

diamond for comparison, and only one study has dealt with those of the eclogite suite. Ten eclogitic garnets included in Finsch diamonds have moderately anomalous  $\delta^{18}\text{O}$  values ( $\delta^{18}\text{O} = +5.7$  to  $+8.0\%$ ) relative to garnet in peridotite xenoliths ( $\delta^{18}\text{O} = +5.35 \pm 0.18\%$ ), but these data are within the range of published oxygen isotope values for garnet in mantle eclogite xenoliths<sup>22</sup>. That is, the Finsch eclogite-suite inclusions are somewhat anomalous relative to typical mantle values or 'pristine' basalts, but not unusual compared to mantle eclogite xenoliths with moderately elevated oxygen isotope values ( $\delta^{18}\text{O} < +9.2\%$ ). Lowry *et al.*<sup>22</sup> concluded that, although fluids associated with diamond formation in eclogites are significantly  $^{18}\text{O}$  enriched ( $> +7\%$ ), "the diamondiferous xenoliths have not undergone any gross exchange of  $\delta^{18}\text{O}$  since the time of diamond growth and inclusion entrapment".

We have confirmed that eclogite diamond formation is associated with elevated  $\delta^{18}\text{O}$  values. We have also documented that  $\delta^{18}\text{O}$  values do in fact exist in members of the subcratonic eclogite suite that are equivalent to the extreme  $\delta^{18}\text{O}$  values found in low-temperature altered oceanic basalts and their ophiolitic equivalents (Fig. 1). The fact that the coesite oxygen isotope ratios are outside the range of  $\delta^{18}\text{O}$  known from eclogite xenoliths, however, suggests that following diamond formation, 'exposed' minerals have undergone oxygen isotope exchange with the surrounding upper mantle, and only the encapsulated minerals preserve the  $\delta^{18}\text{O}$  of diamond formation.

The prospect that disequilibrium or small, but poorly known, fractionation effects can cause small shifts from expected equilibrium  $\delta^{18}\text{O}$  fractionation in upper-mantle materials continues to leave open the possibility that the moderately anomalous oxygen isotope ratios of diamondiferous (and other) eclogites documented to date ( $+6$  to  $+9\%$ ) might be the result of processes other than subduction and prograde metamorphism of hydrothermally-altered oceanic basalt<sup>19,20</sup>. We consider this possibility to be unlikely, but even if it is correct, such shifts would be small<sup>8,22</sup>. Because of the magnitude of the coesite  $\delta^{18}\text{O}$  anomalies, none of these alternative arguments apply to the extreme oxygen isotope ratios that we have documented, and no other mechanisms have been proposed to account for them. The Guaniamo coesite inclusions in diamond clearly represent the 'missing link' between altered ocean-floor basalts and mantle eclogite xenoliths, and they present compelling evidence for the subducted nature of the protoliths of this diamond eclogite suite. □

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## Migration of a Late Cretaceous fish

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Late Cretaceous sediments from the Western Interior of North America yield exceptionally well preserved fossils<sup>1,2</sup> that serve as proxies for the rapidly changing climate preceding the Cretaceous/Tertiary boundary (about 67–65 Myr ago)<sup>3,4</sup>. Here we reconstruct the ontogenetic history of a Maastrichtian-age fish,

*Vorhisia vulpes*<sup>5</sup>, by using the carbon, oxygen and strontium isotope ratios of four aragonite otoliths collected from the Fox Hills Formation of South Dakota. Individuals of *V. vulpes* spawned in brackish water (about 70–80% seawater) and during their first year migrated to open marine waters of the Western Interior Seaway, where they remained for 3 years before returning to the estuary, presumably to spawn and die. The mean  $\delta^{18}\text{O}$  from the marine growth phase of *V. vulpes* yields a seawater temperature of 18 °C, which is consistent with leaf physiognomy and general-circulation-model temperature estimates for the Western Interior during the latest Maastrichtian<sup>4,6,7</sup>.

Modern fish otoliths have proved to be useful proxies for ambient water conditions<sup>8,9</sup>. Biogeochemists have used stable-isotope time series from otoliths to reconstruct detailed ontogenetic histories<sup>10,11</sup> and to characterize ambient life conditions and depositional environments<sup>12,13</sup>. The isotopic analysis of ancient otoliths permits the detailed characterization of ontogenetic histories when skeletal remains are missing and classification is in question.

