

## Using Phylogenies to Test Macroevolutionary Hypotheses of Trait Evolution in Cranes (Gruinae)

Arne Ø. Mooers,<sup>1,2,\*</sup> Steven M. Vamosi,<sup>1</sup> and Dolph Schluter<sup>1</sup>

1. Department of Zoology and Centre for Biodiversity Research, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada;

2. Department of Biology and Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

Submitted July 28, 1998; Accepted March 18, 1999

---

*Keywords:* speciation, phylogeny, macroevolution, Gruinae.

---

We present a novel phylogenetic approach to the study of trait evolution. We compare explicit models describing the evolution of phenotypic characters over the phylogeny of a group, employing a method that avoids point estimates of ancestor reconstructions (see Maddison 1990, 1995; Losos and Miles 1994; Schluter et al. 1997; Zhang and Nei 1997; see also Pagel 1997). We contrast well-characterized models of change in species-specific phenotype: gradual change, in which differences in trait values among species are expected to be proportional to the time available for change; genetic change, in which the differences between species in the tested trait are correlated with the number of inferred substitutions that have accumulated between them (Omland 1997; Pagel 1997); and speciation change (Rohlf et al. 1990; Martins and Garland 1991; see Diaz-Uriarte and Garland 1996; Pagel 1997), in which differences are proportional to the number of speciation events separating species. We also consider a new nonhistorical model in which changes are completely independent of history, such that differences among species are expected to be the same, regardless of relationship. These four models are in turn contrasted to a fifth, null hypothesis (the free model), whereby individual lineages accrue phenotypic change at their own idiosyncratic rates.

We illustrate this method by examining a number of traits presumed to be involved in sexual signaling in the

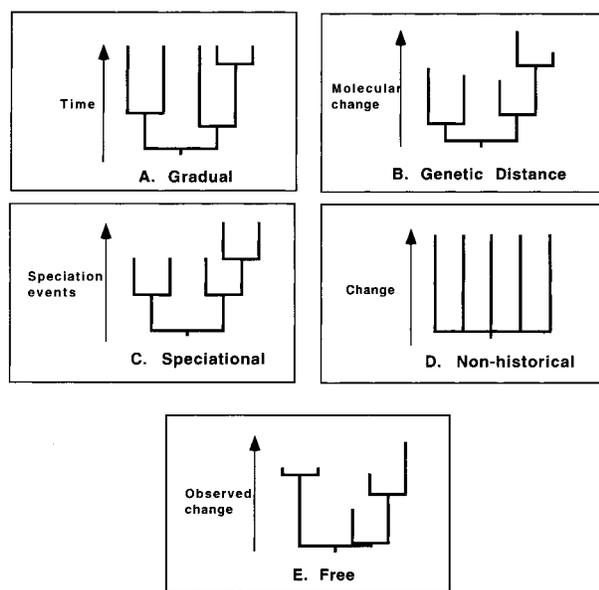
bird family Gruidae (cranes): plumage characters, unison call characters, and the shape of the bones that house the convoluted trachea presumed responsible for the species-specific calls. If sexual selection is intimately associated with the speciation process, then changes in sexually selected traits will be closely associated with speciation events, rather than changing gradually or idiosyncratically. Theory (Lande 1981, 1982; Schluter and Price 1993; Turner and Burrows 1995), comparative evidence (West-Eberhard 1983; Turner 1994; Barraclough et al. 1995; Mitra et al. 1996), and field observations and experiments (Baker and Baker 1990; Houde and Endler 1990; Endler and Houde 1995; studies reviewed in Carson 1997; Saetre et al. 1997) all point to a correlation between sexual selection and speciation. If the traits we model are involved in mate choice, we predict that the speciation change model will offer a better fit to the data than the alternatives. If trait changes are caused by continuous selection pressures that change direction often (Felsenstein 1981, 1988) or drift in large populations (Lande 1976; Lynch 1990), the gradual model should outperform the others. If trait evolution is governed by the same processes causing molecular substitutions, then the genetic model should be a good descriptor. If the evolution of the characters is not constrained by phylogenetic history, then the nonhistorical model will offer the best description. Finally, if change has a strong historical component but is highly idiosyncratic, the free hypothesis should do best. This last free model also offers a detailed description of how the traits have actually evolved over the history of the group, informing us about other possible processes besides those suggested here.

### Methods

#### *Hypotheses*

Figure 1 depicts the five models, which are all based on a Brownian motion process. In each, the branch lengths represent the a priori expectation for the amount of change in the modeled traits. The gradual model (fig. 1A) is the one most commonly employed for change in continuous

\* E-mail: mooers@bio.uva.nl.



**Figure 1:** Characterization of five models for the evolution of quantitative traits. Branch lengths represent the expected amount of change occurring along that lineage. *A*, Gradual model, in which change is correlated with time. This mirrors the inferred phylogenetic tree. *B*, Genetic distance model, in which each branch is proportional to the amount of genetic change that has occurred along it in the gene(s) used to make the tree. *C*, Speciation change model, in which change is correlated with speciation events. *D*, Nonhistorical model, in which there is no phylogenetic component to trait evolution, but each tip is equally divergent from all others. *E*, Free model, in which the branches represent the best fit of the observed data to the topology, rather than a priori hypotheses.

characters (Felsenstein 1985; Harvey and Pagel 1991; for examples, see Berrigan et al. 1993; Martins 1994; Bauwens et al. 1995; Pianka 1995; Pagel 1997; Poiani and Pagel 1997). Here, time predicts the amount of change that has occurred. In simulations, this model performs well as an approximation even when the true data-generating process is quite different (Martins and Garland 1991; Diaz-Uriarte and Garland 1996). Importantly, the process of speciation has no effect on the rate of change of the trait.

Evidence for a correlation between molecular and phenotypic evolution has recently been documented (Omland 1997). Figure 1*B* is a depiction of the genetic model, where the branch lengths represent the amount of genetic change that has accrued. If speciation is accompanied by bottlenecks, theory predicts that the rate of molecular substitution will initially increase, as preexisting slightly deleterious genetic variation is more likely to fix in small populations (Nei 1976). If the rate of change of phenotypic characters also increases during such speciation events, then we might expect the phylogenetic hypothesis taken from the genetic-distance tree (e.g., a phenogram on ge-

netic distance or a variable-rate maximum-likelihood tree) to offer a better fit to the data than hypotheses that include time or speciation events (Pagel 1994). Other factors might influence the rates of both morphological and molecular evolution—for example, body size, generation time, or selective sweeps (see, e.g., Mooers and Harvey 1994; Bromham et al. 1996; Omland 1997).

