Journal of Mammalian Evolution, Vol. 10, No. 4, December 2003 (© 2004)

Is Sequence Heterochrony an Important Evolutionary Mechanism in Mammals?

Olaf R. P. Bininda-Emonds,[1,2] Jonathan E. Jeffrey,[1] and Michael K. Richardson[1]

It is postulated widely that changes in developmental timing (i.e., heterochrony) represent a major mechanism of evolutionary change. However, it is only with recent methodological advances that changes in the order in which development proceeds (sequence heterochrony) can be identified and quantified. We apply these techniques to examine whether heterochrony in the early embryonic (organogenetic) period has played an important role in the diversification of mammals. Although we find clear instances of sequence heterochrony in mammals, particularly between eutherians and marsupials, the majority of mammalian lineages that we could examine (those within the major clades Euarchontoglires and Laurasiatheria) show few or no heterochronic changes in the 116 events examined (e.g., Artiodactyla, Euarchonta, Fereuungulata, Glires, Primates, Rodentia). This is in contrast with the timing shifts reported between and within other tetrapod clades. Our results suggest that sequence heterochrony in embryonic stages has not been a major feature of mammalian evolution. This might be because mammals, and perhaps amniotes in general, develop for an extended time in a protected environment, which could shield the embryos from strong diversifying selection. Our results are also consistent with the view that mammal embryos are subject to special developmental constraints. Therefore, other mechanisms explaining the diversity of extant mammals must be sought.

KEY WORDS: development, event-pair, heterochrony, Mammalia, Eutheria, event-pair cracking.

INTRODUCTION

Mammals are a diverse clade of tetrapods divided into about 26 major lineages ("orders"; Wilson and Reeder, 1993). From its origin approximately 195 million years ago (Luo *et al.*, 2001), the ancestral mammalian (adult) body plan of a small shrew-like animal has evolved many distinct morphologies, including forms adapted for flying (bats), swimming (cetaceans and pinnipeds), burrowing and digging (moles and anteaters), climbing and brachiation (primates), and running (artiodactyls), among others (Nowak, 1999). It is largely agreed that the major eutherian lineages arose in a rapid adaptive radiation, although molecular phylogeneticists and paleontologists tend to disagree about whether

[1] Institute of Biology, Leiden University, Leiden The Netherlands.
[2] To whom correspondence should be addressed at Lehrstuhl für Tierzucht, Technical University of Munich, D-85354 Freising-Weihenstephan, Germany. E-mail: Olaf.Bininda@tierzucht.tum.de

1064-7554/03/1200-0335/0

this radiation occurred before or after the Cretaceous-Tertiary boundary, respectively (see Alroy, 1999; Springer *et al.*, 2003).

The evolutionary mechanism behind the adaptive radiation of mammals is unclear. Certainly, an increase in average adult body size has occurred during the evolution of mammals, but this appears to be a post-Cretaceous event associated with the mass extinction at the Cretaceous-Tertiary boundary (the "KT event") that removed many competing large-bodied forms, particularly the dinosaurs (Alroy, 1999). One possible mechanism might be changes in developmental timing (heterochrony), which are held widely to represent a major mechanism of evolutionary change (Gould, 1977, 1982; Shubin and Alberch, 1986; McKinney and McNamara, 1991; Raff, 1996). Most studies of heterochrony have concentrated on changes in growth rates (allometric heterochrony). However, recent methodological advances (Mabee and Trendler, 1996; Smith, 1996; Jeffery *et al.*, 2002b) allow us to test the hypothesis that changes in the order in which events occur in development (sequence or event heterochrony) have been important in mammalian evolution.

Previous studies of sequence heterochrony within mammals have focused on differences between marsupial and eutherian mammals, where obvious changes in developmental timing have long been recognized (e.g., McCrady, 1938). It has been shown that the two groups differ largely in the relative timings of the development of the central nervous systems and craniofacial structures (Smith, 1997; Nunn and Smith, 1998), although methods for localizing the lineage in which any changes have occurred and which of the two systems they involve have been lacking until recently (Jeffery *et al.*, 2002b). However, heterochrony may be more widespread among mammals. In a previous study examining sequence heterochrony within tetrapods (Jeffery *et al.*, 2002a), we documented unexpected heterochronic shifts within eutherian mammals such as a possible delay in eye development in most groups.

We therefore sought to characterize sequence heterochronies in mammals more fully, specifically those involving structures from the organogenetic period. This was done both to understand the evolution of the group better, and also as part of our ongoing efforts (e.g., Jeffery *et al.*, 2002a; Richardson and Oelschläger, 2002) to quantify the prevalence of sequence heterochrony in vertebrate evolution.

METHODS AND MATERIALS

The Data Set

Detailed developmental timing information was obtained from the literature, primarily from the series of "Normal Tables" (Normentafeln) edited by Keibel (1897–1938). Initially, information for all "major" developmental events was recorded for each of 22 mammalian species using the descriptions provided in the source studies (Table I). However, timing information for many of these 809 events was restricted to very few species. Thus, the data set was pruned according to two guidelines. First, only well-defined events of certain homology, within the limits of the provided descriptions, were included. Second, preference was given to events for which timing information was present for as many species as possible, while attempting to represent both the entire organogenetic

Table I. Species Examined and Literature Source of Developmental Data[a]

Taxon	Species	Common Name	Source
"Reptilia"	*Lacerta agilis*	Sand lizard	Peter (1904)
Aves	*Gallus gallus*	Chicken	Keibel and Abraham (1900)
Mammalia	*Didelphis virginiana*	Virginia opossum	McCrady (1938)
	Erinaceus europaeus	Western European hedgehog	Jacobfeuerborn (1908)
	Suncus murinus	Asian house shrew	Yasui (1992, 1993)
	Talpa europaea	European mole	Heape (1883a,b)
	Oryctolagus cuniculus	European rabbit	Minot and Taylor (1905)
	Cavia porcellus	Guinea pig	Harman and Prickett (1932); Harman and Prickett Dobrovolny (1933)
	Cavia porcellus	Guinea pig	Scott (1937)
	Spermophilus citellus	European ground squirrel	Völker-Brünn (1922)
	Mus musculus	House mouse	Theiler (1989)
	Rattus norvegicus	Brown rat	Henneberg (1937)
	Manis javanica	Malayan pangolin	Huisman and de Lange (1937)
	Delphinus delphis, Phocoena phocoena, Stenella attenuata, and Stenella longirostris	"Dolphins" (Delphinidae)	Sterba *et al.* (2000)
	Capreolus capreolus	Western roe deer	Sakurai (1906)
	Ovis aries	Mouflon (sheep)	Bryden *et al.* (1972)
	Sus scrofa	Wild boar	Keibel (1897)
	Tupaia javanica	Javan tree shrew	de Lange and Nierstrasz (1932)
	Loris tardigradus	Slender loris	Hubrecht and Keibel (1907)
	Tarsius spectrum	Spectral tarsier	Hubrecht and Keibel (1907)
	Homo sapiens	Human	Keibel and Elze (1908)
	Homo sapiens	Human	O'Rahilly and Muller (1987)
	Macaca mulatta	Rhesus monkey	Gribnau and Geijsberts (1981)

[a] Mammalian nomenclature follows Wilson and Reeder (1993) and may not match that in the source study.

period and the various organ systems as completely as possible. The final developmental sequence thus consisted of 116 events (Appendix 1), the vast majority of which (100) were present in at least half of the mammalian species examined. All 41 events examined by Jeffery *et al.* (2002a) are included in this set of 116. The least represented event was "first post-otic somite pair present" (event no. 1), which was present in six species. The next least represented events were "scapula cartilaginous" and "primitive streak no longer present" (events no. 98 and 114, respectively), which were present in eight species apiece.

The data set was pruned further by removing species for which data on less than half of the developmental sequence were available: *Erinaceus europaeus*, *Suncus murinus*, *Talpa europaea*, the "Harman" sequence for *Cavia porcellus*, the four species of Delphinidae, *Ovis aries*, and *Macaca mulatta* (see Table II). Doing so unfortunately removed the only representatives of the major mammal lineage Afrotheria (no suitable data exist for any member of the Xenarthra), but reduced the amount of missing data dramatically. For example, 78 of the 116 events were present in at least 75% of the remaining 15 species, compared to only 28 before. In pruning these seven taxa, we are not making any statement as to the quality of the developmental information contained, merely that the quantity was insufficient for the present study. An extended discussion on the effects of

Table II. Descriptive Statistics for Developmental Timing Data Set (See Appendices 1 and 2)

Species	Number of Events Present	System	Stages		Stage Span[a]	Number Used
			Lowest	Highest		
Lacerta agilis	92 (79.3%)	Stages	31	122	92	49 (53.3%)
Gallus gallus	81 (69.8%)	Stages	4	81	78	51 (65.4%)
Didelphis virginiana	86 (74.1%)	Stages	23	36[b]	14	14 (100.0%)
Erinaceus europaeus	37 (31.9%)	Individual embryos	1	24	37[c]	19 (51.4%)
Suncus murinus	51 (44.0%)	Stages (Carnegie)	9	12.9	10	10 (100.0%)
Talpa europaea	19 (16.4%)	Stages	4	10	7	6 (85.7%)
Oryctolagus cuniculus	102 (87.9%)	Stages	2	24	23	20 (87.0%)
Cavia porcellus (Harman)	31 (26.7%)	Time (hours)	14	31	18	10 (55.6%)
Cavia porcellus (Scott)	72 (62.1%)	Time (hours)	12.7	26.1	21[d]	13 (61.9%)
Spermophilus citellus	110 (94.8%)	Stages	25	134	109	54 (49.5%)
Mus musculus	91 (78.4%)	Stages	11	25	15	14 (93.3%)
Rattus norvegicus	109 (94.0%)	Stages	22	125	104	60 (57.7%)
Manis javanica	83 (71.6%)	Stages	1	22	22	20 (90.9%)
Delphinidae	31 (26.7%)	Stages	3	8	6	6 (100.0%)
Capreolus capreolus	103 (88.8%)	Stages	9	57	49	41 (83.7%)
Ovis aries	23 (19.8%)	Time (days)	15	29	15	13 (86.7%)
Sus scrofa	93 (80.2%)	Stages	1	94	94	46 (48.9%)
Tupaia javanica	99 (85.3%)	Stages	1	24	24	21 (87.5%)
Loris tardigradus	83 (71.6%)	Stages	1	10	10	10 (100.0%)
Tarsius spectrum	103 (88.8%)	Stages	1	35	35	31 (88.6%)
Homo sapiens (Keibel)	109 (94.0%)	Stages	1	80	80	49 (61.3%)
Homo sapiens (O'Rahilly)	94 (81.0%)	Stages (Carnegie)	6	24	19	16 (84.2%)
Macaca mulatta	40 (34.5%)	Stages (Carnegie)	10	23	14	14 (100.0%)

[a] Stage span is the number of stages between the lowest and highest stage in the source study; it need not equal the difference between these two values (e.g., if the staging system is not restricted to whole numbers).

