Experimental and comparative studies suggest that a major determinant of increased ossification of the mandibular symphysis is elevated masticatory stress related to a mechanically challenging diet. However, the morphology of this joint tracks variation in dietary properties in only some mammalian clades. Extant anthropoid primates are a notable exception: synostosis is ubiquitous in this speciose group, despite its great age and diverse array of feeding adaptations. One possible explanation for this pattern is that, once synostosis evolves, reversion to a lesser degree of fusion is unlikely or even constrained. If correct, this has important implications for functional and phylogenetic analyses of the mammalian feeding apparatus. To test this hypothesis, we generated a molecular tree for 76 vespertilionoid and noctilionoid chiropterans using Bayesian phylogenetic analysis and examined character evolution using parsimony and likelihood ancestral-state reconstructions along with the binary state speciation and extinction (BiSSE) model. Results indicate that reversals have occurred within Vespertilionoidea. In contrast, noctilionoids exhibit an anthropoid-like pattern, which suggests that more detailed comparisons of the functional and developmental bases for fusion in these bat clades may provide insight into why fusion is maintained in some lineages but not in others. Potential functional and developmental explanations for the lack of reversal are discussed.

**KEY WORDS:** Chiroptera, constraint, irreversibility, mammals, mandible, Primates, symphysis.
IS MANDIBULAR SYMPHYSEAL FUSION REVERSIBLE?

Figure 1. Diagrammatic representations of symphyseal character states in transverse cross section (anterior is up): (A) unfused—smooth, opposing dentaries loosely connected by fibrous tissue, a fibrocartilage pad, and ligaments (amphiarthrosis); (B) simple and (C) complex partial fusion—a continuum of increasingly more tightly bound joints with greater sutural complexity consisting of interlocking rugosities and numerous variably calcified ligaments (symarthrosis); (D) a fully ossified, or fused, joint (synostosis). See also Scapino (1981).

proposed determinant in at least two ways: (1) by predisposing homologous traits in different taxa to respond to the same environmental selective regime (i.e., external selection) in distinct ways or (2) by opposing such selection pressures. In both of these cases, the constraint results in the absence of an anticipated evolutionary outcome in some clades (Maynard Smith et al. 1985; McKitrick 1993).

Within the order Primates, complete ossification, or fusion, of the mandibular symphyseal joint (i.e., the joint between the left and right hemimandibles, or dentaries) may represent an example of a character that is evolutionarily constrained (Ravosa 1999; Lockwood 2007). Whereas the first primates were characterized by unfused joints with smooth symphyseal plates (e.g. Fig. 1A), fusion is a synapomorphy of all living and fossil members of the crown anthropoid radiation (Kay et al. 1997; Ravosa 1999; Williams et al. 2010; but see Simons and Rasmussen 1996; Seiffert et al. 2004; Simons 2004), the highly speciose clade comprising living and extinct New and Old World monkeys, apes, and humans (approximately 280 species in 45 genera; Groves 2005).

Fusion has also evolved in fossil strepsirrhine primates (relatives of extant lemuris, lorisises, and galagos), including multiple times in Eocene adapids and the recently extinct (subfossil) giant lemuris of Madagascar, as well as convergently in at least one group of Oligocene stem anthropoids (species closely related to but outside of the crown anthropoid clade) (Beecher 1983; Ravosa 1991; Doughty 1996; Dumont 1999; Ravosa and Hylander 1994; Ravosa and Simons 1994). In contrast, decreases in ossification have yet to be documented in primates (Ravosa 1996, 1999). The absence of reversals in crown anthropoids is particularly notable, given the great age of this clade (>35 Ma; e.g., Hodgson et al. 2009; Jameson et al. 2011) and its diverse array of feeding adaptations (e.g., Rosenberger 1992).