An alternate and popular model of evolution is speciation change (fig. 1*C*; for examples, see Rohlf et al. 1990; Harvey and Purvis 1991, box 2; Martin and Badyaev 1996; Bell and Mooers 1997; Poiani and Pagel 1997). Here, change is assumed to occur rapidly at, or shortly after, speciation, and stasis follows until the next speciation event. Though we predict that the speciation change model will be the best description for traits directly involved in speciation, this pattern may also arise for other reasons—for example, when speciation and trait evolution are associated with niche shifts, which may happen commonly in adaptive radiations (Schluter and Nagel 1995).

Under the nonhistorical model (fig. 1*D*), closely related species are no more likely to be similar than any two species picked at random. We model this using the Brownian motion process by setting all internode distances to 0 and setting all terminal branches to unit length, creating a star or pitchfork phylogeny. Significant improvement would suggest that phylogeny is not important to the evolution of the trait, perhaps because the rate of change has been rapid enough to erase past phylogenetic history (Cheverud et al. 1985; Gittleman and Kot 1990; see also Price et al. 1997).

The final scenario tested (fig. 1*E*), the free model, is qualitatively different from the other four and may be viewed as a null model. Here, new species inherit their trait values from their immediate ancestors but subsequently evolve at a new, independent rate. Though the topology is constrained in this model, as in the others, the branch lengths are not. The fit returned is a description of the variation among species, constrained only by the Brownian motion model and the tree topology. A significantly better fit of this hypothesis over the others would indicate that none of our four simpler models accurately reflects the true pattern of character macroevolution. Because this model lets the data choose the branch lengths and so allows a new parameter to be estimated for each branch, it is likely overparameterized (cf. Hey 1992; Mooers and Heard 1997; Mooers and Schluter 1998).

#### *Analysis*

The goal of the analysis is to determine which hypothesis of character change best describes the evolution of the trait(s) in question. The approach applied here uses random walks of continuous traits in continuous time (Ed-

wards and Cavalli-Sforza 1964; Felsenstein 1973, 1981, 1988; Bookstein 1987; McKinney 1990; Maddison 1991; Pagel 1997; Schluter et al. 1997) to try to capture the essence of the evolutionary process. The process is simple and tractable, having few parameters and known statistical properties. Because it is stochastic, it is inherently noisy. Change is continuous, reversals in direction are frequent, and evolution is unbounded (Felsenstein 1985). Changes are independent of each other and independent in different parts of the tree. Felsenstein (1981) presents equations for the likelihood of observing a set of quantitative characters at the nodes and tips of a particular bifurcating tree topology under Brownian motion, which can be recast as

$$L = \prod_{i,i'} \frac{1}{\sqrt{2\pi\beta t_i}} \exp\left[-\frac{(x_i - x_{i'})^2}{2\beta t_i}\right]. \quad (1)$$

Here,  $i' = 1$  and  $i' = 2$  are descendant nodes (or tips) emanating from the ancestor node  $i$ ;  $x_i$  and  $x_{i'}$  are the states at the nodes (or tips), and the opportunity for change along a branch (its length) is the product of the rate of change  $\beta$  times the time available for change  $t_i$  between  $i$  and  $i'$ . The product is taken over  $i = 1$  to  $N - 1$  ancestral nodes, and so over all  $2N - 2$  ancestor/direct descendant pairs in a tree with  $N$  tips. Equation (1) is a description of the likelihood for a particular set of ancestral states. Point estimates of ancestral states are often uncertain (Schluter et al. 1997), however. Furthermore, Felsenstein (1973, 1981) introduced his restricted maximum-likelihood (REML) algorithm for evaluating trees constructed using continuous characters to deal with the problem of inconsistency that arose when individual ancestral states were to be estimated. We make use of the REML algorithm of continuous maximum likelihood (CONTML; Felsenstein 1973, 1981), which integrates across all possible ancestor states. The REML algorithm, further, ignores the root node, such that the likelihood returned is for an unrooted tree (Felsenstein 1973, 1981). We have no information on the possible states at the root of our tree (see Pagel 1994, 1997), and the models we tested do not assume directionality. Finally, the use of the REML algorithm means that zero-length internal branches (corresponding to polytomies) can easily be accommodated, such that the nonhistorical hypothesis can be evaluated in the same framework as the others.

The fits of alternative hypotheses (fig. 1) were compared using the differences in their corresponding log likelihoods. For the first four models, only one parameter (corresponding to a single rate  $\beta$ ) is free to vary, since the  $t$ 's in equation (1) are specified a priori. Any pair of these hypotheses may be considered different when the difference in their likelihoods is approximately seven (Edwards