[b] Postpartum development was not staged by McCrady (1938), but given in absolute time. The single postpartum event examined was thus given a stage number one greater than the highest used by McCrady.

[c,d] The stage span was taken as the number of embryos examined by Jacobfeuerborn (1908) and Scott (1937), respectively.

missing data on analyses of developmental sequences can be found in Bininda-Emonds *et al.* (2002).

The data set therefore contains information for two of the four major clades of mammals, Euarchontoglires and Laurasiatheria (together, the Boreotheria) and two developmental sequences for *Homo sapiens* that are derived from separate studies. The final data set (Appendix 2) was produced by adding information for two outgroup species, *Lacerta agilis* and *Gallus gallus*. Timing information was available for the majority of events for both species (92 and 81, respectively), although some mammal-specific events (e.g., nos. 38, 70, 108, and 109) were inapplicable and coded as missing data (see Bininda-Emonds *et al.*, 2002).

Analysis

Raw timing data, whether in the form of absolute chronological age or developmental stages, are highly species-specific. Instead, to allow comparisons among species, the relative timing of developmental events should be used (Bininda-Emonds *et al.*, 2002). Therefore, the raw timing data within each species were encoded by the method of event-pairing (Mabee and Trendler, 1996; Smith, 1996; Velhagen, 1997). Event-pairing records the relative timing relationship between all possible pairwise combinations of events. For instance, for the hypothetical events A and B, A can occur either before (coded as 0), simultaneous with (1), or after (2) event B. If no timing data exist for either or both events, the event-pair is coded as "?". The comparisons are made in one direction only (i.e., A is compared to B, but B is not compared to A because it yields the same information), giving $\frac{1}{2}(n)(n-1)$ event-pairs for a developmental sequence of n events.

As with conventional characters, the individual event-pairs across all species can be mapped on to a phylogenetic tree (e.g., Smith, 1997). Reconstructed apomorphic changes in the state of an event-pair indicate a timing change between the constituent events (i.e., heterochrony). However, changes in a single event-pair cannot reveal which of the two constituent events in the event-pair have actually moved, nor in which direction (early or later in development). It is also possible that both events have moved (see Jeffery *et al.*, 2002b). For instance, if the event-pair "AB" has changed from state 0 to state 2, all we can state is that events A and B have changed their relative timing relationship. This change could have arisen in five possible ways, of which A moving later or B moving earlier in development represent only two possibilities (see Figure 2 in Jeffery *et al.*, 2002b).

Instead, the method of event-pair cracking (Jeffery *et al.*, 2002b) analyzes all the event-pair apomorphies for a given branch en bloc to reveal those events that are more likely to have moved "actively" and which events they have moved relative to. Briefly, the method operates on the principle that actively moving events will be moving relative to a large number of other events and in a consistent direction (e.g., always later in development). Actively moving events are thus identified by having a relative movement score larger than some threshold value determined from the relative movement scores of all events. Again, consider the event-pair "AB" from above. By examining all other apomorphic changes on the same branch, we might determine that event A has also moved relative to events C, D, and E (and in a consistent direction), whereas event B has only moved relative to A. In this case, we would suspect that it is event A only that has actively changed its developmental timing. Event-pair cracking can indicate this via the higher relative movement score of A compared to B. A complete worked example of event-pair cracking can be found in Jeffery *et al.* (2002b).

Therefore, to reconstruct heterochronic changes in mammals, we mapped our developmental timing data on to a phylogeny and applied the method of event-pair cracking. Higher-level relationships among mammals are controversial. We based the underlying phylogeny on the family-level mammal supertree of Liu *et al.* (2001; see Fig. 1) because it is the only recent comprehensive mammal phylogeny based on a rigorous methodology to detail the relationships among all the species in this study. However, we modified the phylogeny to place Euarchonta and Glires as sister taxa (together, the Euarchontoglires) in agreement with numerous recent molecular studies (Madsen *et al.*,

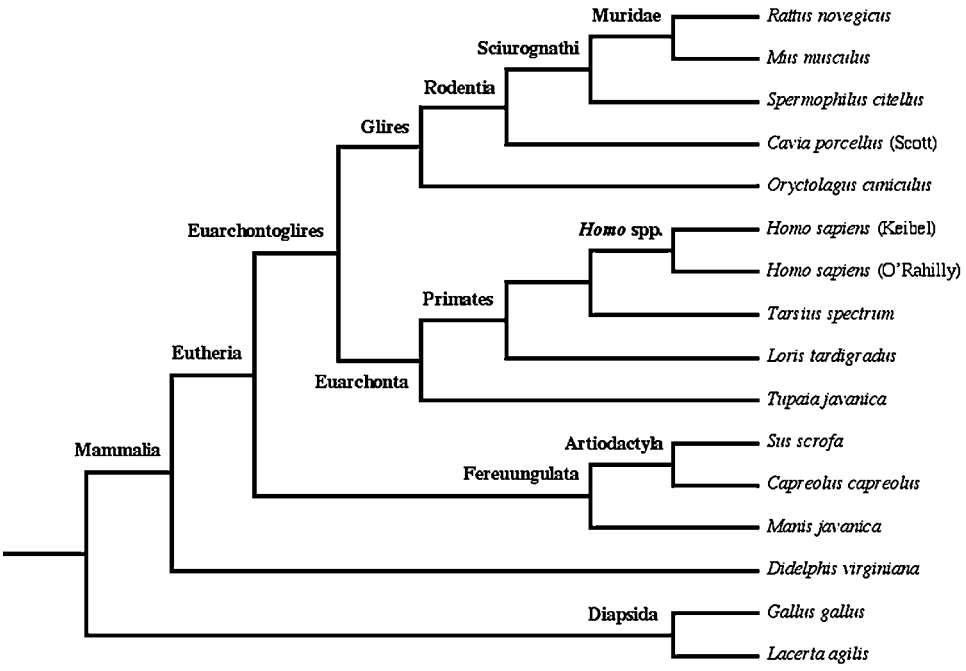

Fig. 1. The phylogeny upon which developmental timing data were mapped (adapted mainly from Liu *et al.*, 2001). Names of higher-level mammalian taxa follow Wilson and Reeder (1993), Waddell *et al.* (1999), and Amrine-Madsen *et al.* (2003).

2001; Murphy *et al.*, 2001a,b; Corneli, 2002; Amrine-Madsen *et al.*, 2003; de Jong *et al.*, 2003; Thomas *et al.*, 2003). We note that this change did not affect the results appreciably.

Inferred instances of heterochrony will depend highly on how the event-pairs are optimized on to the phylogeny. We therefore mapped our data under both ACCTRAN and DELTRAN optimization criteria (Swofford and Maddison, 1987), and cracked each optimized set of event-pair synapomorphies individually. These criteria represent opposite extremes. Given conflicting equally parsimonious reconstructions of a character, ACCTRAN (accelerated transformation) optimization will favor an early origin of the derived state, followed by a reversal to the primitive condition. In contrast, DELTRAN (delayed transformation) optimization will favor later, parallel derivations of the derived state (Swofford and Maddison, 1987). The cracking procedure followed that described in Jeffery *et al.* (2002b), with the "threshold" value to identify actively moving events being the median of the relative movements of all events (see Jeffery *et al.*, 2002b). Only events identified under both ACCTRAN and DELTRAN character optimizations were used in our final hypothesis of event movements. Altogether, this procedure will yield a conservative estimate of the heterochronic changes that have occurred during the evolution of mammals.

To reduce any confusion associated with the distinction between event-pairs and the events that contribute to them, we will use the words apomorphy or plesiomorphy (or

derivatives thereof) to refer to evolutionary transformations in the event-pairs. We restrict our use of heterochronic shifts or timing shifts to changes in the timing of the actual events.

Correcting for Simultaneous Events

Developmental events that occur simultaneously pose a serious problem for studies of sequence heterochrony. Most instances of simultaneity are probably artifactual (Smith, 1997; Nunn and Smith, 1998), arising from a failure to sample an embryo that would resolve the timing difference between two events. However, this apparent timing change (from simultaneous to not simultaneous, and vice versa) has the effect of suggesting real evolutionary differences between species where none exist (see Bininda-Emonds *et al.*, 2002).

Initial results (not shown) displayed this phenomenon, showing a highly skewed distribution of timing shifts. In all cases, the numbers of events inferred to be moving early ("advances") versus late ("delays") on a given branch were about equal. However, far fewer shared changes were inferred to have occurred on internal branches than did unshared changes on terminal branches (on average, 3.3 vs. 22.8 events, respectively). A cogent example was the two *Homo sapiens* sequences. Although these sequences should be similar, numerous heterochronic shifts were inferred along each terminal branch. This was particularly true for the O'Rahilly sequence, which has twice as many simultaneous event-pairs than does the Keibel sequence (366 vs. 183) because it describes the same period of developmental time using far fewer stages (19 vs. 80). In fact, the number of changes along the terminal branches correlates significantly with the number of simultaneous event-pairs for the species in question ($p = 0.0415$).

