Explosive Radiations and the Reliability of Molecular Clocks: Island Endemic Radiations as a Test Case

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Abstract.—The reliability of molecular clocks has been questioned for several key evolutionary radiations on the basis that the clock might run fast in explosive radiations. Molecular date estimates for the radiations of metazoan phyla (the Cambrian explosion) and modern orders of mammals and birds are in many cases twice as old as the palaeontological evidence would suggest. Could some aspect of explosive radiations speed the molecular clock, making molecular date estimates too old? Here we use 19 independent instances of recent explosive radiations of island endemic taxa as a model system for testing the proposed influence of rapid adaptive radiation on the rate of molecular evolution. These radiations are often characterized by many of the potential mechanisms for fast rates in explosive radiations—such as small population size, elevated speciation rate, rapid rate of morphological change, release from previous ecological constraints, and adaptation to new niches—and represent a wide variety of species, islands, and genes. However, we find no evidence of a consistent increase in rates in island taxa compared to their mainland relatives, and therefore find no support for the hypothesis that the molecular clock runs fast in explosive radiations. [Adaptive radiation; Cambrian explosion; molecular dates; phylogeny; speciation; substitution rate.]

The molecular clock is one of the most strikingly useful new tools in evolutionary biology, potentially revealing the evolutionary history of all extant lineages. Molecular data are universal and directly comparable across all organisms, from seaweed to giant squid. Molecular phylogenetic studies span all evolutionary timescales, providing insights from population processes to the deepest branches of the tree of life. Furthermore, both observation and theory suggest a unity of process of molecular evolution. The neutral theory of molecular evolution (Kimura and Ohta, 1971; Kimura, 1983) links the mutation rate in individuals to the changes in allele frequencies in populations to the molecular divergence of species and higher taxonomic divisions. The elegant simplicity of the neutral theory derives, in part, from the observation that the rate of molecular evolution is surprisingly constant over time. For many different systems, from globin proteins in mammalian orders (Zuckerkandl and Pauling, 1962) to cytochrome *b* in Hawaiian birds (Fleischer et al., 1998) to E-gene sequences in the dengue fever virus (Twiddy et al., 2003), a linear relationship has been observed between genetic distance and evolutionary time. This observation suggests that genetic distance could provide a timescale for evolution: if the rate of molecular evolution is known, the amount of time that has passed since the last common ancestor of biological lineages can be estimated from the measurable genetic distance between them.

However, two general observations have cast a shadow over the use of molecular clocks in evolutionary biology. Firstly, it has become clear that there is no universal clock, but that rates of molecular evolution can vary between lineages, not only for comparisons between highly divergent taxa such as endothermic primates and ectothermic sharks (Martin et al., 1992), but also between closely related species, such as different sized members of mammal families (Martin and Palumbi, 1993; Bromham et al., 1996). Studies that compare rates of molecular evolution across lineages continue to reveal potential correlates of the rate of molecular evolution, such as generation length (Mooers and Harvey, 1994; Bromham et al., 1996), metabolic rate (Martin and Palumbi, 1993; Martin, 1999), and species richness (Barraclough and Savolainen, 2001; Webster et al., 2003), suggesting that the rate of accumulation of genetic differences may be shaped by a number of different biological factors.

Secondly, some molecular date estimates are notoriously at odds with other lines of evidence. The most controversial molecular clock dates are those for three key evolutionary radiations. The fossil record suggests half a dozen or more episodes when lineages diversified explosively, producing a great variety of taxa with a high degree of morphological differentiation within a relatively short time period. But molecular clock dates for several of these events—the "Cambrian explosion" of animal phyla and the ordinal-level radiations of modern birds and mammals—are almost twice as old as the available fossil evidence suggests (Hedges et al., 1996; Wray et al., 1996; Cooper and Penny, 1997; Ayala et al., 1998; Bromham et al., 1998; Lynch, 1999; Wang et al., 1999; Madsen et al., 2001). This discrepancy may be due to systematic biases in the fossil record that left particular taxa, regions or periods effectively unrecorded (e.g., Darwin, 1859; Fortey et al., 1997; Bromham et al., 1999). Alternatively, it has been suggested that explosive radiations could speed the molecular clock, causing molecular dates for these radiations to be consistently overestimated (Vermeij, 1996; Wray et al., 1996; Conway Morris, 1998; Archibald, 1999; Knoll and Carroll, 1999; Lee, 1999; Valentine et al., 1999). A recent analysis proposed dramatically higher rates of molecular evolution during the radiation of metazoan phyla (Aris-Brosou and Yang, 2002, 2003), but there has not been any empirical evidence to support the hypothesis of fast rates during explosive radiations. There are, however, a number of potential links between explosive radiations and rate of molecular evolution, in particular, change in body size,

rate of morphological change and increased diversification rate.

The body size trend in molecular evolution rate in vertebrates (Martin and Palumbi, 1993; Mooers and Harvey, 1994; Bromham et al., 1996) has prompted the suggestion that the presumed small body size of stem lineages of major radiations could generate fast early rates (e.g., Vermeij, 1996; Conway Morris, 1998). This could potentially provide a mechanism for overestimated divergence dates for the mammalian radiation: most modern mammal orders increased in average body size from their first appearance in the fossil record, and rates of molecular evolution appear to be negatively associated with body size in mammals (Martin and Palumbi, 1993; Bromham et al., 1996). So as body size of mammalian lineages increased, it is possible that average substitution rates slowed down (Bromham, 2003). However, a body size effect seems unlikely to provide a general explanation for the discrepancy between palaeontological and molecular dates for explosive radiations. The radiation of modern bird orders is not characterized by increase in body size across all orders. For the metazoans, it is not obvious that average body size increased within most phyla, and there is as yet no evidence that body size influences rate of molecular evolution in invertebrates.

The increased pace of phenotypic change that characterizes adaptive radiations has also been put forward as a possible cause of the discrepancy between molecular and palaeontological dates: if rapid phenotypic evolution increases the rate of molecular evolution, then the clock might run fast during explosive radiations (Vermeij, 1996; Conway Morris, 1998; Knoll and Carroll, 1999; Lee, 1999; Valentine et al., 1999). However, there is currently no evidence to support such a link. A wide variety of approaches—including laboratory experiments (Papadopoulos et al., 1999), comparative studies (Wyles et al., 1983; Bromham et al., 2002), and theoretical predictions (Kimura, 1983)—have all suggested that molecular and morphological rates of change are effectively unlinked, at least for the genes typically used in molecular clock studies.

