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Phylogenetic Comparative Methods Strengthen Evidence for Reduced Genetic Diversity among Endangered Tetrapods

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Abstract

The fitness of species with little genetic diversity is expected to be affected by inbreeding and an inability to respond to environmental change. Conservation theory suggests that endangered species will generally demonstrate lower genetic diversity than taxa that are not threatened. This hypothesis has been challenged because the time frame of anthropogenic extinction may be too fast to expect genetic factors to significantly contribute. I conducted a meta-analysis to examine how genetic diversity in 894 tetrapods correlates with extinction threat level. Because species are not evolutionarily independent, I used a phylogenetic regression framework to address this issue. Mean genetic diversity of tetrapods, as assessed by protein heterozygosity, was 29.7–31.5% lower on average in threatened species than in their nonthreatened relatives, a highly significant reduction. Within amphibians as diversity decreased extinction risk increased in phylogenetic models, but not in nonphylogenetic regressions. The effects of threatened status on diversity also remained significant after accounting for body size in mammals. These results support the hypothesis that genetic effects on population fitness are important in the extinction process.

Keywords

effective population size; extinction; life-history evolution; phylogenetic regression; protein heterozygosity

Introduction

Developing effective policy for conservation and species management requires a thorough understanding of the factors that put species at risk of extinction (Mace & Lande 1991). Much research has gone into determining how ecological and genetic factors predispose groups of organisms to extinction (e.g., Lande 1988; Frankham 1995; Hedrick 2001). One technique that could potentially inform species management, but is greatly under-used in conservation biology, is phylogenetic analysis (but see Cardillo et al. 2005; Davies et al. 2008). The advantage of phylogenetic analysis is its ability to assess trait variation among taxa while structuring the data on the basis of the nonindependence that arises from coancestry (Felsenstein 1985). Although these methods are used most commonly to study phenotypic evolution, they can also be applied to studies of the genetic characteristics of populations (Duminil et al. 2007; Jose et al. 2008).

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Supporting Information

All data used in the analyses, relevant citations, tree structure, and the heterozygosity transformation table (Appendix S1) and statistical analyses of diversity and threat level (Appendix S2) are available as part of the online article. The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Reduced genetic diversity may increase extinction risk through several mechanisms. Species that have low genetic diversity often suffer inbreeding depression and lack the evolutionary potential necessary to adapt to a changing environment (Charlesworth & Charlesworth 1987; Reed & Frankham 2003). Low levels of diversity are expected in small populations, including those that have undergone substantial reductions in abundance. Thus, conservation biologists hypothesize that endangered species will usually possess less genetic diversity than species that are not endangered. The competing hypothesis, however, states that stochastic ecological processes are responsible for the majority of extinction events and genetics plays a minor role, if any, in extinction (Lande 1988; Spielman et al. 2004). In a previous meta-analysis designed to address the relation between diversity and extinction, Spielman et al. (2004) showed that in 170 comparisons threatened plant and animal taxa exhibited less genetic diversity than their nonthreatened relatives in 77% of comparisons, a statistically significant majority. Although this analysis incorporated coancestry to some extent through the selection of close relatives, nearly all information provided by the phylogenetic tree was lost in the series of 170 independent comparisons.

Effective Population Size, Variance, and Phylogeny

Populations experience genetic drift at a rate dictated by their effective population size (N_e), defined here relative to census population size (N) (Wright 1931; Hedrick 2005a)

$$\frac{N_e}{N} \approx \frac{2}{1 + \frac{V_k}{\bar{k}}}$$

For a single generation N_e/N is inversely proportional to $V_k/\bar{k}$, where $\bar{k}$ is the mean reproductive output and V_k is the variance in reproductive success (Kimura & Crow 1963; Hedrick 2005b). Crow and Morton (1955) refer to the quotient of $V_k/\bar{k}$ as the “index of variability” and note that when it is greater than unity, $N_e < N$. Kimura and Crow (1963) argue that $V_k/\bar{k}$ is the most important genetic quantity to estimate for natural populations. Over many generations, N_e is also reduced by fluctuating N because long-term N_e is equal to the harmonic mean of the within-generation values of N_e . The cumulative effects of intra- and intergenerational variance in individual reproductive success act together to reduce the N_e/N ratios in populations (Kalinowski & Waples 2002). As a result of the relation between N_e and V_k , unequal sex ratios, large differences in family size, nonrandom mating, and cross-generation fluctuations in census size reduce N_e/N .

