Harvesting a crop of gold in plants

The possibility of turning base metals into gold has intrigued many scientists since the early alchemists, and the discovery of significant gold uptake by plants has long been a 'philosopher's stone'. But background levels of gold in plants are usually very low, rarely exceeding 10 ng per g dry tissue (10 p.p.b.)[1]. Hyperaccumulator plants[2], however, have 100 times the elemental concentrations of normal vegetation, a level of 1 mg per g dry tissue (1 p.p.m.). They can be used in phytoremediation[3], the *in situ* improvement of polluted sites. Hyperaccumulation can be induced by adding a chemical amendment, such as EDTA, to a plant substrate to make soluble an otherwise insoluble target metal, such as lead[4]. Here we have induced plants to accumulate gold from ores by treating the substrate with ammonium thiocyanate. This technique might be used as a form of biological mining (phytomining) for gold[5,6].

We used ammonium thiocyanate as a substrate amendment because it is commonly used for making gold soluble in mining operations. Table 1 compares the ease of gold extraction in four types of ore. Unweathered sulphide gold ore (not shown in Table 1) from Macraes mine in New Zealand had very little extractable gold, and we were unable to induce plants to remove this to any significant degree. Ore from the Waihi Mine has gold mainly in its native form, which we were able to induce plants to remove. To overcome the problem of sulphide occlusion of gold in some ores and possible non-homogeneity in others, a synthetic finely disseminated colloidal gold ore (made from gold chloride) was prepared and planted with *Brassica juncea*, a plant of high biomass and rapid growth rate. Other experiments using this and other species involved the additional use of a synthetic ore prepared from finely divided gold powder (44 μm) as well as the Tui and Waihi ores crushed to a size of 0.5 mm.

All plants were grown in 250-ml pots containing the appropriate substrate. The plants were treated with thiocyanate at rates of 0.00, 0.08, 0.16, 0.32 and 0.62 g per kg dry substrate weight. After seven days, aerial parts of the plants were harvested, dried and analysed for gold by graphite furnace atomic absorption spectroscopy. Gold concentrations in several plants as a function of added thiocyanate are shown in Table 1, and in Fig. 1 (inset) for *Brassica juncea* only.

All plant species showed hyperaccumulation of gold. The highest individual value was 57 μg gold per g dry weight in *B. juncea*. However, values were very variable, perhaps because the higher amendment levels caused necrosis of some plants and the induced gold concentration depended on the time that each plant remained viable. *Brassica* plants grown in the synthetic gold powder substrate (Fig. 1 inset) contained as much gold as those grown in the disseminated substrate. Similar values (9–19 μg per g dry tissue) were obtained for the same species grown in natural Waihi ore.

Induced hyperaccumulation of gold can be used for phytomining, with the resulting auriferous 'bio-ore' sold. The gross value of gold and the chemical costs involved in its extraction using a plant with a biomass of 20 t ha^{-1} (ref. 6) are shown in Fig. 1. Assuming that thiocyanate costs $3 per kg, the figure of $3,627 represents its total cost per hectare when added to a depth of 15 cm at an application rate of 0.64 g per kg (the highest rate of Fig. 1). As the price of gold increases, the operation becomes more favourable economically. At the current world price of about $300 per ounce, we would require a gold concentration of around 17 μg per g dry weight in a crop of *B. juncea*. Several values from the experiments shown in Fig. 1 were either above or very close to 17 μg per g. Some of the other phytomining costs may be recouped by selling the energy of plant combustion, as is done in the sugar-cane industry.

Induced hyperaccumulation of gold appears to be relatively independent of plant species, so it should be possible to use plants (such as chicory) that might be easy to grow on mine tailings. Any residual thiocycanate will be broken down rapidly in the substrate[7].

We believe this is the first evidence of significant gold uptake by any plant. As well as the economic ramifications this technology may have, this ability to make a 'crop of gold' opens up the way for the phytoextraction of other noble metals.