We therefore used simulation to quantify the distribution of developmental events for each species in our data set and thus to judge the level of simultaneity present. The simulation was based on two pieces of data for each species: (1) the number of events present (n_E) and (2) the number of stages between the first and last events (n_S; the "stage span"). For each simulated replicate, the n_E events were assigned randomly to the n_S stages. Thus, some stages may have had no events allocated to it. For each species, the number of stages used and the number of simultaneous event-pairs calculated were averaged over all replicates and compared to the actual values. A significant difference (with $\alpha = 0.05$) was held to occur when the actual values fell more than 1.96 standard deviations from the mean of the simulated values (i.e., fall into either tail of the distribution of simulated values). These differences reveal developmental sequences that might have an excessively clumped distribution of events or are otherwise nonlinear (e.g., due to irregular sampling in time).

The results of our simulation (based on 10,000 replicates) showed significant clumping of events and a significantly high number of simultaneous event-pairs in most species (Table III). Only *Gallus gallus*, *Suncus murinus*, *Rattus norvegicus*, *Ovis aries*, *Tarsius spectrum*, and *Macaca mulatta* did not display a significant difference in either case. Therefore, given the global presence of "significant" (artifactual) simultaneity in the data set, we followed the suggestion of Velhagen (1997) and coded simultaneous events as missing unless strong evidence for true simultaneity existed (which was never the case here).

Table III. Results of a Simulation Study Examining the Distribution of Developmental Events[a]

Species	Number of Events Used			Number of Simultaneous Event-Pairs		
	Actual Value[b]	Simulated Value[c]	Z-Score[d]	Actual Value	Simulated Value[c]	Z-Score[d]
Lacerta agilis	49	56.333 ± 3.011	−2.436*	77	76.601 ± 8.645	0.046
Gallus gallus	51	49.257 ± 2.756	0.632	56	57.426 ± 7.354	−0.194
Didelphis virginiana	14	13.975 ± 0.156	0.16	303	254.546 ± 14.775	3.279*
Erinaceus europaeus	19	23.631 ± 1.875	−2.470*	39	35.181 ± 5.614	0.68
Suncus murinus	10	9.942 ± 0.238	0.244	119	117.381 ± 10.136	0.16
Talpa europaea	6	6.486 ± 0.625	−0.778	34	22.693 ± 4.159	2.719*
Cavia porcellus (Harman)	10	14.312 ± 1.389	−3.103*	55	38.105 ± 6.028	2.803*
Cavia porcellus (Scott)	13	20.318 ± 0.786	−9.313*	249	191.066 ± 12.229	4.737*
Spermophilus citellus	54	68.117 ± 3.202	−4.409*	126	103.286 ± 9.765	2.326*
Mus musculus	14	14.965 ± 0.184	−5.248*	351	261.762 ± 16.240	5.495*
Rattus norvegicus	60	65.799 ± 3.248	−1.786	85	89.103 ± 9.732	−0.422
Oryctolagus cuniculus	20	22.711 ± 0.525	−5.163*	345	247.736 ± 15.378	6.325*
Manis javanica	20	21.473 ± 0.674	−2.187*	262	162.107 ± 12.266	8.144*
Delphinidae	6	5.976 ± 0.153	0.157	92	72.460 ± 7.994	2.444*
Capreolus capreolus	41	42.661 ± 1.987	−0.836	161	118.544 ± 10.406	4.080*
Sus scrofa	46	57.887 ± 2.983	−3.985*	91	85.440 ± 9.254	0.601
Ovis aries	13	11.899 ± 1.199	0.918	14	19.540 ± 4.294	−1.29
Tupaia javanica	21	23.597 ± 0.606	−4.287*	278	217.047 ± 14.153	4.307*
Tarsius spectrum	31	33.146 ± 1.227	−1.749	186	162.402 ± 12.441	1.897
Loris tardigradus	10	9.998 ± 0.045	0.045	480	324.115 ± 18.152	8.588*
Homo sapiens (Keibel)	49	58.886 ± 2.849	−3.470*	183	113.327 ± 10.313	6.756*
Homo sapiens (O'Rahilly)	16	18.875 ± 0.349	−8.248*	366	256.410 ± 14.875	7.367*
Macaca mulatta	14	13.308 ± 0.756	0.915	60	55.488 ± 6.932	0.651

[a] The number of events and stage span for each species are found in Table II.
[b] Simulated values are presented as means ± standard deviation ($n = 10\,000$).
[c] Significant differences are indicated with an asterisk (two-tailed $\alpha = 0.05$).

RESULTS

Distribution of Heterochrony

It is apparent immediately that the distribution of timing shifts within mammals remained highly skewed in favor of unshared changes on terminal branches (Table IV). On average, 1.85 events were inferred to have changed their timing significantly along each internal branch (1.00 advances, 0.85 delays), compared to an average of 9.75 events (4.81 advances, 4.94 delays) along each terminal branch. Several internal branches did not have any heterochronic shifts inferred for them (e.g., the branches leading to Rodentia, Muridae, Fereuungulata, Primates, and *Tarsius* plus *Homo*). Only the branches leading to Eutheria, Euarchonta, and *Homo* showed more than a handful of timing shifts.

Levels of homoplasy are much higher on terminal branches. Of the 22 events (19.0%) that changed their developmental timing on an internal branch, only two (no. 99: humerus chondrification beginning; no. 104: expansion of allantois) changed

Table IV. Inferred Heterochronic Changes along Each Branch in Figure 1[a]

Lineage[b]	Inferred heterochronic changes[c]	
	Events moving early (advances)	Events moving late (delays)
Root → Diapsida/Mammalia	22 (to Diapsida)	22 (to Mammalia)
Mammalia → Eutheria	5, 15, 19, 35, 92	23, 89, 116
Eutheria → Fereuungulata	None	None
Fereuungulata → Artiodactyla	104	None
Eutheria → Euarchontoglires	99	None
Euarchontoglires → Euarchonta	91, 99	16, 58
Euarchonta → Primates	None	None
Primates → *Tarsius* + *Homo*	None	None
Tarsius + *Homo* → *Homo sapiens*	90, 105	17, 43, 45, 86
Euarchontoglires → Glires	106	104
Glires → Rodentia	None	None
Rodentia → Sciurognathi	78	None
Sciurognathi → Muridae	None	None
Diapsida → *Gallus gallus*	4, 15, 22, 67, 73, 100, 102	39, 78, 91, 96, 105
Diapsida → *Lacerta agilis*	12, 23, 27, 37, 41, 42, 44, 45, 84, 91, 104	7, 15, 30, 59, 74, 80, 81, 93, 94, 95
Mammalia → *Didelphis virginiana*	23, 28, 32, 42, 47, 56, 61, 85, 94, 95	84, 103, 105, 114
Fereuungulata → *Manis javanica*	70	74, 104
Artiodactyla → *Capreolus capreolus*	36, 63, 65, 66, 105	25, 35, 48, 109
Artiodactyla → *Sus scrofa*	7, 16, 35, 75	36, 39, 53
Euarchonta → *Tupaia javanica*	30, 35, 41, 91, 102	39, 68, 85, 114
Primates → *Loris tardigradus*	89	77
Tarsius + *Homo* → *Tarsius spectrum*	98, 100	31, 48, 90, 93, 106
Homo sapiens → *Homo sapiens* (O'Rahilly)	10, 16, 18, 85, 87	17, 45, 76, 86, 89, 115
Homo sapiens → *Homo sapiens* (Keibel)	89, 104, 106	5, 63, 87
Glires → *Oryctolagus cuniculus*	21, 31, 59, 83, 100	10, 12, 13, 46, 47, 67, 81, 91, 113, 115
Rodentia → *Cavia porcellus*	51, 103, 113	78, 90
Sciurognathi → *Spermophilus citellus*	11, 13, 14, 16, 37, 60, 64, 66, 91	4, 26, 31, 35, 53, 54, 59, 83, 100, 104
Muridae → *Mus musculus*	25, 35, 41, 80	9, 12, 63, 74, 88
Muridae → *Rattus norvegicus*	12, 28	13, 41, 82, 91

[a] Tree statistics for event-paired data: length = 4196 steps, CI = 0.543 (0.413 excluding uninformative characters), RI = 0.302 and RC = 0.164.
[b] Lineages on internal branches are presented first, followed by those on terminal branches.
[c] Developmental events are listed by their numbers as given in Appendix 1.

more than once (each showing two changes). In contrast, 50 of the 81 events inferred to change along terminal branches changed along more than one branch. Many of these events changed their relative timing up to four times, with one event (no. 91; anlage of adrenal cortex) moving earlier or later in development a total of six times. The high degree of homoplasy is reflected in the low values of various goodness-of-fit statistics (Table IV). The value for CI in particular is lower than the value expected for a study with 16 taxa (0.602; derived from Sanderson and Donoghue, 1989).

Heterochrony in Mammals

We focus largely on heterochronic shifts inferred along the internal branches of the tree. This is both because the shared changes are more interesting from a macroevolutionary perspective and because terminal changes are difficult to interpret objectively. The species examined represent exemplars for various higher-level taxa, and with the exception of the two *Homo sapiens* sequences, are not each other's closest relatives among extant mammals. Therefore, at least some terminal shifts may represent shared changes at a higher level within mammals. However, it is impossible to determine which changes these are.

Apart from the eutherian–metatherian comparison, the inferred heterochronic shifts do not reveal any obvious developmental modules (*sensu* Wagner, 1996), such as a possible delay in eye development (Jeffery *et al.*, 2002a), or have an obvious functional role. Some inferred shifts also seem contradictory. For instance, despite possessing proportionately the largest brain and especially forebrain of any mammal (Jerison, 1973), *Homo sapiens* is characterized by a delay in the formation of the cerebral hemispheres. In fact, except for an advanced appearance of the epiphysis in *Homo*, there are no advances in brain development among primates. The latter two observations contradict the idea that the delayed onset of organ development results in the organ being ultimately of a reduced size (see Gould, 1982), but agrees with the observed rapid postembryonic growth of the forebrain in humans (Langman, 2000). An analogous mechanism was also inferred to account for the relatively large eyes in *Tarsius spectrum* despite an observed general delay in the onset of eye development in mammals (Jeffery *et al.*, 2002a).