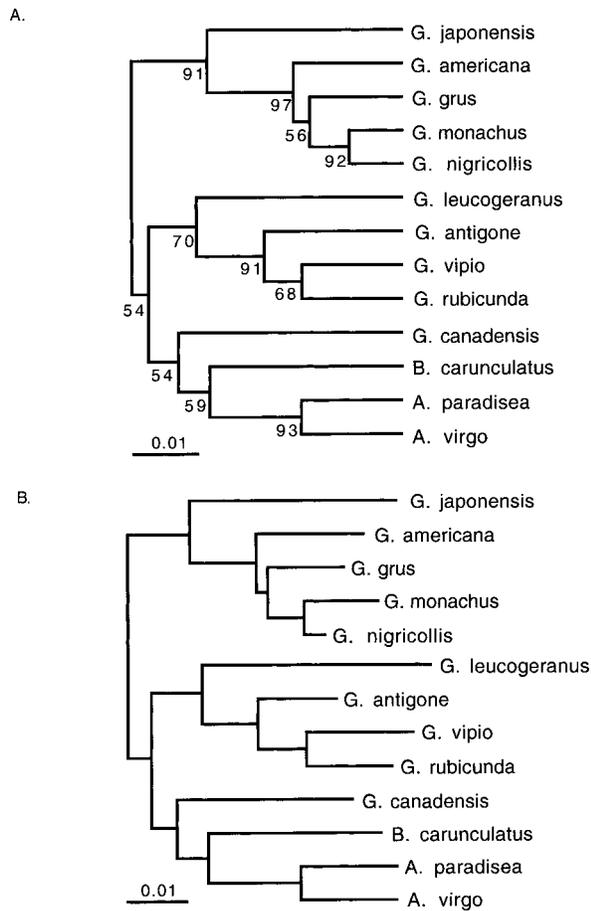
1992, pp. 180 ff.). The fifth scenario is qualitatively different. The trait values themselves are used to optimize the branch lengths, which are unconstrained by a priori hypotheses. Neither  $\beta$  nor the  $t$ 's are specified, but, as these two terms are only present as a product in the likelihood calculations (cf. eq. [1]), they cannot be estimated separately. This means that the free scenario has  $2N - 4$  extra parameters to estimate (the  $2N - 3$  branches for an unrooted tree with  $N$  tips versus the single  $\beta$  calculated for the simple models). All the simpler models are nested within the free model, allowing a log-likelihood ratio test (Goldman 1993).

The scenarios tested are very simple: more complex scenarios are intractable (e.g., trends: Diaz-Uriarte and Garland 1996; Holland and Rice 1998), are of limited relevance to the specific hypotheses tested (e.g., the Ornstein-Uhlenbeck process: Lande 1976; Felsenstein 1988; Garland et al. 1993; Martins 1994; Hansen 1997), or may require more parameter estimates than are warranted by the data (e.g., evolution within boundaries: Diaz-Uriarte and Garland 1996; see Mooers and Schluter 1998 for a discussion).

All analyses were performed using a modified version of the CONTML program of PHYLIP (version 3.0; Felsenstein 1993). The modified program (written for DOS platforms), which makes the analysis independent of scale, is available from the authors.

### Phylogeny

We chose a phylogeny that contained at least  $N - 1$  of the  $N$  known species of the in-group (a "complete tree" sensu Mooers 1995). This requirement is necessary for a valid test of the speciation change hypothesis of morphological evolution. Complete trees do not include information on extinct lineages, of course, so we must assume that there has been negligible extinction (Hey 1992) or that extinctions have been random on the tree (Harvey and Purvis 1991). Little formal work has been done on the effects of extinction on the speciation change model of trait evolution. Krajewski and colleagues (Krajewski and Fetzner 1994; Krajewski and King 1996) have published extensive phylogenetic analyses of all 15 species of cranes (family Gruidae), based on cytochrome *b* sequence data. We reconstructed the phylogeny using PHYLIP 3.5c (Felsenstein 1995) from sequence data taken from GENBANK. For the 13 species in the in-group Gruinae (sensu Sibley and Monroe 1990), with the substitution model suggested by Krajewski and King (1996:  $tr/b = 6.5$ , two categories of substitution), the molecular clock cannot be rejected (DNAML(K) version 3.572 of PHYLIP:  $2\Delta\ln[\text{like}]$  for clock vs. no clock = 13.91,  $X^2_{0.05[11]} = 19.67$ ,  $P = .24$ ). The tree we used is given in figure 2 and is identical in topology to that presented by Krajewski and King (1996). The grad-



**Figure 2:** A maximum-likelihood estimate of the phylogeny of the Gruinae based on cytochrome *b* variation (sequence data from Krajewski and King 1996). *G.* = *Grus*; *B.* = *Bufo*; *A.* = *Anthropoides*. Scale is expected number of substitutions per 100 base positions. The topology is identical to that found by Krajewski and King (1996). *A.*, Maximum-likelihood tree assuming a molecular clock (program DNAMLK of PHYLIP 3.56c); numbers refer to bootstrap support based on 100 samples. *B.*, Maximum-likelihood tree not assuming a molecular clock (program DNAML of PHYLIP 3.56c). For both trees,  $tr/tw = 6.5$ , with two categories of substitution rate in a ratio of 2 : 1.

ual hypothesis was tested using the DNAMLK tree from PHYLIP, and the genetic hypothesis was tested using the DNAML tree, keeping all model parameters the same. Because the clock model fits the cytochrome data well, we do not expect to find large differences in the performances of the gradual and genetic models using this gene. The crowned cranes, the sister group to the Gruinae, show a significantly higher average rate of substitution (Krajewski and Fetzner 1994; A. Ø. Mooers, unpublished observations) and so could not be included in the analysis, as no clock tree could be constructed.

## Data

Cranes (family Gruidae) are large, monomorphic birds with bright, highly contrasting plumage and intricate courtship behavior (Johnsgard 1983). We analyzed three suites of traits associated with intersexual signaling in the subfamily Gruinae: plumage patterns, unison calls, and skeletal traits associated with unison calls. We used mean values of these traits for each species as our data, assuming no sampling error in these measurements. Not enough data are available to incorporate sampling error. To best meet the assumptions of Brownian motion, we substituted scores from a principal components analysis (PCA) on the correlation matrix among traits for the trait values themselves. All scores were made positive by adding the minimum score on each axis to individual values for that axis.

The courtship dances of cranes involve prominent displays of plumage patterns (Johnsgard 1983). We used the lightness/darkness measures from 22 areas of the body, scored by Wood (1976), as a complex measure of plumage coloration. The scores from the first three axes of a principal components analysis based on the correlation matrix accounted for 78% of the total variance.

Unison calls are species specific, and their primary function is interpair sexual communication (Archibald 1976). Archibald (1976) found the calls to be so species specific that they could be used as taxonomic data. He measured the following parameters for 11 species of Gruinae: mean basal frequency of introduction and regular elements, mean length and mean number of harmonics for regular elements (for both males and females), and the mean length of a combined unison call. We obtained unison calls for a twelfth species (*Grus leucogeranus*) from a captive pair at the International Crane Foundation (Baraboo, Wis.) and measured the same parameters using the Canary program (Cornell Laboratory of Ornithology, version 1.2.1), with clipping level set at  $-80$  dB and the fast-Fourier transform set to 1,024. We found no recordings for the thirteenth species, *Grus nigricollis*. The scores from the first three principal components based on the correlation matrix represented 72% of the total variance.

