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Supplementary Information for

Developmental influence on evolutionary rates and the origin of placental mammal tooth complexity

Aidan M. C. Couzens, Karen E. Sears, and Martin Rücklin

Corresponding Author: Aidan M. C. Couzens
Email: acouzens@ucla.edu

This PDF file includes:

Figures S1 to S11
Tables S1 to S4

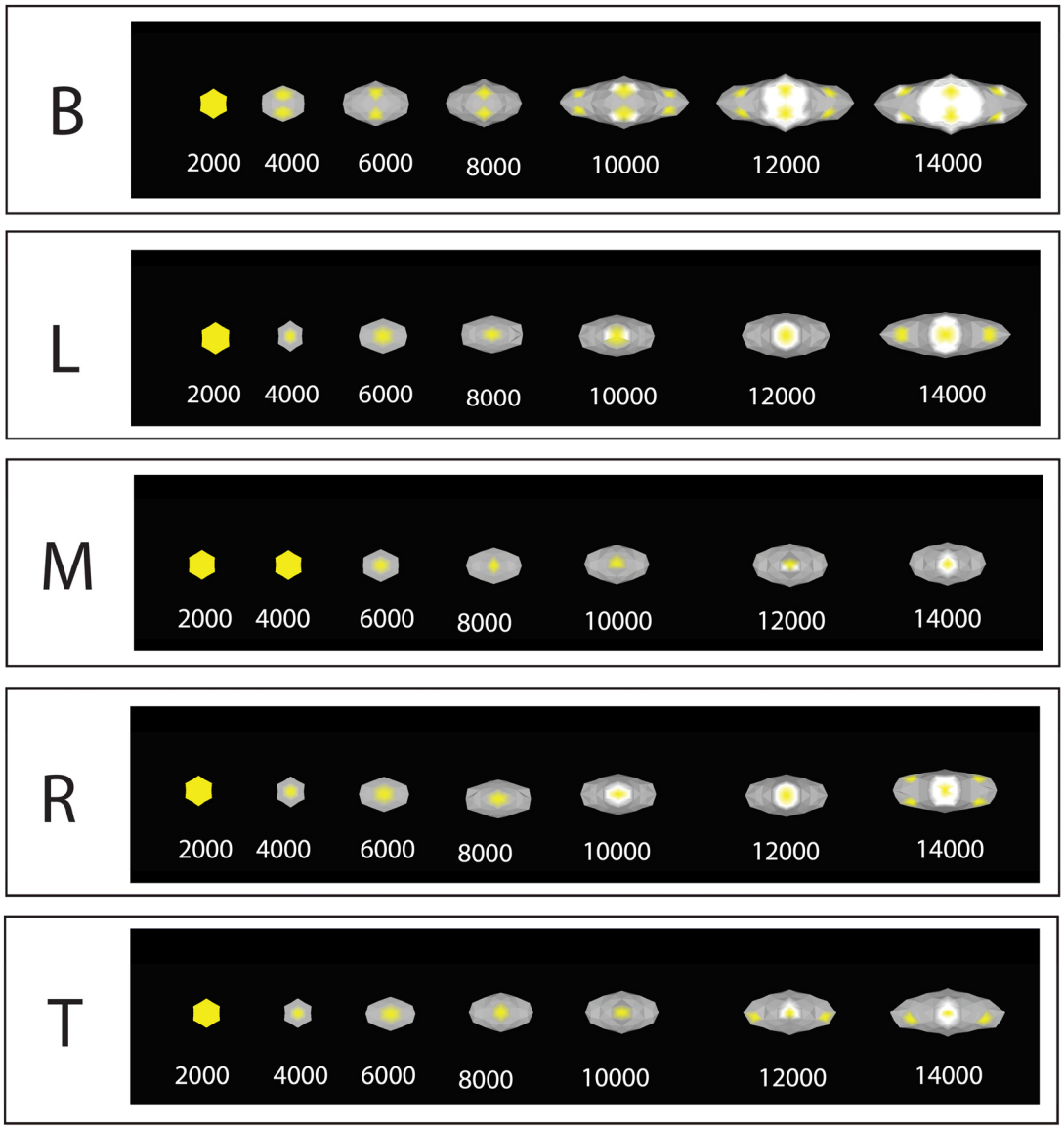

Figure S1. Occlusal view of enamel knot patterning histories. Enamel knots are indicated by yellow zones of epithelial differentiation. Numbers are model iterations (range 0–14000), a proxy for in silico developmental time. Knot pattern abbreviations: 'B', buccolingual (lateral); 'L', longitudinal; 'M', monocuspid; 'R', radial; 'T', triangular.