The general developmental timing shifts we observed between eutherians and marsupials (see Table V) largely match those found by Smith and colleagues, who summarized the shift as involving features of the central nervous system relative to those of the craniofacial apparatus. Eutherians display a relative advance in the former, while marsupials display a

Table V. Inferred Heterochronic Changes in the Lineages Leading to Marsupials[a] and Eutherians[b]

Taxon	Advances	Delays
Eutheria	**Mesencephalic flexure first indicated**	*Optic stalk invaded by retinal nerve fibers*
	Septum primum broken through	*Infundibular groove forms*
	1st aortic arch formed	First ribs appearing
	Anlage of utricle	
	Pericardial coelom beginning	
Marsupialia	*Optic stalk invaded by retinal nerve fibers*	**Posterior neuropore closed**
	Eyelid anlage appears	Allantoic diverticulum appears
	Otocyst detached from ectoderm	Amniopore closed
	Nasal placodes depressed	Primitive streak no longer present
	Anlage of nasolacrimal duct	
	2nd visceral pouch contacts ectoderm	
	Mandible beginning to ossify	
	Rhombomeres start to appear	
	Lung buds first visible	
	Forelimb bud first visible	

[a] Represented by *Didelphis virginiana.*
[b] Changes listed in bold face agree with the general pattern identified by Smith and colleagues (Smith, 1997; Nunn and Smith, 1998), those in italics run counter to this pattern.

relative advance in the latter (Smith, 1997; Nunn and Smith, 1998), with the latter being determined independently as the actual heterochronic shift (Jeffery *et al.*, 2002b). Additionally, *Didelphis virginiana* displays an advance in the first appearance of the forelimb relative to eutherian mammals (McCrady, 1938; Sánchez-Villagra, 2002), although more detailed observations suggest that the initial appearance of both sets of limb buds is relatively synchronous in marsupials followed by accelerated development of the forelimb (Jeffery *et al.*, unpublished data). Altogether, these observations have clear functional correlates with the life history strategies in each group (see also McCrady, 1938), particularly with marsupials being born and commencing suckling at a relatively earlier stage.

It is not possible to assess which changes occurring along the branch leading to *Didelphis virginiana* are specific to this species and which characterize marsupials as a whole. We note that several heterochronic changes also occur along the branch leading to eutherians, disagreeing with previous suggestions based on a more restricted data set that the differences between the two groups are due exclusively to heterochronic changes within marsupials (Jeffery *et al.*, 2002b). Our more comprehensive data set also suggests that the heterochronic changes differentiating eutherian and marsupial mammals extend beyond central nervous system and craniofacial structures.

DISCUSSION

Macroevolutionary Changes

Our findings indicate that embryonic sequence heterochrony has not played an important role in the diversification of at least those mammalian lineages we could examine (also Sánchez-Villagra, 2002). Many of these lineages are characterized by few or no timing shifts (e.g., primates, rodents, or artiodactyls). Instead, heterochrony among mammals appears to have occurred much more recently in evolutionary time, possibly implying an increased role in speciation. However, these terminal changes seem to show no concerted pattern such that they become swamped, erased, or diluted with time to no longer define most of the major lineages (with a few notable exceptions such as the origins of Eutheria and Marsupialia).

We would suggest that the low number of heterochronic shifts inferred along internal branches, with its implications for the macroevolutionary history of mammals, is reasonably accurate. The diversity of mammals belies the conservativeness of the underlying mammalian body plan (e.g. with respect to phalangeal and vertebral count, and the morphology of the wrist, cranium, ribs, and pectoral and pelvic girdles), from which there are few significant departures. Instead, our results agree with the hypothesis that many of the differences among mammals can be accounted for by differential postembryonic and postnatal growth (Vogel, 1973; Bard, 1977; Raff, 1996; see Alberch, 1985), a form of allometric heterochrony (see Gould, 1977). The one clear example of sequence heterochrony in mammals, that between eutherians and marsupials, derives largely from the vastly different life history strategies of each group, rather than from different adult body plans. Important sequence heterochronies may typify the evolution of clades such as Cetacea (Richardson and Oelschläger, 2002) or Chiroptera (Adams, 1992) that diverge more sharply from the ancestral body plan. Unfortunately, detailed developmental information for both groups is

lacking. But, available evidence suggests that even the wings of bats are again the result of allometric, rather than sequence heterochrony (Adams, 1992; Raff, 1996; van Leeuwen *et al.*, unpublished data).

The number of timing changes we observed along internal branches within mammals was also proportionately smaller than we found in our more restricted (only 41 events) previous study examining tetrapods (Jeffery *et al.*, 2002a). However, in that study, we reconstructed character evolution using ACCTRAN optimization only; using the consensus of ACCTRAN and DELTRAN optimizations decreases the number of changes to a comparable level (results not shown). The delay in several eye events from Jeffery *et al.* (2002a) was also not apparent here. This was true regardless of the position of *Rattus norvegicus* (as a member of Glires) in the phylogeny, a factor that affected the presence of this putative developmental module in the previous study (see Jeffery *et al.*, 2002a).

A Comparison with Other Tetrapods

Of more interest, perhaps, is the number of heterochronic events in mammals compared to other tetrapods. To address this question, we added the additional mammal species examined herein to the data set of Jeffery *et al.* (2002a) and mapped the 41 events on to a combined tree (results not shown) following the methodology herein. We compared all possible paired combinations of the following three groups for both the total number of internal and terminal changes: mammals, diapsids (Aves + *Lacerta agilis*), and amphibians. The only significant difference was that mammals show fewer changes along internal branches than do amphibians ($t_{14.4} = -3.344$; $p = 0.0041$; $\alpha = 0.05$, corrected for multiple comparisons). This correlates with the diversity of body plans seen in living amphibians together with the well-documented instances of heterochrony within this group (see Duellman and Trueb, 1994). However, although the amphibian and mammalian crown groups are of comparable ages (Wake, 1997; Luo *et al.*, 2001), the possible effect of differences in the timescales of the internal branches between mammals and amphibians (and also the diapsids) was not taken into account.

Mammals (and amniotes in general) may show comparatively fewer heterochronic shifts because the organogenetic period in this group is spent entirely in the protected environment of the amniotic egg, shielding the embryo from diversifying selection. Wolpert (1994) described such embryos as being evolutionarily "privileged" and predicted low phenotypic diversity among amniote embryos as a consequence. In mammals, there is also a direct (biochemical) connection between the mother and embryo during embryonic development, meaning that any heterochronic changes in the embryo may require concomitant changes in the mother. In contrast, because amphibians hatch at a comparatively earlier stage, the juvenile phenotype is more subject to strong diversifying selection.

Although tentatively supported by our results, the privileged embryo hypothesis may not be absolute. Richardson (1999) has pointed out that amniote embryos are only shielded from diversifying selection acting on larval adaptations (i.e., those associated with the free-living stages of many anamniotes). He contended that "privileged" amniote embryos are not shielded necessarily from diversifying selection acting on adult characters. Thus, snake embryos show elongation of the primary axis and acceleration

of somitogenesis even at early embryonic stages when they are protected in the egg (Richardson *et al.*, 1998). These changes are presumably driven by selection for adult body elongation.

Simultaneous Events and Inferring Sequence Heterochrony

Interestingly, most of the species that did not display either a significant clumping of events or a significantly high number of simultaneous event-pairs (*Gallus gallus*, *Suncus murinus*, *Rattus norvegicus*, *Ovis aries*, *Tarsius spectrum*, and *Macaca mulatta*) were "data poor" species. This suggests that there may be a maximum number of events that can reasonably be examined for a given developmental time span. Even examining an increased number of embryos (e.g., as for *Lacerta agilis*, *Spermophilus citellus*, and the Keibel *Homo sapiens* sequence) might not overcome the problems associated with embryo sampling and intraspecific variation, both of which will generate instances of simultaneity (see Bininda-Emonds *et al.*, 2002). Further support for this conjecture is provided by the tetrapod data set of Jeffery *et al.* (2002a), which covers approximately the same developmental time span as the current study but examines fewer events. Only three species out of 14 had more simultaneous event-pairs than expected by chance: *Lacerta agilis*, *Gallus gallus*, *Rattus norvegicus* (results not shown). Interestingly, the latter two species showed no significant differences in the current study, reinforcing the fact that data set quality is highly dependent on the exact events being examined (Wheeler, 1990; Bininda-Emonds *et al.*, 2002). About the same proportion of species ($\sim$50%) for both data sets used significantly fewer stages than expected by chance, suggesting that development events may in fact be slightly clumped in their distribution.

Concluding Thoughts

Most of the mammalian lineages that we examined here are characterized by few or no changes in the sequence of key developmental events. Two exceptions include the eutherian–marsupial split and the lineage leading to the common ancestor of Euarchonta. For the former, many of the sequence heterochronies that we identify agree with historical and more recent observations; however, other instances of heterochrony are also apparent. Compared to other tetrapods, mammals display an equal number of sequence heterochronies as do other amniotes, but significantly fewer than amphibians. Therefore, we suggest that sequence heterochrony has not been a key factor contributing to mammalian diversity in general, although it may still play an important role in the origin of some lineages that we could not examine in detail (e.g. Cetacea or Chiroptera). For instance, Richardson and Oelschläger (2002) provide evidence that the cetacean flipper with its characteristic hyperphalangy may have evolved, at least in part, through delayed offset of outgrowth in the embryonic limb bud.

It is surprising that the extraordinary diversification of adult mammals has not forced significant changes on early developmental stages. One explanation is that mammals are subject to special developmental constraints not present in other vertebrate clades (e.g., Galis, 1999). If this is the case, then the adult diversity of mammals may be explained more readily by changes in postembryonic growth (allometric heterochrony). Further work is required to examine these ideas.

