Population size and molecular evolution on islands

Megan Woolfit[1,2,*] and Lindell Bromham[2]

[1]*Department of Genetics, Trinity College, University of Dublin, Dublin 2, Ireland*
[2]*Centre for the Study of Evolution, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK*

The nearly neutral theory predicts that the rate and pattern of molecular evolution will be influenced by effective population size (N_e), because in small populations more slightly deleterious mutations are expected to drift to fixation. This important prediction has not been widely empirically tested, largely because of the difficulty of comparing rates of molecular evolution in sufficient numbers of independent lineages which differ only in N_e. Island endemic species provide an ideal test of the effect of N_e on molecular evolution because species restricted to islands frequently have smaller N_e than closely related mainland species, and island endemics have arisen from mainland lineages many times in a wide range of taxa. We collated a dataset of 70 phylogenetically independent comparisons between island and mainland taxa, including vertebrates, invertebrates and plants, from 19 different island groups. The rate of molecular evolution in these lineages was estimated by maximum likelihood using two measures: overall substitution rate and the ratio of non-synonymous to synonymous substitution rates. We show that island lineages have significantly higher ratios of non-synonymous to synonymous substitution rates than mainland lineages, as predicted by the nearly neutral theory, although overall substitution rates do not differ significantly.

Keywords: comparative method; molecular evolution; substitution rates; effective population size; d_N/d_S; molecular clock

1. INTRODUCTION

The nearly neutral theory of molecular evolution (Ohta & Kimura 1971; Ohta 1992) has become one of the most important foundations of modern sequence analysis. Although generally considered to be theoretically sound, it is not universally accepted (e.g. Gillespie 1991, 1999), and there have been few general empirical tests of some predictions arising from the theory. One such prediction which is amenable to further testing is the influence of effective population size (N_e) on the rate and pattern of molecular evolution. A decrease in N_e should lead to an increase in the rate of fixation of nearly neutral mutations—that is, mutations with selection coefficients on the order of the inverse of the effective population size—as selection is less effective in small populations (Wright 1931; Kimura 1983). Thus, if a substantial proportion of mutations are nearly neutral, overall substitution rate should increase in species with small N_e compared to those with larger N_e, provided mutation rates are similar in both species (Ohta 1972a). The ratio of non-synonymous to synonymous substitution rates (ω) is also predicted to increase in these species (Ohta 1993), as many non-synonymous mutations are expected to be nearly neutral, while synonymous mutations are more likely to be neutral and thus fix at a rate unaffected by N_e.

There have been relatively few empirical studies that have directly tested these predictions, due partly to the difficulty of identifying comparisons between species which differ only in effective population size. For example, while comparisons of rates of evolution in primates and rodents support the predictions (Wu & Li 1985; Weinreich

2001), these lineages differ in many other aspects of their biology which could affect substitution rates, such as generation time, metabolic rate and DNA repair mechanisms (Bromham *et al.* 1996). Similarly, endosymbiotic species of bacteria and fungi with small effective population sizes have significantly higher substitution rates and ω values than their free-living relatives (Moran 1996; Woolfit & Bromham 2003), but other biological changes associated with adoption of a symbiotic lifestyle may contribute to this pattern (Johnson & Seger 2001).

Comparisons between island endemic species and closely related mainland lineages offer an ideal test of the effect of N_e on the rate and pattern of evolution (Ohta 1972b; Llopart & Aguade 1999; Johnson & Seger 2001). Endemics are likely to have undergone both a severe population bottleneck during the initial colonization of an island and subsequent long-term reduction in census population size due to range restriction. Together, these factors are expected to frequently lead to a reduction in N_e relative to mainland species (e.g. Frankham 1997; Eldridge *et al.* 1999). Island endemics have arisen independently many times, in species as diverse as raspberries, weevils, toads and finches, and on islands ranging from tiny sub-Antarctic islets such as Campbell Island to the large, tropical island of Madagascar. Island and mainland relatives often share similar morphology, life history and ecology (e.g. Johnson & Seger 2001), and where they do not, the differences between island and mainland species are not expected to be systematically biased across a wide range of taxa (for example, trends for both increase and decrease in body size have been shown for island species; Clegg & Owens 2002). Any consistent trend in the rate or pattern of molecular evolution between island and mainland lineages is, therefore, likely to be due

* Author for correspondence (woolfitm@tcd.ie).

to differences in effective population size rather than some other aspect of their biology.