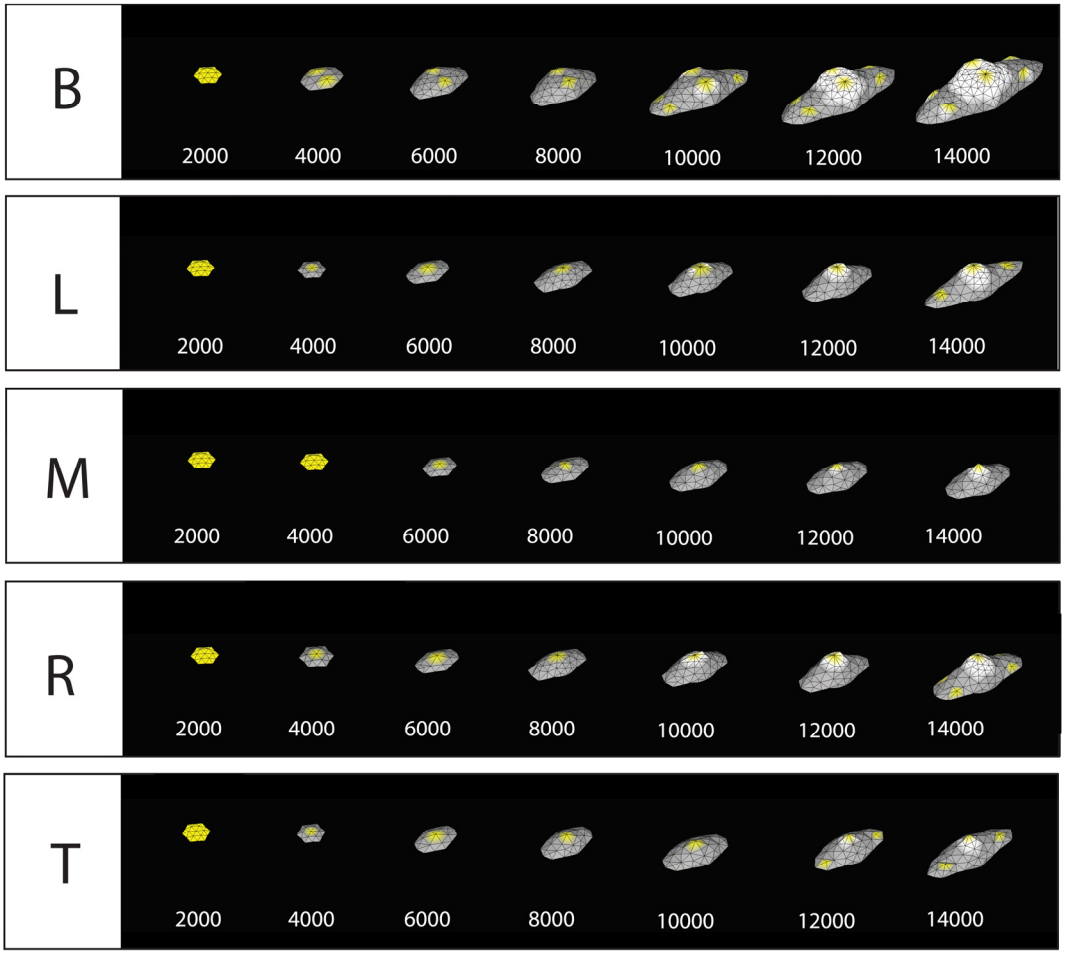

Figure S2. Oblique view of enamel knot patterning histories. Enamel knots are indicated by yellow zones of epithelial differentiation. Numbers are model iterations (range 0–14000), a proxy for developmental time. Knot pattern abbreviations: ‘B’, buccolingual (lateral); ‘L’, longitudinal; ‘M’, monocuspid; ‘R’, radial; ‘T’, triangular.

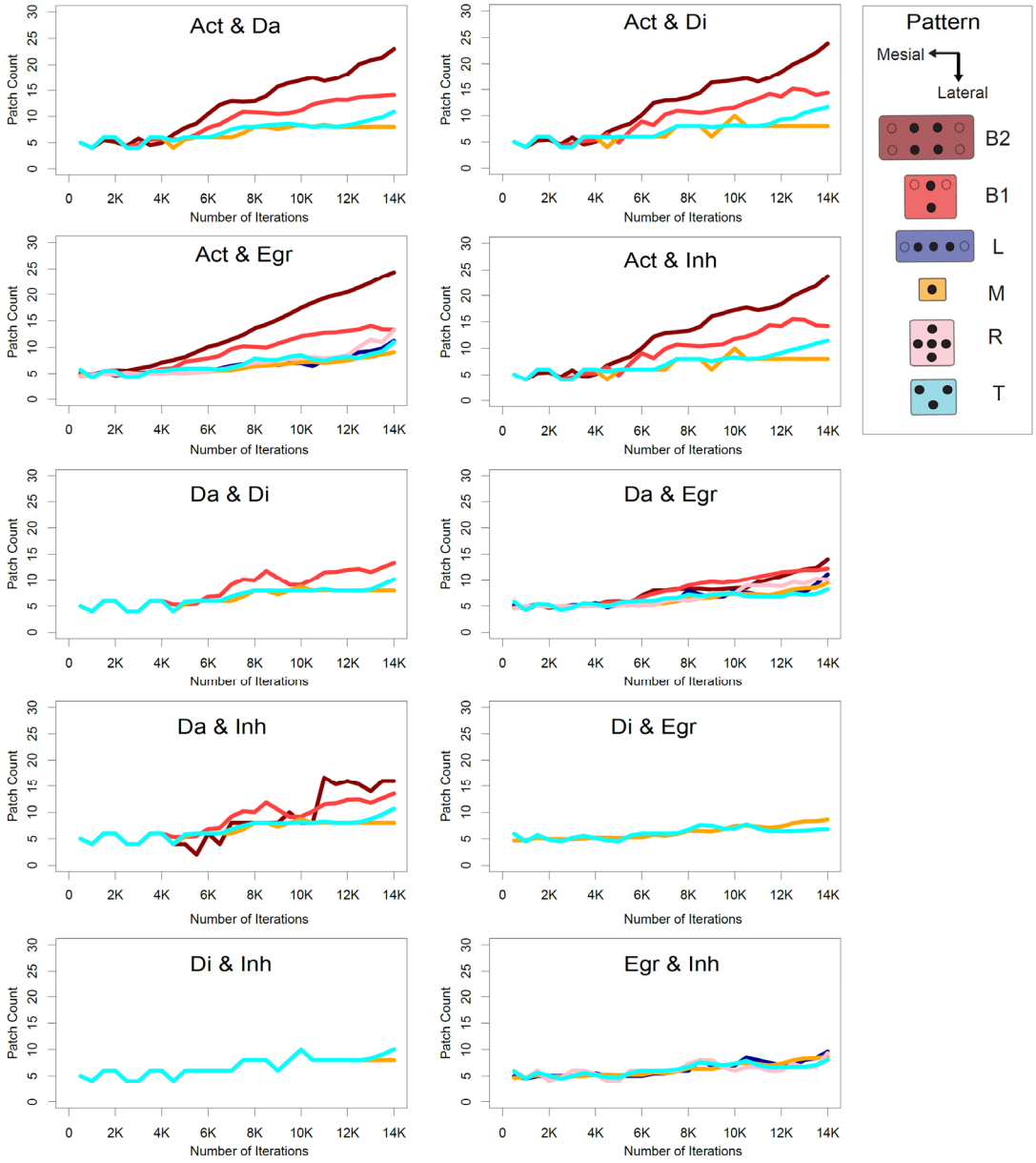

Figure S3. Developmental trajectories of tooth complexity for different enamel knot patterns. Tooth complexity measured with orientation patch count. Parameter abbreviations: Activator self-regulation (Act), activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and strength of inhibition (Inh).