As a third trait complex, we considered anatomical features associated with the form of the convoluted trachea. The tracheal form is said to contribute to differences among species in calls (Archibald 1976; Johnsgard 1983), and Wood (1976) identified and measured five sternum and keel traits of cranes associated with differences in tracheal form. Our suite of tracheal traits was represented as the scores on the first three axes of the principal components analysis and represented over 98% of the total variance. We found no skeletal data for *G. nigricollis*, and so this analysis was restricted to 12 species. Table 1 contains all raw data used in the analyses.

Table 1: Appearance, call, and trachea data

Species	Appearance traits																Unison call characteristics						Sternum/keel measures														
	CH	AU	NA	DA	RU	UT	RE	TH	BR	AB	CR	SN	PR	SC	IS	GP	CG	MA	AL	IG	BE	GU	MIB	FIB	MRB	FRB	MH	FH	LE	MCL	FCL	SW	KW	TH	SD	TI	
<i>paradisea</i>	5	5	6	6	6	4	3	6	6	6	6	2	2	2	4	6	6	2	6	7	4	.61	1.21	.86	1.24	1	1	3.46	.24	.16	8.25	6.96	11.93	40.57	57.44		
<i>virgo</i>	2	9	6	6	6	6	4	2	6	6	6	2	2	2	4	3	6	6	3	6	2	2	.74	1.53	.84	1.4	1	1	3.81	.21	.17	6.74	3.96	2.03	32.43	39.52	
<i>carunculatus</i>	8	8	8	2	2	2	2	8	8	2	2	8	2	2	3	2	7	5	2	4.1	3	8	.8	1.2	1.4	1.7	5	5	5.07	.11	.15	8.66	6.86	1.00	30.04	46.83	
<i>americana</i>	1	9	9	9	9	9	9	9	9	9	9	2	9	9	2	9	9	2	9	9	9	.56	.61	.79	.69	5	6	8.45	.45	.28	22.22	17.86	19.23	63.52	179.00		
<i>antigone</i>	1	5.9	6.9	5.2	6.2	6.2	5.9	7.8	5.2	5	5.2	7.5	3	6.2	6.2	4	6	6	3	6	1	1	.74	.89	1.09	1.27	4	4	14.53	.45	.1	20.44	18.54	15.05	56.8	109.90	
<i>canadensis</i>	7.7	6.6	6.6	5	4.9	5	4.2	6.8	5.4	5.5	5.6	6.5	3.1	5	5	3.8	5.8	5	4.3	5	2	7.6	.49	1.05	.54	.91	8	3	8.82	.32	.09	10.28	9.95	10.46	44.15	64.53	
<i>grus</i>	3.7	8	8	5.7	6	4.8	3	3.3	6.1	6.1	6.1	3.4	1.6	1.6	2.6	1.9	4.2	6	2.8	4.2	8	3.3	.75	.95	.68	1.01	6	4	6.7	.58	.13	11.95	15.45	13.73	50.57	150.50	
<i>japonensis</i>	3.4	3.4	3.2	9	9	9	9	3.5	9	9	9	3.3	9	2	2	9	9	9	9	2	9	5	.86	.55	1.03	1.1	3	2	11	.4	.14	17.78	19.72	16.65	62.4	198.75	
<i>leucogeranus</i>	9	9	9	9	9	9	9	9	9	9	9	9	2	9	9	2	9	9	2	9	9	.75	1.01	.78	1.045	8	5	23	.3	.3	6.82	5.72	1.00	32.33	45.63		
<i>monachus</i>	8.1	8.1	8.1	4	4	3.1	3	8.1	3.3	4	4	8.1	3	3	3	3.2	4	4	4	3.9	8.1	8.1	.77	.79	.77	1.15	5	5	6.52	.56	.2	7.5	11.9	9.90	43.6	139.00	
<i>nigricollis</i>	2.2	2.2	2.2	7	7.1	7	2.1	2	7	7	7	2	2	2	2	2	7	7	2.1	2.5	8	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>rubicunda</i>	2.6	4	6	6.1	6	6	5.1	6.1	6.1	6.1	6.1	6	2	6	5	3.1	6	6	3	5	3	1.9	.52	.49	.57	.88	7	5	12.61	.3	.1	17.93	17.7	12.34	52.92	122.17	
<i>vipio</i>	1	3.7	8.9	3	5	5	3	3	3	4	5	3	2	3	8	3	7	6	2	7	1	8	.77	1.06	.77	1.27	6	5	8.52	.2	.1	14	16.43	14.49	58.64	168.50	

Note: Species data for 13 species of Gruinae. Traits, abbreviations, units and sources are as follows. Genetic designations follow fig. 1. Appearance measures are from Wood (1976, app. A), lightness score based on Munsell scale. CH, cheek; AU, ear coverts; NA, nape; DA, back; RU, rump; UT, upper tail coverts; RE, rectrices; TH, throat; BR, breast; AB, abdomen; CR, crissum; SN, side of neck; PR, primaries; SC, outer secondaries; IS, inner secondaries; GP, greater primary coverts; CG, outer greater secondary coverts; MA, marginal coverts; AL, alula; IG, inner greater secondary coverts; BE, behind eye; GU, gular. Unison call characteristics are from Archibald (1976), except *G. leucogeranus*, measured for this study. Data are missing for *G. nigricollis*. Frequencies are in kHz, times in seconds. MIB, introductory call basal frequency (male); FIB, introductory call basal frequency (female); MRB, regular call basal frequency (male); FRB, regular call basal frequency (female); MH, number of harmonics (male); FH, number of harmonics (female); LE, length of unison call bout; MCL, length of unison call (male); FCL, length of unison call (female); Sternum-keel measures are from Scott-Wood (1976, app. B). Data are missing for *G. nigricollis*. Units are mm. SW, sternal head width; KW, keel width; TH, tracheal hump width; SD, sternal head depth; TI, tracheal invagination.

