

Unexpected resilience of species with temperature-dependent sex determination at the Cretaceous–Palaeogene boundary

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It has been suggested that climate change at the Cretaceous–Palaeogene (K–Pg) boundary, initiated by a bolide impact or volcanic eruptions, caused species with temperature-dependent sex determination (TSD), including dinosaurs, to go extinct because of a skewed sex ratio towards all males. To test this hypothesis, the sex-determining mechanisms (SDMs) of Cretaceous tetrapods of the Hell Creek Formation (Montana, USA) were inferred using parsimony optimizations of SDMs on a tree, including Hell Creek species and their extant relatives. Although the SDMs of non-avian dinosaurs could not be inferred, we were able to determine the SDMs of 62 species; 46 had genotypic sex determination (GSD) and 16 had TSD. The TSD hypothesis for extinctions performed poorly, predicting between 32 and 34 per cent of survivals and extinctions. Most surprisingly, of the 16 species with TSD, 14 of them survived into the Early Palaeocene. In contrast, 61 per cent of species with GSD went extinct. Possible explanations include minimal climate change at the K–Pg, or if climate change did occur, TSD species that survived had egg-laying behaviour that prevented the skewing of sex ratios, or had a sex ratio skewed towards female rather than male preponderance. Application of molecular clocks may allow the SDMs of non-avian dinosaurs to be inferred, which would be an important test of the pattern discovered here.

Keywords: Cretaceous; temperature-dependent sex determination; extinction

1. INTRODUCTION

Geological evidence indicates that a large bolide hit the Yucatan Peninsula at the Cretaceous–Palaeogene (K–Pg) boundary (*ca* 65.5 Ma) [1,2], whereas other evidence indicates that massive volcanic eruptions on the

Indian plate peaked slightly before the K–Pg boundary [3]. It has been widely suggested that one or both events were responsible for the mass extinction at the K–Pg boundary and that each had profound effects on global climate. The impact vapourized large quantities of evaporite minerals, and the resulting sulphate aerosols probably seeded clouds that reflected solar radiation [4]. The volcanic eruptions, which formed the Deccan Traps of India, released large quantities of CO₂ into the atmosphere and may have initiated global warming [5]. Those species that had temperature-dependent sex determination (TSD) are expected to have been negatively impacted by these climate changes [6,7].

In TSD, the sex of the embryo is determined by the incubation temperature of the eggs. Incubation at the pivotal temperature(s) yields a 1 : 1 sex ratio, small temperature deviations yield an unbalanced sex ratio and larger deviations yield single-sex clutches [8]. In genotypic sex determination (GSD), a sex-determining gene activates a downstream cascade of other genes that are responsible for testis or ovarian development. The specific chromosomes and genes in GSD have evolved independently in numerous lineages, suggesting that it has adaptive benefits [7]. Therian mammals employ an XX–XY, male heterogametic system that evolved from autosomes when the *SRY* (testis-determining) gene emerged on what became the Y chromosome. Exactly when this transformation occurred depends on homologies of the complicated monotreme XX–XY system [9]. In contrast, birds employ a female heterogametic (ZZ–ZW) system that according to recent experimental evidence [10] leads to testicular development with a double dosage of the Z-linked gene *DMRT1*.

It has been suggested that non-avian dinosaurs had TSD, that temperature change at the K–Pg boundary led to male-dominated populations of non-avian dinosaurs via TSD and that this imbalance led to their extinction [6,7]. Although elements of this hypothesis have been criticized (i.e. [11]), there has been no systematic attempt to test whether Cretaceous species with TSD were more prone to extinction than their GSD cohorts. Thus, in the present study, we are determining whether Cretaceous species of tetrapods with TSD experienced substantially more extinctions than those with GSD at the K–Pg boundary. Ideally, several regions would be examined, but unfortunately only the Cretaceous Hell Creek Formation and Early Palaeocene Tullock Formation display near-continuous non-marine sedimentation across the K–Pg boundary, have an unambiguous K–Pg boundary and have rich and intensively studied fossil faunas above and below the boundary [12].

2. MATERIAL AND METHODS

Survivorship at the K–Pg boundary was based on table 5.1 of Archibald [12], which was compiled from the Hell Creek and Tullock formations of Garfield and McCone Counties, Montana, USA. Minor modifications were made to this table to incorporate studies published since 1996 (see the electronic supplementary material). Explanatory power of the TSD hypothesis is determined by counting the number of taxa whose extinction or survival is correctly predicted. Taxa that have TSD are predicted to have gone extinct, whereas taxa that have GSD are predicted to have survived. The statistical significance of differences in extinction frequencies between species with TSD and those with GSD was determined using Fisher's exact test of independence [13] in the computer application SPSS.