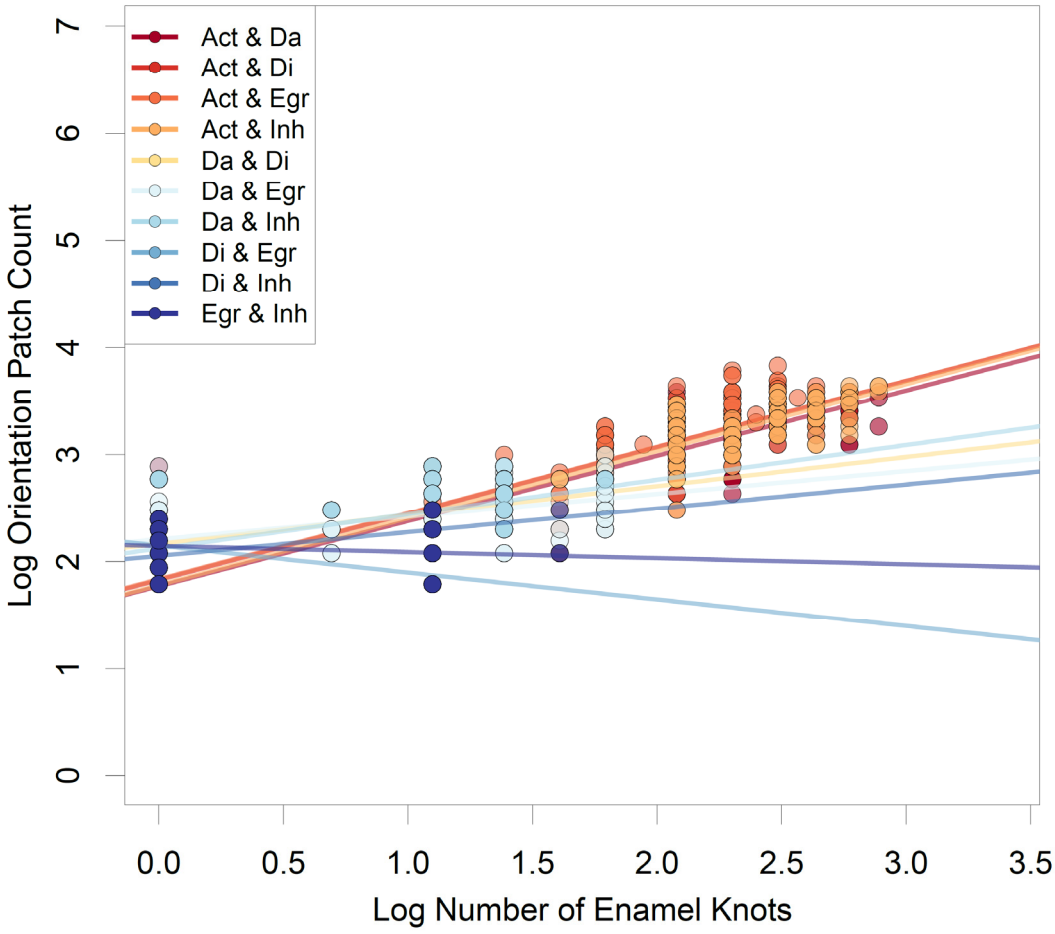

Figure S4. Log–Log major axis regressions of enamel knot number and orientation patch count for simulated tooth shapes (r score=0.69). Colors denote different developmental landscapes. Parameter abbreviations: Activator self-regulation (Act), activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and strength of inhibition (Inh).

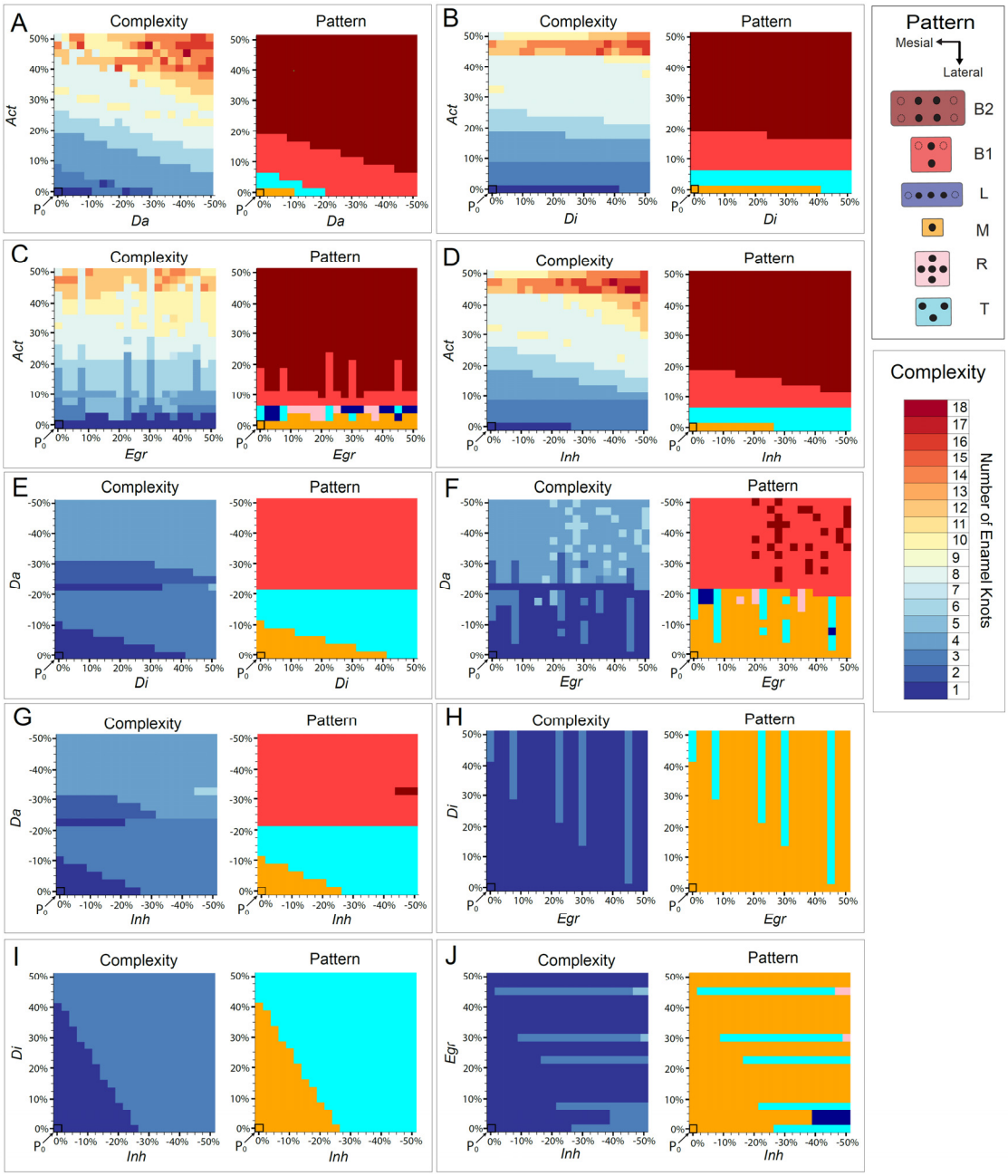

Figure S5. Heatmap representations of enamel knot number and pattern variation across different developmental landscapes. The starting parameter state (P_0) is indicated in the bottom left corner. Axis values denote percentage changes (±) in the parameter values relative to the ancestral state. Axis tick-marks denote the center of grid cells. Parameter abbreviations as follows: Activator self-regulation (Act), Activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and strength of inhibition (Inh).

