than a novel one¹⁵.

With the exception of trimming, all chicks were handled in a similar manner and were held in captivity for about 30 min. Trimmed chicks did not appear appreciably smaller as a result of the removal of the orange feather tips. To ensure further that the removal of feathers *per se* did not affect the viability of chicks and thus confound our experiment, we used a double set of control broods. In black control broods, we trimmed the orange feather tips from all chicks in the brood, and in orange control broods all chicks were left with their natural plumage intact. If the orange plumes are strictly ornamental, chicks in these two control groups should not differ in any measure of parental care or fitness. We found no difference between the two control groups in any of the measures (Fig. 2a–c; Mann-Whitney *U* tests in all cases); feeding rates, $U' = 85$, $P = 0.24$; growth rates, $U' = 59$, $P = 0.78$, or chick survival, $U' = 91.5$, $P = 0.94$. The almost identical feeding rates and viabilities of chicks in the two control groups confirm the validity of our experiment and demonstrate that the colourful plumes are strictly ornamental.

Within experimental broods, orange chicks were fed at a higher rate than black chicks (Fig. 2d; Wilcoxon test, $z = 3.84$, $P < 0.001$), demonstrating that parents prefer ornamented chicks. As a result of their enhanced feeding rate, orange chicks grew more rapidly (Fig. 2e; Wilcoxon test, $z = 2.83$, $P < 0.005$) and enjoyed higher survival rates than black chicks in the same brood (Fig. 2f; Wilcoxon test, $z = 2.77$, $P < 0.01$). Parental preference clearly translates into increased fitness for ornamented offspring.

The survival benefits from increased parental care did not accrue to all orange chicks in experimental broods, but depended strongly on a chick's position in the hatching order (Fig. 3). Late-hatched orange chicks had dramatically higher survival than late-hatched black chicks, but there was little difference in survival between early-hatched black and orange chicks (Fig. 3). This result is predicted, given the patterns of parental care in American coots. After brood division occurs, each parent has one or two ‘favourite’ chicks that receive most of the feeding. These are primarily the late-hatched chicks (B.E.L., unpublished data). Accordingly, any influence of ornamentation on parental feeding should be most strongly evidenced in the late-hatched chicks, as we found. Ornamental plumes thus appear to increase