We have analysed four Late Cretaceous (Maastrichtian) utricular otoliths (*Vorhisia vulpes*) collected from a tidal-channel lag deposit in the Iron Lightning Member (Colgate Lithofacies) of the Fox Hills Formation of South Dakota (Fig. 1). The Iron Lightning Member was deposited in coastal estuaries that developed behind a series of barrier islands along the margin of an extensive delta platform<sup>14–16</sup> and is overlain there by fluvial deposits of the Hell Creek Formation. These channel deposits represent the distal portion of the Hell Creek fluvial distributary system, where it flowed into and mixed with the Fox Hills Sea. Isotope analyses were conducted on these otoliths to understand the life history of these fish and to characterize the riverine, estuarine and marine environments of the type area of the Fox Hills Formation in the Missouri Valley Region of South and North Dakota during the latest Cretaceous<sup>17</sup>.

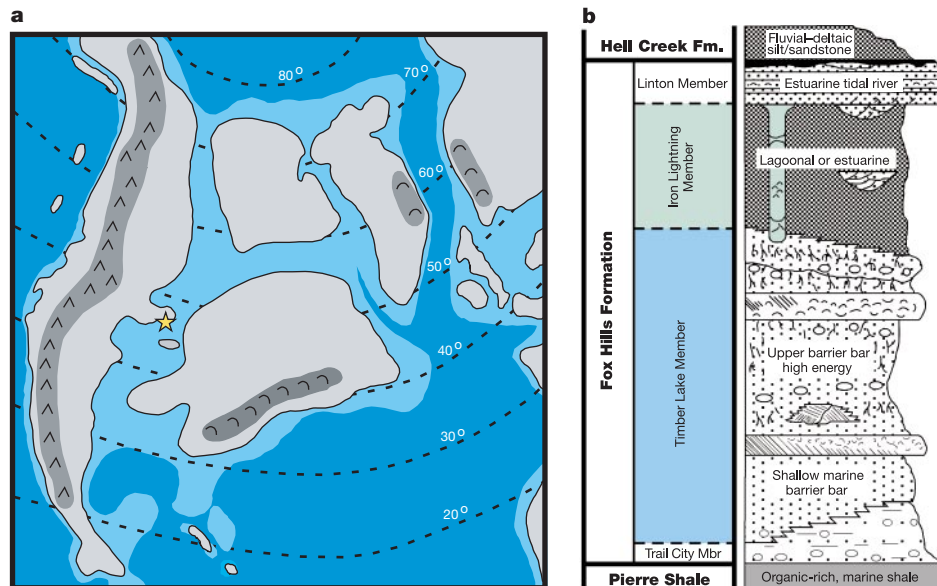
A discussion of the taxonomic assignment of the otoliths is appropriate because they have been left in open nomenclature by recent revisers<sup>18</sup>. These utricular otoliths were originally described from the Fox Hills Formation of South Dakota by Frizzell<sup>5</sup> as *V. vulpes*, and have been cited as such in the regional literature. On the basis of presumably conspecific material from the Severn Formation in Maryland, they have recently been assigned<sup>18</sup> to a form genus 'Ariidarum' without further description or evidentiary discussion. We choose to employ the original appellation<sup>5</sup> for three

reasons: the present work is not intended to influence classificatory decisions regarding these specimens; we are using material from the type area of the species *V. vulpes* and clearly conspecific with it; and the name is well known regionally.

Carpenter *et al.*<sup>1</sup> described the exceptional preservation of molluscan aragonite contained within carbonate cemented concretions from the Timber Lake Member of the Fox Hills Formation and characterized  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of those marine molluscs. Otoliths analysed here, composed of fibrous aragonite, are equally well preserved, having undergone no diagenetic alteration, cementation or dissolution. One specimen (SLU FR478) is abraded, probably by subaqueous transport. Concentric growth bands are visible on all specimens (Fig. 2). Ultraviolet fluorescence petrography reveals an overall high organic matter (otolin?) content within each otolith and a concentration along growth bands (Supplementary Information).