A number of previous tests of the effect of N_e on rates of molecular evolution have utilized data from island endemic species. Comparisons of ω values in a small number of island and mainland *Drosophila* lineages have yielded inconsistent results, possibly due to variation in codon usage bias among species (e.g. Ohta 1993; Llopart & Aguade 1999). A recent analysis of sequence data from island and mainland species of ducks and doves included a larger number of species, and found a significant increase in non-synonymous substitution rates in the island lineages (Johnson & Seger 2001). However, the datasets used in each of these previous analyses were taxonomically restricted, limiting the generality of the conclusions.

In order to conduct a general test of the influence of N_e on the rates and patterns of molecular evolution, we have collated a dataset of 70 phylogenetically independent comparisons between island and mainland species, with sequence data (including mitochondrial and nuclear, protein-coding and rRNA genes) from over 600 vertebrate, invertebrate and plant species, from 19 different island groups.

2. MATERIAL AND METHODS
(a) *Data*
We identified published datasets of DNA sequence data from closely related island and mainland species. To ensure that all species identified as 'island' species were true endemics, we confirmed the known ranges of all island species using a combination of recent literature, handbooks (e.g. del Hoyo *et al*. 1992) and online databases (e.g. http://www.nature serve.org).

Sequences for each dataset (consisting of island, mainland and outgroup species) were obtained from Genbank (http://www.ncbi.nlm.nih.gov/Genbank), and aligned by eye using Se-Al (Rambaut 1996). Where more than one gene was available for a dataset, the genes were concatenated and analysed together, as change in N_e resulting from demographic effects is predicted to affect genome-wide rates of substitution. Maximum likelihood (ML) phylogenetic trees were constructed using PAUP* (Swofford 2003), with an HKY+Γ model of nucleotide substitution (Hasegawa *et al*. 1985; Yang 1994).

Phylogenetically independent comparisons between island and mainland species were chosen from the ML trees (see Electronic Appendix table S1 for species included in each comparison). To be statistically independent, the paths connecting these comparisons on the tree must neither meet nor cross, to ensure that no data are used more than once in the analysis (Harvey & Pagel 1991). We used established taxonomy and previously published phylogenies based on morphological or DNA hybridization data, where available, to ensure that the comparisons chosen were consistent with accepted phylogenetic relationships.

(b) *Analysis of ω*
For each of the 44 comparisons containing protein-coding sequence data (see table 1), we used the CODEML program from PAML 3.13 (Yang 1997) to test whether island species had a higher ratio of non-synonymous to synonymous substitution rates (ω) than their mainland relatives. Branches on the tree were designated as belonging to island, mainland

or outgroup classes (figure 1), and the value of ω was then estimated for each of these branch classes. These analyses were performed on unrooted trees (Yang 1997), but outgroup species were included in this analysis to increase the amount of data available for parameter estimation. The comparison was scored as positive if ω was higher for island branches than mainland branches, negative if it was lower. Each comparison contributed one point to a one-tailed Wilcoxon signed-ranks test across all comparisons. This test takes into account the magnitude of the difference in ω between island and mainland lineages as well as the sign of each comparison (Sokal & Rohlf 1995).

(c) *Analysis of overall substitution rate*
For each of the 70 comparisons we compared the rate of substitution in island and mainland lineages using the BASEML program from PAML. The local clock model we implemented allowed branches in the rooted tree to take one of two substitution rates (Yoder & Yang 2000). A 'basal' substitution rate was estimated for all branches in the outgroup and mainland lineages, and a second substitution rate was then estimated for the branches in the island lineage relative to the basal rate. The comparison was scored as positive if the relative rate of the island branches was greater than one (i.e. faster than the mainland), or negative if it was less than one. Each comparison contributed one point to a one-tailed Wilcoxon signed-ranks test.