The life histories of closely related species are generally more similar than those of distantly related species. Life-history traits such as life span, reproductive events per lifetime, number of offspring per reproductive event, time to reproductive maturity, and mating strategies are all phylogenetically nonrandom (Stearns 1983; Dunham & Miles 1985; Harvey et al. 1991). Assuming that closely related species are more likely to have similar values of N than distantly related species, each of these life-history traits has the potential to influence the value of N_e due to their effects on the variance in reproductive success. For example, species that exhibit type III survivorship curves can have much higher variance in reproductive success than species that exhibit type I survivorship curves and have high parental investment (Hedrick 2005b). When relatively few parents are capable of repopulating whole demes (i.e., sweepstakes reproduction), N_e can be orders of magnitude lower than N (Hedrick 1994). Not all species with a type III life-history have low N_e/N ratios (Turner et al. 2006). Nevertheless, the potential for non-random mortality to greatly reduce N_e/N is high in type III species, and a recent review by Palstra and Ruzzante (2008) suggests N_e/N is generally lower in type III than in type I species.

Because it is expected that genetic diversity and phylogeny are correlated, a phylogenetic statistical framework is needed to detect a correlation between decreased diversity and increased extinction risk. Not considering the effects of phylogenetic relations could lead to inaccurate conclusions. Figure 1 shows two hypothetical phylogenies with extinction risk and genetic diversity at the tree tips. The phylogenetic tree at the top is a star phylogeny that assumes all species are equally related, but the tree at the bottom shows hierarchical species relations. Nonphylogenetic statistical methods treat species as independent units related in the way depicted in the star phylogeny. When the data shown for the species tree were analyzed on the basis of the star phylogeny, a *t* test showed that the mean genetic diversity of the species categorized as least concern and threatened were not different ($p = 0.8763$). Nevertheless, when the data were analyzed with a Grafen's rho phylogenetic regression, which used the topology of the species tree to account for correlation in the data, threatened species were significantly less genetically diverse ($p = 0.0194$) than species categorized as of least concern. This simple example shows how ignoring phylogeny can result in an incomplete understanding of the data. In the species tree, threatened species always have less genetic diversity than their nearest phylogenetic neighbors. If independence is assumed, that association is missed because the threatened species are in a portion of the tree with higher overall diversity.

Methods

Data Collection

I used mean heterozygosities from published allozyme studies of 894 tetrapod species from 32 orders and 125 families in four classes (Mammalia [434 species], Aves [177], Reptilia [90], and Amphibia [193]). Much of the data came from Nevo et al. (1984), Evans (1987), Nevo and Beiles (1991), Makarieva (2001), and Spielman et al. (2004). (All data and citations are in Supporting Information.) Allozyme data comprise the most complete data set available across a wide range of species; however, allozyme analyses are susceptible to the confounding effects of selection. In this case, selection could introduce a systematic bias to the analysis if threat status of species and selection are correlated. Nevertheless, it seems unlikely that threat status is correlated broadly with pre-existing selection on allozymes or linked loci. I did not use microsatellites because fewer studies are available and loci are often selected for either high or low diversity, depending on the purpose of the study. Abundant mtDNA data are available, but recently Bazin et al. (2006) showed that allozymes are likely better predictors of genetic diversity than mtDNA. Although nuclear sequence data would be the best option for this type of analysis, the current available sequences would have limited the number of taxa I could examine.