To infer the SDMs of Hell Creek tetrapods, three character states for SDM (XX/XY, ZZ/ZW and TSD) were mapped onto a

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phylogeny including extant taxa and Hell Creek species in MAC-CLADE v. 4.08 [14]. Most parsimonious reconstructions were considered for each internal branch, and SDMs were treated as an unordered, multistate character. In the absence of total evidence, comprehensive phylogenies that include Hell Creek and extant taxa, SDMs were reconstructed on a well-supported molecular tree of tetrapods, with fossil taxa placed next to their closest extant relatives, as determined by morphological studies (see the electronic supplementary material). For extant taxa, we used modified versions of the SDM dataset and phylogeny employed by Organ & Janes [15] (see the electronic supplementary material).

3. RESULTS

The TSD hypothesis was originally developed to explain the extinction of non-avian dinosaurs, but unfortunately the SDM of non-avian dinosaurs remains speculative because birds, their closest extant relatives [16], have GSD whereas crocodylians, their next closest relatives [17], have TSD [6]. The GSD of extant birds evolved somewhere on the branch leading from their common ancestor with crocodylians, so that it is equally parsimonious to infer that all non-avian dinosaurs and basal avialans like *Anchiornis* and *Archaeopteryx* had TSD or that all dinosaurs, including extinct birds, had GSD (figure 1).

For the 68 non-dinosaur tetrapods of the Hell Creek Formation, SDMs can be inferred in 62 of them (figure 1). GSD is inferred to have occurred in 46 of these species, including all mammals, amphibians and lizards; and TSD is inferred to have occurred in 16 of these species, 11 turtles and all crocodylians. The TSD hypothesis is a very poor predictor of extinction (table 1); of the 32 extinctions and 30 survivals of the K–Pg event in eastern Montana, only 32.3 per cent are explained. If rare taxa are ignored, which may mitigate pseudoextinctions, the performance of the TSD hypothesis is still dismal; only 34 per cent of 16 extinctions and 25 survivals are explained. Our results are also robust to alternate tree topologies and to the inclusion of less-reliable SDM data (see the electronic supplementary material).

Even more remarkable is that of the 16 taxa inferred to have had TSD (table 1), only two of them (i.e. *Basilemys sinuosa* and *Brachychampsa montana*) went extinct. In contrast, species with GSD suffered many more extinctions; 28 out of 46 species went extinct (61%). Metatherians (11 species) were entirely wiped out as were all but one undetermined species of eutherian (one species of *Cimolestes* is tentatively assumed to have evolved into *Cimolestes simpsoni*). Multituberculates did somewhat better, with five out of nine species surviving. Only three out of 10 squamates, all of which are inferred to have had GSD, survived into the Palaeocene. The differences in rates of survival of taxa with TSD as compared with those with GSD are statistically significant ($p = 0.001$).

4. DISCUSSION

Although we do not have a compelling explanation for why taxa with TSD fared better than those with GSD at the K–Pg boundary, the apparent immunity of species with TSD is easier to explain. One possibility is that the abiotic event that precipitated the K–Pg mass extinction did not change global temperatures. Oxygen isotopes in foraminifera and tree leaf-margin