Oxygen isotope time series from four specimens of *V. vulpes* (Fig. 2), collected from the same bedding-plane surface, yield similar patterns of oxygen isotope variation that result from migration during the ontogeny of each fish. Although these specimens do not yield geochemical data from synchronous growth, they represent specimens that probably lived within tens or hundreds of years of each other. We assume that these data are representative of the habitats and ontogenetic history of *V. vulpes*. The rapid growth rates of *V. vulpes* lapilli (1.1–1.25 cm maximum length; 0.4–0.5 g total mass) for a maximum age of 4 years, would place them among the highest of modern teleosts (Supplementary Information).

Aragonite precipitated in the kernel (first ~1.5 mm of growth) of all otoliths analysed has relatively low  $\delta^{18}\text{O}$  values (from –6.2‰ to –3.4‰), typical of growth in an estuarine habitat. Between 2 and 4 mm of growth,  $\delta^{18}\text{O}$  values increase abruptly to a mean of –1.1‰, typical of otolith growth in a marine environment. The marine phase of otolith growth is characterized by a sinusoidal variation in  $\delta^{18}\text{O}$  values about this mean (Fig. 2). We interpret these sinusoidal patterns as seasonal temperature variation in the shallow marine waters of the western margin of the Fox Hills Sea, and conclude that *V. vulpes* lived in open marine water for about 3 years. <sup>87</sup>Sr/<sup>86</sup>Sr ratios of 0.70778 to 0.70779 for the marine portions of *V. vulpes* otoliths are consistent with those of other marine molluscs from the Western Interior Seaway and with an age of between 67 and 65.5 Myr ago (Supplementary Information)<sup>2</sup>.



**Figure 1** Palaeogeography and stratigraphy of the Fox Hills Formation of North and South Dakota. **a**, Palaeogeographic map after ref. 16. The palaeolatitude of this location is 46° N. **b**, Schematic stratigraphic column of the Pierre–Fox Hills–Hell Creek Formations of

North and South Dakota. Modified from ref. 1. *V. vulpes* specimens were collected from the estuarine tidal channel (green) in the Iron Lightning Member. The marine Timber Lake Member is shown in blue.