In cases where multiple heterozygosity values were found for the same species, I calculated the heterozygosity from a weighted mean of the number of individuals and number of loci used for each estimate. When available, I recorded both expected heterozygosity (H_e) and observed heterozygosity (H_o) from the cited source. In cases where H_e was not given in the source but allele frequencies were available, I calculated H_e as Nei's (1973) gene diversity. If I could not determine whether estimates of heterozygosity were provided as H_o or H_e and I could not locate original references, I assumed values of heterozygosity were H_o . I made this assumption for 8.8% of the data. To create a directly comparable data set, observations with only one estimate of heterozygosity (H_o or H_e) were transformed following Nevo et al. (1984) to obtain estimates of both heterozygosity values (Supporting Information). The transformation was based on best estimates of the relation between H_o and H_e values. If H_o and H_e were both available, I did not transform any heterozygosity values.

The IUCN (International Union for Conservation of Nature) categorizes species' probabilities of extinction from least concern to extinct in the wild and extinct (IUCN 2008).

I used the IUCN categorization to separate the species in my analysis into three categories: least concern (included least concern and near threatened species); threatened (species that are vulnerable, endangered, critically endangered, and extinct in the wild); and not evaluated (species listed as data deficient by IUCN and species that are not on the IUCN Red List). This general strategy of binning threat categories follows the IUCN hierarchy and the clustering of Spielman et al. (2004). Additional regressions that did not bin species but were instead based on all categorizations in order of increasing threat were also conducted (Supporting Information).

Phylogeny Construction

Using my best estimates of topology from previously published trees, I constructed a phylogenetic tree for tetrapods in Mesquite (version 2.6) (Maddison & Maddison 2009) (Supporting Information). Analyses were performed with branch lengths set to one, and with an arbitrarily ultrametricized tree. Any divergence for which no data were available was left as an unresolved soft polytomy resulting in a tree with 346 internal nodes. When necessary for analyses polytomies were randomly resolved with branch lengths of zero. I did not adjust the number of degrees of freedom in the generalized least-squares (GLS) on the basis of the number nodes in the tree, which may result in liberal tests (Rohlf 2006; Lavin et al. 2008). Although branch length estimates are certainly inaccurate in this reconstruction, and certain aspects of the topology are still contested, phylogenetic methods based on imperfect “working phylogenies” should still outperform statistical methods based on independence (Freckleton et al. 2002). Errors in topology will generally downwardly bias phylogenetic signal (Ives et al. 2007; Revell et al. 2008). Data analyzed with no tree can also be thought of as assuming a star phylogeny where all taxa are equally related to one another. Therefore, even an obviously imperfect tree, of the type employed in this review, provides more information about statistical dependence than no tree at all (Rohlf 2006).

Statistical Analyses

With transformation to the arcsine square root, the distribution of mean heterozygosity was approximately normal (Sokal & Rohlf 1995). I plotted transformed H_o as function of taxonomic class and threat category (Fig. 2) and used analysis of variance with type I sums of squares to assess significance. To test for the presence of phylogenetic signal in the diversity data without incorporating threat category, I followed the methods of Blomberg et al. (2003). I calculated the K statistic for the presence of phylogenetic signal in the R package “picante,” with 1000 replicates for each tree and heterozygosity combination (R Core Development Team 2008; Kembel et al. 2009). The observed versus expected variance of phylogenetically independent contrasts are the basis for the K statistic. Under Brownian motion evolution $K = 1$. Values significantly different from zero, as assessed by randomizing tip data, demonstrate phylogenetic signal.

To analyze the relation between threat category and genetic diversity, I implemented a series of GLS models in R packages “ape” and “nlme” (Paradis et al. 2004; Pinheiro et al. 2008). In these models, parameter values for tree transformation and coefficients of threat category were estimated simultaneously with maximum likelihood. A simple GLS model with no covariance in error terms was compared with models with phylogenetically based correlation structures, including Brownian motion, Grafen’s rho, Pagel’s lambda, and the Ornstein–Uhlenbeck (OU) model (Felsenstein 1985; Grafen 1989; Martins & Hansen 1997; Freckleton et al. 2002; Blomberg et al. 2003). The rho, lambda, and OU models incorporate additional parameters and branch length transformations to best describe the covariance structure in the model residuals. I used Akaike’s information criterion (AIC) and the Bayesian information criterion (BIC) to compare GLS models. All models tested whether mean heterozygosity changed as a function of threat category.