Evidence for a relationship between diversification rate (as measured by extant species diversity) and rate of molecular evolution for some phylogenies (Barraclough et al., 1996, 1998; Barraclough and Savolainen, 2001; Webster et al., 2003) provides another potential link between explosive radiations and the molecular clock. The generality and underlying cause of the relationship have not been established. Direct action of natural selection on incipient species seems unlikely to provide the explanation as it would be unlikely to influence the rate of change in housekeeping genes typically used in phylogenies (Wray et al., 1996; Bromham and Hendy, 2000). Faster diversification could drive higher substitution rate by repeated subdivision of the population, potentially accelerating the rate of fixation of nearly-neutral substitutions through a reduction in average effective population size (Ohta, 1993; Bromham and Hendy, 2000; Woolfit and Bromham, 2003). Alternatively, lineages with high rates of molecular evolution may be predisposed to speciation,

either through the generation of more genetic variation, which may then be selected for in incipient species, or because higher rates of molecular evolution may hasten the development of hybrid incompatibility (e.g., Wu, 2001).

As well as the potential effect on rates of molecular evolution, explosive radiations might also influence the patterns of substitutions, such as the relative proportions of nonsynonymous (amino acid) and synonymous (silent) substitutions. There are three ways that explosive radiations could increase the ratio of nonsynonymous to synonymous substitutions. Firstly, speciation may be associated with a reduction in effective population size (N_e) either temporarily (e.g., species formed from small peripheral isolates) or permanently (e.g., lower carrying capacity of new niche). Reduced N_e should lead to less efficient selection, so more slightly deleterious amino acid substitutions could go to fixation through drift, rather than being removed from the population by selection (see Ohta and Kimura, 1971; Ohta, 1995, 2002). Secondly, species evolving into new niches might be released from previous constraints, and therefore might experience a reduction in selection pressure, making more amino acid changes effectively neutral or nearly neutral, increasing the relative rate of fixation of nonsynonymous substitutions. Thirdly, the process of rapid adaptation to new niches may promote the positive selection of amino acid changes (this is likely to affect only those genes associated with adaptive traits, which may be a relatively small proportion of the genome), or favor those lineages with a higher rate of generation of genetic novelty.

The approach we have taken to testing whether explosive radiations influence the rate and pattern of molecular evolution is to identify lineages that have undergone rapid diversification and to compare their rate of molecular evolution to closely related lineages that have not shown an increase in diversification rate. With sufficient comparisons between "exploding" and "nonexploding" lineages, we should be able to detect any influence of explosive radiation on the rate of molecular evolution. Island endemic radiations provide an ideal case study. They are associated with several evolutionary processes that could affect rate of molecular evolution: genetic bottlenecks as populations are initiated from a small number of colonists, rapid rate of phenotypic evolution as species are released from the constraints of mainland ecosystems (such as predators or competing species), and novel adaptations as they evolve into a range of new niches. In addition, because island radiations have long been a focus of studies on diversification, there is a wealth of sequence data for a wide range of island endemic clades. Molecular data have been a popular tool for investigating island endemic radiations because they remove the circularity of studying the evolution of adaptation using a phylogeny based on the same morphological characters (Givnish, 1997).

We have gathered DNA sequence data from island endemic radiations with an evidently greater rate of ecological or morphological diversification than their mainland relatives. For example, Darwin's finches have

TABLE 1.   Island endemic radiations included in this study, ordered by island and taxon.

Islands	Taxon	Radiation	Species IS[a]	Species ML[a]	Sequence[b]	Type[c]	Length[d]	Reference
Hawaii	Flies	Drosophilids	20	3	Yp1	nuc	831 (0)	Kambysellis et al., 1995
	Birds	Honeycreepers	10	5	cytb	mt	789 (0)	Feldman et al., 1997
	Plants	Silverswords	18	4	ITS1+2	nuc	647 (0)	Baldwin and Sanderson, 1998
	Spiders	Thomisidae	9	2	COI	mt	440 (0)	Garb, 1999
		Orsonwelles	12	6	COI	mt	438 (0)	Hormiga et al., 2003
			12	6	16S	mt	444 (0)	Hormiga et al., 2003
	Damselflies	*Megalarion*	21	1	EF1α	nuc	735 (0)	Jordan et al., 2003
			21	1	COII	mt	666 (0)	Jordan et al., 2003
Galapagos	Birds	Darwin's finches	13	3	CR	mt	1117 (20)	Sato et al., 2001
			13	3	cytb	mt	921 (0)	Sato et al., 2001
Macronesia	Spiders	*Dysdera*	9	3	COI	mt	471 (0)	Arnedo and Ribera, 1999
	Plants	*Echium*	21	10	cp spacer 1	cp	691 (0)	Böhle et al., 1996
			21	10	cp intron	cp	496 (0)	Böhle et al., 1996
			21	10	cp spacer 2	cp	326 (0)	Böhle et al., 1996
			21	10	ITS1	nuc	233 (0)	Böhle et al., 1996
		Sempervivoideae	22	2	ITS2	nuc	217 (18)	Mes et al., 1996
			22	2	cp spacer	cp	270 (0)	Mes et al., 1996
		Sonchus	26	17	ITS1+2	nuc	484 (22)	Kim et al., 1996
	Beetles	*Tarphius*	6	8	COI	mt	420 (6)	Emerson et al., 2000
		Pimelia	7	2	COI	mt	364 (0)	Juan et al., 1996
Juan Fernandez	Plants	*Robinsonia*	6	2	ITS1+2	nuc	490 (0)	Sang et al., 1995
Madagascar	Birds	Vangas	11	3	12S	mt	401 (0)	Yamagishi et al., 2001
			11	4	16S	mt	466 (0)	Yamagishi et al., 2001
	Birds	Songbirds	8	5	cytb	mt	888 (0)	Cibois et al., 2001
	Primates	Lemurs	7	3	cytb	mt	1137 (0)	Yoder et al., 1996
			7	3	IRBP	nuc	939 (0)	Yoder and Irwin, 1999; Yoder et al., 2001
Caribbean	Lizards	*Anolis*	7	7	ND2	mt	1035 (0)	Jackman et al., 1999
Bonin	Snails	Mandarina	12	7	16S	mt	576 (210)	Chiba, 1999

[a] Number of species (sequences) for the island (IS) and mainland (ML) clades.