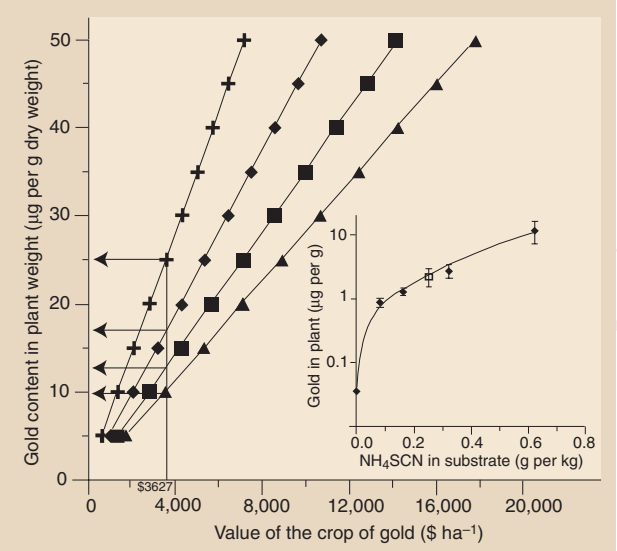

Figure 1 From gold leaves to gold leaf: the economic value of a gold crop as a function of world prices and concentrations in plant material. Prices: $200 (crosses), $300 (diamonds), $400 (squares), $500 (triangles). Inset, thiocyanate-induced uptake of gold by *Brassica juncea* from a 5 μg per g finely disseminated synthetic gold ore (diamonds) and natural ore (square).

Table 1 **Conditions and hyperaccumulation data**

Species	Substrate	Extraction* (%)	Thiocyanate (g per kg)	Plants	Gold yield (μg per g)†
Brassica juncea	Waihi ore‡	1.8	0.50	4	9.27–19.34
Chicory	Tui mine§	22.6	0.64	5	0.07–1.19
Impatiens sp.	Waihi ore‡	1.8	0.20	1	3.09
Arrhenatherum elatius	Waihi ore‡	1.8	0.50	4	0.07–1.43
B. juncea	Disseminated gold in sand	9.2	0.64	12	2.13–57.32
B. juncea	Fine gold powder in sand	–	0.25	8	0.37–6.48

*Percentage of gold extracted from 1 g of substrate into 10 ml of 2 g l^{-1} ammonium thiocyanate in 24 h.
†Gold values are for dry matter and are whole-plant analyses except for *B. juncea* on fine gold powder 44 (μm) in sand, which are for leaves only.
‡Natural colloidal gold.
§Acid sulphide mine tailings.

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Salinity history of the Earth's early ocean

It is commonly thought that the oceans are becoming saltier with time as sodium and chlorine are weathered out of continental rocks and transported to the sea. Here we argue that the salinity of the early ocean was 1.5 to 2 times the modern value, and that it did not decline significantly until surprisingly late in the Earth's history. If correct, this theory could help explain why the evolution of higher life took so long.

Volatile compounds such as HCl and H_2O were probably outgassed from the interior of the Earth early in its history[1]. Chloride ions would have accumulated in the initial ocean with sodium ions leached from surrounding rocks. The only known processes that can remove NaCl from the oceans in significant amounts are the evaporative deposition of salt and the sequestration of brine as deep groundwater on continents. The brine originates from the dissolution of salt and/or as remnants of partly evaporated sea water. Before the continental crust developed, the salt and brine currently found on the continents would have been entirely in the ocean. The oceans became less saline as continental platforms assembled and brine and salt began to accumulate amid a cycle of deposition and erosion within giant sedimentary basins.

If all the known subsurface salt deposits were returned to the sea, salinity would rise by 30% (ref. 1). More chlorine may currently occur in saline groundwater than in salt, perhaps 2-3 times as much[2]. If this chlorine had come from the sea, the initial salinity could have been twice the present level.

There was some continental crust 3.5 billion years ago (Gyr), but large-scale riverine input of ^{87}Sr derived from granite on emergent continents does not appear in the marine sedimentary record until 2.5 Gyr (Fig. 1). Using a recent model of continental growth[4], one continental mass existed at 3.0 Gyr and another developed at about 2.5 Gyr (Fig. 1). Two further masses appear at about 2.0 Gyr, from which time the history is dominated by assembly and break-up of continents. If this model is correct, large-scale deposition of NaCl on continents could not have begun before 2.5 Gyr.