3. RESULTS
Island lineages had significantly higher ω values than mainland lineages (27 positive: 17 negative comparisons; $T_s = 338$; $p = 0.03$; median island ω/mainland $\omega = 1.20$; table 1). This result is based on data from vertebrates and invertebrates only. Protein-coding sequence data was available for five plant datasets, but each of these trees contained branches with non-synonymous but no synonymous substitutions, and we could not include the resulting undefined values of ω in the analysis.

The trend towards increased ω in island lineages was consistently observed across both vertebrates and invertebrates, nuclear and mitochondrial genes and radiating and non-radiating lineages (Bromham & Woolfit 2004), although the trend was non-significant for most subsets of the data (see Electronic Appendix table S2 for statistics). There were no obvious differences in results for lineages from different islands, although too few comparisons were available from each island to test this formally. We found no significant association between island size and ω (Spearman's correlation $r_s = 0.08$, $p = 0.37$).

When overall substitution rate was examined for all 70 comparisons, no significant difference was observed between island and mainland lineages (38 positive: 32 negative; $T_s = 1088$; $p = 0.18$; median island rate relative to mainland rate = 1.04; table 1). We also performed a signed-ranks test on the substitution rate estimates using only those 44 comparisons with protein-coding sequence data that were included in the analysis of ω. Again, there was no significant pattern of rate increase in island species ($p = 0.29$). Results were not qualitatively different when we reanalysed the data by comparing island rate relative to the outgroup with mainland rate relative to the outgroup.

Table 1. Relative substitution rates (island/mainland) and ratios of non-synonymous to synonymous substitution rates for island (IS ω) and mainland (ML ω) lineages for 70 independent comparisons (see Electronic Appendix for full list of species names). (Some taxa provide more than one independent comparison (e.g. *Acrocephalus* 1 and 2), each of which represents a separate colonization of an island from the mainland. Genes used in the analyses include mitochondrial genes (cytochrome *b* (*cytb*), NADH dehydrogenase subunit 2 (*ND2*), cytochrome oxidase subunit I (*COI*), cytochrome oxidase subunit II (*COII*), 12S small subunit ribosomal RNA (*12S*), 16S small subunit ribosomal RNA (*16S*)), nuclear genes (interphotoreceptor-binding protein (*IRBP*), von Willebrand factor (*vWF*), yolk protein 1 (*YP1*), elongation factor 1α (*EF1α*), internal transcribed spacer region (*ITS*), external transcribed spacer region (*ETS*)) and chloroplast genes (NADH dehydrogenase subunit F (*ndhF*) and ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*)).)