[b] Sequences used are yolk protein gene (*Yp1*), cytochrome *b* (cytb), internal transcribed spacer regions 1 and 2 (ITS1+2), cytochrome oxidase I (COI), cytochrome oxidase II (COII), 16S rRNA (16S), 12S rRNA (12S), elongation factor 1-α (EF1α), mitochondrial control region (CR), interphotoreceptor retinoid binding protein (IRBP), and NADH dehydrogenase subunit 2 (ND2). Chloroplast spacer (cp spacer) and intron sequences (cp intron): Sempervivoideae, *trnL*(UAA)–*trnF*(GAA); *Echium* cp spacer 1 = chloroplast *trnT*(UGU)–*trnL*(UAA), cp spacer 2 = *trnL*(UAA)–*trnF*(GAA), cp intron = chloroplast *trnL*(UAA).

[c] Type of DNA sequence: nuc (nuclear), mt (mitochondrial), and cp (chloroplast).

[d] Final alignment length after excision of saturated bases, with number of bases deleted given in parentheses.

clearly diversified ecologically compared to their mainland seed-eating relative, with 14 island endemic species adapted to a range of novel niches, including cactus eaters and blood-drinking finches (Sato et al., 2001). Similarly, the 30 island endemic species of Hawaiian silverswords are adapted to a wide range of habitats, including exposed lava, dry scrub, mesic forests, wet forests, and bogs, and display a variety of reproductive morphologies and growth forms, including rosette plants, cushion plants, shrubs, trees, and vines (Baldwin and Sanderson, 1998). The nearest mainland relatives of the silverswords are tarweeds, most of which are ephemeral herbs growing at low elevations (Baldwin and Wessa, 2002). In many cases, the island endemic radiations also have a greater net rate of diversification (greater species richness): for example, the Hawaiian drosophilids constitute 25% of the species in this genus, despite the small size and young age of the islands (Kambysellis et al., 1995). These and other island endemic radiations are model systems for explosive radiations—a large number of diverse lineages generated in a relatively short period of evolutionary time as they radiate into empty niche space.

We identified 19 suitable, phylogenetically independent comparisons between explosive island endemic radiations and their mainland relatives, including a wide range of islands, taxa, and genes (see Table 1). We used maximum likelihood methods to compare both the rate (number of substitutions per site) and pattern (relative proportion of nonsynonymous changes) of molecular evolution in the "explosive" island radiation to those of the mainland relative. We found no evidence of consistent differences in the patterns or rates of molecular evolution in the island endemic radiations.

METHODS

Data Selection

We used the following criteria in selecting data sets for this study:

1. The island endemic radiation must show a marked increase in ecological diversification compared to its mainland relative, as evident from greater morphological and/or ecological disparity than the mainland clade. For example, Darwin's finches on the Galapagos Islands have a wider variety of niches and more variation in morphological characters, particularly beak size and shape, than the mainland lineage (Sato et al., 2001). In particular, we used only those studies where the authors specified that the group was

a clear example of an endemic radiation (i.e., diversified after the lineage colonized the island) and the study included details of morphological and/or ecological diversity found in the island lineages but not in any mainland lineages. We erred on the side of caution, including only those radiations for which clear evidence was presented in the literature of a greater rate of ecological diversification than the mainland lineages. (We did not necessarily establish that the island had more lineages, as this may be subject to sampling bias, if all of the island lineages are identified but only some of the mainland relatives.)

2. The island clade must be the same age or younger than the mainland clade, so that we can be sure that the island lineage has undergone a more rapid rate of morphological or ecological diversification, rather than simply having had a longer time to diversify. Where the comparison consists of sister groups (i.e., the island and mainland species form two monophyletic clades that are each other's closest relatives), the island and mainland lineages are the same age. For cases where the island radiation is nested within the mainland clade, the island radiation is younger than the paraphyletic mainland clade.

3. There must be DNA sequences available (on GenBank: www.ncbi.nlm.nih.gov) for at least two species of the island endemic radiation, for at least one related mainland species, and for an outgroup taxon (Fig. 1). Where an outgroup taxon was included in the original published study, it was used in our analysis. Otherwise, GenBank was searched for a suitable closely related sequence.

4. The comparisons must be phylogenetically independent (i.e., nonoverlapping; Harvey and Pagel, 1991; Harvey and Purvis, 1991).

Using these criteria, 19 data sets were selected (Table 1). Sequences for each comparison were aligned by eye using the Se-Al alignment editor (Rambaut, 1996)—any regions that could not be confidently aligned were excluded from the analysis (see Table 1 for de-

tails). For some comparisons (e.g., Darwin's finches: see Table 1), several gene sequences were available. Each gene was aligned and analyzed separately (alignments are available at http://www.lifesci.sussex.ac.uk/CSE/members/lbromham/lbromham.htm). In addition, a concatenated alignment of all sequences for the comparison was produced and analyzed (details given below in "Statistical Analysis"). For each alignment, a maximum likelihood (ML) phylogeny was estimated with an HKY+Γ substitution model (Hasegawa et al., 1985; Yang, 1994), with parameters of the model (gamma shape parameter and transition/transversion ratio) estimated from the data. Heuristic searches were conducted using the TBR (tree bisection and reconnection) algorithm as implemented in PAUP* (Swofford, 1999).

Each phylogeny was checked to make sure that the island taxa formed a monophyletic clade. In three estimated phylogenies where the island taxa did not form a single monophyletic clade (*Anolis*, *Dysdera*, and Thomisidae: see Table 1), any island taxa falling outside the main island endemic clade were pruned from the tree. The maximum likelihood branch lengths were then reestimated for the pruned tree to ensure that each datapoint consisted only of a single colonization and radiation.

Comparison of Rates

To compare the estimated substitution rate for the island lineages with that of their mainland relatives, we conducted a likelihood ratio test using the program Rhino (Rate Heterogeneity across InterNOdes: available from Andrew Rambaut at http://evolve.zoo.ox.ac.uk). For each molecular phylogeny (representing one alignment, whether a single gene or a concatenated alignment, for a single island endemic radiation), lineages falling within the island radiation were designated "island," and all other lineages (including the outgroups) were designated "mainland" (see Fig. 1). Note that because substitution rates are estimated for each category of lineages—island and mainland—these rates can be directly compared. It is not necessary for the island and mainland lineages to be sister groups for this analysis.