## Results

Table 2 summarizes the results. The nonhistorical model fits the plumage coloration data much better than do either the gradual, speciation change, or genetic models, while the free model offers a vastly better fit than any of the simple models tested (more than  $5.3 \times 10^8$  times better;  $P < .02$ , based on the log-likelihood ratio test). Figure 3 shows the fit of the free model to the tree topology for plumage coloration.

The unison call data show a different pattern (table 2). Here, genetic and gradual hypotheses fit significantly better than either the speciation scenario (10- and 13-fold, respectively) or the nonhistorical scenario (80- and 100-fold, respectively). In contrast to plumage coloration, the free model (depicted in fig. 3B) offers only modest improvement, not enough to warrant the extra estimated parameters ( $P < .19$ , based on the log-likelihood ratio test).

Finally, the tracheal data is fit poorly by the nonhistorical hypothesis, while the other three simple models all do equally well and significantly better (at least 81 times better; table 2). The many extra parameters available to the free scenario improve the fit to the data by approximately  $10^7$  times over the nonhistorical model, a significant increase ( $P < .05$ , based on the log-likelihood ratio test). There is no significant improvement over the simple phylogenetic models ( $1.3 \times 10^5$  times;  $P < .26$ , based on log-likelihood ratio test).

## Discussion

The study of trait evolution and diversification has relied on the fossil record (see, e.g., Simpson 1944; Valentine 1973; Foote 1996; Jernvall et al. 1996), on comparisons between fossil and extant forms (Lynch 1990; Cheetham et al. 1994), among pairs or groups of extant species and estimated divergence times (Avice and Ayala 1975; Avice 1977; Lynch 1990; Cheetham et al. 1993), and among groups of extant organisms in the context of a phylogeny (see, e.g., Losos 1992). This last endeavor often involves

reconstructing ancestral states on phylogenetic trees according to some evolutionary model or set of assumptions (e.g., Brownian motion, maximum parsimony) with data from extant species. These estimates are then used to test hypotheses of trait evolution (Leman and Freeman 1989; Mindell et al. 1989, 1990; Brooks and McLennan 1991; Losos 1992; Martins 1994; Martins and Hansen 1997; Murphy and Lovejoy 1998; see also Garland 1992). This approach greatly expands the database but suffers from the need to infer point estimates of ancestral states. There are often many plausible reconstructions (e.g., equally parsimonious reconstructions) on a tree, each of which may suggest different evolutionary scenarios (Maddison 1990; Losos and Miles 1994). Also, ancestral state estimates are expected to have wide confidence limits under maximum parsimony (Maddison 1995) or Brownian motion (Schluter et al. 1997; Zhang and Nei 1997). Our approach avoids point estimates of ancestral states by integrating across all possible states. Though several specific model-based methods that avoid ancestral reconstruction are available (Ferris et al. 1979; Pagel 1994, 1997), these require log-likelihood ratio tests even when considering the simplest hypotheses, and they do not offer the free model as a way of graphically representing the pattern of trait evolution.

The plumage coloration traits (table 2; fig. 3) were fit very poorly by all but the nonhistorical and free hypotheses. Repeating the comparison using only the first axis or all nine axes had little effect (results not shown). This suggests that neither constant drift in large populations (or constantly varying selection pressures) nor speciation-induced change is a good description of the evolution of this complex. Figure 3A, which depicts the free model, is a novel way of representing changes in character traits on a phylogeny. The branch lengths represent the most likely amount of change that has occurred in the suite of traits given a Brownian motion process and topology. The many zero-length internal branches are consistent with the notion that most of the variation in plumage coloration that we see today occurred after the most recent recorded speciation events. This pattern also explains the good per-

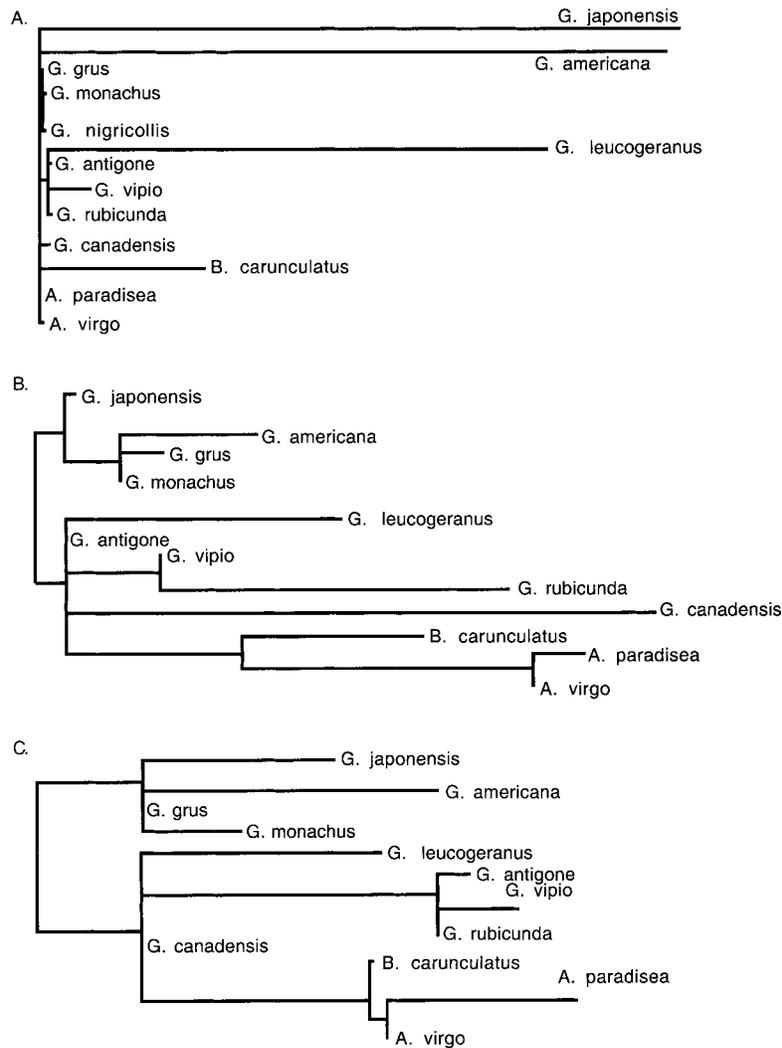
**Table 2:** Log-likelihood fits of continuous traits for cranes to five models of macroevolution

Trait	$N^a$	Gradual	Genetic	Speciation	Nonhistorical	Free <sup>b</sup>
Plumage	13	.00	.36	.45	3.36	23.46
Unison call	12	4.36	4.64	2.06	.00	12.70
Trachea	12	4.40	4.47	4.43	.00	16.92

Note: Log likelihoods are scaled to the poorest-fit scenario, which is given score 0.00.