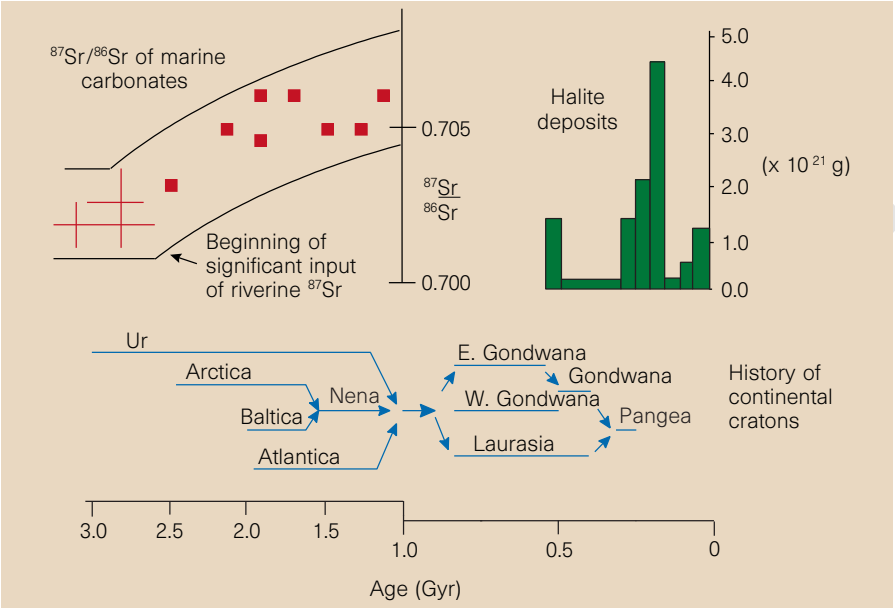

Figure 1 History of continents (after ref. 4), record of salt deposition for the past 540 million years (after ref. 2) and Sr isotope record of marine sediments (after ref. 3). The time axis applies to all three panels and is expanded for the past 1.0 Gyr. Salt deposits older than those shown have not been inventoried but probably total less than 0.5×10^{21} g because of recycling and/or non-deposition. The progressive increase in ^{87}Sr is most readily interpreted as input of riverine Sr from the weathering of continents that emerged largely after 2.5 Gyr.

Direct evidence for this highly saline early ocean may exist in chemical data[5] for fluid inclusions in quartz crystals formed in a deep marine hydrothermal environment of 3.2 Gyr. Chloride contents were 165% of the modern value, so the authors inferred that evaporative concentration of sea water had occurred before hydrothermal circulation. An alternative interpretation is that the inclusions are samples of a more saline early ocean.

If large-scale salt deposition and brine storage began at 2.5 Gyr and proceeded at the net rate of the last 540 million years (Fig. 1), the decrease in salinity could have been complete by ~2 Gyr. However, unusual (even improbable) geographic, geologic, climatic, oceanographic and depositional conditions are required for large salt deposits to form. The rate of accumulation was almost certainly lower until continental platforms were widespread at ~2 Gyr. Also, approximately 50% of the known salt was deposited in an interval of 100 million years (Fig. 1). If the conditions that gave rise to this extraordinary accumulation never existed previously, then the decrease in salinity may have persisted until about 1 Gyr, or even to the time of the Cambrian 'explosion' of marine life at 0.54 Gyr.

Most forms of modern macroscopic life cannot tolerate salinities above 50‰ (ref. 6). Cyanobacteria are more salt tolerant than most organisms, and these dominate the Precambrian fossil record. This may be, in part, because higher salinities were an impediment to the evolution of more complicated life forms. For example, the increase in dissolved oxygen from marine photosynthesizers is commonly invoked to explain the evolution of metazoans[7]. Oxygen solubility in sea water decreases significantly as salinity and temperature increase[8], so the rise in oxygen level would have been retarded in a more saline ocean. This would be especially true if early Earth temperatures were as high as suggested by isotope data for early sedimentary rocks[9].

It is commonly assumed that the oceans were the exclusive site of early evolution. However, the lack of fossil constraints, the increasing indications of microbial activity in Precambrian non-marine environments[10] and the inferred higher ocean salinity suggest that this is not necessarily the case. In the present model, the earliest life was either tolerant of salt or was restricted to the more dilute waters of estuaries or entirely non-marine environments. Either way, if continents were not fully developed early on there is a major salinity problem that must be considered when discussing the history of life on Earth or on any object with an overall Cl:H_2O ratio similar to that of Earth.