Substitution rates were then estimated for each phylogeny using two (nested) models of substitution rates across lineages (both models use an HKY+Γ model of substitution). For the single-rate model, the substitution rate was averaged along all island, mainland and outgroup lineages. The likelihood of the one-rate model was compared to that of a two-rate model in which average substitution rates were estimated separately for two categories of lineages, island and mainland + outgroup. A likelihood ratio test was used to compare the fit of these two models. In common with previous studies that used likelihood ratio tests of differences in substitution rate, we have assumed that the test statistic follows a chi-square distribution (e.g., Rambaut and Bromham [1998]; alternatively, the null distribution of the test statistic could be generated by parametric simulations, e.g., Huelsenbeck et al. [1996]).
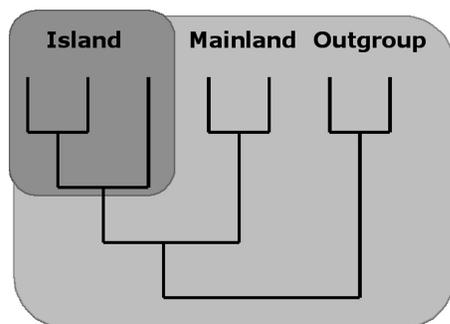

FIGURE 1. Maximum likelihood phylogenies were used to estimate substitution rates separately for island lineages, and for all other (mainland and outgroup) lineages using the program Rhino (see Methods for details). The comparison of ratios of nonsynonymous to synonymous substitutions in PAML between island and mainland lineages did not use an outgroup.

To compare the one-rate and two-rate models, twice the difference of the log-likelihoods of the two models was compared to a chi-squared distribution with one degree of freedom. The two substitution rates estimated under the second model were compared to determine if the island radiation had a faster average rate of molecular evolution that the mainland and outgroup lineages.

In addition to estimating average substitution rates in island and mainland taxa, we looked for differences in the patterns of substitution by comparing the ratio of nonsynonymous to synonymous substitution rates (ω) for island and mainland species in each comparison. We used the program PAML 3.1 (Yang, 1997, 2001) to compare ω in island and mainland lineages, following the same basic procedure as described above: a likelihood ratio test was used to determine whether a two-ratio model (where island lineages had a different ω to mainland lineages) was a significantly better fit to the data than a one-ratio model. The values of ω estimated under the two-ratio model were compared to determine whether the island lineages had a significantly higher value of ω than the mainland lineages (Yang and Bielawski, 2000).

Statistical Analysis

Because the likelihood ratio tests performed in Rhino and PAML identify any significant differences in substitution rates between island and mainland lineages, each island radiation represents an independent test of the hypothesis that adaptive radiations should speed molecular evolution. We can also ask if there is an overall trend, by asking whether the island lineages have a faster rate in more comparisons than expected by chance. Note that this test across comparisons does not require that each individual comparison shows a significant difference in rate—even very small differences may reveal a bias towards faster rates in island lineages when all comparisons are considered together.

We have used two statistical tests to look for an overall trend across all comparisons. Firstly, we have used a Wilcoxon signed-ranks test, which compares the distribution of both signs and magnitudes of differences (Sokal and Rohlf, 1995). However, the Wilcoxon test is ideally applied to observations made without error, rather than to estimates with variance associated with them. Secondly, we have used a very simple statistical test that makes no assumptions about the distribution of variance: the sign test.

Three separate analyses of the difference in rates across comparisons were conducted: (1) substitution rates for single genes; (2) substitution rates for concatenated alignments; (3) ratio of nonsynonymous to synonymous rates (ω) for the single longest protein-coding gene. Although there are multiple genes available for some comparisons, it would violate the assumptions of statistical independence to include more than one data point per comparison in a single statistical test. Each island radiation represents one independent incidence of an "explosive" radiation, so each island radiation should only appear once in any analysis. Two separate analyses were conducted, one where differences in rate of molecular evolution were calculated for the single longest available gene for each radiation, and one where differences in rate were calculated for concatenated alignment of all available genes. Different genes can have different average substitution rates, just as sites within a gene can have different substitution rates, so we allowed for variation in rates across all sites in the alignment by using a gamma parameter in the substitution model (the gamma shape parameter was estimated for each alignment using maximum likelihood). Because the hypothesis being tested concerns genome-wide rates of substitution, concatenation of sequences is an appropriate analysis of multigene data. In addition, we assessed the power of our comparative study by testing for an association between island age and rate difference, and between number of variable sites per comparison and rate difference, using Spearman rank correlations.

RESULTS

Of the 19 comparisons of substitution rates between island and mainland lineages, 6 showed a significant difference in estimated substitution rate for the single gene alignments (4 significant differences for the concatenated alignments: Table 2). However, the island clade had the faster substitution rate in only half of these significant comparisons, so the Rhino results do not support the hypothesis that explosive island radiations should have a faster rate of molecular evolution. Wilcoxon signed-ranks tests across all comparisons were not significant for single longest genes (10 positive comparisons/9 negative comparisons) or concatenated alignments (9/10). The sign test gave similar results for both single longest gene (10/9) and the concatenated alignment (9/10).

There was no apparent influence of genome (nuclear, mitochondrial, chloroplast), sequence type (protein-coding or non–protein-coding), or taxon (vertebrate, invertebrate, or plant) on direction of the rate difference for these comparisons. There was no significant association between island age and rate difference, nor between number of variable sites per comparison and rate difference. Comparisons with more lineages did not appear more likely to give a significant result, as the mean number of island taxa (14) and mainland taxa (5) for the significant comparisons was similar to the mean for all comparisons (13 island, 5 mainland).

The difference in ratio of nonsynonymous to synonymous substitutions (ω) for island and mainland lineages was significant for only three comparisons; in two of these comparisons, the island lineages had the higher ratio. Wilcoxon ranked-signs test across all comparisons was not significant (6 positive comparisons/6 negative comparisons), nor was a sign test (6/6).

DISCUSSION

The discrepancy between molecular and palaeontological dates for three explosive radiations—metazoans, birds, and mammals—has engendered a widely held reluctance to trust the molecular clock. The molecular dates

TABLE 2. Estimated relative substitution rates and ratios of nonsynonymous to synonymous substitutions, for the comparisons between island and mainland lineages listed in Table 1.