<sup>a</sup> Number of species.

<sup>b</sup> Log-likelihood ratio test statistic for free vs. other models:  $2\Delta(\log\text{-likelihood})$  with  $2N - 4$  df.  $\chi^2$  critical values: df = 20,  $\alpha = 0.05$ ,  $\chi^2 = 31.41$ ; df = 22,  $\alpha = 0.05$ ,  $\chi^2 = 33.92$ ; df = 22,  $\alpha = 0.01$ ,  $\chi^2 = 40.29$ .



**Figure 3:** Free model trees. *G.* = *Grus*; *B.* = *Bugeranus*; *A.* = *Anthropoides*. *A.*, The best-fit set of branch lengths for the plumage coloration suite of traits (principal components analysis [PCA] scores on first three axes representing variation in brightness of 22 separate areas). The tree topology is the same as in figure 1. These branch lengths fit the plumage data significantly better than the branch lengths representing time. *B.*, The best-fit set of branch lengths for the unison call suite of traits (PCA scores on the first three axes of variation for six call characteristics). These branch lengths do not describe the data significantly better than branch lengths representing time. *C.*, The best-fit set of branch lengths for the tracheal traits (PCA scores on the first three axes representing five skeletal measures of the sternum and keel). These branch lengths do not describe the data significantly better than branch lengths representing time.

formance of the nonhistorical model. Under this scenario, evolution has been so rapid that history is erased.

The free model results suggest further tests. If we consider only the 13 terminal branches from figure 3A (all the internal branches being indistinguishable from 0), a correlation between plumage evolution (branch lengths from fig. 3A) and inferred time (branch lengths from fig. 2A) is not significant ( $r = 0.40$ ,  $P < .17$ ,  $N = 13$ ), but a correlation between plumage evolution and genetic change

(branch lengths from fig. 2B) is ( $r = 0.58$ ,  $P < .04$ ,  $N = 13$ ). A model 1 regression indicates that the inferred amount of genetic change that has occurred after the most recent speciation event leading to a species predicts the amount of change in plumage for that species (slope:  $t = 2.37$ ,  $P < .04$ ; intercept:  $t = 0.73$ ,  $P < .5$ ,  $N = 13$ ). Indeed, the relationship remains strong even after controlling for the effects of time in a stepwise multiple regression (partial  $F = 4.72$ ,  $df = 1, 10$ ,  $P < .06$ ,  $N = 13$ ). Coupled

with Omland's (1997) recent report of a positive correlation between morphological and genetic change for taxa ranging from birch tree species to members of the Carnivora, this result indicates a scenario in which increased substitution rates at the cytochrome *b* locus are coupled with high rates of plumage pattern evolution in cranes (indeed, so high that history is erased). Other molecules and other traits should be investigated to test this hypothesis, as well as renewed theoretical consideration of the forces that might allow such correlated evolution (Omland 1997).

The unison calls show a striking pattern (table 2). The speciation change model performs very poorly in comparison with the others tested, which strongly indicates that changes in unison calls are not associated with speciation events. Archibald (1976) reports evidence of secondary hybrids between *Grus rubicunda* and *Grus antigone* in Australia, two species whose calls are well differentiated on our PCA axes (unpublished results). *Grus rubicunda* and *G. antigone* are not sister species, and so the unison calls might still have functioned as initial species-isolating mechanisms. The trait is well modeled as drifting through time, however, which suggests highly variable selection pressures or constant genetic drift in large populations.

Tracheal morphology, thought to be associated with species-specific unison calls (Wood 1976, 1979), performed as well as might be expected of any useful phylogenetic character and did not seem to evolve at vastly different rates in different lineages (table 2). Because the variation in this trait was very well explained by the first axis (indeed, only the first axis has an eigenvalue  $>1$ ), we repeated the analysis using only scores from this axis, and the results did not change. In contrast to the unison calls themselves, both the gradual and speciation models are equally good at describing the evolution of this suite. The difference in the performance of the speciation model for tracheal morphology and the unison call indicates that they are not evolving in step. Indeed, there is no positive correlation between the branch lengths returned by the free model for the two traits ( $r = -0.09$ ,  $P < .70$ ,  $N = 21$ ). We might therefore expect that tracheal form governs pitch and harmonic characteristics but not duration. If we construct a free model tree based on a principal components analysis of the unison call that excludes duration parameters (not shown), however, the correlation of its branch lengths with the tracheal free tree remains poor ( $r = -0.14$ ,  $P < .52$ ,  $N = 21$ ). If our analysis captures the essence of the evolution of these two suites, then they are evolving independently, in contrast with the notion that interspecific tracheal morphology variation is correlated with interspecific call variation (Wood 1979; Johnsgard 1983).

More data on the biological significance of plumage coloration and unison calls, and on other aspects of crane

signaling (e.g., intensity of skin coloration [Voss 1976], courtship dances [Johnsgard 1983]), and more sophisticated models may be required to explain the evolution of the cranes' striking plumage and haunting mating calls. Our approach offers a novel way to test hypotheses of trait evolution in a phylogenetic framework. Furthermore, through the application of the free model, we can observe both the pattern of change of complex continuous traits on phylogenetic trees and investigate the correlated evolution of different complexes.

#### Acknowledgments

We thank J. Felsenstein for continuous insight and assistance. We are grateful to S. B. Heard, D. Kapan, D. Ludwig, M. J. MacInnis, M. Pagel, and especially S. Otto for valuable input. We thank G. Archibald, G. Dietzman, and A. Reinhardt, all at the International Crane Foundation, for recording the Siberian crane calls, providing library help, and offering encouragement. V. Deecke helped us analyze our crane calls. C. Cunningham, M. Pagel, and an anonymous referee carefully reviewed an earlier version of the manuscript. This study is based in large part on D. S. Wood's extensive master's thesis work from the 1970s. A.Ø.M. was supported by the Hamilton Foundation through an E. B. Eastburn Post-Doctoral Fellowship at the University of British Columbia. We all acknowledge the continued support of the National Sciences and Engineering Research Council of Canada.