ACKNOWLEDGMENTS

We thank Marcelo Sánchez-Villagra for kindly providing a preprint of his paper and for his comments on this paper. We also thank Mark Springer and an anonymous referee for their helpful comments. The programs used in this paper are available for download at http://www.tierzucht.tum.de/Bininda-Emonds or on request from the first author. Funding for this project was provided by the van der Leeuw Fonds (OBE and MKR) and the Leverhulme Trust (JEJ).

LITERATURE CITED

Adams, R. A. (1992). Stages of development and sequence of bone formation in the little brown bat, *Myotis lucifigus. J. Mammal.* **73:** 160–167.

Alberch, P. (1985). Problems with the interpretation of developmental sequences. *Syst. Zool.* **34:** 46–58.

Alroy, J. (1999). The fossil record of North American mammals: Evidence for a Paleocene evolutionary radiation. *Syst. Biol.* **48:** 107–118.

Amrine-Madsen, H., Koepfli, K. P., Wayne, R. K., and Springer, M. S. (2003). A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. *Mol. Phylogenet. Evol.* **28:** 225–240.

Bard, J. B. L. (1977). A unity underlying the different zebra striping patterns. *J. Zool.* **183:** 527–539.

Bininda-Emonds, O. R. P., Jeffery, J. E., Coates, M. I., and Richardson, M. K. (2002). From Haeckel to event-pairing: The evolution of developmental sequences. *Theory Biosci.* **121:** 297–320.

Bryden, M. M., Evans, H. E., and Binns, W. (1972). Embryology of the sheep. I. Extraembryonic membranes and the development of body form. *J. Morphol.* **138:** 169–186.

Corneli, P. S. (2002). Complete mitochondrial genomes and eutherian evolution. *J. Mammal. Evol.* **9:** 281–305.

de Jong, W. W., van Dijk, M. A., Poux, C., Kappe, G., van Rheede, T., and Madsen, O. (2003). Indels in protein-coding sequences of Euarchontoglires constrain the rooting of the eutherian tree. *Mol. Phylogenet. Evol.* **28:** 328–340.

de Lange, D., Jr., and Nierstrasz, H. F. (1932). *Tabellarische Übersicht der Entwicklung von Tupaia javanica Horsf*, A. Oosthoek Verlag A.G., Utrecht.

Duellman, W. E., and Trueb, L. (1994). *Biology of Amphibians*, Johns Hopkins University Press, Baltimore.

Galis, F. (1999). Why do almost all mammals have seven cervical vertebrae? Developmental constraints, Hox genes, and cancer. *J. Exp. Zool.* **285:** 19–26.

Gould, S. J. (1977). *Ontogeny and Phylogeny*, Belknap Press, Cambridge, MA.

Gould, S. J. (1982). Change in developmental timing as a mechanism of macroevolution. In: *Evolution and Development*, J. T. Bonner, ed., pp. 333–346, Springer-Verlag, New York.

Gribnau, A. A. M., and Geijsberts, L. G. M. (1981). *Developmental Stages in the Rhesus Monkey (Macaca mulatta)*, Springer-Verlag, Berlin.

Harman, M. T., and Prickett, M. (1932). The development of the external form of the guinea-pig (*Cavia cobaya*) between the ages of eleven days and twenty days of gestation. *Amer. J. Anat.* **49:** 351–378.

Harman, M. T., and Prickett Dobrovolny, M. (1933). The development of the external form of the guinea-pig (*Cavia cobaya*) between the ages of 21 days of and 35 days of gestation. *J. Morphol.* **54:** 493–519.

Heape, W. (1883a). The development of the mole (*Talpa europea*). The formation of the germinal layers and early development of the meduallry groove and notochord. *Q. J. Microscop. Sci.* **23:** 412–452.

Heape, W. (1883b). The development of the mole (*Talpa europea*). Stage E to *J. Q. J. Microscop. Sci.* **26:** 123–163.

Henneberg, B. (1937). *Normentafel zur Entwicklungsgeschichte der Wanderratte (Rattus norvegicus Erxleben*), Verlag von Gustav Fischer, Jena.

Hubrecht, A. A. W., and Keibel, F. (1907). *Normentafel zur Entwicklungsgeschichte des Koboldmaki (Tarsius spectrum) und des Plumplori (Nycticebus tardigradus)*, Verlag von Gustav Fischer, Jena.

Huisman, F. J., and de Lange, D., Jr. (1937). *Tabellarische Übersicht der Entwicklung von Manis javanica Desm*, A. Oosthoek Verlag A.G., Utrecht.

Jacobfeuerborn, H. (1908). Die intrauterine Ausbildung der äußeren Körperform des Igels (*Erinaceus europaeus* L.) mit Berücksichtigung der Entwicklung der wichtigeren inneren Organe. *Z. Wiss. Zool.* **91:** 382–420.

Jeffery, J. E., Bininda-Emonds, O. R. P., Coates, M. I., and Richardson, M. K. (2002a). Analyzing evolutionary patterns in vertebrate embryonic development. *Evol. Dev.* **4:** 292–302.

Jeffery, J. E., Richardson, M. K., Coates, M. I., and Bininda-Emonds, O. R. P. (2002b). Analyzing developmental sequences within a phylogenetic framework. *Syst. Biol.* **51:** 478–491.

Jerison, H. J. (1973). *Evolution of the Brain and Intelligence*, Academic Press, New York.
Keibel, F. (1897). *Normentafel zur Entwicklungsgeschichte des Schweines (Sus scrofa domesticus)*, Verlag von Gustav Fischer, Jena.
Keibel, F., ed. (1897–1938). *Normentafeln zur Entwicklungsgeschichte der Wirbelthiere*, Verlag von Gustav Fischer, Jena.
Keibel, F., and Abraham, K. (1900). *Normentafel zur Entwicklungsgeschichte des Huhnes (Gallus domesticus)*, Verlag von Gustav Fischer, Jena.
Keibel, F., and Elze, C. (1908). *Normentafel zur Entwicklungsgeschichte des Menschen*, Verlag von Gustav Fischer, Jena.
Langman, J. (2000). *Langman's Medical Embryology*, 8th ed., Lippincott Williams & Wilkins, Philadelphia.
Liu, F.-G. R., Miyamoto, M. M., Freire, N. P., Ong, P. Q., Tennant, M. R., Young, T. S., and Gugel, K. F. (2001). Molecular and morphological supertrees for eutherian (placental) mammals. *Science* **291:** 1786–1789.
Luo, Z.-X., Crompton, A. W., and Sun, A.-L. (2001). A new mammaliform from the Early Jurassic and evolution of mammalian characteristics. *Science* **292:** 1535–1540.
Mabee, P. M., and Trendler, T. A. (1996). Development of the cranium and paired fins in *Betta splendens* (Teleosti: Percomorpha): Intraspecific variation and interspecific comparisons. *J. Morphol.* **227:** 249–287.
Madsen, O., Scally, M., Douady, C. J., Kao, D. J., DeBry, R. W., Adkins, R., Amrine, H. M., Stanhope, M. J., de Jong, W. W., and Springer, M. S. (2001). Parallel adaptive radiations in two major clades of placental mammals. *Nature* **409:** 610–614.
McCrady, E., Jr. (1938). *The Embryology of the Opossum*, The Wistar Institute of Anatomy and Biology, Philadelphia.
McKinney, M. L., and McNamara, K. J. (1991). *Heterochrony: The Evolution of Ontogeny*, Plenum Press, New York.
Minot, C. S., and Taylor, E. (1905). *Normal Plates of the Development of the Rabbit (Lepus cuniculus L.*) Verlag von Gustav Fischer, Jena.
Murphy, W. J., Eizirik, E., Johnson, W. E., Zhang, Y. P., Ryder, O. A., and O'Brien, S. J. (2001a). Molecular phylogenetics and the origins of placental mammals. *Nature* **409:** 614–618.
Murphy, W. J., Eizirik, E., O'Brien, S. J., Madsen, O., Scally, M., Douady, C. J., Teeling, E., Ryder, O. A., Stanhope, M. J., de Jong, W. W., and Springer, M. S. (2001b). Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* **294:** 2348–2351.
Nowak, R. M. (1999). *Walker's Mammals of the World*, 6th ed., The John Hopkins University Press, Baltimore.
Nunn, C. L., and Smith, K. K. (1998). Statistical analyses of developmental sequences: The craniofacial region in marsupial and placental mammals. *Amer. Nat.* **152:** 82–101.
O'Rahilly, R., and Müller, F. (1987). *Developmental Stages in Human Embryos*, Carnegie Institute of Washington, Meriden, CT.
Peter, K. (1904). *Normentafel zur Entwicklungsgeschichte der Zauneidechse (Lacerta agilis)*, Verlag von Gustav Fischer, Jena.
Raff, R. A. (1996). *The Shape of Life: Genes, Development, and the Evolution of Animal From*, University of Chicago Press, Chicago.
Richardson, M. K. (1999). Vertebrate evolution: The developmental origins of adult variation. *BioEssays* **21:** 604–613.
Richardson, M. K., Allen, S. P., Wright, G. M., Raynaud, A., and Hanken, J. (1998). Somite number and vertebrate evolution. *Development* **125:** 151–160.
Richardson, M. K., and Oelschläger, H. A. (2002). Time, pattern and heterochrony: A study of hyperphalangy in the dolphin embryo flipper. *Evol. Dev.* **4:** 435–444.
Sakurai, T. (1906). *Normentafel zur Entwicklungsgeschichte des Rehes (Cervus capreolus)*, Verlag von Gustav Fischer, Jena.
Sánchez-Villagra, M. R. (2002). Comparative patterns of postcranial ontogeny in therian mammals: an analysis of relative timing of ossification events. *J. Exp. Zool. (Mol. Dev. Evol.)* **294:** 264–273.
Sanderson, M. J., and Donoghue, M. J. (1989). Patterns of variation in levels of homoplasy. *Evolution* **43:** 1781–1795.
Scott, J. P. (1937). The embryology of the guinea pig I. A table of normal development. *Amer. J. Anat.* **60:** 397–432.
Shubin, N. H., and Alberch, P. (1986). A morphogenetic approach to the origin and basic organisation of the tetrapod limb. In: *Evolutionary Biology*, M. K. Hecht, B. Wallace, and G. I. Prance, eds., pp. 319–387, Plenum Press, New York.
Smith, K. K. (1996). Integration of craniofacial structures during development in mammals. *Amer. Zool.* **36:** 70–79.
Smith, K. K. (1997). Comparative patterns of craniofacial development in eutherian and metatherian mammals. *Evolution* **51:** 1663–1678.
Springer, M. S., Murphy, W. J., Eizirik, E., and O'Brien, S. J. (2003). Placental mammal diversification and the Cretaceous-Tertiary boundary. *Proc. Natl. Acad. Sci. U.S.A.* **100:** 1056–1061.