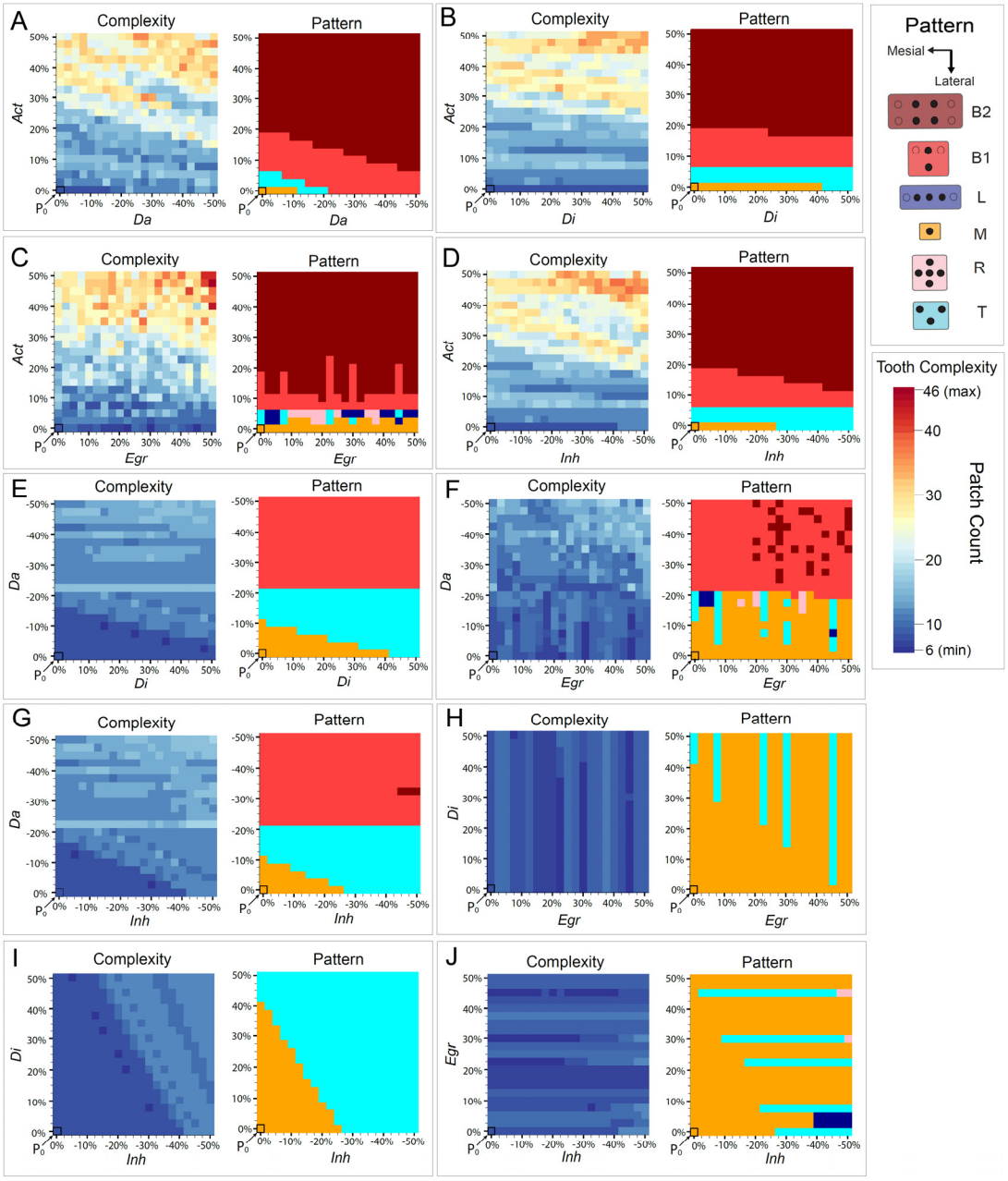

Figure S6. Heatmap representation of tooth surface complexity (orientation patch count) and knot pattern across different developmental landscapes. The starting parameter state (P_0) is indicated in bottom left corner. Axis values denote percentage changes (±) in parameter values relative to the ancestral state. Axis tick-marks denote the center of grid cells. Parameter abbreviations as follows: Activator self-regulation (Act), Activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and the strength of inhibition (Inh).

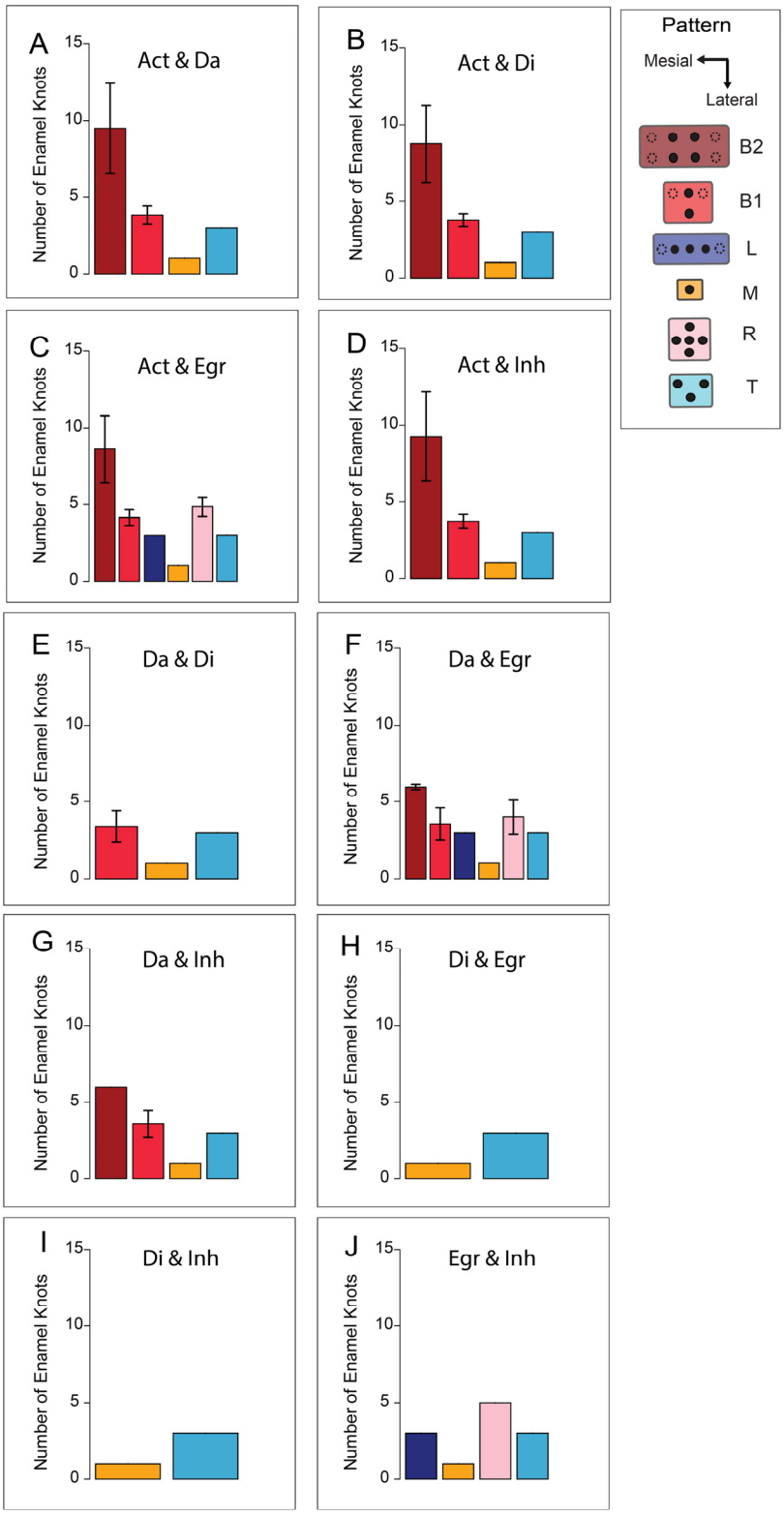

Figure S7. Bar plots of enamel knot number and pattern for different developmental landscapes. Error bars represent one standard deviation. Parameter abbreviations as follows: Activator self-regulation (Act), activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and the strength of inhibition (Inh).