taxon	island(s)	gene(s)	relative substitution rate	IS ω	ML ω
vertebrates					
Acrocephalus 1	Macaronesia	*cytb*	1.4715	0.0816	0.2062
Acrocephalus 2	Madagascar, Seychelles	*cytb*	0.8076	0.0277	0.0123
Anairetes	Juan Fernandez	*cytb, ND2*	1.0513	0.0643	0.04
Anas 1	Subantarctic	*cytb, ND2*	8.4771	0.0819	0.0378
Anas 2	Madagascar	*cytb, ND2*	0.9232	0.0215	0.0236
Anas 3	Phillipines	*cytb, ND2*	2.8793	0.0281	< 0.0001
Anas 4	Hawaii, Madagascar	*cytb, ND2*	1.7029	0.0691	< 0.0001
Anolis	West Indies	*cytb*	0.5631	0.0704	0.1183
Bufo	West Indies	*cytb, 12S, 16S*	1.8487	0.0033	0.0029
Chalcides	Macaronesia	*cytb, 12S, 16S*	2.1278	0.0107	0.0279
Drepanidinae	Hawaii	*cytb*	1.1726	0.0019	< 0.0001
Falco	Madagascar, Mauritius, Seychelles	*cytb*	0.9896	0.0261	0.0647
Geospizinae	Galapagos	*cytb*	0.5986	0.0605	0.0245
Lacerta	Macaronesia	*cytb, COI, 12S*	0.7057	0.039	0.0196
Lemuridae	Madagascar	*cytb, IRBP*	0.7775	0.0977	0.0856
Mabuya 1	Socotra	*cytb, COI, 12S*	1.5738	0.0119	0.0092
Mabuya 2	Macaronesia	*cytb, COI, 12S*	32.0482	0.0196	0.0124
Myadestes 1	West Indies	*cytb*	0.7764	< 0.0001	0.0142
Myadestes 2	West Indies	*cytb*	1.0709	0.0106	0.0024
Nesomyinae	Madagascar	*cytb*	0.9537	0.0138	0.016
Regulus	Macaronesia	*16S*	2.1198	—	—
Sylviidae	Madagascar	*cytb*	0.8656	0.0207	0.0212
Tarentola 1	Macaronesia	*cytb, 12S*	1.3683	0.0468	0.03
Tarentola 2	Macaronesia	*cytb, 12S*	0.4980	0.04	0.0573
Tarentola 3	Macaronesia	*cytb, 12S*	0.5222	0.0526	0.0744
Tenrecidae	Madagascar	*vWF, 12S, 16S*	0.6540	0.2085	0.0619
Vangidae	Madagascar	*12S, 16S*	4.9250	—	—
Xantusiidae 1	California Channel	*cytb, 12S*	0.7275	0.0517	0.0545
Xantusiidae 2	West Indies	*cytb, 12S*	0.8533	0.0532	0.0458
Zenaida 1	Socorro	*cytb, ND2*	1.9165	0.1569	0.0362
Zenaida 2	Galapagos	*cytb, ND2*	2.4174	0.0587	0.012
invertebrates					
Brachyderes	Macaronesia	*COII*	9.9626	< 0.0001	0.0061
Celatoblatta	Chatham	*COI*	0.7309	0.0047	0.0019
Drosophila	Hawaii	*YP1*	2.1216	0.3037	0.1108
Dysdera	Macaronesia	*COI*	1.5143	0.0119	0.0175
Galapaganus	Galapagos	*COI*	0.3768	0.0325	0.0688
Geodorcus	Chatham	*COI*	0.2095	0.0044	0.0023
Gonepteryx	Macaronesia	*COI*	2.0660	0.1467	0.0306
Mandarina	Bonin	*16S*	0.6424	—	—
Megalagrion	Hawaii	*COII, EF1α*	0.2580	0.036	0.0327
Meladema	Macaronesia	*COI*	1.8450	0.0143	0.01
Orsonwelles	Hawaii	*COI, 16S*	0.7334	0.0128	0.0112
Pimelia	Macaronesia	*COI*	2.8382	0.0041	0.0108
Steganacarus	Macaronesia	*COI*	3.7297	0.0096	0.0128
Talitropsis	Chatham	*COI*	2.2770	0.0044	0.0023
Tarphius	Macaronesia	*COI*	13.9775	0.0165	0.0133
Thomisidae	Hawaii	*COI*	0.4193	0.003	0.0055
plants					
Asteriscus	Macaronesia	*ITS, ETS*	1.3181	—	—
Bencomia alliance	Macaronesia	*ITS*	5.8705	—	—
Bidens 1	Hawaii	*ITS*	0.1354	—	—
Bidens 2	Starbuck	*ITS*	0.9856	—	—

(*Continued.*)