Sterba, O., Klima, M., and Schildger, B. (2000). *Embryology of Dolphins*, Springer, Berlin.
Swofford, D. L., and Maddison, W. P. (1987). Reconstructing ancestral character states under Wagner parsimony. *Math. Biosci.* **87:** 199–229.
Theiler, K. (1989). *The House Mouse: Atlas of Embryonic Development*, Springer Verlag, New York.
Thomas, J. W., *et al.* (70 other authors). (2003). Comparative analysis of multi-species sequences from targeted genomic regions. *Nature* **424:** 788–793.
Velhagen, W. A. (1997). Analyzing developmental sequences using sequence units. *Syst. Biol.* **46:** 204–210.
Vogel, P. (1973). Vergleichende Untersuchung zum Ontogenesemodus einheimischer Soriciden (*Crocidura russula, Sorex araneus und Neomys fodiens). Rev. Suisse Zool.* **79:** 1201–1332.
Völker-Brünn, O. (1922). *Normentafel zur Entwicklungsgeschichte des Ziesels (Spermophilus citillus)*, Verlag von Gustav Fischer, Jena.
Waddell, P. J., Okada, N., and Hasegawa, M. (1999). Towards resolving the interordinal relationships of placental mammals. *Syst. Biol.* **48:** 1–5.
Wagner, G. P. (1996). Homologues, natural kinds and the evolution of modularity. *Amer. Zool.* **36:** 36–43.
Wake, M. H. (1997). Amphibian locomotion in evolutionary time. *Zoology* **100:** 141–151.
Wanek, N., Muneoka, K., Holler-Dinsmore, G., Burton, R., and Bryant, S. V. (1989). A staging system for mouse limb development. *J. Exp. Zool.* **249:** 41–49.
Wheeler, Q. D. (1990). Ontogeny and character phylogeny. *Cladistics* **6:** 225–268.
Wilson, D. E., and Reeder, D. M., eds. (1993). *Mammal Species of the World: Ataxonomic and Geographic Reference*, Smithsonian Institution Press, Washington.
Wolpert, L. (1994). The evolutionary origin of development: Cycles, patterning, privilege and continuity. *Development Supplement* 79–84.
Yasui, K. (1992). Embryonic development of the house shrew (*Suncus murinus*). I. Embryos at stages 9 and 10 with 1 to 12 pairs of somites. *Anat. Embryol.* **186:** 49–65.
Yasui, K. (1993). Embryonic development of the house shrew (*Suncus murinus*). II. Embryos at stages 11 and 12 with 13 to 29 pairs of somites, showing limb bud formation and closed cephalic neural tube. *Anat. Embryol.* **187:** 45–65.

APPENDIX 1

List of Developmental Events Examined in This Study[a]

No.	System	Subsystem	Event
1*	External	Somites	1 post-otic somite pair
2			4 post-otic somite pairs
3			13 post-otic somite pairs
4			23 post-otic somite pairs
5		Miscellaneous	Mesencephalic (cephalic) flexure first indicated
6			Externally visible tailbud beginning (all three germ layers need not be present)
7*	Cardiovascular	Heart	Endocardial anlage
8*			Endocardial tubes start to fuse
9*			Heart becoming bent or S-shaped (endocardial tubes no longer straight)
10			Interventricular septum just beginning
11*			Trabeculae carneae in ventricles of heart
12*			Atrioventricular canal indicated by constriction
13*			Endocardial cushions of atrioventricular canal just beginning
14*			Septum primum of atrium just beginning
15			Septum primum broken through (foramen ovale/secundum)
16*			Proximal outflow tract cushions appearing
17			Truncus arteriosus initially divided (= initial fusion of distal bulbar cushions)
18*			Semilunar (aortic and pulmonary) valves first appearing
19*		Associated vessels	1st aortic arch formed
20			2nd aortic arch (hyoidean) formed
21*	Eye	Optic vesicle	(Primary) optic vesicle beginning as distinct lateral evagination from neural tube (not sulcus opticus)
22*			(Primary) optic vesicle starts to invaginate to form optic cup (secondary optic vesicle)
23			Optic stalk beginning to be invaded by retinal nerve fibres
24*			Retinal pigmentation beginning
25*		Lens	Lens placode appears
26*			Lens placode depressed (formation of optic pit)
27*			Lens vesicle pinches off from surface ectoderm
28		External	Eyelid anlage appears
29*	Ear	Otic placode	Otic placode appears
30*			Otic placode depressed (formation of otic pit)
31*			Otocyst closed but still connected with surface ectoderm
32*			Otocyst detached from ectoderm
33*			Endolymphatic appendage appears
34			Auricular tubercles (hillocks) become distinct
35			Anlage of utricle
36			Anlage of cochlea
37		Semicircular canals	Superior (vertical or anterior) semicircular canal pinched-off as a tube
38		Ossicles	Malleus appears as mesenchymal condensation
39	Facial region	Oropharyngeal membrane	Oropharyngeal (buccopharyngeal/oral) membrane formed

APPENDIX 1 Continued

No.	System	Subsystem	Event
40			Oropharyngeal membrane becomes perforated
41*		Nasal pits	Nasal placodes appear as ectodermal thickenings
42*			Nasal placodes depressed (formation of olfactory pit)
43			Primitive choanae (posterior nares) open
44			Closure of nostrils by epitrichial plugs
45			Jacobson's organ beginning as a diverticulum
46			Conchae (turbinates) developing
47			Anlage of nasolacrimal duct (as maxillary and lateral nasal processes start to fuse)
48*		Miscellaneous	Anlage of hypophysis (Rathke's pouch)
49			Lateral palatine processes appearing on either side of tongue
50			Dental lamina forming
51			Anlagen of tooth germs appearing in dental lamina
52			Parotid gland first indicated
53*	Pharynx	Thyroid	Thyroid (or endostyle) depression appears in floor of pharynx
54			Anlage of ultimobranchial bodies (of pharyngeal pouch V) (lateral thyroid; parafollicular cells of thyroid)
55		Pharyngeal pouches	1st visceral pouch contacts ectoderm
56*			2nd visceral pouch contacts ectoderm (formation of hyoid arch)
57*			3rd visceral pouch contacts ectoderm (formation of first branchial arch)
58			Cervical sinus formed
59			Anlage of thymus (of pharyngeal pouch III)
60		Visceral skeleton	Meckel's cartilage cartilaginous
61			Mandible beginning to ossify
62*	Urogenital	Wolffian (mesonephric) duct	Wolffian duct appears as a thickening with no lumen
63*			Wolffian ducts open into cloaca
64		Kidneys	Mesonephric (Wolffian) tubules appearing, but still solid
65			Ureteric bud just forming from Wolffian duct
66			Anlagen of metanephric tubules
67		Gonad	Anlage of gonad (gonadal ridge) appears as a thickening of the coelomic epithelium
68*		Mullerian (paramesonephric) duct	Anlagen of Mullerian ducts appear
69			Mullerian ducts fusing caudally
70		Cloaca	Cloacal partition just completed
71		External genitalia	Genital tubercle appears
72*	Intestinal tract	Intestine	Anterior intestinal portal beginning as a diverticulum
73			Postanal (tail-) gut no longer present
74			(Future) cloacal membrane becomes distinct at the caudal end of primitive streak
75			Cloacal membrane breaks through (perforated)
76*		Associated glands	Liver diverticulum appears
77*			Liver cords forming
78*			Gall bladder beginning as a diverticulum
79*			Dorsal pancreas beginning as a diverticulum
80*			Ventral pancreas beginning as a diverticulum

APPENDIX 1 Continued

No.	System	Subsystem	Event
81*			Spleen anlage beginning as thickening of peritoneal epithelium and/or mesenchymal proliferation
82*	Nervous	Neural tube	Neural folds first beginning to fuse
83			Anterior neuropore closed
84			Posterior neuropore closed
85		Brain	Rhombomeres start to appear
86			Cerebral hemisphere anlagen of telencephalon just beginning (as lateral expansions
87			Anlage of optic chiasma
88		Miscellaneous	Trigeminal ganglion (of cranial nerve V) becomes distinct as cellular aggregate
89			Infundibular groove (not recess) forms
90			Anlage of epiphysis
91			Anlage of adrenal cortex (interrenal gland) as mesodermal proliferation from peritoneum
92	Coelom	Coelom	Pericardial coelom beginning
93	Respiratory	Respiratory	Laryngotracheal (part of the median pharyngeal) groove indicated
94*			Lung buds as distinct evaginations
95*	Limbs	Forelimbs	Wanek *et al.* (1989) stage 1 (limb bud first visible)
96			Wanek *et al.* (1989) stage 6 (constricted wrist/dorsoventrally flattened hand plate)
97			Wanek *et al.* (1989) stage 8 (initial indentations between digits)
98			Scapula cartilaginous
99			Humerus chondrification beginning
100		Hind limbs	Wanek *et al.* (1989) stage 1 (limb bud first visible)
101			Wanek *et al.* (1989) stage 6 (constricted wrist/dorsoventrally flattened hand plate)
102			Wanek *et al.* (1989) stage 8 (initial indentations between digits)
103	Extraembryonic membranes	Allantois	Allantoic diverticulum beginning (mesodermal and endodermal components)
104			Expansion of allantois (anlage of bladder)
105		Amnion	Amniopore closed (=amnion complete)
106		Umbilical cord	Umbilical hernia beginning
107			Umbilical hernia totally reduced
108	Integument	Mammary glands	Milk line appears
109			Anlagen of mammary glands (papillae) appear
110		Hair	Hair papilla appears over eye
111		Claws	Anlagen of claws
112	Axial skeleton	Skull	Petrosal bone (periotic capsule) as mesenchymal condensation
113		Vertebrae	Vertebral bodies are mesenchymal condensations around notochord
114		Notochord	Primitive streak no longer present
115			Notochord starts separating from alimentary endoderm
116		Ribs	Some ribs are appearing as mesenchymal condensations

[a] Events are subdivided according to organ system and subsystem. Events marked with an asterisk were also used by Jeffery *et al.* (2002a).