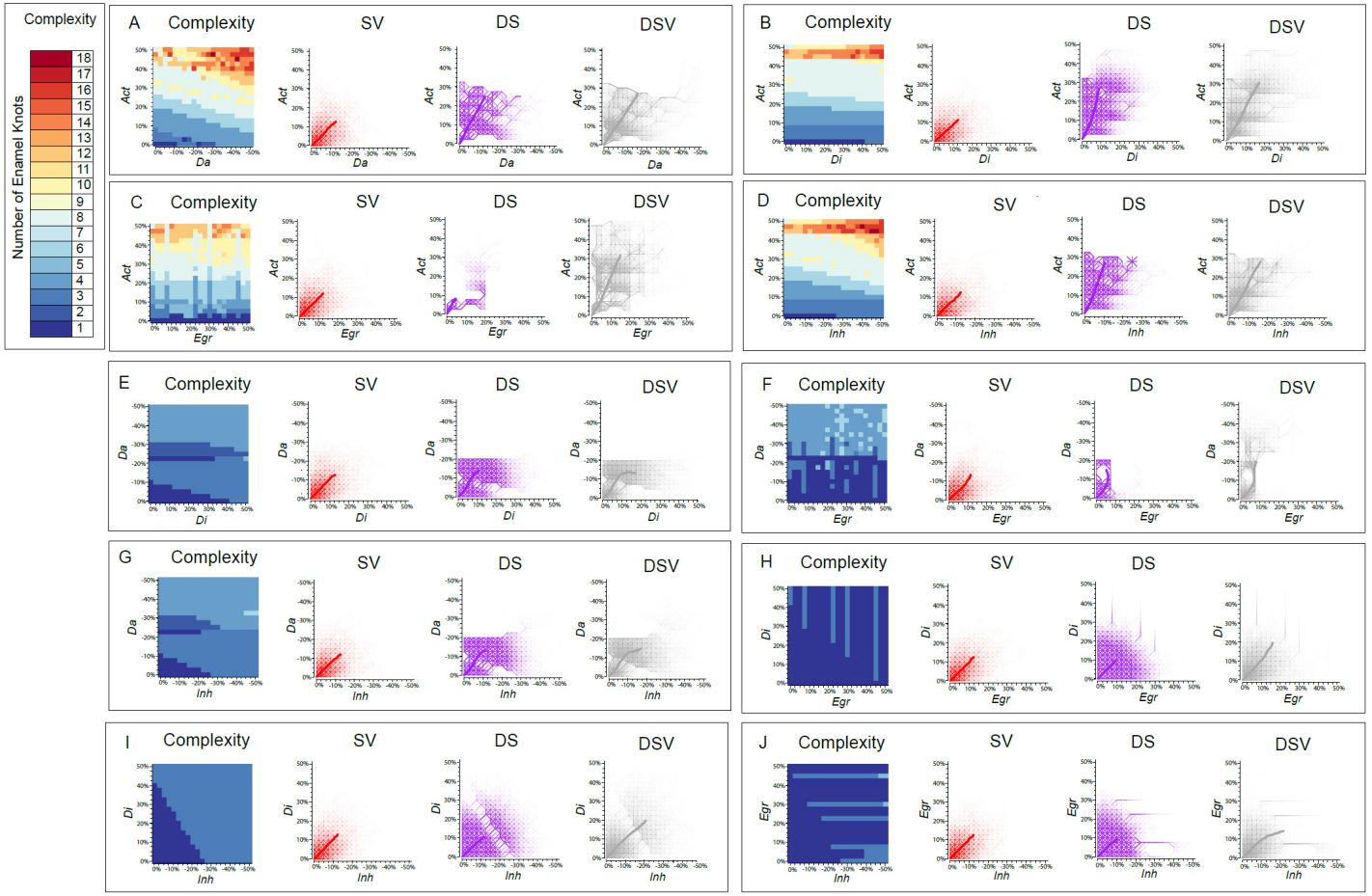

Figure S8. Evolutionary trajectories across different developmental landscapes under three evolutionary models. Bold lines are the average trajectory through the landscape (10,000 simulations, each run for a maximum of 30 generations). Landscapes are ordinated with the starting state (P_0) in the lower left corner. Faint lines are a random sample of 5,000 trajectories. Parameter abbreviations as follows: Activator self-regulation (Act), activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and strength of inhibition (Inh).

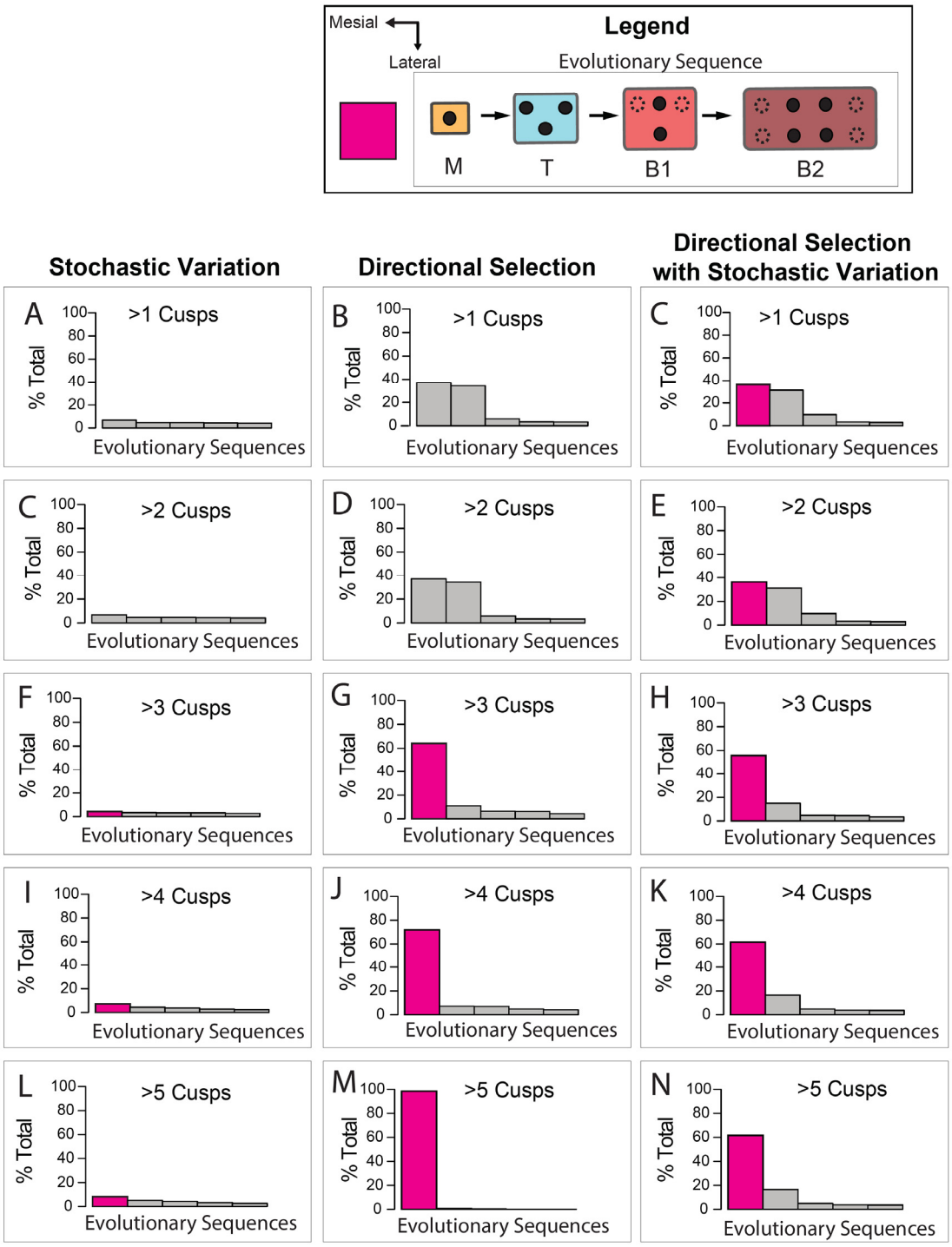

Figure S9. Frequency distributions of the most common evolutionary sequences for each evolutionary model at different tooth complexity levels: (A, C, F, I, L) stochastic variation (SV); (B, D, G, J, M) directional selection (DS), (C, E, H, K, N) directional selection combined with stochastic variation (DSV). Magenta highlights 'M>T>B1>B2' transformation sequence.