Table 1 (*Continued.*)

| taxon	island(s)	gene(s)	relative substi-tution rate	IS ω	ML ω
Coreocarpus	California Baja	*ITS*	1.0359	—	—
Erigeron	Juan Fernandez	*ITS*	0.2384	—	—
Genista	Macaronesia	*ITS*	0.2330	—	—
Geranium	Hawaii	*rbcL*	1.2503	—	—
Hesperomannia	Hawaii	*ndhF*	1.0294	—	—
Inuleae 1	Macaronesia	*ITS*	0.5137	—	—
Inuleae 2	Macaronesia	*ITS*	1.0224	—	—
Lavatera 1	Macaronesia	*ITS*	0.4470	—	—
Lavatera 2	Macaronesia	*ITS*	0.5278	—	—
Madieae	Hawaii	*ITS*	1.5509	—	—
Pericallis	Macaronesia	*ITS*	0.3512	—	—
Phylica	Mauritius, St Helena, Tristan da Cunha	*ndhF*	0.9212	—	—
Robinsonia	Juan Fernandez	*ITS*	0.5760	—	—
Rubus 1	Hawaii	*ndhF*	4.1627	—	—
Rubus 2	Hawaii	*ndhF*	1.6679	—	—
Sideritis	Macaronesia	*ITS*	2.2418	—	—
Sonchus	Macaronesia	*ITS*	2.8499	—	—
Teline	Macaronesia	*ITS*	4.9128	—	—
Viola	Hawaii	*ITS*	4.0606	—	—
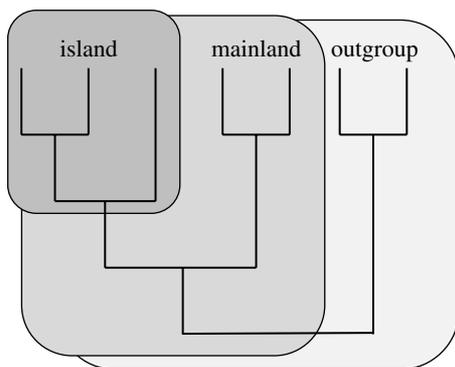
Figure 1. For the CODEML analysis, branches were assigned to one of three classes—island, mainland or outgroup (different rate classes are indicated using different shades of grey). The non-synonymous to synonymous substitution rate ratio (ω) was then estimated for each class. For the BASEML analysis, a single rate was estimated for mainland and outgroup lineages, and a second rate for island lineages.

4. DISCUSSION

We have shown that island species tend to have significantly higher ratios of non-synonymous to synonymous substitution rates than their mainland relatives. This difference is best explained by an increase in nearly neutral mutations drifting to fixation in small island populations. The pattern of increase in ω we detected was consistent across genes, taxa and island groups. There is no *a priori* reason to believe that these diverse island endemic lineages differ systematically from their mainland relatives in generation time, body size, or other life history trait thought to affect molecular evolution. It is likely, however, that most island lineages experience a reduction in N_e. Reduced levels of heterozygosity and other population genetic markers of decreased N_e have been found in a broad range of island endemic vertebrates, invertebrates and plants relative to mainland species (Ellstrand & Elam 1993; Frankham 1997). Direct estimates of N_e were not available for a great majority of

the datasets included in this analysis. However, even if a number of our datasets did include island taxa with N_e greater than or equal to that of their mainland relatives, this would not bias our results. These lineages should experience a reduction or no change in ω, and so would simply decrease the significance of the trend we see towards increased ω in island endemics across datasets.

This increase in ω is unlikely to be due to the adaptation of island endemic species to novel environments. The vast majority of the genes included in our analysis are housekeeping genes, which are more likely to be evolving under purifying selection, to maintain their essential functions, than positive selection (Zhang & Li 2004). Furthermore, the diversity of the data in our analysis makes it unlikely that our results could be due to positive selection: we have included data from genes coding for components of different metabolic pathways, from many species filling a vast range of ecological niches.