APPENDIX 2

Developmental Timing Information[a]

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Lacerta agilis	35	40	53	65	?	60	47	?	49	?	84	49	85	85	118
Gallus gallus	4	9	23	38	?	43	10	20	23.5	59.6	?	?	59.6	52	69.6
Didelphis virginiana	?	23	25	?	26	29	?	25	26	30	?	?	30	30	32
Erinaceus europaeus	?	1	?	?	4	9	1	2	8	?	?	?	?	?	?
Suncus murinus	9	10.3	11.3	12.3	9	?	?	9	10.3	12.6	10.3	?	12.3	12.6	?
Talpa europaea	5	?	?	?	8	?	6	8	?	?	?	?	?	?	?
Cavia porcellus (Harman)	14	14	?	16	16	?	15	15	15	?	?	?	?	?	?
Cavia porcellus (Scott)	?	?	15.5	16.5	?	16.5	14.5	?	15.5	?	?	?	?	18.5	19.7
Spermophilus citellus	?	35	56	76	35	68	25	36	49	84	48	57	70	53	107
Mus musculus	12	12	14	15	?	16	12	13	16	?	?	17	15	19	19
Rattus norvegicus	27	31	52	57	46	54	26	35	37	62	43	41	70	68	71
Oryctolagus cuniculus	?	?	?	9	5	?	?	6	6	15	11	13	15	12	?
Manis javanica	?	1	4	?	3	9	3	6	?	?	?	?	?	?	?
Delphinidae	?	?	?	?	?	?	?	?	?	?	4	?	?	?	?
Capreolus capreolus	?	10	?	21	20	21	11	?	13	22	23	21	23	22	36
Sus scrofa	?	15	43	?	?	53	1	29	32	62	55	54	62	60	69
Ovis aries	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tupaia javanica	?	?	4	7	?	6	?	4	6	15	7	8	?	12	15
Tarsius spectrum	?	?	?	11	2	4	?	2	5	17	13	?	?	17	20
Loris tardigradus	?	1	?	?	?	4	?	?	2	6	4	4	?	6	8
Homo sapiens (Keibel)	?	?	6	7	5	6	?	4	4	12	10	10	22	11	25
Homo sapiens (O'Rahilly)	?	10	11	12	9	?	?	10	10	11	11	12	13	12	15
Macaca mulatta	?	?	?	?	13	?	?	?	?	?	?	?	?	?	?

APPENDIX 2 Continued

Species	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Lacerta agilis	85	115	122	51	64	42	61	103	91	56	63	73	112	49	51
Gallus gallus	59.6	78	?	37.5	39.5	13	32.5	?	64	31	37	47.5	?	18	19
Didelphis virginiana	?	31	34	25	24	24	30	30	35	28	29	30	32	24	25
Erinaceus europaeus	?	?	?	?	?	1	?	?	?	?	15	?	24	?	1.27
Suncus murinus	?	?	?	9	10.6	10.9	12.9	?	?	11.9	12.6	?	?	10.6	10.9
Talpa europaea	?	?	?	8	?	6	?	?	?	?	?	?	?	8	10
Cavia porcellus (Harman)	?	?	?	?	?	15	16	?	19	?	16	?	24	16	16
Cavia porcellus (Scott)	?	21.6	23.7	15.5	15.5	?	18.5	?	?	17.5	18.5	19.7	23.7	?	?
Spermophilus citellus	77	?	123	33	51	49	85	118	109	79	90	107	120	49	49
Mus musculus	18	21	21	12	15	13	16	?	19	14	16	19	22	12	14
Rattus norvegicus	79	86	110	29	43	37	65	105	?	57	64	84	94	40	40
Oryctolagus cuniculus	?	16	20	4	9	2	13	19	15	11	12	14	19	5	7
Manis javanica	?	?	?	5	5	4	12	18	17	10	11	15	18	5	8
Delphinidae	?	?	6	?	?	?	?	?	?	?	?	?	5	?	?
Capreolus capreolus	28	39	39	13	14	15	31	50	39	32	34	41	52	17	19
Sus scrofa	62	77	?	?	?	30	68	?	74	67	69	84	91	30	39
Ovis aries	?	?	?	?	?	?	?	?	?	19	?	?	26	17	19
Tupaia javanica	17	14	19	?	?	4	12	20	14	?	12	?	20	?	3
Tarsius spectrum	27	19	28	2	2	4	16	29	20	14	16	19	28	5	6
Loris tardigradus	?	?	9	?	?	2	7	?	?	4	7	?	?	?	2
Homo sapiens (Keibel)	33	28	57	3	7	6	13	61	28	10	13	33	66	4	6
Homo sapiens (O'Rahilly)	13	17	16	9	10	11	14	19	15	13	14	15	18	9	10
Macaca mulatta	?	?	?	?	?	?	21	15	13	13	16	19	10	?	

APPENDIX 2 Continued

Species	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Lacerta agilis	71	79	72	110	?	101	112	?	?	64	49	63	111	112	91
Gallus gallus	46	?	53	?	?	?	79.5	?	31	46	42	46	76	78.5	?
Didelphis virginiana	28	28	29	31	35	?	32	?	24	29	?	28	31	?	33
Erinaceus europaeus	?	?	?	18	?	?	?	?	?	?	?	14	?	?	?
Suncus murinus	11.9	12.9	12.9	?	?	?	?	?	10.3	10.9	11.3	12.9	?	?	?
Talpa europaea	?	?	?	?	?	?	?	?	10	?	?	?	?	?	?
Cavia porcellus (Harman)	17	17	18	21	?	?	?	?	?	?	16	16	?	?	?
Cavia porcellus (Scott)	?	15.5	19.1	?	19.1	19.7	26.1	23.7	14.5	?	16.5	17.5	23.7	26.1	21.6
Spermophilus citellus	80	?	80	109	127	108	118	117	35	68	75	85	120	128	104
Mus musculus	15	15	16	19	19	20	?	?	11	14	14	16	21	?	19
Rattus norvegicus	60	69	66	69	92	82	103	96	29	42	62	68	99	118	80
Oryctolagus cuniculus	9	11	12	15	18	?	19	?	?	9	11	13	19	20	14
Manis javanica	9	14	11	?	17	16	?	19	4	9	9	11	16	18	17
Delphinidae	3	?	4	?	?	?	?	?	?	?	?	3	?	7	?
Capreolus capreolus	24	29	32	39	52	36	48	47	14	22	27	35	47	52	43
Sus scrofa	63	69	70	77	74	86	85	?	46	64	64	69	87	94	76
Ovis aries	?	20	?	25	?	?	?	?	?	?	22	22	?	?	?
Tupaia javanica	8	10	12	15	14	15	?	19	6	8	7	12	18	21	14
Tarsius spectrum	15	?	14	22	?	27	?	28	3	9	12	18	26	32	20
Loris tardigradus	5	6	5	9	?	9	?	?	?	5	5	7	?	9	8
Homo sapiens (Keibel)	8	10	10	49	37	27	56	61	3	7	10	24	59	67	36
Homo sapiens (O'Rahilly)	13	13	13	16	?	14	18	17	9	11	11	14	18	?	18
Macaca mulatta	?	13	13	15	18	17	18	?	?	?	13	14	19	22	18

APPENDIX 2 Continued

Species	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Lacerta agilis	108	114	63	109	111	?	?	62	?	49	55	64	104	107	?
Gallus gallus	75	75	32.5	?	?	?	?	43	54	26	30	43	?	71.5	81
Didelphis virginiana	32	28	?	33	35	?	?	27	?	24	24	28	?	?	?
Erinaceus europaeus	?	15	?	?	?	?	?	?	?	?	3.31	9	13	?	?
Suncus murinus	?	?	12.6	?	?	?	?	9	?	10.3	10.9	11.9	12.3	11.9	?
Talpa europaea	?	?	?	?	?	?	?	?	?	10	?	?	?	?	?
Cavia porcellus (Harman)	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Cavia porcellus (Scott)	?	23.7	15.5	?	?	20.7	?	16.5	?	16.5	16.5	16.5	15.5	?	?
Spermophilus citellus	110	113	65	120	120	125	120	78	113	49	54	69	80	109	120
Mus musculus	?	?	15	21	20	22	?	13	20	13	?	?	16	19	22
Rattus norvegicus	82	68	55	93	94	122	99	36	69	42	54	64	68	80	107
Oryctolagus cuniculus	21	20	9	20	21	22	19	9	13	5	7	9	11	12	21
Manis javanica	18	?	9	18	?	?	19	8	?	?	?	?	?	9	20
Delphinidae	?	4	?	?	4	7	?	?	?	?	?	?	3	?	?
Capreolus capreolus	?	39	26	50	50	57	50	20	39	17	20	23	28	39	54
Sus scrofa	84	81	59.3	93	90	93	?	62	68	30	43	60	68	77	?
Ovis aries	?	26	?	?	?	?	?	?	?	?	17	18	21	?	?
Tupaia javanica	18	17	9	18	18	20	20	7	16	5	6	8	14	16	20
Tarsisu spectrum	29	22	13	27	27	31	28	11	17	5	7	11	20	20	?
Loris tardigradus	9	8	4	?	?	9	9	5	5	2	2	3	7	7	?
Homo sapiens (Keibel)	55	26	6	56	61	73	53	7	19	6	6	7	21	19	58
Homo sapiens (O'Rahilly)	?	16	10	17	?	?	?	10	12	?	11	12	12	13	?
Macaca mulatta	22	16	12	17	22	23	?	?	?	?	?	?	?	?	19