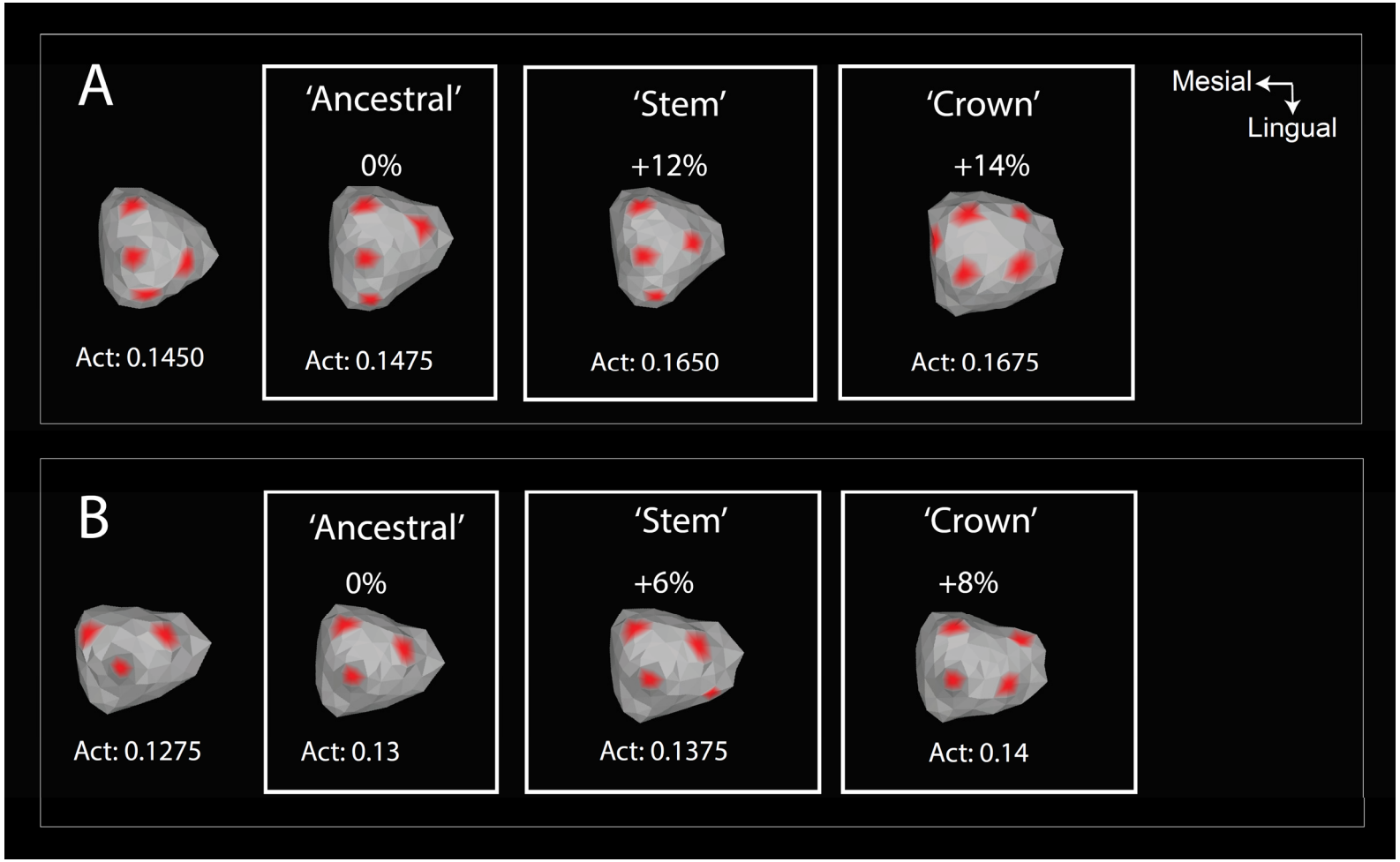

Figure S10. Sensitivity of different tooth bud growth biases to increases in activation (Act). (A) Enamel knot patterns originating from an 'ancestral' tooth bud with low levels of anterior growth, low levels of posterior growth, and a strong lingual bias in activator expression. (B) Enamel knot patterns originating from ancestral parameters as with 'A', but with more posterior growth. Red denotes growth factor concentration at view threshold=0.2.