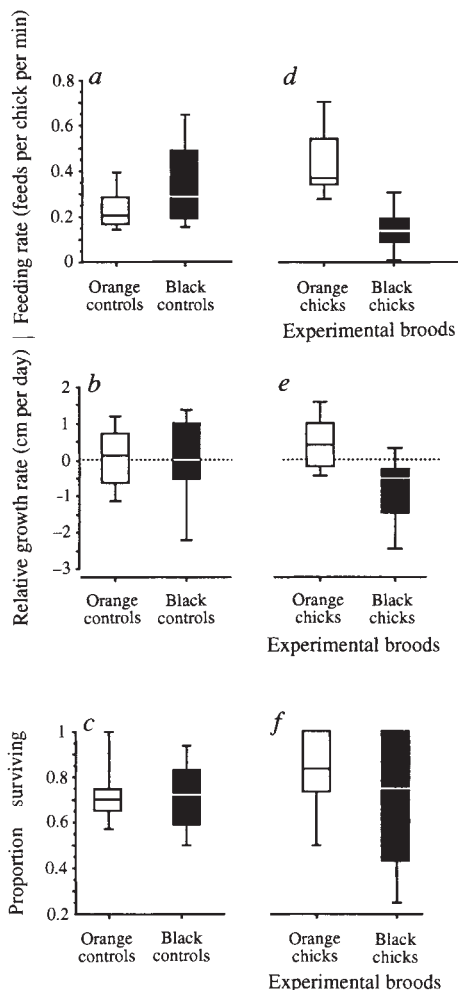


FIG. 2 Feeding rates, growth rates and survival of chicks in control (a–c) and experimental (d–f) broods. Values shown are medians, interquartile ranges, and 10–90 percentiles. To avoid pseudoreplication, a single mean value was calculated over all chicks in each control brood and each mean was then used as an independent datum in two sample comparisons. For experimental broods, mean values were calculated separately over all orange chicks and all black chicks, respectively, within each brood. Matched-paired comparisons were then used to compare these values for each treatment within broods. Plumage on chicks were trimmed within a day of hatching. To control for the confounding influence of hatching order²¹, the first chick to hatch in each experimental brood was randomly assigned to a treatment and thereafter treatments were alternated with hatching order. Chicks in each brood were individually colour-marked and broods were observed from floating blinds to estimate feeding rates, growth and survival²¹. All observation periods for each brood were pooled to calculate one overall mean feeding rate (average of 1.4 h observation per brood at 12 orange control broods, 11 black control broods, 21 experimental broods). To estimate growth rate, swimming chicks were photographed at known distances and body length at waterline was estimated from projected slides²². This measure of body size is strongly correlated with body mass²² ($r=0.97$, $n=43$). Residual values from a regression of body length on chick age were used as an index of relative growth rate, and mean brood values were calculated for the 11 orange control, 10 black control and 13 experimental broods. Survival comparisons are based on 15 orange control, 12 black control and 25 experimental broods. Sample sizes differ among comparisons because some broods could not be monitored for feeding and/or growth rates. Sixteen broods (9 experimental, 4 orange controls, 3 black controls) were excluded from the analysis because they adopted chicks or donated to chicks other broods, but the results are unchanged if these broods are included.

the likelihood that a chick will be chosen as a favourite chick once brood division takes place.

An important issue in the evolution of exaggerated traits through a mechanism such as female choice or parental choice is whether the preference is relative or absolute^{7,8}. Our experiment confirms that parental preference in coots is relative. Non-ornamented chicks were only disadvantaged when ornamented chicks were also present in the brood (experimental broods). In black control broods, for which there was no variation from which parents could choose, non-ornamented chicks ate, grew and survived as well as the ornamented chicks in orange control broods. This finding is important because it allows us to reject the hypothesis that non-ornamented chicks fare poorly in experimental broods simply because they are not recognized as coot chicks by their parents.

Given that parental preference is relative, an alternative explanation for the observed pattern is possible—perhaps trimming feathers somehow influenced chick begging behaviour so that parents responded to behavioural differences rather than plumage differences. Begging differences between trimmed and non-trimmed chicks might result in higher feeding rates for non-trimmed chicks in experimental broods, but no difference would be expected between the two groups of controls owing to a lack of variation within these broods. However, we could detect no difference in the behaviour of trimmed and non-trimmed chicks in observations conducted within a day or two of the trimming. Moreover, we cannot envisage a mechanism by which the removal of the thin, highly modified feather tips would affect chick begging behaviour sufficiently to produce the large differences observed in parental feeding rates. A second possibility is that trimming made black chicks appear smaller than orange chicks and parents responded to the apparent size of chicks rather than plumage colour. This hypothesis is rejected by the observation that parents preferentially feed the smallest chicks—black chicks should therefore have been fed more, not less, if this mechanism applied. We conclude that the trait under selection by parental choice is the ornamental plumage of coot chicks.

Our study now raises the question of why parents prefer ornamented chicks in the first place. We suggest three possibilities. First, ornamental plumes may provide an honest signal of a

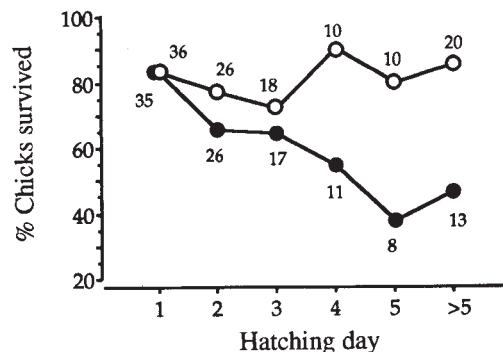


FIG. 3 The relationship between hatching day and survival of orange chicks (open circles) and black chicks (filled circles) within experimental broods. Hatching in American coots is asynchronous; day 1 represents the day on which the first chicks in a brood hatched. Sample sizes (number of chicks) are shown beside each point. Analysis of log likelihood revealed a significant interaction effect between hatching order and plumage colour on the survival of chicks (Wald $\chi^2=4.92$, d.f. = 1, $P<0.03$). Post-hoc comparisons indicate no effect of plumage colour on the survival of early chicks (days 1–3 pooled, G-test with Williams' correction, $G_w=0.69$, d.f. = 1, $P>0.50$ after Bonferroni correction), but a significant effect of plumage colour on the survival of late chicks (days 4+ pooled; $G_w=11.85$, d.f. = 1, $P<0.003$ after Bonferroni correction).