APPENDIX 2 Continued

Species	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Lacerta agilis	?	49	77	49	101	120	94	108	?	?	108	38	118	118	121
Gallus gallus	?	18	58	30	67.6	79.5	47.6	76.3	?	?	75	4	67.3	?	?
Didelphis virginiana	33	24	30	?	31	35	30	107	?	34	?	24	34	?	34
Erinaceus europaeus	?	9	?	?	?	?	?	?	?	?	23	1	?	?	?
Suncus murinus	?	11.6	?	12.3	?	?	?	?	?	?	?	?	?	11.3	?
Talpa europaea	?	?	?	?	?	?	?	?	?	?	?	4	?	8	?
Cavia porcellus (Harman)	?	?	?	?	?	?	?	?	?	?	?	14	?	?	?
Cavia porcellus (Scott)	23.7	16.5	19.1	16.5	19.1	?	20.7	23.7	?	?	23.7	14.5	?	?	?
Spermophilus citellus	123	49	86	48	92	110	108	114	134	123	111	34	119	35	?
Mus musculus	23	14	22	16	17	21	17	21	?	21	?	11	?	14	?
Rattus norvegicus	120	42	69	55	73	115	72	101	122	111	79	28	85	35	109
Oryctolagus cuniculus	20	3	13	9	13	18	17	19	24	19	17	3	18	3	19
Manis javanica	?	4	9	8	13	18	11	18	20	15	13	2	14	9	18
Delphinidae	6	?	4	?	4	8	4	7	?	?	5	?	?	?	?
Capreolus capreolus	55	11	20	20	27	46	39	45	57	52	39	9	?	9	57
Sus scrofa	?	23	65	32	69	90	75	80	?	90	75	2	84	1	90
Ovis aries	?	?	?	?	?	?	?	?	?	?	26	?	?	?	?
Tupaia javanica	21	5	?	7	15	20	14	20	24	19	16	1	19	4	20
Tarsius spectrum	31	4	14	7	18	31	18	22	33	29	22	1	27	1	31
Loris tardigradus	9	2	5	2	5	9	6	8	9	8	8	2	8	?	?
Homo sapiens (Keibel)	69	?	15	6	14	61	27	45	79	61	35	2	46	1	63
Homo sapiens (O'Rahilly)	18	11	13	11	14	?	14	16	23	?	?	9	?	6.5	18
Macaca mulatta	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

APPENDIX 2 Continued

Species	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
Lacerta agilis	61	71	77	65	86	110	40	61	45	56	74	108	?	?	74
Gallus gallus	37	46	66.3	46	47	67.6	13	23.5	34	28	46	76.3	?	?	46
Didelphis virginiana	28	30	30	29	30	33	25	28	30	24	?	?	24	24	?
Erinaceus europaeus	9	?	?	?	?	?	?	6	?	?	9	?	11	?	?
Suncus murinus	10.9	?	11.9	12.6	12.9	?	10.6	11.9	?	10.3	11.9	?	10.9	12.9	?
Talpa europaea	?	?	?	?	?	?	7	10	?	?	?	?	?	10	?
Cavia porcellus (Harman)	?	?	?	?	?	?	15	?	?	?	18	?	?	?	?
Cavia porcellus (Scott)	16.5	?	19.7	17.5	17.5	?	14.5	15.5	16.5	15.5	17.5	?	15.5	18.5	26.1
Spermophilus citellus	55	69	70	77	?	107	30	69	70	48	79	120	50	92	113
Mus musculus	14	15	15	15	15	18	12	14	16	12	17	?	16	18	20
Rattus norvegicus	42	53	?	54	69	?	37	51	60	42	65	89	42	69	98
Oryctolagus cuniculus	9	11	13	12	13	18	4	6	9	?	12	?	6	14	18
Manis javanica	9	15	12	12	13	16	3	9	9	11	12	18	?	?	18
Delphinidae	?	?	?	4	4	?	?	?	3	?	3	7	?	?	7
Capreolus capreolus	16	20	?	22	22	40	12	22	22	29	38	50	28	39	40
Sus scrofa	56	62	65	64	64	81	28	59	59.6	?	75	?	64	77	?
Ovis aries	?	?	?	?	?	?	16	18	20	?	?	?	?	?	?
Tupaia javanica	6	8	8	9	9	12	4	7	7	10	12	20	?	14	20
Tarsius spectrum	7	11	12	14	16	?	2	8	14	9	13	31	4	20	30
Loris tardigradus	3	6	5	5	5	7	1	4	5	5	5	9	?	4	9
Homo sapiens (Keibel)	6	7	9	11	11	21	4	7	11	6	16	74	7	13	25
Homo sapiens (O'Rahilly)	12	12	12	12	14	13	10	11	12	9	15	10	10	16	15
Macaca mulatta	?	?	?	?	?	?	?	11	12	13	14	20	?	14	17

APPENDIX 2 Continued

Species	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
Lacerta agilis	92	44	83	85	74	105	118	?	120	74	105	118	?	55	53
Gallus gallus	79	?	?	46	44	77	79	81	79	44	73	76	?	54.6	51.5
Didelphis virginiana	32	25	25	27	26	?	32	?	?	29	33	?	28	31	29
Erinaceus europaeus	?	1	?	?	9	18	24	?	?	13	23	24	10	15	13.37
Suncus murinus	?	?	9	12.6	12.3	?	?	?	?	11.6	?	?	?	?	?
Talpa europaea	?	10	?	?	?	?	?	?	?	?	?	?	6	?	8
Cavia porcellus (Harman)	?	?	?	?	18	?	24	?	?	18	?	?	?	?	?
Cavia porcellus (Scott)	23.7	15.5	?	?	16.5	20.7	23.7	?	?	18.5	21.6	23.7	12.7	26.1	?
Spermophilus citellus	99	31	65	77	71	111	118	120	120	86	118	125	49	128	48
Mus musculus	20	12	14	16	15	19	21	?	21	16	20	21	12	?	11
Rattus norvegicus	105	24	55	60	53	83	96	?	?	63	96	104	?	84	22
Oryctolagus cuniculus	20	2	9	12	9	15	19	20	19	9	17	19	7	20	9
Manis javanica	19	2	9	9	9	15	?	?	?	9	16	19	?	19	9
Delphinidae	?	?	?	?	?	4	?	5	5	?	?	?	?	?	?
Capreolus capreolus	53	9	?	23	23	?	54	54	54	24	?	54	10	18	9
Sus scrofa	89	1	62	64	60	85	91	?	?	64	87	92	15	43	34
Ovis aries	?	?	?	?	20	24	27	?	?	20	24	29	?	?	15
Tupaia javanica	8	1	8	9	8	16	19	20	18	11	18	19	?	?	?
Tarsius spectrum	14	?	13	14	11	24	28	27	22	11	24	30	?	17	7
Loris tardigradus	5	1	?	5	5	8	?	9	9	5	8	?	2	?	5
Homo sapiens (Keibel)	28	2	7	7	10	34	49	56	44	11	45	65	?	10	1
Homo sapiens (O'Rahilly)	?	9	10	12	13	15	17	?	17	13	17	19	7	15	6
Macaca mulatta	?	?	?	?	13	16	19	?	?	14	18	20	?	?	?

APPENDIX 2 Continued

Species	106	107	108	109	110	111	112	113	114	115	116
Lacerta agilis	?	?	?	?	?	121	113	94	?	31	?
Gallus gallus	?	?	?	?	?	?	?	72	44	5	?
Didelphis virginiana	?	34	34	34	?	34	31	32	30	23	32
Erinaceus europaeus	24	?	19	21.39	23	?	?	?	?	?	?
Suncus murinus	?	?	?	?	?	?	?	?	?	10.3	?
Talpa europaea	?	?	?	?	?	?	?	?	?	7	?
Cavia porcellus (Harman)	21	?	24	27	24	31	?	?	?	?	?
Cavia porcellus (Scott)	19.7	?	21.6	?	23.7	?	?	18.5	?	14.5	?
Spermophilus citellus	107	133	109	114	123	130	107	106	?	47	111
Mus musculus	17	25	?	?	21	?	20	19	?	?	?
Rattus norvegicus	69	125	57	93	101	125	89	89	?	36	94
Oryctolagus cuniculus	15	24	16	18	19	?	19	18	11	9	?
Manis javanica	?	?	15	?	19	22	?	12	9	5	17
Delphinidae	5	7	?	?	?	?	?	3	?	?	3
Capreolus capreolus	50	?	?	53	52	?	47	42	?	10	47
Sus scrofa	?	?	80	86	89	?	?	?	61	6	?
Ovis aries	?	?	?	?	?	?	?	?	?	?	?
Tupaia javanica	?	24	16	16	20	22	18	16	10	4	16
Tarsius spectrum	28	35	22	25	28	32	25	20	13	4	22
Loris tardigradus	?	10	8	9	9	9	8	8	?	2	8
Homo sapiens (Keibel)	27	?	21	36	80	?	28	27	8	4	28
Homo sapiens (O'Rahilly)	17	24	?	17	?	?	15	?	11	11	?
Macaca mulatta	?	?	?	?	20	?	?	?	?	?	?

[a] Information is listed for each of the species in Table I for the 116 events in Appendix 1. Values for each species are given in the staging system listed in Table II. "?" indicates no timing data available.