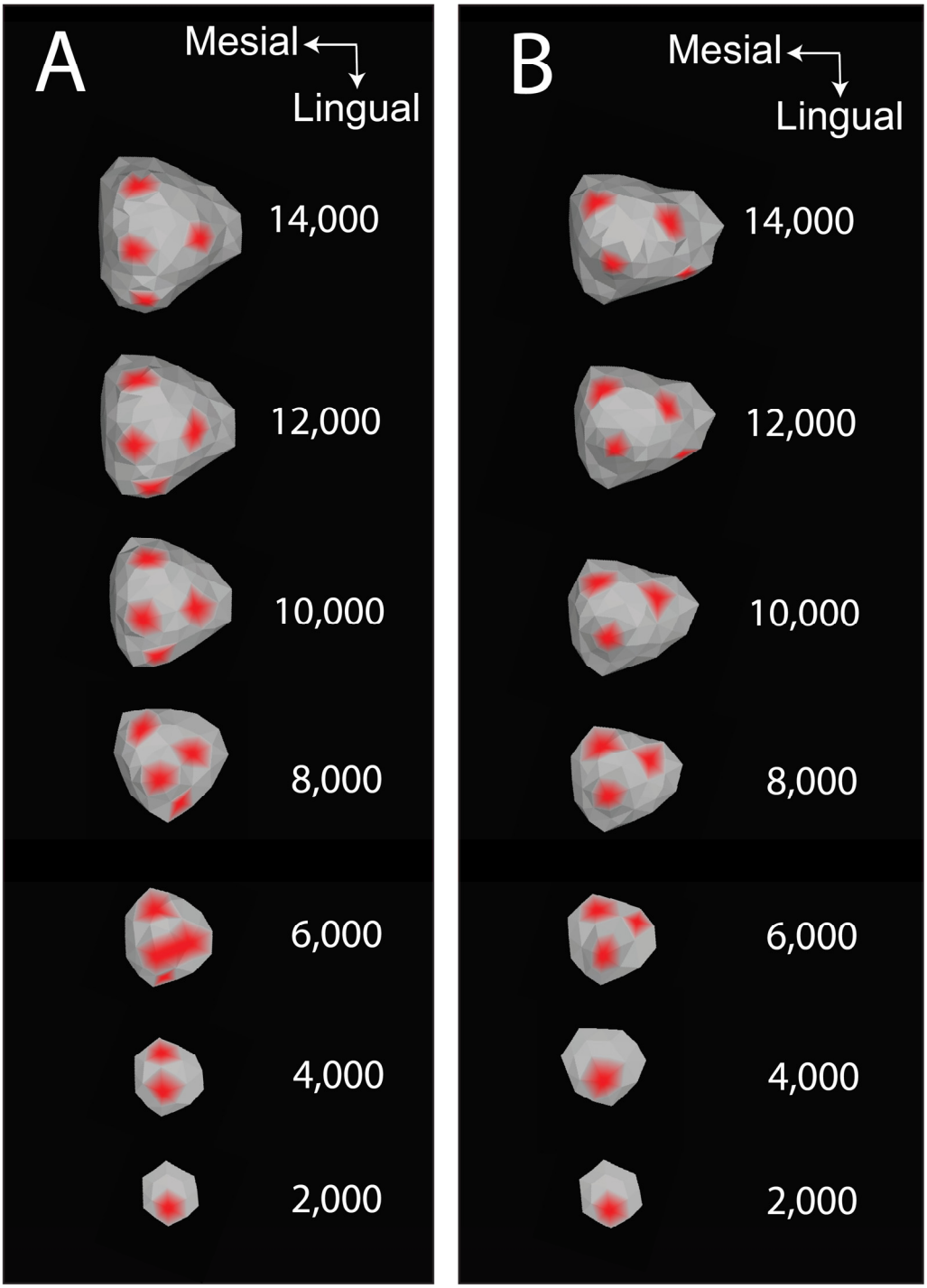

Figure S11. Patterning changes leading to distinct origins of posterolingual knots in ToothMaker simulations. (A) Formation of a lingual cingulum-like structure on an ancestral tooth subject to low-levels of anterior and posterior growth, and a strong lingual bias in activator expression. (B) As for (A), but with increased posterior growth. Red denotes growth factor concentration at view threshold=0.2. Numbers indicate the model iteration number, a proxy for developmental time.

Table S1. ToothMaker parameter settings used to generate the initial tooth state (P_0) for constructing the developmental landscapes and for the ancestral state in trait evolution simulations. Blue rows denote parameters that were varied in the pairwise simulations.

Parameter	Abbreviation	Class	Starting Value
Protein degradation rate	Deg	Degradation	0.076
Growth factor threshold	Set	Growth factor	0.95
Growth factor secretion rate	Sec	Growth factor	0.14
Growth factor diffusion rate	Ds	Growth factor	1.0
Activator self-regulation	Act	Activator	0.1
Activator diffusion rate	Da	Activator	0.05
Buccal bias	Bbi	Activator	0.1
Lingual bias	Lbi	Activator	0.1
Initial activator concentration	Ina	Activator	0.0
Initial inhibitor threshold	Int	Inhibitor	0.19
Inhibition of activator	Inh	Inhibitor	10.0
Inhibitor diffusion rate	Di	Inhibitor	1.0
Epithelial proliferation rate	Egr	Epithelium	0.01
Downward vector of growth	Dgr	Epithelium	10500.0
Differentiation rate	Dff	Epithelium	0.0002
Traction between neighboring cells	Adh	Epithelium	0.005
Young's modulus (stiffness)	Rep	Epithelium	1.5
Mechanical traction from the borders to the nucleus	Ntr	Epithelium	0.000013
Anterior bias	Abi	Epithelium	5.0
Posterior bias	Pbi	Epithelium	5.0
Mesenchymal proliferation rate	Mgr	Mesenchyme	1000.0
Border growth, amount of mesenchyme in the anterior–posterior region.	Bgr	Mesenchyme	0.9
Mesenchyme mechanical resistance	Boy	Mesenchyme	0.1
Radius of initial conditions	Rad	Initial size	3.0
Distance from 0 where the borders are defined	Swi	Initial size	1.4
Width of border	Bwi	Initial size	2.4

Table S2. Combinations of ToothMaker parameters used to construct developmental landscapes (blue). Parameter abbreviations: Activator self-regulation (Act), activator diffusion rate (Da), inhibitor diffusion rate (Di), epithelial growth rate (Egr), and inhibition of activation (Inh). P_{min}: minimum parameter value; P_{max}: maximum parameter value; Step: step size increment for each grid cell. Underlined values represent initial conditions (P_0).

Parameter	**Act** P_{min}: <u>0.1</u> P_{max}: 0.15 Step:2.5×10^{-3}	**Da** P_{min}: 2.5×10^{-2} P_{max}: <u>5×10^{-2}</u> Step: 1.25×10^{-3}	**Di** P_{min}: <u>1</u> P_{max}: 1.5 Step: 2.5×10^{-2}	**Egr** P_{min}: <u>1.0×10^{-2}</u> P_{max}: 1.5×10^{-2} Step:2.5×10^{-4}	**Inh** P_{min}: 5 P_{max}:<u>10</u> Step:0.25
Act P_{min}: <u>0.1</u> P_{max}: 0.15 Step:2.5×10^{-3}					
Da P_{min}: 2.5×10^{-2} P_{max}: <u>5×10^{-2}</u> Step: 1.25×10^{-3}					
Di P_{min}: <u>1</u> P_{max}: 1.5 Step: 2.5×10^{-2}					
Egr P_{min}: <u>1.0×10^{-2}</u> P_{max}: 1.5×10^{-2} Step:2.5×10^{-4}					
Inh P_{min}: 5 P_{max}:<u>10</u> Step:0.25					

Table S3. Variation in cusp pattern and cusp number on the upper second molar for 93 species of fossil and extant mammalian taxa. PPM=Protocone–Paracone–Metacone.