Island	Radiation	Ratio of substitution rates (Rhino)[a]				d_N/d_S (PAML)[b]		
		Single Gene	P	Concatenated	P	Island ω	Mainland ω	P
Hawaii	Drosophilids	2.45	**	2.45	**	0.415	0.095	**
	Honeycreepers	1.27		1.27		0.002	0.005	
	Silverswords	1.49		1.49				
	Thomisidae	0.52		0.52		0.010	0.014	
	Orsonwelles	0.99		0.86		0.012	0.011	
	Megalarion	0.23	*	0.52	*	0.056	0.016	
Galapagos	Darwin's finches	1.79		0.99		0.060	0.024	*
Macronesia	*Dysdera*	1.18		1.18		0.015	0.017	
	Echium	1.35	*	0.66				
	Sempervivoideae	1.15		1.23				
	Sonchus	1.59		1.59				
	Tarphius	6.05	**	6.05	**	0.015	0.012	
	Pimelia	1.68		1.68		0.004	0.010	
Juan Fernandez	*Robinsonia*	0.58		0.58				
Madagascar	Vangas	0.96	*	2.80				
	Lemurs	0.96		0.81		0.032	0.029	
	Songbirds	0.89		0.89		0.021	0.021	
Caribbean	*Anolis*	0.56	**	0.56	**	0.080	0.132	**
Bonin	Mandarina	0.71		0.71				

[a]Substitutions per site estimated by maximum likelihood in Rhino (see Methods). The ratio of island to mainland substitution rates is given for the single longest gene and the concatenated alignment of all sequences.
[b]Ratio of nonsynonymous to synonymous substitutions (ω) was estimated for island and mainland lineages using PAML for the single largest protein-coding gene for each radiation (see Table 1). It was not possible to estimate ω for the seven radiations for which no protein-coding genes were available. Significant differences between island and mainland lineages (P) are marked by asterisks: $^{*}P \leq 0.05$; $^{**}P \leq 0.005$.

have been defended by highlighting potential deficiencies in the palaeontological data that could result in a systematic gap in the fossil record. Is it also possible for molecular dates to be systematically biased? If the molecular clock runs fast in lineages undergoing rapid evolutionary diversification, then dates for explosive radiations could be consistently overestimated. This study has aimed to test this hypothesis using multiple independent incidences of explosive radiations on islands: if rapid radiation speeds the molecular clock, these island endemic lineages should have faster rates of molecular evolution than their nonexploding mainland relatives. We found no evidence that a lineage undergoing rapid adaptive radiation has a faster rate of molecular evolution, for the taxa and genes examined here. How confident can we be that these data represent an appropriate model for the adaptive radiations of the past?

Have we looked at the right sequences? Positive selection during an adaptive radiation might generate a burst of substitutions in specific genes associated with diversification (e.g., Gillespie, 1991). The genes included in this study were originally sequenced for their phylogenetic utility, typically genes for basic metabolic functions that would be unlikely to be under direct selection in an adaptive radiation (see Table 1). Although these housekeeping genes are not ideal for examining the more general question of whether rapid adaptation can influence molecular evolution, they are the correct focus for a study of the reliability of molecular clocks. Molecular date estimates for the metazoan, avian, and mammalian radiations have been based primarily on housekeeping genes, precisely because they are considered less likely to be influenced by variable evolutionary pressure, and are therefore deemed the most reliable keepers of evolu-

tionary time. Moreover, although there is wide variation in published date estimates for these explosive radiations, studies based on a broad range of sequences all point to divergences long before the first fossils (e.g., Runnegar, 1982; Wray et al., 1996; Ayala et al., 1998; Bromham et al., 1998; Lynch, 1999; Wang et al., 1999). So only a process that affected genome-wide rates of molecular evolution—not just the substitution rate in genes under direct selection—could account for a systematic bias in published molecular date estimates for the metazoan, avian, and mammalian radiations. We would expect to detect such a genome-wide bias in the genes used in this study.

Do we have sufficient data to detect an effect of explosive radiations on molecular evolution? The statistical power of each Rhino test (i.e., the comparison of rates between island and mainland for a single alignment for a single island radiation) is related to the 'sample size' of observed substitutions, which is determined by such factors as the sequence length, number of variable sites, rates of substitution, and the age of the radiation. However, there is no apparent association between the number of variable sites per comparison and the difference in rate between island and mainland lineages, suggesting that statistical power of each comparison is not preventing this study from revealing an effect of adaptive radiation on molecular evolution rates. The number of comparisons may also limit the statistical power of this study. However, the 19 comparisons were evenly divided between positive and negative rate differences, so there is no hint of a weak trend that could become significant if more data was added.

Are the comparisons too recent to have allowed for the accumulation of sufficient differences in number of

substitutions? Although the radiations included in this study are much younger than the mammalian, avian (>50 Mya), and metazoan (>500 Mya) radiations, the study should still be able to detect any increase in substitution rate in the radiating lineages. The gene sequences used in this study will have been specifically selected to have a substitution rate appropriate to the estimation of phylogenies of the island endemic radiations, and so should provide sufficient genetic differences. The older island radiations do not show a greater tendency to have faster rates, suggesting that the analysis is not limited by insufficient time for the island taxa to accumulate a greater number of substitutions. In any case, the relevant factor is not the age but the duration of the radiation: the length of time during which "explosive" processes might increase the number of DNA substitutions. For example, although the Cambrian explosion occurred over half a billion years ago, the period of hypothesized increased rates may have been as little as 10 million years (Bowring et al., 1993), in which case the total increase in DNA substitutions might not be any greater than a modern radiation of the same duration. Unless, of course, the number of substitutions added is influenced by the magnitude of the phenotypic change.

Some researchers may be unconvinced that recent island radiations provide an appropriate model for the more dramatic radiations of the past, when a high degree of morphological disparity was generated in an apparently short period of time. The Cambrian explosion has fascinated biologists because of the apparent evolution of phylum-level differences in body plan—such as change in the organization of body segments—in as little as 10 million years. Modern mammals and birds apparently radiated into a great variety of forms and niches from a more generalized ancestor in a similarly short period. Although some of the island radiations included here do show dramatic differences in morphology, such as the Hawaiian silverswords, most show more modest variation from their mainland relatives. Can the modifications of beak shape and size in Darwin's finches compare to variation in number of limbs or body segments of the metazoan phyla?

It has been argued that the radiations of animal phyla and other higher taxa involve fundamentally different kinds of evolutionary changes: in particular that new phyla originated through modifications of *Hox* gene expression patterns that generated new body plans, rather than by the gradual accumulation of incremental changes to morphology (Gellon and McGinnis, 1998). This argument is macroevolutionary—that the evolution of higher taxa is qualitatively different from the microevolutionary changes that drive evolution of species-level differences—and contrary to the widely accepted neo-Darwinian model of evolution. Whether or not this model of evolution can be proven, we can ask: if this were true, would it influence the speed of the molecular clock? If a higher-order explosive radiation involved few genetic changes, each of large phenotypic effect, then the few genetic changes that occurred would be unlikely to have occurred in the housekeeping genes, and therefore

these genes would be expected to accumulate changes at the normal rate. This would apply whether the significant changes were in the form of substitutions within key genes, gene duplication events, or changes to the developmental architecture. None of these changes would be expected to directly influence the molecular clock as it is currently applied, based on nucleotide substitutions in housekeeping genes, such as those coding for ribosomal RNAs or metabolic enzymes.