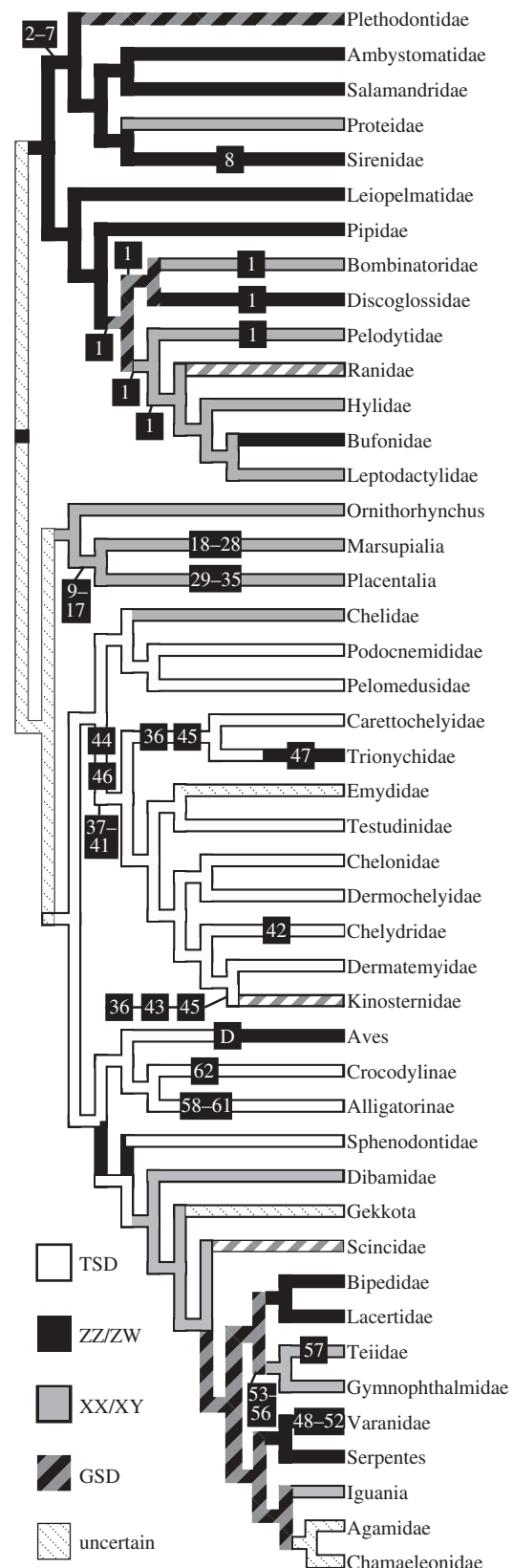


Figure 1. Most parsimonious optimization of sex-determining mechanisms on a phylogeny of extant tetrapods. Numbers indicate where extinct taxa of the Hell Creek Formation attach to this tree (see the first column of table 1). D, Dinosauria. Hatched branches indicate polymorphism (terminal branches) or ambiguity (internal branches). See the electronic supplementary material for studies that support this topology, the placement of extinct taxa, and for taxa whose SDM could not be reconstructed.

Table 1. List of species from the Hell Creek Formation of Garfield and McCone Counties Montana, USA, their survivorship across the K–Pg boundary (mainly from [12]) and whether individual species fates are predicted by the TSD hypothesis.