Island endemics may alternatively experience relaxed selective constraints, allowing the fixation of more slightly deleterious mutations, and thus increasing ω. Many niches may be vacant during the early stages of colonization of an island, reducing competition for resources. Colonizing populations tend to carry only a fraction of the parasites hosted by the originating population (Mitchell & Power 2003; Torchin *et al.* 2003), and island endemics may also encounter fewer herbivores or predators, leading to relaxed selection on many kinds of defence mechanisms (e.g. Bowen & Van Vuren 1997). Other island endemic species, however, experience strong competition for limited resources, harsh environmental conditions and heavy mortality (e.g. Wright 1999). Any relaxation of purifying selection which does occur is, in any case, unlikely to act across a broad range of genes and taxa, as would be required to explain the pattern of increased ω that we observe.

Although an increase in substitution rate has previously been demonstrated for a number of lineages with reduced N_e (e.g. Kliman *et al.* 2000; Woolfit & Bromham 2003), we

did not detect any significant change in overall substitution rate in island endemic lineages. It is possible that, in these datasets, the signal of a consistent small change in the rate of fixation of nearly neutral mutations is being overwhelmed by the 'noise' of random variation in mutation rate between island and mainland lineages. Such variation in mutation rate is not expected to affect the analysis of ω. Selection on synonymous sites (Akashi 1999; Comeron *et al*. 1999) may reduce the sensitivity of the analysis of ω, but would not bias our results: such selection would reduce the likelihood of detecting a significant increase in ω in island species, and our test is therefore a conservative one. It seems likely that the increase in ω values in island lineages is a robust result, and the absence of significant change in overall substitution rate, while not supporting the nearly neutral theory, is not in conflict with it.

5. CONCLUSIONS

We have shown, for a diverse group of vertebrate and invertebrate lineages, that island endemic species have significantly higher non-synonymous to synonymous substitution rate ratios than their mainland relatives. This increase in ω is most likely caused by increased fixation of nearly neutral mutations due to reduction in effective population sizes in island endemic lineages. Our results support a key assertion of the nearly neutral theory, that the fixation of neutral and nearly neutral mutations by drift is a significant force in molecular evolution.

REFERENCES

Akashi, H. 1999 Inferring the fitness effects of DNA mutations from polymorphism and divergence data: statistical power to detect directional selection under stationarity and free recombination. *Genetics* **151**, 221–238.

Bowen, L. & Van Vuren, D. 1997 Insular endemic plants lack defenses against herbivores. *Conserv. Biol.* **11**, 1249–1254. (doi:10.1046/j.1523-1739.1997.96368.x.)

Bromham, L. & Woolfit, M. 2004 Explosive radiations and the reliability of molecular clocks: island endemic radiations as a test case. *Syst. Biol.* **53**, 758–766. (doi:10.1080/10635150490522278.)

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

Clegg, S. M. & Owens, I. P. F. 2002 The 'island rule' in birds: medium body size and its ecological explanation. *Proc. R. Soc. B* **269**, 1359–1365. (doi:10.1098/rspb.2002.2024.)

Comeron, J. M., Kreitman, M. & Aguade, M. 1999 Natural selection on synonymous sites is correlated with gene length and recombination in *Drosophila*. *Genetics* **151**, 239–249.

del Hoyo, J., Elliott, A. & Sargatal, J. 1992 *Handbook of the birds of the world: ostrich to ducks*, vol. 1. Barcelona: Lynx Edicions.

Eldridge, M. D. B., King, J. M., Loupis, A. K., Spencer, P. B. S., Taylor, A. C., Pope, L. C. & Hall, G. P. 1999 Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. *Conserv. Biol.* **13**, 531–541. (doi:10.1046/j.1523-1739.1999.98115.x.)

Ellstrand, N. C. & Elam, D. R. 1993 Population genetic consequences of small population size: implications for plant conservation. *Annu. Rev. Ecol. Syst.* **24**, 217–242. (doi:10.1146/annurev.es.24.110193.001245.)

Frankham, R. 1997 Do island populations have less genetic variation than mainland populations? *Heredity* **78**, 311–327. (doi:10.1038/sj.hdy.6880980.)

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

Gillespie, J. H. 1999 The role of population size in molecular evolution. *Theor. Popul. Biol.* **55**, 145–156. (doi:10.1006/tpbi.1998.1391.)