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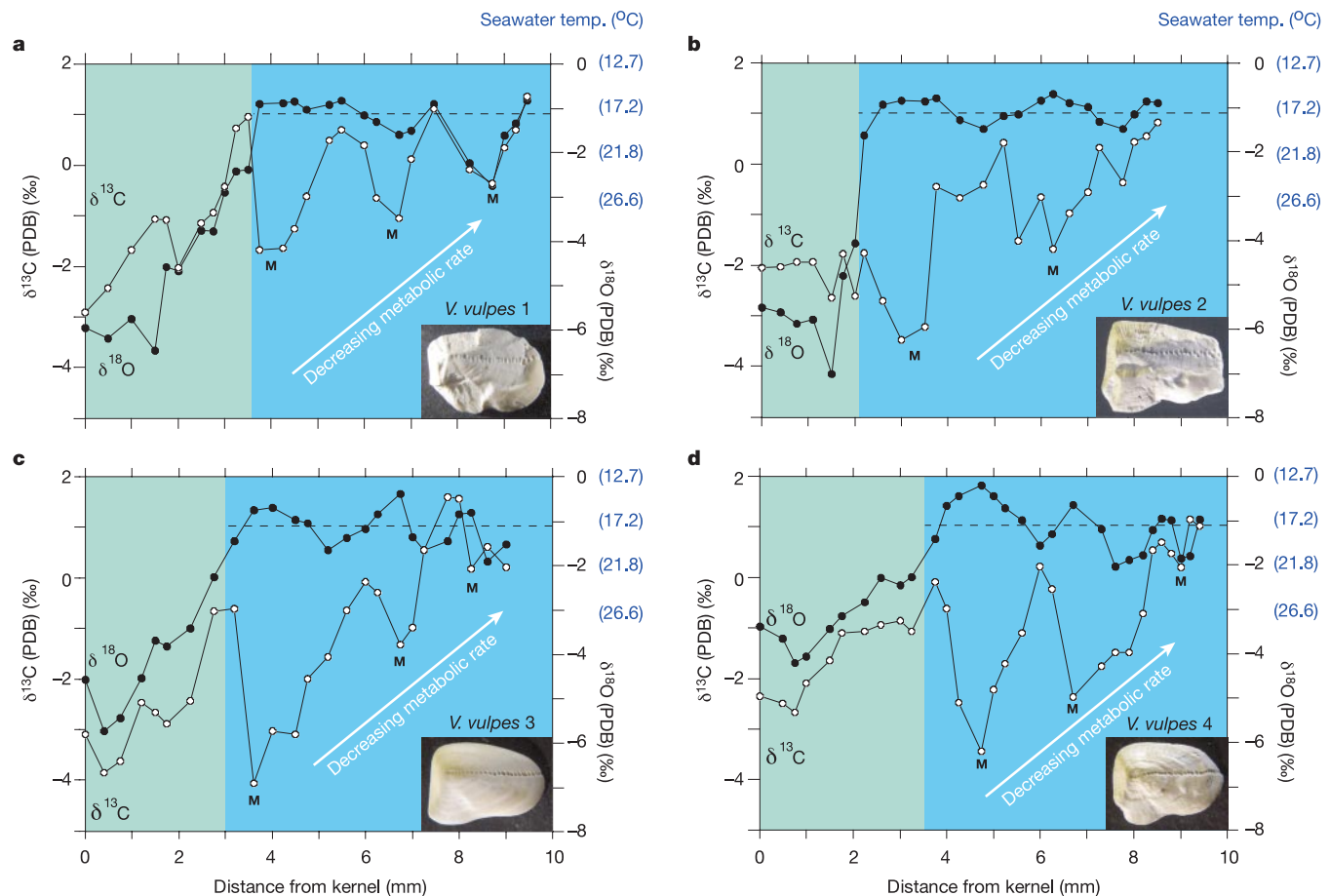
Using the fractionation factor of Patterson *et al.*<sup>8</sup> for modern fish otoliths and a seawater  $\delta^{18}\text{O}$  of  $-1\text{‰}$  (standard mean ocean water; SMOW) (for an ice-free world<sup>19</sup>), the temperature range for all four otoliths would be  $14\text{--}25\text{ °C}$ . An otolith  $\delta^{18}\text{O}$  of  $-1.1\text{‰}$  yields a temperature of  $18\text{ °C}$  (Fig. 2). These temperature estimates are significantly lower than those calculated from the Thorrold *et al.*<sup>9</sup> relation (otolith  $\delta^{18}\text{O}$  of  $-1.1\text{‰}$  yields a temperature of  $22\text{ °C}$  and an annual temperature range of  $18\text{--}30\text{ °C}$ ). As temperatures calculated from the Thorrold *et al.*<sup>9</sup> relation seem high for this palaeo-latitude, we have used the fractionation factor of Patterson *et al.*<sup>8</sup> (Fig. 2). Although high relative to modern mid-latitude temperatures, a seawater temperature of  $18\text{ °C}$  is consistent with temperature estimates for  $46^\circ\text{N}$  during the Late Cretaceous<sup>3</sup>. This temperature is several degrees lower than general circulation model results for this latitude in the mid-Cretaceous period (atmospheric  $\text{CO}_2$  levels fourfold modern levels)<sup>7</sup> but is consistent with leaf physiognomy data projecting mean annual temperatures of  $15\text{--}22\text{ °C}$  for the Western Interior during the late Maastrichtian<sup>4,6</sup>.

The death of each fish occurred without precipitation of a final spring–summer growth increment. We therefore conclude that the return of *V. vulpes* to estuarine waters (presumably to spawn) occurred in the autumn. As these otoliths were discovered in known estuarine beds<sup>14–16</sup> and because isotope ratios of the juvenile phase of otolith growth indicate precipitation in an estuary, we infer that death occurred in their natal waters. Because the return of *V. vulpes* to estuarine waters was not recorded in the  $\delta^{18}\text{O}$  values of

otolith outer margins, we suggest that there might have been no otolith growth during the physiologically demanding (and short-lived) spawning interval.