species	no. in figure 1	classification	SDM	K/Pg extinction	TSD hypothesis
<i>Scotiophryne pustulosa</i>	1	Lissamphibia	XY or ZW	survived	predicted
<i>Opisthotriton kayi</i>	2	Lissamphibia	ZW	survived	predicted
<i>Prodesmodon copei</i>	3	Lissamphibia	ZW	survived	predicted
<i>Albanerpeton nexuosus</i>	4	Lissamphibia	ZW	survived	predicted
<i>Lisserpeton bairdi</i>	5	Lissamphibia	ZW	survived	predicted
cf. <i>Piceoerpeton</i> sp.	6	Lissamphibia	ZW	survived	predicted
<i>Scapherpeton tectum</i>	7	Lissamphibia	ZW	survived	predicted
<i>Habrosaurus dilatus</i>	8	Lissamphibia	ZW	survived	predicted
<i>Cimolodon nitidus</i>	9	Mammalia: Multituberculata	XY	survived	predicted
<i>Cimolomys gracilus</i>	10	Mammalia: Multituberculata	XY	extinct	unpredicted
<i>Meniscoessus robustus</i>	11	Mammalia: Multituberculata	XY	extinct	unpredicted
<i>Cimexomys minor</i>	12	Mammalia: Multituberculata	XY	survived	predicted
<i>Essonodon browni</i>	13	Mammalia: Multituberculata	XY	extinct	unpredicted
<i>Paracimexomys priscus</i>	14	Mammalia: Multituberculata	XY	extinct	unpredicted
<i>Mesodma formosa</i>	15	Mammalia: Multituberculata	XY	survived	predicted
<i>Mesodma hensleighi</i>	16	Mammalia: Multituberculata	XY	survived	predicted
<i>Mesodma thompsoni</i>	17	Mammalia: Multituberculata	XY	survived	predicted
<i>Didelphodon vorax</i>	18	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Glasbius twitchelli</i>	19	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Pediomys cooki</i>	20	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Pediomys elegans</i>	21	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Pediomys florencae</i>	22	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Pediomys hatchery</i>	23	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Pediomys krejci</i>	24	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Alphadon marshi</i>	25	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Alphadon wilsoni</i>	26	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Protalphadon lulli</i>	27	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Turgidodon rhaister</i>	28	Mammalia: Metatheria	XY	extinct	unpredicted
<i>Gypsonictops hypoconus</i>	29	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Gypsonictops illuminatus</i>	30	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Batodon tenuis</i>	31	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Cimolestes cerberoides</i>	32	Mammalia: Eutheria	XY	survived	predicted
<i>Cimolestes incisus</i>	33	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Cimolestes propalaeoryctes</i>	34	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Cimolestes stirtoni</i>	35	Mammalia: Eutheria	XY	extinct	unpredicted
<i>Adocus</i> sp.	36	Reptilia: Testudines	TSD	survived	unpredicted
<i>Eubaena cephalica</i>	37	Reptilia: Testudines	TSD	survived	unpredicted
<i>Neurankylus</i> cf. <i>eximus</i>	38	Reptilia: Testudines	TSD	survived	unpredicted
<i>Peckemys brinkman</i>	39	Reptilia: Testudines	TSD	survived	unpredicted
<i>Stygiochelys estesi</i>	40	Reptilia: Testudines	TSD	survived	unpredicted
<i>Palatobaena cohen</i>	41	Reptilia: Testudines	TSD	survived	unpredicted
<i>Protochelydra</i> sp.	42	Reptilia: Testudines	TSD	survived	unpredicted
<i>Emarginochelys cretacea</i>	43	Reptilia: Testudines	TSD	survived	unpredicted
' <i>Clemmys</i> ' <i>backmani</i>	44	Reptilia: Testudines	TSD	survived	unpredicted
<i>Basilemys sinuosa</i>	45	Reptilia: Testudines	TSD	extinct	predicted
<i>Compsemys victa</i>	46	Reptilia: Testudines	TSD	survived	unpredicted
<i>Apalone</i> sp.	47	Reptilia: Testudines	ZW	survived	predicted
<i>Odaxosaurus piger</i>	48	Reptilia: Squamata	ZW	survived	predicted
<i>Paraderma bogerti</i>	49	Reptilia: Squamata	ZW	extinct	unpredicted
<i>Parasaniwa wyomingensis</i>	50	Reptilia: Squamata	ZW	extinct	unpredicted
<i>Palaeosaniwa canadensis</i>	51	Reptilia: Squamata	ZW	extinct	unpredicted
<i>Exostinus lancensis</i>	52	Reptilia: Squamata	ZW	survived	predicted
<i>Contogenys sloani</i>	53	Reptilia: Squamata	XY or ZW	survived	predicted
<i>Chamops segnis</i>	54	Reptilia: Squamata	XY or ZW	extinct	unpredicted
<i>Haptochamops placodon</i>	55	Reptilia: Squamata	XY or ZW	extinct	unpredicted
<i>Leptochoamops denticulatus</i>	56	Reptilia: Squamata	XY or ZW	extinct	unpredicted
<i>Peneteius aquilonius</i>	57	Reptilia: Squamata	XY	extinct	unpredicted
<i>Brachychampsia montana</i>	58	Reptilia: Crocodylia	TSD	extinct	predicted
undescribed alligatoroid A	59	Reptilia: Crocodylia	TSD	survived	unpredicted
undescribed alligatoroid B	60	Reptilia: Crocodylia	TSD	survived	unpredicted
<i>Leidyosuchus sternbergi</i>	61	Reptilia: Crocodylia	TSD	survived	unpredicted
<i>Thoracosaurus neocessariensis</i>	62	Reptilia: Crocodylia	TSD	survived	unpredicted

analyses [18] do not support an abrupt change in temperatures at the K–Pg boundary. Instead, they support a cooling that began 100 ka before and then persisted through the K–Pg boundary. That said, a cold snap, such as one induced by a bolide impact, may have been too brief [1] to be recorded in the geological record. Second, it is possible that TSD species can effectively respond to temperature changes by shifting pivotal temperatures [19], altering their choice of nest sites [20] or by rapid habitat tracking [21]. If any of the above suggestions are true, we would expect that TSD and GSD species would have similar extinction rates, but then what can explain the relatively low percentage of extinctions in TSD taxa? We speculate that traits that co-occur with TSD in turtles and crocodylians, such as ectothermy, may have allowed a higher percentage of TSD taxa to survive the K–Pg extinction.

Two aspects of the apparent survivorship of TSD species during the K–Pg mass extinction warrant further investigation. If molecular clocks can be applied to the sex chromosomes of birds, then it may be possible to estimate when GSD evolved on the dinosaur branch leading to birds and which Hell Creek dinosaurs had GSD. Second, it would be important to test survivorship of TSD species at other intervals of Earth history where climate change occurred. Turtles and crocodylians, which primarily have TSD, also fared well during the Palaeocene–Eocene thermal maximum [22,23]. These same groups experienced drops in diversity during Late Eocene cooling, although the drop in crocodylians maybe a sampling artefact [23] and turtle extinctions were concentrated among aquatic taxa, suggesting that aridification, not cooling, was to blame [24]. Recently, there has been concern about the future of TSD species if global temperatures continue to rise (e.g. [25]). Our findings offer the hope that in a world facing human-induced climate change, species with TSD may still find a way to survive.

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