Taxon	Order/Clade	Status	Cusp Pattern	Cusp Number	PPM Angle
Abdounodus hamdii	Paenungulatomorpha	Fossil	T	6	64
Adapis parisiensis	Primates	Fossil	T	4	66
Ailurus fulgens	Carnivora	Extant	B2	13	85
Ailuropoda melanoleuca	Carnivora	Extant	B2	>13	88
Amblysomus hottentotus	Afrosoricida	Extant	T	4	65
Apheliscus insidiosus	Macroscelidea	Extant	B1	3	93
Archaeonycteris brailloni	Chiroptera	Fossil	B1	5	88
Archaeotherium mortoni	Artiodactylamorpha	Fossil	T	5	75
Artiocetus clavis	Artiodactyla	Fossil	T	3	NA
Basilosaurus cetoides	Artiodactyla	Fossil	L	6	NA
Bos taurus	Artiodactyla	Extant	B2	8	89
Cantius abditus	Primates	Fossil	T	8	64
Caperea marginata	Artiodactyla	Extant	E	0	NA
Castor canadensis	Rodentia	Extant	B2	7	90
Cavia porcellus	Rodentia	Extant	B2	5	90
Cocomys lingchaensis	Glires	Fossil	B1	8	81
Cymbalophus cuniculus	Perissodactyla	Fossil	T	4	76
Cynocephalus volans	Dermoptera	Extant	T	8	67
Dasypus novemcinctus	Cingulata	Extant	M	1	NA
Dawsonolagus antiquus	Glires	Fossil	T	4	70
Diacodexis antunesi	Artiodactyla	Fossil	B1	5	80
Didelphis virginiana	Didelphimorphia	Extant	T	7	67
Didolodus multicuspis	Meridiungulata	Fossil	T	6	70
Dormaalocyon latouri	Carnivoraformes	Fossil	B1	5	99
Dromiciops gliroides	Microbiotheria	Extant	B1	6	82
Echinops telfairi	Afrosoricida	Extant	B1	4	95
Ectocion parvus	Euungulata	Fossil	T	8	60
Equus caballus	Perissodactyla	Extant	B2	9	97
Erinaceus europaeus	Eulipotyphla	Extant	T	5	55
Eritherium azzouzorum	Tethytheria	Fossil	B2	5	97
Galeopterus variegatus	Dermoptera	Extant	T	8	65
Gomphos elkema	Glires	Fossil	T	4	62
Gracilocyon solei	Carnivoraformes	Fossil	B1	6	86
Haldanodon exspectatus	Docodonta	Fossil	T	2	NA
Hapalops elongatus	Pilosa	Fossil	M	1	NA
Henkelotherium guimarotae	Dryolestida	Fossil	T	4	43
Hippopotamus amphibius	Artiodactyla	Extant	B2	7	81
Homo sapiens	Primates	Extant	T	4	70

Hyopsodus paulus	Perissodactyla	Fossil	T	6	56
Icaronycteris menui	Chiroptera	Fossil	B1	5	88
Ictidomys tridecemlineatus	Rodentia	Extant	B2	3	87
Lama glama	Artiodactyla	Extant	B2	7	91
Lemur catta	Primates	Extant	T	5	78
Leptictis dakotensis	Leptictida	Fossil	B1	4	80
Loxodonta africana	Proboscidea	Extant	B2	17	95
Lycophocyon hutchisoni	Carnivora	Fossil	T	3	72
Maelestes gobiensis	Cimolesta	Fossil	B1	4	91
Manis pentadactyla	Pholidota	Extant	E	0	NA
Mesohippus bairdi	Perissodactyla	Fossil	T	5	73
Mesonyx obtusidens	Mesonychia	Fossil	T	4	67
Metacheiromys marshi	Palaeanodonta	Fossil	E	0	NA
Moeritherium lyonsi	Proboscidea	Fossil	B2	5	81
Morganucodon watsoni	Morganucodonta	Fossil	L	3	NA
Myotis lucifugus	Chiroptera	Extant	T	6	73
Notharctus tenebrosus	Primates	Fossil	T	7	65
Nycteris thebaica	Chiroptera	Extant	T	7	76
Ocepeia grandis	Paenungulatomorpha	Fossil	T	5	63
Orycteropus afer	Tubulidentata	Extant	B2	4	90
Oryctolagus cuniculus	Lagomorpha	Extant	B2	4	89
Paramys delicatus	Rodentia	Fossil	T	4	47
Phenacodus intermedius	Euungulata	Fossil	T	6	62
Phosphatherium escuillei	Proboscidea	Fossil	B2	5	90
Plesiadapis ploegi	Plesiadapiformes	Fossil	T	6	61
Procavia capensis	Hyracoidea	Extant	T	5	62
Protictis microlestes	Carnivoramorpha	Fossil	T	6	66
Protolipterna ellipsodontoides	Litopterna	Fossil	T	6	63
Protungulatum donnae	Eutheria	Fossil	T	6	67
Pteronotus parnellii	Chiroptera	Extant	B2	7	84
Pteropus giganteus	Chiroptera	Extant	B1	2	NA
Ptilocercus lowii	Scandentia	Extant	T	3	60
Pucadelphys andinus	Marsupialiformes	Fossil	B1	9	80
Purgatorius titusi	Primatomorpha	Fossil	T	4	79
Rattus norvegicus	Rodentia	Extant	B2	6	NA
Rhinopoma hardwickii	Chiroptera	Extant	B1	7	91
Rhombomylus turpanensis	Glires	Fossil	T	5	50
Rhynchocyon cirnei	Macroscelidea	Extant	T	4	73
Saccopteryx bilineata	Chiroptera	Extant	B2	7	101
Saimiri sciureus	Primates	Extant	T	4	70
Solenodon paradoxus	Euliptophyla	Extant	B1	6	88

Sorex araneus	Euliptophyla	Extant	T	7	74
Sus scrofa	Artiodactyla	Extant	B2	6	80
Tachyglossus aculeatus	Monotremata	Extant	E	0	NA
Talpa europaea	Euliptophyla	Extant	B2	6	80
Tamandua tetradactyla	Pilosa	Extant	E	0	NA
Tarsius syrichta	Primates	Extant	T	3	78
Todralestes variabilis	Eutheria	Fossil	T	4	78
Tribosphenomys minutus	Glires	Fossil	T	4	74
Trichechus manatus	Sirenia	Extant	B2	6	87
Tupaia glis	Scandentia	Extant	T	6	70
Tursiops truncatus	Artiodactyla	Extant	M	1	NA
Uintacyon rudis	Carnivoraformes	Fossil	B1	4	83
Vulpavus ovatus	Carnivoraformes	Fossil	T	3	69
Zalambdalestes lechei	Eutheria	Fossil	T	4	78

Table S4. ToothMaker parameters states used to generate 'ancestral' placental tooth shapes. 'Ancestral state 1' = Fig. 5A, 'Ancestral state 2' = Fig. 5B.

Parameter	Abbreviation	Ancestral State 1	Ancestral State 2
Protein degradation rate	Deg	0.076	0.076
Growth factor threshold	Set	0.95	0.95
Growth factor secretion rate	Sec	0.14	0.14
Growth factor diffusion rate	Ds	1.0	1.0
Activator self-regulation	Act	0.1475	0.13
Activator diffusion rate	Da	0.06	0.06
Buccal bias	Bbi	0.00001	0.00001
Lingual bias	Lbi	0.5	0.5
Initial activator concentration	Ina	0.0	0.0
Initial inhibitor threshold	Int	0.19	0.19
Inhibition of activator	Inh	11.0	11.0
Inhibitor diffusion rate	Di	1.50	1.50
Epithelial proliferation rate	Egr	0.012	0.012
Downward vector of growth	Dgr	10500	10500
Differentiation rate	Dff	0.0002	0.0002
Traction between neighboring cells	Adh	0.005	0.005
Young's modulus (stiffness)	Rep	1.5	1.5
Mechanical traction from the borders to the nucleus	Ntr	0.00013	0.00013
Anterior bias	Abi	0.2	0.2
Posterior bias	Pbi	2.5	3.0
Mesenchymal proliferation rate	Mgr	1000	1000
Border growth, amount of mesenchyme in the anterior–posterior region.	Bgr	0.9	0.9
Mesenchyme mechanical resistance	Boy	0.1	0.1
Radius of initial conditions	Rad	3.0	3.0
Distance from 0 where the borders are defined	Swi	1.5	1.5
Width of border	Bwi	3.0	3.0