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology*. Oxford: Oxford University Press.

Hasegawa, M., Kishino, H. & Yano, T. A. 1985 Dating of the human ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**, 160–174.

Johnson, K. P. & Seger, J. 2001 Elevated rates of nonsynonymous substitution in island birds. *Mol. Biol. Evol.* **18**, 874–881.

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

Kliman, R. M., Andolfatto, P., Coyne, J. A., Depaulis, F., Kreitman, M., Berry, A. J., McCarter, J., Wakeley, J. & Hey, J. 2000 The population genetics of the origin and divergence of the *Drosophila simulans* complex species. *Genetics* **156**, 1913–1931.

Llopart, A. & Aguade, M. 1999 Synonymous rates at the *RpII215* gene of *Drosophila*: Variation among species and across the coding region. *Genetics* **152**, 269–280.

Mitchell, C. E. & Power, A. G. 2003 Release of invasive plants from fungal and viral pathogens. *Nature* **421**, 625–627. (doi:10.1038/nature01317.)

Moran, N. A. 1996 Accelerated evolution and Muller's rachet in endosymbiotic bacteria. *Proc. Natl Acad. Sci. USA* **93**, 2873–2878. (doi:10.1073/pnas.93.7.2873.)

Ohta, T. 1972*a* Evolutionary rate of cistrons and DNA divergence. *J. Mol. Evol.* **1**, 150–157. (doi:10.1007/BF01659161.)

Ohta, T. 1972*b* Population size and the rate of evolution. *J. Mol. Evol.* **1**, 305–314. (doi:10.1007/BF01653959.)

Ohta, T. 1992 The nearly neutral theory of molecular evolution. *Annu. Rev. Ecol. Syst.* **23**, 263–286. (doi:10.1146/annurev.es.23.110192.001403.)

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

Ohta, T. & Kimura, M. 1971 On the constancy of the evolutionary rate of cistrons. *J. Mol. Evol.* **1**, 18–25. (doi:10.1007/BF01659391.)

Rambaut, A. 1996 Se–Al: sequence alignment editor v2.0. Available at http://evolve.zoo.ox.ac.uk/..

Sokal, R. & Rohlf, F. J. 1995 *Biometry*. New York: W.H. Freeman.

Swofford, D. L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, v. 4. Sunderland, MA: Sinauer Associates.

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J. & Kuris, A. M. 2003 Introduced species and their missing parasites. *Nature* **421**, 628–630. (doi:10.1038/nature01346.)

Weinreich, D. M. 2001 The rates of molecular evolution in rodent and primate mitochondrial DNA. *J. Mol. Evol.* **52**, 40–50.

Woolfit, M. & Bromham, L. 2003 Increased rates of sequence evolution in endosymbiotic bacteria and fungi with small effective population sizes. *Mol. Biol. Evol.* **20**, 1545–1555. (doi:10.1093/molbev/msg167.)

Wright, S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159.

Wright, P. C. 1999 Lemur traits and Madagascar ecology: coping with an island environment. *Am. J. Phys. Anthropol.* **29**(Suppl.), 31–72. (doi:10.1002/(SICI)1096-8644(1999) 110:29+<31::AID-AJPA3>3.0.CO;2-0.)

Wu, C. I. & Li, W. H. 1985 Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl Acad. Sci. USA* **82**, 1741–1745.

Yang, Z. H. 1994 Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites—approximate methods. *J. Mol. Evol.* **39**, 306–314. (doi:10.1007/BF00160154.)

Yang, Z. H. 1997 PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* **13**, 555–556.

Yoder, A. D. & Yang, Z. H. 2000 Estimation of primate speciation dates using local molecular clocks. *Mol. Biol. Evol.* **17**, 1081–1090.

Zhang, L. & Li, W. H. 2004 Mammalian housekeeping genes evolve more slowly than tissue-specific genes. *Mol. Biol. Evol.* **21**, 236–239. (doi:10.1093/molbev/msh010.)