On the assumption that *V. vulpes* spawned in the fall, the early record of otolith growth occurred during the following spring. The time associated with the increase in otolith  $\delta^{18}\text{O}$  values during the estuarine–marine transition is difficult to determine because the change in the isotopic composition of ambient waters may obscure seasonal temperature variation. However, as transitional  $\delta^{18}\text{O}$  values adjoin the marine sinusoidal pattern during the summer temperature maximum ( $\delta^{18}\text{O}$  value minimum; Fig. 2), the time that the juvenile *V. vulpes* spent in the estuary is less than 1 year. Migration to seawater during the first summer of life is common in modern chinook and sockeye salmon and requires the attainment of a size capable of withstanding salinity changes<sup>20,21</sup>. High rates of otolith growth are required to record this short-lived migration. Growth curves consistent with this interpretation are presented in Supplementary Information.

$\delta^{13}\text{C}$  values from the juvenile phase of otolith growth (estuarine) are low ( $-7\text{‰}$  to  $-4\text{‰}$ ) and the latest phases of otolith growth (marine) are relatively high (more than  $0\text{‰}$ ). The marine growth phase of *V. vulpes* has  $\delta^{13}\text{C}$  values that are comparable with those reported for marine bivalves<sup>1</sup>. This overall increase in otolith  $\delta^{13}\text{C}$  values during ontogeny is consistent with a transition from estuarine to marine conditions, yet marked seasonal variation superimposed on an overall trend towards higher  $\delta^{13}\text{C}$  values



**Figure 2**  $\delta^{18}\text{O}$  (filled circles) and  $\delta^{13}\text{C}$  (open circles) values of *Vorthisia vulpes* otolith specimens 1 (SLU FR476) (a), 2 (SLU FR477) (b), 3 (SLU FR478) (c) and 4 (SLU FR479) (d). Photographs of each specimen are found in the lower right corner of each plot. The maximum lengths of each otolith are: 1, 1.25 cm; 2, 1.10 cm; 3, 1.15 cm; 4, 1.20 cm. The green shaded area represents estuarine conditions; the blue shaded area represents marine conditions. Seawater temperature estimates using the oxygen isotope

fractionation factor of ref. 8 and a seawater  $\delta^{18}\text{O}$  of  $-1\text{‰}$  are next to the highest oxygen isotope axis labels. Temperature estimates apply to the marine  $\delta^{18}\text{O}$  values in the blue shaded areas. The horizontal dashed line represents the mean  $\delta^{18}\text{O}$  and temperature of the marine phase of *V. vulpes* ( $-1.1\text{‰}$ ,  $17.6\text{ °C}$ ).  $\delta^{13}\text{C}$  minima indicated by M are interpreted as having been caused by annual migrations within the basin.

suggests that this environmental change does not fully explain the observed variation. Carbon-isotope time series collected from *V. vulpes* must be interpreted in terms of environment, diet, metabolic activity and migration (Fig. 2).

Dietary changes associated with a change from feeding at lower trophic levels to feeding at higher trophic levels during ontogeny might account for some of the increase in *V. vulpes* otolith  $\delta^{13}\text{C}$  values with increasing age<sup>22</sup>. However, trophic level changes in  $\delta^{13}\text{C}$  values are typically modest (up to 1‰ per trophic level)<sup>22</sup> and would probably not account for all of the ontogenetic variation observed in *V. vulpes* otoliths (Fig. 2). Otoliths of juvenile chinook salmon from hatchery-raised fish have significantly higher  $\delta^{13}\text{C}$  values than those of naturally spawned 'wild' juveniles. This reflects the concomitant differences in  $\delta^{13}\text{C}$  values of hatchery diets between those that include a large marine component and those that include freshwater aquatic insects consumed by wild young (with lower  $\delta^{13}\text{C}$  values) (W. P. Patterson, personal communication).