#### Literature Cited

- Archibald, G. W. 1976. The unison call of cranes as a useful taxonomic tool. Ph.D. diss. Cornell University, Ithaca, N.Y.
- Avise, J. C. 1977. Is evolution gradual or rectangular? evidence from living fishes. *Proceedings of the National Academy of Sciences of the USA* 74:5083–5087.
- Avise, J. C., and F. J. Ayala. 1975. Genetic change and rates of cladogenesis. *Genetics* 81:757–773.
- Baker, M. C., and A. E. M. Baker. 1990. Reproductive behavior of female buntings—isolating mechanisms in a hybridizing pair of species. *Evolution* 44:332–338.
- Barraclough, T. G., P. H. Harvey, and S. Nee. 1995. Sexual selection and taxonomic diversity in passerine birds. *Proceedings of the Royal Society of London B, Biological Sciences* 259:211–215.
- Bauwens, D., T. J. Garland, A. M. Castilla, and R. Van Damme. 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* 49:848–863.
- Bell, G. A., and A. Ø. Mooers. 1997. Size and complexity

- among multicellular organisms. *Biological Journal of the Linnean Society* 60:345–363.
- Berrigan, D., A. Purvis, E. L. Charnov, and P. H. Harvey. 1993. Phylogenetic contrasts and the evolution of mammalian life histories. *Evolutionary Ecology* 7:270–278.
- Bookstein, F. L. 1987. Random walk and the existence of evolutionary rates. *Paleobiology* 13:446–464.
- Bromham, L., A. Rambaut, and P. H. Harvey. 1996. Determinants of rate variation in mammalian DNA sequence variation. *Journal of Molecular Evolution* 43:610–621.
- Brooks, D. R., and D. McLennan. 1991. *Phylogeny, ecology and behavior: a research program in comparative biology*. University of Chicago Press, Chicago.
- Carson, H. L. 1997. Sexual selection: a driver of genetic change in Hawaiian *Drosophila*. *Journal of Heredity* 88:343–352.
- Cheetham, A. H., J. B. C. Jackson, and L. C. Hayek. 1993. Quantitative genetics of bryozoan phenotypic evolution. 1. Rate tests for random change versus selection in differentiation of living species. *Evolution* 47:1526–1538.
- . 1994. Quantitative genetics of bryozoan phenotypic evolution. 2. Analysis of selection and random change in fossil species using reconstructed genetic parameters. *Evolution* 48:360–375.
- Cheverud, J. M., M. M. Dow, and W. Leutenegger. 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. *Evolution* 39:1335–1351.
- Diaz-Uriarte, R., and J. T. Garland. 1996. Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviations from Brownian motion. *Systematic Biology* 45:27–47.
- Edwards, A. W. F. 1992. *Likelihood*. Johns Hopkins University Press, Baltimore.
- Edwards, A. W. F., and L. L. Cavalli-Sforza. 1964. Reconstruction of evolutionary trees. Pages 67–76 in *Phenetic and phylogenetic classification*. Systematics Association, London.
- Endler, J., and A. Houde. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456–468.
- Felsenstein, J. 1973. Maximum likelihood estimates of evolutionary trees from continuous characters. *American Journal of Human Genetics* 25:471–492.
- . 1981. Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. *Evolution* 35:1229–1242.
- . 1985. Phylogenies and the comparative method. *American Naturalist* 125:1–15.
- . 1988. Phylogenies from molecular sequences: inferences and reliability. *Annual Review of Genetics* 22:521–565.
- . 1993. PHYLIP (phylogeny inference package). Version 3.0. Seattle.
- . 1995. PHYLIP (phylogeny inference package). Version 3.5c. Seattle.
- Ferris, S. D., S. L. Portnoy, and G. S. Whitt. 1979. The roles of speciation and divergence time in the loss of duplicate gene expression. *Theoretical Population Biology* 15:114–139.
- Foote, M. 1996. Ecological controls on the evolutionary recovery of post-Paleozoic crinoids. *Science (Washington, D.C.)* 274:1492–1495.
- Garland, T. J. 1992. Rate tests for phenotypic evolution using phylogenetically independent contrasts. *American Naturalist* 140:509–519.
- Garland, T. J., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* 39:265–292.
- Gittleman, J. L., and M. Kot. 1990. Adaptation: statistics and a null model for estimating phylogenetic effects. *Systematic Zoology* 39:227–241.
- Goldman, N. 1993. Statistical tests of models of DNA evolution. *Journal of Molecular Evolution* 36:182–198.
- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51:1341–1351.
- Harvey, P. H., and M. D. 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 (London)* 351:619–624.
- Hey, J. 1992. Using phylogenetic trees to study speciation and extinction. *Evolution* 46:627–640.
- Holland, B., and W. R. Rice. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* 52:1–7.
- Houde, A. E., and J. A. Endler. 1990. Correlated evolution of female mating preference and male color patterns in the guppy *Poecilia reticulata*. *Science (Washington, D.C.)* 248:1405–1408.
- Jernvall, J., J. P. Hunter, and M. Fortelius. 1996. Molar tooth diversity, disparity, and ecology in Cenozoic ungulate radiations. *Science (Washington, D.C.)* 274:1489–1492.
- Johnsgard, P. A. 1983. *Cranes of the world*. Indiana University Press, Bloomington.
- Krajewski, C., and J. W. Fetzner, Jr. 1994. Phylogeny of cranes (Gruiformes: Gruidae) based on cytochrome-B DNA sequences. *Auk* 111:351–365.
- Krajewski, C., and D. G. King. 1996. Molecular divergence and phylogeny: rates and patterns of cytochrome *b* evolution in cranes. *Molecular Biology and Evolution* 13:21–30.

- Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30:314–334.
- . 1981. Models of speciation by natural selection. *Proceedings of the National Academy of Sciences of the USA* 78:3721–3725.
- . 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* 36:213–223.
- Leman, C. A., and P. W. Freeman. 1989. Testing macroevolutionary hypotheses with cladistic analysis: evidence against rectangular evolution. *Evolution* 43:1538–1554.
- Losos, J. 1992. The evolution of convergent structure in Caribbean *Anolis* communities. *Systematic Biology* 41:403–420.
- Losos, J. B., and D. B. Miles. 1994. Adaptation, constraint, and the comparative method. Pages 60–98 in P. C. Wainwright and S. M. Reilly, eds. *Ecological morphology: integrative organismal biology*. University of Chicago Press, Chicago.
- Lynch, M. 1990. The rate of morphological evolution in mammals from the standpoint of neutral expectation. *American Naturalist* 136:727–741.
- Maddison, W. P. 1990. A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44:539–557.
- . 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Zoology* 40:304–314.
- . 1995. Calculating the probability distributions of ancestral states reconstructed by parsimony on phylogenetic trees. *Systematic Biology* 44:474–481.
- Martin, T. E., and A. V. Badyaev. 1996. Sexual dichromatism in birds: importance of nest predation and nest location for females versus males. *Evolution* 50:2454–2460.
- Martins, E. P. 1994. Estimating the rate of phenotypic evolution from comparative data. *American Naturalist* 144:193–209.
- Martins, E. P., and T. J. Garland. 1991. Phylogenetic analyses of the correlated evolution of continuous characters. *Evolution* 45:534–557.
- Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information in the analysis of interspecific data. *American Naturalist* 149:646–667.
- McKinney, M. L. 1990. Classifying and analysing evolutionary trends. Pages 28–58 in K. J. McNamara, ed. *Evolutionary trends*. University of Arizona Press, Tucson.
- Mindell, D. P., J. W. Sites, and D. Graur. 1989. Speciation evolution—a phylogenetic test with allozymes in *Sceloporus* (Reptilia). *Cladistics* 5:49–61.
- . 1990. Assessing the relationship between speciation and evolutionary change. *Cladistics* 6:393–398.
- Mitra, S., H. Landel, and S. Pruett-Jones. 1996. Species richness covaries with mating system in birds. *Auk* 113:544–551.
- Mooers, A. Ø. 1995. Tree balance and tree completeness. *Evolution* 49:379–384.
- Mooers, A. Ø., and P. H. Harvey. 1994. Metabolic rate, generation time, and the rate of molecular evolution in birds. *Molecular Phylogenetics and Evolution* 3:344–350.
- Mooers, A. Ø., and S. B. Heard. 1997. Inferring evolutionary process from phylogenetic tree shape. *Quarterly Review of Biology* 72:31–54.
- Mooers, A. Ø., and D. Schluter. 1998. Fitting macroevolutionary models to phylogenies: an example using vertebrate body sizes. *Contributions to Zoology* 68:3–18.
- Murphy, R. W., and N. R. Lovejoy. 1998. Punctuated equilibrium or gradualism in the lizard genus *Sceloporus*? lost in plesiomorphs and a forest of trees. *Cladistics* 14:95–103.
- Nei, M. 1976. Mathematical models of speciation and genetic distance. Pages 723–766 in S. Karlin and E. Nevo, eds. *Population genetics and ecology*. Academic Press, New York.
- Omland, K. E. 1997. Correlated rates of molecular and morphological evolution. *Evolution* 51:1381–1393.
- Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London B, Biological Sciences* 255:37–45.
- . 1997. Inferring evolutionary processes from phylogenies. *Zoologica Scripta* 26:331–348.
- Pianka, E. R. 1995. Evolution of body size: varanid lizards as a model system. *American Naturalist* 46:398–414.
- Poiani, A., and M. Pagel. 1997. Evolution of avian cooperative breeding: comparative tests of the nest predation hypothesis. *Evolution* 51:226–240.
- Price, T. D., A. J. Helbig, and A. D. Richman. 1997. Evolution of breeding distributions in the Old World leaf warblers (genus *Phylloscopus*). *Evolution* 51:552–561.
- Rohlf, F. J., W. S. Chang, R. R. Sokal, and J. Kim. 1990. Accuracy of estimated phylogenies: effects of tree topology and evolutionary model. *Evolution* 44:1671–1684.
- Saetre, G.-P., T. Moum, S. Bures, M. Kral, M. Adamjan, and J. Moreno. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature (London)* 387:589–592.
- Schluter, D., and L. Nagel. 1995. Parallel speciation by natural selection. *American Naturalist* 146:292–301.
- Schluter, D., and T. Price. 1993. Honesty, perception and population divergence in sexually selected traits. *Pro-*

- ceedings of the Royal Society of London B, Biological Sciences 253:117–122.
- Schluter, D., T. Price, A. Ø. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699–1711.
- Sibley, C. G., and B. L. Monroe. 1990. *Distribution and taxonomy of the birds of the world*. Yale University Press, New Haven, Conn.
- Simpson, G. G. 1944. *Tempo and mode in evolution*. Columbia University Press, New York.
- Turner, G. F. 1994. Speciation mechanisms in Lake Malawi cichlids: a critical review. *Ergebnisse der Limnologie* 44: 139–160.
- Turner, G. F., and M. T. Burrows. 1995. A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society of London B, Biological Sciences* 260: 287–292.
- Valentine, J. W. 1973. *Evolutionary paleoecology of the marine biosphere*. Prentice Hall, Upper Saddle River, N.J.
- Voss, K. S. 1976. Behavior of the greater sandhill crane. Ph.D. diss. University of Wisconsin, Madison.
- West-Eberhard, M. J. 1983. Sexual selection, social competition and evolution. *Quarterly Review of Biology* 58: 155–183.
- Wood, D. S. 1976. Phenetic relationships within the family Gruidae. MS thesis. University of Oklahoma, Norman.
- . 1979. Phenetic relationships within the family Gruidae. *Wilson Bulletin* 91:384–399.
- Zhang, Y., and M. Nei. 1997. Accuracies of ancestral amino acid sequences inferred by parsimony, likelihood, and distance methods. *Journal of Molecular Biology* 44(suppl.):S139–S146.

Associate Editor: Mark Pagel