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Receptor that leaves a sour taste in the mouth

The ability to detect taste stimuli results from the activation of taste receptors located in taste-bud cells. There are several gustatory transduction mechanisms, involving membrane receptors, guanine-nucleotide-binding proteins (G proteins), second messengers and ion channels[1], but genes encoding taste receptors have not yet been identified. Here we identify a complementary DNA that encodes a receptor for sour tastes.

There are five basic tastes: sour, salty, bitter, sweet and umami (the taste of glutamate and similar naturally occurring substances)[1]. Sour-tasting substances, evoked by acids, interact directly with ion channels in taste-receptor cells[1]. In rats, amiloride-sensitive cation channels may directly mediate part of sour-taste transduction[2].

To isolate a sour-taste receptor, we constructed a rat circumvallate papilla cDNA library and screened it using combined homology and functional expression approaches. We screened the library at low stringency with oligonucleotide probes encoding proteins that shared significant homology with the second putative transmembrane domain of the amiloride-sensitive/degenerin cation-channel family. We obtained 18 independent cDNA clones, restriction-enzyme analysis of which produced several digestion patterns.

To find candidate sour-taste receptors, we expressed these clones in *Xenopus laevis* oocytes and analysed them using a two-electrode voltage clamp. Injecting a mixture of 5′-capped complementary RNA transcripts from all positive clones into *Xenopus* oocytes resulted in no amiloride-sensitive current at pH 7.5, but altering the extracellular pH to 5.5 produced a large inward current, which could be partly blocked by amiloride. We detected almost no current in water-injected oocytes with the same pH alterations.

Each clone from the pool was then transcribed independently and the cRNA products were injected separately into *Xenopus* oocytes. Only one clone showed the characteristics of the proton-gated amiloride-sensitive cation channel (data not shown). Simultaneous *in situ* hybridization showed that this clone was expressed in the taste buds in the circumvallate papilla of the rat tongue. Sequence analysis showed that this clone was identical to mammalian degenerin-1 (MDEG1)[3–5], although minor nucleotide differences were found in non-coding regions.

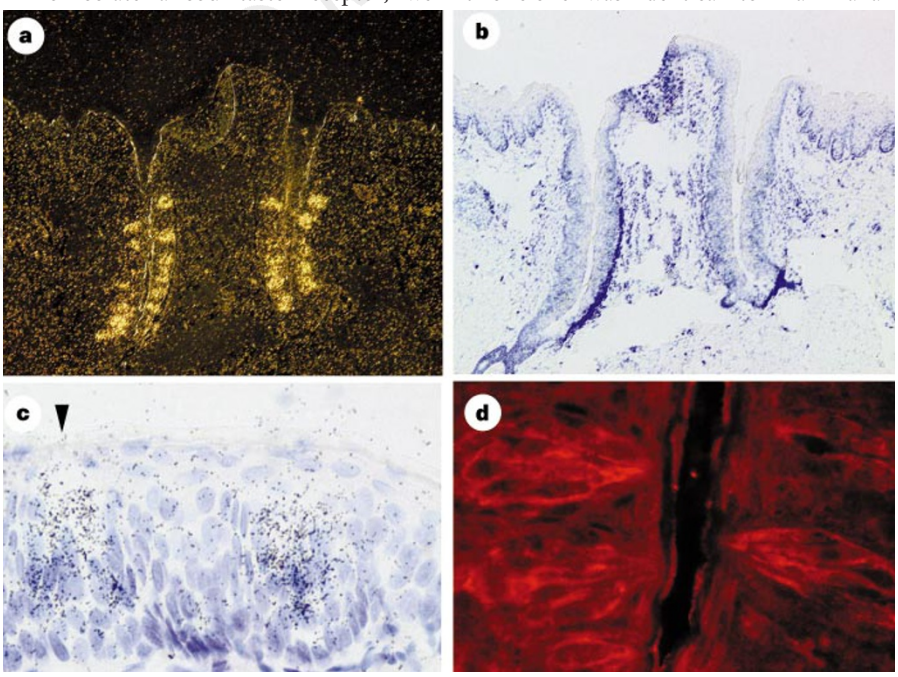

Figure 1 Expression and localization of MDEG1. **a–c,** Expression of MDEG1 mRNA in the rat circumvallate papilla by *in situ* hybridization. **a, b,** The circumvallate papilla, stained with an antisense probe, in dark-field (**a**) and bright-field (**b**) photomicrographs. **c,** Taste buds stained with an antisense probe, under high magnification. The dense hybridization signals for MDEG1 mRNA accumulate only in the central portion of the taste buds. The arrowhead indicates a taste pore. **d,** Localization of MDEG1 by immunohistochemistry in the circumvallate taste buds. Intense MDEG1 immunoreactivity was seen at the plasma membrane and the apical part of the MDEG1-positive cells in the circumvallate taste buds.