I conducted additional statistical analyses for the 193 amphibian species alone. For amphibians, I used a simple GLS model to determine the relation between threat category and transformed H_e without including phylogenetic relations. Using the amphibian portion of the phylogenetic tree, I also applied the rho model to only the amphibian data. I used the rho model because results from this model depended only on tree topology and not on branch lengths. Because the simple GLS model is nested within the rho model, I used a likelihood-ratio test to compare model fits.

Finally, to test potential confounding effects of body size on the analyses, I compiled mean adult body mass for 429 mammals in Nowak's (1999) data set (Supporting Information). I used the mean body mass of males and females and the center of the range of potential masses if a range was provided. For species that did not have a specific body mass, I used mean body mass of the genus. This is an imperfect analysis because it fails to capture some potentially important within-genus variation. Nevertheless, the variation of body mass within a genus should be much smaller than the variation across all genera, which spanned seven orders of magnitude.

I used ordinary least-squares analysis of variance to determine the relation between $\log_{10}$ body mass and threat category. I also regressed $\log_{10}$ body mass on H_e . To determine the influence of phylogeny, I applied the OU model in same manner as for the previously described GLS models, but I included $\log_{10}$ body mass along with threat category to predict genetic diversity. I ran an additive model with both terms, a model without the mass term, and a model without threat category. I compared the results of nested models with likelihood-ratio tests.

Results

Taxonomic class and threat category were highly significant predictors of H_o and H_e , but explained only about 10% of the variance in the data (H_e : $F_{11,882} = 9.741$, $p < 2.2E^{-16}$, adjusted $R^2 = 0.0972$; H_o : $F_{11,882} = 10.26$, $p < 2.2E^{-16}$, adjusted $R^2 = 0.1024$) (Table 1). Tests for the presence of phylogenetic signal in genetic diversity data independent of threat category resulted in K values that were significantly different from 0, but less than 1, which suggests a phylogenetic signal was present in the data but not as a pure process of Brownian motion (H_e K : ultrametric = 0.1107, branch length of 1 = 0.2196; H_o K : ultrametric = 0.1127, branch length of 1 = 0.2050). For both H_e and H_o , the trees with branch lengths of 1 had higher K values than the ultrametric trees.

The simple GLS model and the Brownian motion model fit the data poorly relative to the other GLS models (Table 2). In the case of the ultrametric trees, the simple GLS model provided a better fit to the data than the Brownian motion model on the basis of AIC and BIC. In all cases the rho model, the lambda model, and the OU model were significantly better fits than the Brownian motion model. For each tree and heterozygosity combination, the OU model provided the best fit. Nevertheless, differences between the rho, lambda, and OU models were small.

For all models, there was no statistically significant difference in species diversity between species of least concern and species that have not been evaluated. Similarly, all models showed that threatened species had significantly less genetic diversity than species that are not threatened. Estimates of coefficients of threat category were consistent across models (Table 3). For H_e on trees with a branch length of 1 and on ultrametric trees, the threatened category was associated with average reductions in heterozygosity of 29.7% and 30.3%, respectively. For H_o on these trees, the threatened category was associated with reductions in heterozygosity of 30.9% and 31.5%, respectively. For both H_e and H_o , phylogenetic

regressions were associated with higher estimates of diversity loss for threatened species. Estimates of diversity loss with nonphylogenetic GLS models were 23.4% and 25.9% for H_e and H_o , respectively.