Explosive radiation could only bias molecular clock estimates if it influenced genome-wide rates of molecular evolution. This study was designed to detect such an influence by looking at a range of genes and taxa in many independent adaptive radiations, all characterized by high rates of morphological change. These island radiations provide an informative test case because they combine a number of factors that could influence rate of molecular evolution in adaptive radiations, such as reduced population size, adaptation to new niches, and release from previous constraints. The relatively young age of the island radiations should not bias the results, as they are comparable to the projected duration of explosive radiations of the past, and gene sequences were selected that would provide a sufficient sample of substitutions. Even small increases in the substitution rate should be sufficient to be detected across such a wide range of comparisons, and yet there is no sign of an influence of adaptive radiation on molecular evolution rates in these sequences. Although these results do not prove that the molecular clock is "safe" for explosive radiations, they do suggest that dismissals of molecular date estimates on the grounds that the clock runs fast in adaptive radiations should be reassessed in light of empirical evidence.

REFERENCES

Archibald, J. D. 1999. Pruning and grafting on the mammalian phylogenetic tree. Acta Palaeontol. Pol. 44:220–222.

Aris-Brosou, S., and Z. Yang. 2002. Effects of models of rate evolution on estimation of divergence dates with special reference to the metazoan 18S ribosomal RNA phylogeny. Syst. Biol. 51:703–714.

Aris-Brosou, S., and Z. Yang. 2003. Bayesian models of episodic evolution support a late precambrian explosive diversification of the metazoa. Mol. Biol. Evol. 20:1947–1954.

Arnedo, M. A., and C. Ribera. 1999. Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. J. Arachnol. 27:604–662.

Ayala, F. J., A. Rzhetsky, and F. J. Ayala. 1998. Origin of the metazoan phyla: Molecular clocks confirm palaeontological estimates. Proc. Natl. Acad. Sci. U.S.A. 95:606–611.

Baldwin, B. G., and M. J. Sanderson. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. U.S.A. 95:9402–9406.

Baldwin, B. G., and B. L. Wessa. 2002. Origin and relationships of the tarweed-silversword lineage (Compositae-Madiinae). Am. J. Bot. 87:1890–1908.

Barraclough, T. G., P. H. Harvey, and S. Nee. 1996. Rate of *rbc*L gene sequence evolution and species diversification in flowering plants (Angiosperms). Proc. R. Soc. Lond. B 263:589–591.

Barraclough, T. G., and V. Savolainen. 2001. Evolutionary rates and species diversity in flowering plants. Evolution 55:677–683.

Barraclough, T. G., A. P. Vogler, and P. H. Harvey. 1998. Revealing the factors that promote speciation. Phil. Trans. R. Soc. Lond. B 353:241–249.

Böhle, U. R., H. H. Hilger, and W. F. Martin. 1996. Island colonization and evolution of the insular woody habit in *Echium* L (Boraginaceae). Proc. Natl. Acad. Sci. U.S.A. 93:11740–11745.

Bowring, S. A., J. P. Grotzinger, C. E. Isachsen, A. H. Knoll, S. M. Pelechaty, and P. Kolosov. 1993. Calibrating rates of early Cambrian evolution. Science 261:1293–1298.

Bromham, L. 2003. Molecular clocks and explosive radiations. J. Mol. Evol. 57:S13–S20.

Bromham, L., A. Rambaut, R. Fortey, A. Cooper, and D. Penny. 1998. Testing the Cambrian explosion hypothesis by using a molecular dating technique. Proc. Natl. Acad. Sci. U.S.A. 95:12386–12389.

Bromham, L., A. Rambaut, and P. H. Harvey. 1996. Determinants of rate variation in mammalian DNA sequence evolution. J. Mol. Evol. 43:610–621.

Bromham, L., M. Woolfit, M. S. Y. Lee, and A. Rambaut. 2002. Testing the relationship between morphological and molecular rates of change along phylogenies. Evolution 56:1921–1930.

Bromham, L. D., and M. D. Hendy. 2000. Can fast early rates reconcile molecular dates to the Cambrian explosion? Proc. R. Soc. Lond. B 267:1041–1047.

Bromham, L. D., M. J. Phillips, and D. Penny. 1999. Growing up with dinosaurs: Molecular dates and the mammalian radiation. Trends Ecol. Evol. 14:113–118.

Chiba, S. 1999. Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. Evolution 53:460–471.

Cibois, A., B. Slikas, T. S. Schulenberg, and E. Pasquet. 2001. An endemic radiation of Malagasy songbirds is revealed by mitochondrial DNA sequence data. Evolution 55:1198–1206.

Conway Morris, S. 1998. Early metazoan evolution: Reconciling paleontology and molecular biology. Am. Zool. 38:867–877.

Cooper, A., and D. Penny. 1997. Mass survival of birds across the Cretaceous-Tertiary boundary: Molecular evidence. Science 275:1109–1113.

Darwin, C. 1859. The origin of species by means of natural selection. John Murray, London.

Emerson, B. C., P. Oromi, and G. M. Hewitt. 2000. Tracking colonization and diversification of insect lineages on islands: Mitochondrial DNA phylogeography of *Tarphius canariensis* (Coleoptera: Colydiidae) on the Canary Islands. Proc. R. Soc. Lond. B. 267:2199–2205.

Feldman, R. A., L. A. Freed, J. G. Groth, and R. L. Cann. 1997. Convergent evolution in Hawaiian honeycreepers: Unpublished sequences.

Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 1998. Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K-Ar based ages of the Hawaiian islands to estimate molecular evolutionary rates. Mol. Ecol. 7:533–545.

Fortey, R. A., D. E. G. Briggs., and M. A. Wills. 1997. The Cambrian evolutionary "explosion" recalibrated. BioEssays 19:429–434.

Garb, J. E. 1999. An adaptive radiation of Hawaiian Thomisidae: Biogeographic and genetic evidence. J. Arachnol. 27:71–78.

Gellon, G., and W. McGinnis. 1998. Shaping animal body plans in development and evolution by modulation of Hox expression patterns. BioEssays 20:116.

Gillespie, J. H. 1991. The causes of molecular evolution. Oxford University Press, Oxford.