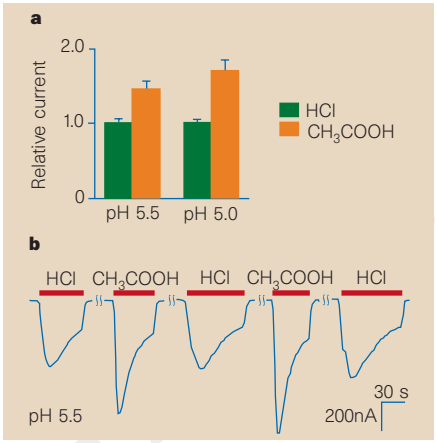

Figure 2 Expression of MDEG1 in *Xenopus* oocytes. **a,** Whole-cell currents of oocytes expressing MDEG1 at −70 mV. Data are plotted relative to the current elicited by ND140 solution adjusted with HCl. Values are mean ± s.e.m. from seven different oocytes. **b,** Representative current trace of an oocyte injected with cRNA encoding MDEG1. ND140 solution (containing (in mM) 140 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES, pH 5.5) adjusted with HCl or CH_3COOH was applied alternately.

We localized MDEG1 in the taste buds of the circumvallate papillae by using *in situ* hybridization. There were specific hybridization signals for MDEG1 messenger RNA in most of the taste buds on both sides of deep clefts in the rat circumvallate papilla, but none in any surrounding tissue (Fig. 1a, b). Under high magnification, the dense hybridization signals are seen to accumulate only in the central portion of the taste buds, indicating that this mRNA was not expressed in the basal cells (Fig. 1c). A ribonuclease protection assay confirmed that MDEG1 mRNA is expressed in circumvallate papillae (data not shown). Thus, MDEG1 mRNA is expressed in the taste buds of the rat circumvallate papilla.

We used immunohistochemistry to determine the subcellular localization of MDEG1 protein in the taste buds. We prepared a polyclonal antiserum against MDEG1 by immunizing rabbits. MDEG1 immunoreactive cells occurred only in the taste buds, not in surrounding tissues.

At the subcellular level, intense MDEG1 immunoreactivity was seen at the plasma membrane and the apical part of the immunoreactive taste-bud cells (Fig. 1d). Immunoelectron microscopic analysis showed that MDEG1-positive taste-bud cells had the characteristics of type III cells (primary taste-receptor cells) (data not shown)[1,6,7].

Protons cause acidic taste, and they activate sour-taste receptors[1]. However, acetic acid, the main ingredient of vinegar, is more sour than hydrochloric acid at equal

pH. In an attempt to explain this phenomenon, we applied ND140 solutions adjusted to pH 5.5 with acetic acid or hydrochloric acid to oocytes injected with cRNAs encoding MDEG1. Stimulation by acetic acid generated larger inward currents than those induced by hydrochloric acid at equal pH (Fig. 2a, b). These response patterns of MDEG1 to the two acids are similar to characteristics of sour-taste sensation of the acids *in vivo*.

Our molecular biological, morphological and electrophysiological analyses indicate that MDEG1 acts as a receptor for sour tastes in taste-bud cells.

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Over-water dispersal of lizards due to hurricanes

The possibility and probability of over-water dispersal as a mechanism to explain the distribution of terrestrial animal species in the Caribbean has been hotly debated since the early part of this century[1,2]. Each theory that has been proposed — including land bridges and over-water dispersal — has involved over-water dispersal to some extent in the distribution of animals. Yet many people remain sceptical of over-water dispersal, believing that the use of rafts is improbable, unobservable and consequently untenable. Here we present evidence to support over-water dispersal as the mechanism by which green iguanas colonized Anguilla.