Heterozygosity of amphibians in the categories of least concern and threatened were not significantly different on the basis of a simple GLS model ($p = 0.401$), but the mean genetic diversity of threatened species was 9.7% greater than the mean genetic diversity of species of least concern. The model that incorporated tree structure fit the data better (likelihood-ratio test, $\chi^2 = 21.29$, $df = 1$, $p < 0.0001$). Furthermore, when tree structure was incorporated into the analysis, the heterozygosity of species of least concern was approximately 11.3% greater than that of threatened taxa. Although, the difference between these groups was not significant ($p = 0.412$).

In the mammalian body size analysis, as predicted $\log_{10}$ body mass was significantly different among the three threat categories ($F_{2,426} = 39.25$, $p = 2.27E^{-16}$, $R^2 = 0.1516$). Threatened species were an average of 1.5 orders of magnitude larger than species of least concern. The relation between body mass and genetic diversity was significant, but explained a very small portion of the variance ($F_{1,427} = 7.39$, $p = 0.0068$, $R^2 = 0.0147$). The OU GLS model that included threat category but not mass did not fit the data significantly worse than the full model ($\chi^2 = 0.2228$, $df = 1$, $p = 0.6369$). Nevertheless, the fit of the model with the mass term but without threat category was significantly worse than the fit of the full model ($\chi^2 = 12.938$, $df = 1$, $p = 0.0016$).

Discussion

Diversity and Extinction

The role of genetic diversity in the extinction process has been debated for years. If genetic diversity is phylogenetically nonrandom, as hypothesized, then informed statistical methods are required to address whether genetics affects the extinction process. In a previous meta-analysis, Spielman et al. (2004) compared threatened species and their close nonthreatened relatives to account for species relatedness. My study accounts for deeper evolutionary relations by including the tetrapod phylogeny directly in the analysis.

As I hypothesized, genetic diversity data had a significant phylogenetic signal as demonstrated by the K statistic. This signal likely arose from similar life-history characteristics and values of N in closely related species. The N and V_k determine the rate at which genetic diversity is lost through drift in a population. These two characteristics are expected to be correlated because at stable N , species with relatively high maximum fecundity will have an increased V_k if any individuals in the population approach full reproductive capacity. In a sexually reproducing species with replacement-level reproduction of 2/female, if any individual female has 50 offspring that survive to reproduce, then over the long term at least 24 females must have <2 reproducing offspring. Instances of closely related taxa with very different N exist, but the phylogenetic conservatism of population size is just as important at the deeper nodes of the tree separating major clades. Overall, the expected additional genetic diversity of a higher value of N is dampened by increased variance in V_k . Across major evolutionary lineages the value of N_e/N should vary in a phylogenetically nonrandom way. The use of population-genetic statistics to draw conclusions about genetic diversity in comparative studies should account for the influence of this effect and for phylogeny in general.

The significant relation between threat category and heterozygosity contradicts the hypothesis that genetic effects are unimportant in extinctions. Across the tetrapod phylogeny, threat category, and genetic diversity were significantly correlated. This effect of

threatened status corresponded to an average of 29.7–31.5% less genetic diversity in threatened than in nonthreatened species. These heterozygosity differences are not as large as those of Spielman et al. (2004), who found a mean reduction in heterozygosity of 35% in a series of independent comparisons between closely related threatened and non-threatened vertebrate species. Our results are not directly comparable, however, because Spielman et al.'s (2004) analysis included some fish species and mixed types of genetic markers, including some micro- and minisatellites. My analyses are correlational and do not permit conclusions about causation in this process. It may be that the diminishing population sizes that precede extinction are accompanied by a loss of genetic diversity or, conversely, that species with low genetic diversity are more likely to decline in abundance in response to environmental perturbations and to be listed as threatened by the IUCN.

The influence of human-induced population bottlenecks on genetic diversity has remained contentious because heterozygosity is lost in bottlenecked populations at a rate of $1/2N_e$ per generation (Wright 1931). This means a large number of generations must pass for a substantial component of heterozygosity to be lost in all but severely bottlenecked population. The length of time required may be indirect evidence that the relation between diversity and endangerment is based on species with low initial diversity being more susceptible to environmental change. Nevertheless, bottlenecks of diversity can also occur when movement among previously connected subpopulations is constrained and results in range fragmentation and contraction. The loss of genetic diversity in these instances may be due to the fractionation of populations into local demes with low N_e , rather than the direct loss of many individuals from the subpopulations. Ultimately, the question of whether human impacts cause low genetic diversity remains equivocal given my results, but the existence of a significant relation between diversity and endangerment is clear.