Givnish, T. J. 1997. Adaptive radiation and molecular systematics: Issues and approaches. Pages 1–54 *in* Molecular evolution and adaptive radiation (T. J. Givnish and K. J. Sytsma, eds.). Cambridge University Press, Cambridge.

Harvey, P. H., and M. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford.

Harvey, P. H., and A. Purvis. 1991. Comparative methods for explaining adaptations. Nature 351:619–624.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21:160–174.

Hedges, S. B., P. H. Parker, C. G. Sibley, and S. Kumar. 1996. Continental breakup and the diversification of birds and mammals. Nature 381:226–229.

Hormiga, G., M. Arnedo, and R. G. Gillespie. 2003. Speciation on a conveyor belt: Sequential colonization of the Hawaiian islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Syst. Biol. 52:70–88.

Huelsenbeck, J. P., D. M. Hillis, and R. Nielsen. 1996. A likelihood-ratio test of monophyly. Syst. Biol. 45:546–558.

Jackman, T. R., A. Larson, K. de Queiroz, and J. B. Losos. 1999. Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. Syst. Biol. 48:254–285.

Jordan, S., C. Simon, and D. Polhemus. 2003. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata: Coenagrionidae). Syst. Biol. 52:89–109.

Juan, C., K. M. Ibrahim, P. Oromi, and G. M. Hewitt. 1996. Mitochondrial DNA sequence variation and phylogeography of *Pimelia* darkling beetles on the island of Tenerife (Canary islands). Heredity 77:589–598.

Kambysellis, M. P., K. F. Ho, E. M. Craddock, F. Piano, M. Parisi, and J. Cohen. 1995. Pattern of ecological shifts in the diversification of Hawaiian *Drosophila* inferred from a molecular phylogeny. Curr. Biol. 5:1129–1139.

Kim, S. C., D. J. Crawford, J. FranciscoOrtega, and A. SantosGuerra. 1996. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: Molecular evidence for extensive radiation. Proc. Natl. Acad. Sci. U.S.A. 93:7743–7748.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge.

Kimura, M., and T. Ohta. 1971. On the rate of molecular evolution. J. Mol. Evol. 1:1–17.

Knoll, A. H., and S. B. Carroll. 1999. Early animal evolution: Emerging views from comparative biology. Science 284:2129–2137.

Lee, M. S. Y. 1999. Shortening the phylogenetic fuse. Trends Ecol. Evol. 13:323–323.

Lynch, M. 1999. The age and relationships of the major animal phyla. Evolution 53:319–325.

Madsen, O., M. Scally, C. J. Douady, D. J. Kao, R. W. DeBry, R. Adkins, H. M. Amrine, M. J. Stanhope, W. W. de Jong, and M. S. Springer. 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409:610–614.

Martin, A. P. 1999. Substitution rates of organelle and nuclear genes in sharks: Implicating metabolic rate (again). Mol. Biol. Evol. 16:996–1002.

Martin, A. P., G. Naylor, and S. R. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357:153–155.

Martin, A. P., and S. R. Palumbi. 1993. Body size, metabolic rate, generation time and the molecular clock. Proc. Natl. Acad. Sci. U.S.A. 90:4087–4091.

Mes, T. H. M., J. van Brederode, and H. 't Hart. 1996. Origin of the woody Macaronesian Sempervivoideae and the phylogenetic position of the east African species of *Aeonium*. Bot. Acta 109:477–491.

Mooers, A. Ø., and P. H. Harvey. 1994. Metabolic rate, generation time and the rate of molecular evolution in birds. Mol. Phylog. Evol. 3:344–350.

Ohta, T. 1993. Amino acid substitution at the ADH locus of *Drosophila* is facilitated by small population size. Proc. Natl. Acad. Sci. U.S.A. 90:4548–4551.

Ohta, T. 1995. Synonymous and nonsynonymous substitutions in mammalian genes and the nearly neutral theory. J. Mol. Evol. 40:56–63.

Ohta, T. 2002. Near-neutrality in evolution of genes and gene regulation. Proc. Natl. Acad. Sci. U.S.A. 99:16134–16137.

Ohta, T., and M. Kimura. 1971. On the constancy of the evolutionary rate of cistrons. J. Mol. Evol. 1:18–25.

Papadopoulos, D., D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, and M. Blot. 1999. Genomic evolution during a 10,000-generation experiment with bacteria. Proc. Natl. Acad. Sci. U.S.A. 96:3807–3812.

Rambaut, A., and L. Bromham. 1998. Estimating divergence dates from molecular sequences. Mol. Biol. Evol. 15:442–448.

Rambaut, A. E. 1996. Se-Al, version 1.0. University of Oxford. http://evolve.zoo.ox.ac.uk/software/Se-Al.

Runnegar, B. 1982. A molecular-clock date for the origin of the animal phyla. Lethaia 15:199–205.

Sang, T., D. J. Crawford, T. F. Stuessy, and M. S. O. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). Syst. Bot. 20:55–64.

Sato, A., H. Tichy, C. O'HUigin, P. R. Grant, B. R. Grant, and J. Klein. 2001. On the origin of Darwin's finches. Mol. Biol. Evol. 18:299–311.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: The principles and practice of statistics in biological research, 3rd edition. W.H. Freeman, San Francisco.

Swofford, D. L. 1999. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods): Version 4. Sinauer Associates, Sunderland, Massachusetts.

Twiddy, S. S., E. C. Holmes, and A. Rambaut. 2003. Inferring the rate and time-scale of dengue virus evolution. Mol. Biol. Evol. 20:122–129.

Valentine, J., D. Jablonski, and D. Erwin. 1999. Fossils, molecules and embryos: New perspectives on the Cambrian explosion. Development 126:851–859.

Vermeij, G. 1996. Animal origins. Science 274:525–526.

Wang, D. Y.-C., S. Kumar, and S. B. Hedges. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. Proc. R. Soc. Lond. B. 266:163–171.

Webster, A. J., R. J. H. Payne, and M. Pagel. 2003. Molecular phylogenies link rates of evolution and speciation. Science 301:478.

Woolfit, M., and L. Bromham. 2003. Increased rates of sequence evolution in endosymbiotic bacteria and fungi with small effective population sizes. Mol. Biol. Evol. 20:1545–1555.

Wray, G. A., J. S. Levigton, and L. H. Shapiro. 1996. Molecular evidence for deep Precambrian divergences among metazoan phyla. Science 274:568–573.