For over-water dispersal to be considered a realistic explanation for the distribution of species in the Caribbean, it must be demonstrated that a viable population could be established. This can be accomplished by the invasion of either a pregnant individual, an asexually reproducing individual or several individuals of both sexes. Animals have been observed on rafting flotsam[3], but most of these are small organisms, such as insects[4,5]. The few accounts of vertebrates found on rafts[6,7] have reported only single individuals (but see ref. 8), and do not provide convincing evidence that a population can become established once landfall is reached.

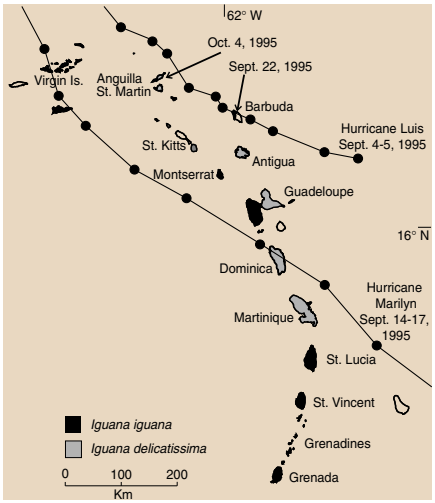

Figure 1 Tracks of hurricane Luis and hurricane Marilyn through the islands of the Lesser Antilles in the Caribbean. Dates with arrows indicate the first sightings of iguanas.

On 4 October 1995, at least 15 individuals of the green iguana, *Iguana iguana*, appeared on the eastern beaches of Anguilla in the Caribbean. This species did not previously occur on the island. They arrived on a mat of logs and uprooted trees, some of which were more than 30 feet long and had large root masses. Local fishermen say the mat was extensive and took two days to pile up on shore. They reported seeing iguanas on both the beach and on logs in the bay.

Dispersal events are often assumed to be caused by large storms, such as hurricanes[9]. The 1995 hurricane season had above normal activity, with 11 hurricanes and 8 tropical storms[10]. On 4 and 5 September 1995, hurricane Luis moved through the eastern Caribbean (Fig 1). The storm was rated category 4 on the Saffir/Simpson Hurricane Scale (SSHS). Hurricane-force winds were reported as far south as Guadeloupe. A week and half later, on 14–17 September, hurricane Marilyn (SSHS category 2) followed a parallel path slightly south of hurricane Luis, and many of the same islands once again experienced hurricane-force winds (Fig. 1). Approximately a month after the first of these hurricanes, iguanas reached the shores of Anguilla.

A survey was established within a month of the invasion. We preserved one individual (CM 145848) and marked seven others. Another iguana escaped before processing, and three more were caught by the fishermen but died shortly afterwards. A further three iguanas were sighted on Scrub Island, 0.5 km northeast of Anguilla, and green iguanas were also reported on the northeast coast of Barbuda, 150 km southeast of Anguilla (D. V. Nicholson, personal communication).

Captured individuals (three males and five females) ranged in snout–vent length from 276 to 400 mm. Iguanas were captured between December 1995 and March 1998. Our most recent sighting of *I. iguana* on Anguilla, on 11 March 1998 (29 months after the invasion), was of a female with enlarged ovarian follicles (possibly oviductal eggs). Because both males and females invaded the island, survived and appear to be healthy, with a female in reproductive condition, the likelihood of reproduction is high.

There are two species of *Iguana* in the Lesser Antilles: *I. iguana* and *I. delicatissima* (Fig. 1). *I. iguana* is widespread, occurring from Mexico through Central America into South America as far south as northern Paraguay, and is also found on many of the southern islands of the Lesser Antilles. *I. delicatissima* occurs on most islands in the Lesser Antilles where *I. iguana* is absent. The track of hurricanes Luis and Marilyn (Fig. 1), the current distribution of *I. iguana* in the Lesser Antilles (Fig 1), and the general west-northwest ocean currents in the region suggest that these iguanas originated on the island of Guadeloupe.

The probability that a species will successfully colonize an island depends on both the probability that it will reach the island and the probability that it will survive once it arrives. Our observations confirm that raft dispersal can occur successfully, and document the over-water dispersal of a group of large vertebrates and their persistence and possible reproduction after landfall.

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