The overall increase in  $\delta^{13}\text{C}$  values in *V. vulpes* otoliths is the result of decreasing metabolic activity<sup>23–25</sup> and potentially of trophic level changes<sup>23,24</sup> during ontogeny. Atlantic croaker otolith  $\delta^{13}\text{C}$  values have been described<sup>9</sup> that are 3–8‰ lower than ambient dissolved inorganic carbon of seawater. Ontogenetic variation similar to that observed in *V. vulpes* (Fig. 2) has been observed in the freshwater drum<sup>25</sup>. These trends have been interpreted as a diminished contribution of respired  $\text{CO}_2$  to endolymph  $\text{HCO}_3^-$  as a result of decreasing metabolic activity during ontogeny<sup>25</sup>. If we assume that the timing of a maximum otolith  $\delta^{13}\text{C}$  value marks sexual maturation as in Atlantic cod<sup>23</sup>, maturation of *V. vulpes* occurred just before spawning. Although high  $\delta^{13}\text{C}$  values are achieved, *V. vulpes* otoliths do not attain a  $\delta^{13}\text{C}$  plateau as seen in cod and drum otoliths, probably because of the short life span of *V. vulpes* (about 4 years) and the rigors of a final spawning migration soon after sexual maturation.

Superimposed on this ontogenetic trend are periodic marked decreases in  $\delta^{13}\text{C}$  values at 2–5, 5–7 and 7–9 mm (Fig. 2). With the exception of otolith no. 1, which has a poorly defined sinusoidal trend in  $\delta^{18}\text{O}$  during marine growth, these  $\delta^{13}\text{C}$  minima are coincident with annual temperature minima. Such decreases (1–4‰) seem too large to be caused solely by seasonal variation in primary productivity. Seasonal carbon isotope variation in North Atlantic cod otoliths<sup>24</sup> is smaller than that of freshwater drum otoliths<sup>25</sup> (1–2‰ compared with 4–5‰), presumably owing to the greater impact that primary productivity has on the  $\delta^{13}\text{C}$  of ambient dissolved inorganic carbon in freshwater environments.  $\delta^{13}\text{C}$  variation in *V. vulpes* (about 2–4‰; Fig. 2) might, in part, reflect the shallow nature of the Fox Hills Sea and its sensitivity to seasonal changes in productivity.

Periodic  $\delta^{13}\text{C}$  minima in *V. vulpes* are interpreted as episodes of elevated metabolism or catabolism associated with migration or elevated predation stress and an increased contribution of respired  $\text{CO}_2$  to the endolymph calcifying fluid (Fig. 2). Physiological changes associated with migration into seawater (osmoregulatory stress and/or increased metabolic activity)<sup>26</sup> are the likely cause of the first of these  $\delta^{13}\text{C}$ -lowering events during ontogeny (at 2–5 mm; Fig. 2). A correlation between low temperatures and low  $\delta^{13}\text{C}$  values runs counter to the positive correlation between temperature and metabolic activity, which generally lowers otolith  $\delta^{13}\text{C}$  values<sup>9</sup>.

Decreases in otolith  $\delta^{13}\text{C}$  values coincident with temperature minima suggest that *V. vulpes* migrated seasonally within the marine basin, perhaps because of changes in surface-water productivity and the diminished availability of food during the winter months (Fig. 2). Modern chinook salmon achieve migration speeds approaching  $80 \text{ km d}^{-1}$  (ref. 21). Maintenance of such migration speeds for an extended period would probably require a significant loss of body mass through catabolism. Decreases in otolith  $\delta^{13}\text{C}$  values also would be expected during periods of gametogenesis and spawning<sup>27</sup>. However, given that *V. vulpes* otoliths occur in

decidedly estuarine sediments, that they are of uniform size and weight and that their early life history is estuarine, it is likely that periodic  $\delta^{13}\text{C}$  minima do not represent spawning migrations.

Concentrations of *V. vulpes* otoliths of a similar age class in estuarine deposits suggest that *V. vulpes* migrated back to their natal waters to spawn and then die. If spawning had occurred at a variety of ages, a range of otolith sizes would probably be found. *V. vulpes* otoliths collected from the Fox Hills Formation<sup>5,14</sup> and the Severn Formation of Maryland<sup>28</sup> have a similar size distribution, suggesting that this behaviour is typical for this fish.