Wu, C. I. 2001. The genic view of the process of speciation. J. Evol. Biol. 14:851–865.

Wyles, J. S., J. G. Kunkel, and A. C. Wilson. 1983. Birds, behavior, and anatomical evolution. Proc. Natl. Acad. Sci. U.S.A. 80:4394–4397.

Yamagishi, S., M. Honda, K. Eguchi, and R. Thorstrom. 2001. Extreme endemic radiation of the Malagasy vangas (Aves: Passeriformes). J. Mol. Evol. 53:39–46.

Yang, Z. 1994. Maximum likelihood phylogenetic estimate from DNA sequences with variable rates over sites: Approximate methods. J. Mol. Evol. 39:306–314.

Yang, Z. 2001. Phylogenetic analysis by maximum likelihood (PAML), version 3.1. University College, London. http://abacus.gene.ucl.ac.uk/software/paml.html.

Yang, Z. H. 1997. PAML: A program package for phylogenetic analysis by maximum likelihood. CABIOS 13:555–556.

Yang, Z. H., and J. P. Bielawski. 2000. Statistical methods for detecting molecular adaptation. Trends Ecol. Evol. 15:496–503.

Yoder, A. D., M. Cartmill, M. Ruvolo, K. Smith, and R. Vilgalys. 1996. Ancient single origin for Malagasy primates. Proc. Natl. Acad. Sci. U.S.A. 93:5122–5126.

Yoder, A. D., and J. A. Irwin. 1999. Phylogeny of the Lemuridae: Effects of character and taxon sampling on resolution of species relationships within *Eulemur*. Cladistics 15:351–361.

Yoder, A. D., J. A. Irwin, and B. A. Payseur. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50:408–424.

Zuckerkandl, E., and L. Pauling. 1962. Molecular disease, evolution, and genic heterogeneity. Pages 189–225 *in* Horizons in biochemistry (M. Kasha and B. Pullman, eds.). Academic Press, New York.

APPENDIX 1.   Accession numbers for sequences (see Table 1).

Anolis: AF055924–26, AF055928–34, AF055943–94, AF294298, AF294303, AF294304, AF294309, AF294312–7

Drosophila: U51456, U51579, U51580, U51582, U51646–48, U51798–803, U51875–77, U52009–27, U52122–28, U52143, U52361

Dysdera: AF244233–37, AF244243, AF244248, AF244251, AF244253, AF244255, AF244256, AF244260, AF244267, AF244270, AF244272, AF244274–78, AF244280, AF244282, AF244283, AF244285, AF244287, AF244289, AF244290, AF244292, AF244294, AF244296–99, AF244301, AF244303, AF244305, AF244307, AF244310, AF244312, AF244313, AF244315, AF244316, AF244318, AF244321, AF303509, AF303522, AF303524

Echium: L33347, L33348, L33357, L33360, L33363, L43166, L43167, L43169, L43170–72, L43176, L43178, L43179, L43180, L43182, L43183, L43184, L43186, L43187, L43188, L43190, L43191, L43192, L43194–96, L43198, L43199, L43200, L43202–04, L43206–08, L43210–16, L43218–20, L43222–24, L43226–28, L43230–32, L43234–36, L43238, L43239, L43240, L43242, L43243, L43244, L43246–48, L43250–52, L43254–56, L43258–60, L43262, L43263, L43264, L43266–68, L43270–72, L43274–76, L43282, L43284, L43286–88, L43290–92, L43294–96, L43298–300, L43302–04, L43306–08, L43310, L43312, L43314–16, L43318, L43321, L43323

Finches: AF108769, AF108771, AF108774, AF108779, AF108784, AF108787, AF108789, AF108792, AF108794, AF108796, AF108798, AF108802, AF108805, AF108807, AF109015–18, AF109020, AF109021, AF109024, AF109026, AF109027, AF109030, AF109032, AF109038, AF109048, AF109065, AF310041–45, AF310070–74

Honeycreepers: AF006215, AF015754–64, AF171656, AF171659, AF290142, X74258

Lemurs: AF081053–55, AF081057, AF081058, AF081060, AF271414, AF271418, AF271419, AF271421, AF271422, AF271424, U53569, U53570, U53572–75, U53577–82

Songbirds: AF094617, AF094628, AF094629, AF094641, AF094642, AF096457, AF096458, AF199381–83, AF199388–91

Mandarina: AF095823, AF098689–716

Megalarion: AY179039, AY179042, AY179045, AY179047, AY179052, AY179054, AY179056, AY179057, AY179063, AY179065, AY179067, AY179069, AY179072, AY179074, AY179076, AY179078, AY179081, AY179084, AY179086, AY179088, AY179093–101, AY179103, AY179106, AY179109, AY179111, AY179116, AY179118, AY179120, AY179121, AY179127, AY179129, AY179131, AY179133, AY179136, AY179138, AY179140, AY179142, AY179145, AY179148, AY179150, AY179152, AY179157–65

Orsonwelles: AY078660, AY078662–66, AY078689–91, AY078693–96, AY078699, AY078710, AY078711, AY078727–29, AY078732, AY078733, AY078735, AY078737–41, AY078743, AY078745, AY078750, AY078751, AY078754, AY078756, AY078760, AY078762, AY078764, AY078765, AY078767, AY078768, AY078773

Pimelia: X97209, X97222, X97210–15, X97217, X97219, X97220, X97221, X97223, AF267483

Robinsonia: Sequences taken from text in Sang et al. (1995)

Sempervivoideae: X80539–87

Silverswords: AF061882–AF061914

Sonchus: L48115, L48117, L48119, L48121, L48123, L48125, L48127, L48129,L48131, L48133, L48135, L48137, L48139, L48141, L48143, L48145, L48147, L48149, L48151, L48153, L48155, L48159, L48163, L48165, L48167, L48171, L48173, L48287, L48291, L48295, L48297, L48299, L48301, L48303, L48305, L48307, L48309, L48311, L48313, L48315, L48317, L48323, L48325, L48329, L48331, L48333, L48337

Tarphius: AF278545–47, AF278551–54, AF278556, AJ236959, AJ236962, AJ403042, AJ403043, AJ403061, AJ403062, AJ403099–101, AJ403103, AJ403104

Thomisidae: Sequences kindly supplied by Jessica Garb (garb@nature.berkeley.edu)

Vangas: AB042334–42, AB042344, AB042345, AB042347, AB042348, AB042351, AB042356, AB042365, AB042367–75, AB042377, AB042378, AB042380, AB042381, AB042384, AB042389, AB042398