chick's genetic or phenotypic quality^{16,17} which allows parents to invest selectively in high-quality offspring¹⁸. For such a signal to be reliable, it must be costly^{16,17,19}; we cannot yet assess whether this might apply to the bright plumage of coot chicks. Second, the orange plumage may provide an honest signal of age, allowing parents to allocate food optimally among offspring. Finally, the preferences for the orange plumage may involve sensory exploitation of a parental colour preference favoured for other reasons²⁰. For example, the bare skin patch on the chick's head varies in colour over time and may signal off-

spring need¹⁹. At its brightest intensity, the skin is similar in colour to the ornamental plumes (B.E.L. *et al.*, unpublished data). Thus, the initial parental preference for orange colouration may have evolved as a response to a condition-dependent signal of the naked skin, with the elaboration of bright plumage following as a secondary consequence of this sensory bias. We are currently investigating these possibilities, although, as with studies of sexual selection, demonstrating that a preference exists may prove easier than understanding why the choice mechanism itself has been favoured by selection²⁰. □

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1. Darwin, C. *The Descent of Man and Selection in Relation to Sex* (Murray, London, 1871).
2. Dobzhansky, T. *Am. Nat.* **74**, 312–321 (1940).
3. Baker, R. R. & Parker, G. A. *Trans. R. Soc. Lond. B* **287**, 63–130 (1979).
4. West-Eberhard, M. J. *Proc. Am. Phil. Soc.* **132**, 222–243 (1979).
5. West-Eberhard, M. J. *Q. Rev. Biol.* **58**, 155–183 (1983).
6. Zahavi, A. *Anim. Behav.* **42**, 501–503 (1991).
7. Arnold, S. in *Mate Choice* (ed. Bateson, P.) 67–107 (Cambridge Univ. Press, Cambridge, 1983).
8. Zuk, M., Johnson, K., Thornhill, R. & Ligon, J. D. *Evolution* **44**, 477–485 (1990).
9. Harrison, C. *A Field Guide to the Nests, Eggs and Nestlings of North American Birds* (Collins, Toronto, 1984).
10. Boyd, H. J. & Alley, R. *Ibis* **90**, 582–593 (1948).
11. Andersson, M. *Nature* **299**, 818–820 (1982).
12. Kodric-Brown, A. *Behav. Ecol. Sociobiol.* **17**, 199–205 (1985).

13. Lyon, B. *Anim. Behav.* **46**, 911–928 (1993).
14. Ripley, D. *Rails of the World* (Godine, Boston, 1977).
15. Ryan, M. J. & Keddy-Hector, A. *Am. Nat.* **139**, S4–S35 (1992).
16. Zahavi, A. in *International Symposium of Biological Evolution* (ed. Delfino, V. P.) (Adriatica Editrice, Bari, 1987).
17. Grafen, A. *J. Theor. Biol.* **144**, 517–546 (1990).
18. Stephenson, A. G. & Winsor, J. A. *Evolution* **40**, 453–458 (1986).
19. Godfray, H. C. J. *Nature* **352**, 328–330 (1991).
20. Kirkpatrick, M. & Ryan, M. J. *Nature* **350**, 33–38 (1991).
21. Lyon, B. thesis, Princeton Univ. (1992).
22. Lyon, B. *Condor* (in the press).