$\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of marine, estuarine and freshwater biogenic carbonates from the Fox Hills and Hell Creek Formations produce a linear mixing trend interpreted as the mixing of Fox Hills seawater and Hell Creek river water over a range of about 20‰ for  $\delta^{18}\text{O}$  values and about 7‰ for  $\delta^{13}\text{C}$  values (Supplementary Information)<sup>17</sup>. With the use of a two-component mixing model with a seawater  $\delta^{18}\text{O}$  of  $-1\text{‰}$  (SMOW)<sup>19</sup> (marine molluscs and marine phases of *V. vulpes*) and a river-water  $\delta^{18}\text{O}$  of  $-20\text{‰}$  (SMOW)<sup>29</sup> (unionids and *Corbicula*), the lowest  $\delta^{18}\text{O}$  values of the juvenile growth phases of the *V. vulpes* ( $-7.0\text{‰}$ ,  $-6.5\text{‰}$ ,  $-5.7\text{‰}$  and  $-4.2\text{‰}$  (PDB)) indicate that these fish initially grew in waters composed of 68–83% seawater.

The remarkable preservation of *V. vulpes* otoliths provides a detailed record of both the ontogeny of this fish and the palaeo-environmental conditions of its habitat. Extracting such information from Mesozoic-age otoliths might provide important information for phylogenetic assignments of this and other enigmatic taxa. Our data indicate that *V. vulpes* did not live in fresh water as previously suggested<sup>5,14</sup>, but instead preferred shallow marine waters for much of its adult life and spawned in brackish waters. There is no evidence to suggest that *V. vulpes* lived for an extended period in the isotopically distinct freshwaters of the Hell Creek delta platform.

The absence of Late Maastrichtian ammonite biostratigraphic indices in upper Fox Hills–Hell Creek estuarine-fluvial beds enhances the value of isotope data of contained fossils for palaeoenvironmental and chemostratigraphic uses. An improved understanding of surface-water isotope ratios and temperatures (both terrestrial and marine) provides constraints for climate modellers and vertebrate palaeontologists reconstructing habitats of the Western Interior at the close of the Cretaceous. □

## Methods

*V. vulpes* specimens 1–4 (SLU FR476–479 respectively), *Euspira subscassa* (SLU FR480) and *Corbicula* sp. (SLU FR481, FR482) are part of the St Lawrence University Faculty Research Collection collected in the 1960s from the Colgate Lithofacies, Fox Hills Formation, Iron Lightning Badlands (type locality of the Iron Lightning Member)<sup>15</sup>, Redelm NE Quadrangle, Ziebach County, South Dakota. Additional stratigraphic information is provided in the Supplementary Information.

Microsamples of aragonite ( $\sim 0.02\text{--}0.05 \text{ mg}$ ) were milled from the surface of the inner faces of otoliths with a Brasseler UP 200 controller and a UG 12 handpiece fitted with a 0.3-mm tungsten carbide dental bur with observation under a Nikon SMZU microscope. Samples were collected along transects perpendicular to visible growth bands from the posterior to the anterior of each otolith. Samples were analysed on a Finnigan MAT 252 Isotope Ratio Mass Spectrometer with a Kiel III automated carbonate device housed in the Paul H. Nelson Stable Isotope Laboratory at the University of Iowa. Carbonates were reacted with two drops of anhydrous phosphoric acid at  $75^\circ\text{C}$ . Daily analyses of National Institute of Standards and Technology powdered carbonate standards (NBS-18, NBS-19 and NBS-20) and several in-house standards were conducted. Analytical precision on these standards was better than  $\pm 0.1\text{‰}$  for both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ . All results are reported in per ml (‰) relative to V-PDB.

Microsamples of aragonite were dissolved in ultrapure 3 M  $\text{HNO}_3$  and loaded onto chromatography columns containing Sr-specific resin (EiChromM Sr Resin SPS); Sr was recovered in the aqueous eluate. Dried extracts were dissolved in  $3 \mu\text{l}$  of ultrapure dilute  $\text{HNO}_3$  and loaded onto rhenium filaments (with  $\text{Ta}_2\text{O}_5$  in dilute  $\text{H}_3\text{PO}_4$ ). Isotopic ratios were measured on a Finnigan MAT 261 thermal ionization mass spectrometer in the Radiogenic Isotope Laboratory of the University of Texas at Dallas. Each analysis typically involved 160 static multi-collection cycles of all four Sr isotopes in addition to  $^{85}\text{Rb}$ , for monitoring  $^{87}\text{Rb}$ . Samples were analysed over a 4-week period during which replicate measurements of standards were conducted before and after sample analyses. Standard analyses produced the following statistics: NBS-987 ( $n = 55$ ), mean ( $2\sigma_m$ ) =  $0.710242(12)$ , s.d. =  $0.000016$ ; EN-1 ( $n = 10$ ), mean ( $2\sigma_m$ ) =  $0.709180(11)$ ,