Relations within Amphibia

Amphibians in my data set are a good example of why phylogenetic methods are necessary to assess diversity. The raw data showed that threatened amphibians had slightly higher heterozygosity than amphibians that were not threatened. This effect could be attributed to incomplete knowledge of amphibian population dynamics or it could be a byproduct of the ineffectiveness of taxonomic class in describing species relations. A phylogenetic analysis of the 193 amphibians in this data set suggests the latter is likely the case. The better-fitting phylogenetic model revealed a trend consistent with the hypothesis that increased extinction risk is correlated with lower levels of heterozygosity.

Influence of Body Mass

Other characteristics, including body size, influence extinction risk significantly. It is possible that the correlation between extinction risk and genetic diversity is an indirect result of the correlation of each variable to body size. In my analysis, body size did not significantly improve the prediction of genetic diversity when threat category was included in the model. I suspect body size did not significantly improve the fit of the model because it was highly phylogenetically conserved. The majority of effects of body mass in my analysis were removed once the variance–covariance matrix of the phylogenetic tree was included in the model. The level of phylogenetic conservatism in body size is inflated in this model by the necessity of using genus means for some species. Nevertheless, even in the model that included the effects of body size, H_e of threatened species was an average of 38.6% lower than H_e of species of least concern.

Conclusion

My analysis is among the first in which an assembled phylogeny is used to statistically dissect a population genetic characteristic. I used allozymes because these were the most

complete data available to me, but as sequence databases mature DNA-based diversity metrics will be available. Nevertheless, my results underscore an important point of the comparative method: the incorporation of tree structure makes statistical analyses more robust to both type I and type II error. Nonphylogenetic analyses revealed a significant relation between threat category and diversity. These observations, however, are not independent. A common misconception is that comparative methods control for phylogeny in a way that only removes significant relations between variables. My analysis is an example of the opposite effect. Here the incorporation of the phylogenetic covariance in the data resulted in a more accurate and statistically significant relation.

Phylogenetic methods offer a powerful way to think about population–genetic statistics in threatened species. Statistical nonindependence due to coancestry is well recognized in phenotypic analysis; however, population–genetic characteristics can also vary with phylogeny, and I believe they should be analyzed accordingly in comparative studies. My analysis provides one example of how phylogenetic methods can be used to illuminate a contentious issue in conservation biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hypothetical array of trait data on two species trees. The top tree assumes statistical independence of species, and the bottom tree shows species relations (T, threatened species; LC, species of least concern; numbers, levels of genetic diversity).

Figure 2.

Observed heterozygosity transformed to the arcsine square root. Diversity levels are stratified by taxonomic class and threat category (LC, least concern and near threatened species; T, species categorized as vulnerable, endangered, critically endangered, and extinct in the wild [IUCN 2008]; NE, species not evaluated by the International Union for Conservation of Nature).

Table 1

Analysis of variance showing heterozygosity transformed to the arcsine square root as a function of taxonomic class and threat category.

<i>Source</i>	<i>Partial sums of squares</i>	<i>Mean squares</i>	<i>df</i>	<i>F</i>	<i>p</i>
Expected heterozygosity					
taxonomic class	0.7690	0.2563	3	24.84	1.95E-15
threat category	0.1426	0.0713	2	6.91	1.05E-03
taxonomic class*threat category	0.1940	0.0323	6	3.13	4.81E-03
residual	9.1000	0.0103	882		
adjusted R^2	0.0972				
Observed heterozygosity					
taxonomic class	0.6437	0.2146	3	24.79	2.10E-15
threat category	0.1484	0.0742	2	8.57	2.06E-04
taxonomic class*threat category	0.1847	0.0308	6	3.56	1.73E-03
residual	7.6345	0.0087	882		
adjusted R^2	0.1024				

Table 2

Model-selection table of generalized least-squares models for heterozygosity transformed to the arcsine square root as a function of threat category.