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Electrostatic tuning of Mg²⁺ affinity in an inward-rectifier K⁺ channel

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INWARD-RECTIFIER potassium channels conduct K⁺ across the cell membrane more efficiently in the inward than outward direction. This unusual conduction property is directly related to the biological action of these channels^{1–6}. One basis for inward rectification is voltage-dependent blockade by intracellular Mg²⁺ (refs 1, 7–9): strong inward-rectifier channels are so sensitive to intracellular Mg²⁺ that no outward K⁺ current is measurable under physiological conditions; weak inward rectifiers are less sensitive and allow some K⁺ to flow outwards. Background K1 channels and acetylcholine-regulated K⁺ channels from the heart are examples of strong inward rectifiers and ATP-sensitive K⁺ channels are weak rectifiers^{1,7–10}. Here we show that mutations at one position in the second transmembrane segment can alter the Mg²⁺ affinity and convert a weakly rectifying channel (ROMK1) into a strong rectifier. The amino acid at this position exposes its side chain to the aqueous pore and affects Mg²⁺ blockade as well as K⁺ conduction through an electrostatic mechanism.

Figure 1 illustrates two general features of Mg²⁺ blockade in an inward rectifier K⁺ channel, ROMK1. First, blockade due to intracellular Mg²⁺ is highly voltage-dependent. The voltage dependence accounts for the curvature (inward rectification) in the current–voltage (*I*–*V*) graphs (Fig. 1*a*). Second, as the external K⁺ concentration ([K⁺]_o) is raised, Mg²⁺ affinity is decreased: at higher external [K⁺]_o stronger depolarization is required to achieve the same degree of blockade (Fig. 1*b, c*). Taken together, the voltage dependence and effect of extracellular [K⁺]_o argue that Mg²⁺ occludes the ion-conduction pore^{7,9}.

The membrane folding topology of inward-rectifier K⁺ channels is unknown. The homology between voltage-activated and inward-rectifier K⁺ channels suggests that inward rectifiers contain a unit consisting of a P-region flanked by two transmembrane segments^{11,13}. Based on mutagenesis studies in voltage-

activated K⁺ channels, part or all of the unit could be involved in ion conduction^{14–18}. Figure 2*a* shows a sequence alignment of the second membrane-spanning segment of three inward-rectifier K⁺ channels^{11,13,19,20}. IRK1 and GIRK1 are strong rectifiers but ROMK1 is a weak rectifier^{11–13,19–21}. Figure 2 shows that ROMK1 becomes a strong rectifier when aspartate (D), the residue found in IRK1 and GIRK1, is substituted for asparagine (N) at position 171. In cell-attached patch recordings, the wild-type ROMK1 channel shows weak inward rectification (Fig. 2*c*). By contrast, the mutant channel with an aspartate substitution at position 171 (N171D) shows no outward current (Fig. 2*e*). The dramatic effect on the *I*–*V* relationship can be attributed to the carboxyl side chain of aspartate because substituting glutamate (Fig. 2*f*), but not threonine (Fig. 2*d*), has an effect nearly identical to that of aspartate. In these cell-attached recordings, we assume that the affinity for intracellular Mg²⁺ has been significantly increased by placing an acidic residue at position 171.

Sixteen residues were substituted at position 171 and only those with hydrophilic side chains produced currents in oocytes (Fig. 2*b*). All were highly selective for K⁺ over Na⁺ (data not shown). The Mg²⁺ sensitivity of mutant channels in excised, inside-out patches is shown in Fig. 3. Both macroscopic and single-channel records demonstrate a 20-fold increase in Mg²⁺ affinity when Asp is present at position 171 (Figs 1*a* and 3). (In single-channel records Mg²⁺ reduces the amplitude because discrete blocking events are filtered by the relatively slow response time of our recording system (Fig. 3*g*.) Mg²⁺ affinity is altered by the same factor at all voltages, implying that the N171D mutation affects the binding energy, but not the location of the Mg²⁺-binding site in the pore (Fig. 3*e, f*).

If substitution of aspartate or glutamate at position 171 influences Mg²⁺ blockade through an electrostatic mechanism, then placement of a cationic amino acid should dramatically reduce the affinity. Figure 4*a* shows the *I*–*V* curve for the mutant channel containing an arginine residue (N171R). As expected, this mutant channel is insensitive to a high concentration of intracellular Mg²⁺. In addition, the *I*–*V* curve rectifies strongly in the absence of Mg²⁺. To investigate further the Mg²⁺-independent rectification, we substituted histidine at position 171 (Fig. 4*b*). The *I*–*V* relationship is linear at pH 9.0, but begins to rectify like the arginine mutant channel as the pH is lowered to a range where the cationic form of histidine is favoured. This result