s.d. = 0.000014; E&A ( $n = 8$ ), mean ( $2\sigma_m$ ) = 0.708008(13), s.d. = 0.000020. A polynomial least-squares fit to NBS-987 variance during the analysis period was used to drift-compensate measured ratios relative to a baseline of 0.710242. Compensations for these samples ranged from -0.000015 to 0.000009.

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## Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*

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Resistance genes (R-genes) act as an immune system in plants by recognizing pathogens and inducing defensive pathways. Many R-gene loci are present in plant genomes, presumably reflecting the need to maintain a large repertoire of resistance alleles. These loci also often segregate for resistance and susceptibility alleles that natural selection has maintained as polymorphisms within a species for millions of years<sup>1–5</sup>. Given the obvious advantage to an individual of being disease resistant, what prevents these resistance alleles from being driven to fixation by natural selection? A cost of resistance<sup>6</sup> is one potential explanation; most models require a lower fitness of resistant individuals in the absence of pathogens for long-term persistence of susceptibility alleles<sup>7</sup>. Here we test for the presence of a cost of resistance at the *RPM1* locus of *Arabidopsis thaliana*. Results of a field experiment comparing the fitness of isogenic strains that differ in the presence or absence of *RPM1* and its natural promoter reveal a large cost of *RPM1*, providing the first evidence that costs contribute to the maintenance of an ancient R-gene polymorphism.

*RPM1* codes for a peripheral plasma membrane protein<sup>8</sup> that confers the ability to recognize *Pseudomonas syringae* pathogens carrying *AvrRpm1* or *AvrB*<sup>9,10</sup>. Susceptible individuals lack the entire coding region of *RPM1* (ref. 10), so there is a single susceptible allele at this locus. Both resistance and susceptibility alleles frequently occur together within natural populations and are common across the range of *A. thaliana*<sup>3</sup>. Molecular evolutionary analysis of the non-coding DNA flanking *RPM1* indicates that the resistance and susceptibility alleles have coexisted for more than nine million years<sup>3</sup>. The great age of this polymorphism indicates that the susceptibility allele might be maintained by natural selection due to a cost of resistance, because other mechanisms of coexistence can be eliminated. First, heterozygote advantage can be excluded by *A. thaliana*'s high selfing rate<sup>11</sup>. Second, there is no evidence of geographic differentiation between allelic classes<sup>3</sup>. Last, because the susceptibility allele is deleted for the entire locus<sup>10</sup>, it cannot have differential adaptive values in alternative environments.

To test for a fitness cost of *RPM1* expression, we inserted a 3.84-kilobase (kb) region from Columbia containing *RPM1*, its promoter and its full terminator<sup>10</sup> into a susceptible ecotype, Bla-2, to create four independent transgenic lines. Transgenic plants containing this fragment have been shown to respond to infection with pathogenic *P. syringae* DC3000:AvrRpm1 by eliciting the appropriate hypersensitive response (D. Boyes and J. L. Dangl, personal communication). The *RPM1* gene fragment was placed between two *lox* sites<sup>12</sup>. To induce recombinational excision of the *RPM1* transgene, we crossed each replicate line with a Bla-2 line expressing *cre* recombinase and created selfed lines that had lost the *RPM1* transgene. This allowed us to generate independent pairs of homozygous lines with identical genetic backgrounds differing only with respect to the presence or absence of the *RPM1* gene. Individuals within each pair shared a common insertion site for the introduced DNA, but resistant individuals (*RPM1*<sup>+</sup>) expressed *RPM1* and the selectable marker, kanamycin, whereas susceptible individuals (*RPM1*<sup>-</sup>) expressed only kanamycin. The creation of paired lines effectively controls for fitness changes caused by the