	Parameter	ΔAIC^a	ΔBIC^b	$\ln L^c$	Threat category p
Expected heterozygosity					
branch length 1					
simple GLS ^d	–	–	736.13	0.0050	
Brownian motion	68.33	68.33	770.30	0.0001	
Grafen (rho) ^e	0.1075	151.77	146.97	813.02	0.0009
Pagel (lambda) ^e	0.6504	154.41	149.52	814.34	0.0002
Ornstein–Uhlenbeck (d) ^e	0.5032	157.75	152.96	816.01	0.0006
ultrametric					
simple GLS	15.96	15.96	736.13	0.0050	
Brownian motion	–	–	728.16	0.0018	
Grafen (rho) ^e	0.1075	167.72	162.93	813.02	0.0009
Pagel (lambda) ^e	0.5891	154.97	150.17	806.64	0.0003
Ornstein–Uhlenbeck (d) ^e	0.4620	168.90	164.11	813.61	0.0007
Observed heterozygosity					
branch length 1					
simple GLS	–	–	813.16	0.0017	
Brownian motion	70.39	70.39	848.35	<0.0001	
Grafen (rho) ^e	0.1054	152.46	147.66	890.39	0.0004
Pagel (lambda) ^e	0.6559	159.38	154.58	893.84	0.0001
Ornstein–Uhlenbeck (d) ^e	0.4916	161.06	156.27	894.69	0.0002
ultrametric					
simple GLS	4.05	4.05	813.16	0.0017	
Brownian motion	–	–	811.13	0.0009	
Grafen (rho) ^e	0.1054	156.51	151.71	890.39	0.0004
Pagel (lambda) ^e	0.6141	148.51	143.71	886.39	0.0001
Ornstein–Uhlenbeck (d) ^e	0.4464	162.27	157.48	893.27	0.0002

FLIGHT

^a Values correspond to the difference in Akaike's information criterion (AIC) values for the model shown and the worst fitting model. Larger values indicate better model fit.

^b Values are similar to ΔAIC but are based on Bayesian information criterion (BIC) values.

^c Values are the log likelihood (ln L) scores for the models shown.

^d Simple generalized least-squares (GLS) models do not use the correlation structure of the phylogenetic tree.

^e Term in parentheses refers to the additional parameter estimated by the model. Value of the term is in the second column.

Ornstein-Uhlenbeck models for heterozygosity transformed to the arcsine square root as a function of threat category for all tree and heterozygosity combinations.

Table 3

<i>Term</i>	<i>Value</i>	<i>SE</i>	<i>t</i>	<i>p</i>
Expected heterozygosity				
branch length 1				
intercept (LC*)	0.2259	8.71E-03	25.932	<0.0001
threatened	-0.0370	9.58E-03	-3.859	0.0001
not evaluated	-0.0059	1.08E-02	-0.550	0.5824
ultrametric				
intercept (LC)	0.2209	7.37E-03	29.963	<0.0001
threatened	-0.0369	9.66E-03	-3.823	0.0001
not evaluated	-0.0061	1.08E-02	-0.568	0.5704
Observed heterozygosity				
branch length 1				
intercept (LC)	0.2132	8.16E-03	26.121	<0.0001
threatened	-0.0365	8.78E-03	-4.153	<0.0001
not evaluated	-0.0081	9.87E-03	-0.821	0.4120
ultrametric				
intercept (LC)	0.2091	6.90E-03	30.300	<0.0001
threatened	-0.0365	8.85E-03	-4.125	<0.0001
not evaluated	-0.0084	9.84E-03	-0.851	0.3947

* Species of least concern and near threatened species (LC) as determined by the International Union for Conservation of Nature.