On the other hand, variation in symphyseal fusion in extant strepsirrhine primates tracks interspecific differences in dietary mechanical properties, ranging from unfused with relatively smooth symphyseal plates in species that feed on relatively easy-to-process foods, such as insects, ripe fruits, and exudates, to partially fused with complex, interlocking rugosities (e.g., Fig. 1C) in species that rely on more resistant foods, such as seeds and leaves (Beecher 1977a,b, 1979; Ravosa 1991; Ravosa and Hylander 1994; Scott et al. 2012). Moreover, complete fusion characterizes giant subfossil lemuris that are inferred, based on studies of dental wear and morphology (i.e., independently of mandibular form), to have been seed-predators or dedicated folivores (Jungers et al. 2002; Rafferty et al. 2002; Godfrey et al. 2004; Scott et al. 2009). The strepsirrhine pattern thus indicates that, in at least some primates, fusion is an adaptive response to elevated joint stresses associated with processing a mechanically demanding diet (Beecher 1977a,b, 1979; Ravosa 1991, 1999; Ravosa and Hylander 1994; Ravosa and Hogue 2004), and data from other mammalian clades support this idea (Scott et al. 2012). Given the fact that anthropoids are as dietarily diverse as strepsirrhine, these observations suggest that all crown anthropoids have a fused symphyseal character simply because they inherited it from a common ancestor with a diet composed of items that required intensive postcanine processing (Ravosa and Hylander 1994; Ravosa 1999). In other words, symphyseal fusion may have been adaptive at one point early in the history of this clade but is now retained for reasons other than selection related to dietary properties (e.g., developmental canalization or functional integration; Lockwood 2007).

Notably, the issues raised above are not limited to primates. Mammalian clades in which at least some members are characterized by loss of the symphyseal joint include marsupials, xenarthrans, carnivorans, cetartiodactyls, perissodactyls, proscideans, hyracoids, and chiropterans (Beecher 1977a,b, 1979; Scapino 1981; Hogue and Ravosa 2001; Hogue 2004; Ravosa and Hogue 2004; Williams et al. 2008; Davis et al. 2010; Scott et al. 2012). Thus, the possibility that the mandibular symphyseal character is unlikely to become unfused once complete ossification is achieved has important implications with respect to understanding the evolution of the masticatory system and testing phylogenetic hypotheses in a variety of mammalian groups.

Although the contrast between the patterns exhibited by strepsirrhines and crown anthropoids suggests the existence of a constraint in the latter group, the fact that complete fusion (as opposed to partial fusion) is relatively uncommon in strepsirrhines raises questions about the appropriateness of this comparison—that is, because fusion is observed in only a handful of extinct strepsirrhines, and these species are apomorphic for this character in comparison to their sister taxa, the Strepsirrhini may not represent a suitable null model for generating expectations regarding the pattern of symphyseal character-state evolution in crown anthropoids (Schwenk 1995), within which fusion is pleisiomorphic. Thus, in this study, we examine the evolution of symphyseal form in the Chiroptera, a clade that may be a better analogue in terms of evaluating the anthropoid pattern and
addressing questions regarding constraints on symphyseal evolution in mammals. Although chiropterans are much more distantly related to the Anthropoidea than are the Strepsirrhini, complete symphyseal ossification is common in this highly speciose clade without being ubiquitous, which allows us to test the hypothesis that this condition is irreversible. Moreover, chiropterans, like anthropoids, are very ecologically diverse. Dietary variation ranges from widespread insectivory in the Vespertilionidae to the existence of at least six distinct feeding guilds in Phyllostomidae, including sanguinivory, nectarivory, frugivory, insectivory, carnivory, and omnivory. Moreover, the diversity in chiropteran ecology is reflected in the morphology of the masticatory apparatus (e.g., Freeman 1979, 1988, 1995; Dumont 1999, 2004; Davis et al. 2010; Dumont et al. 2012). Examining a more distantly related clade also allows us to evaluate the generality of the anthropoid pattern: is the lack of reversal in the Anthropoidea due to clade-specific factors? Or is reversal unlikely to occur in any clade in which symphyseal fusion evolves?

Materials and Methods

SAMPLE

Our sample consisted of 76 taxa from two superfamilies, the Vespertilionoidea and the Noctilionoidea (Table 1), which are sister clades within the suborder Yangochiroptera (Teeling et al. 2005; also referred to as Vespertilioniformes; Hutcheon and Kirsch 2004). Over half of the sample (n = 44) was provided by R. Beecher, who examined all mammalian genera housed in the collections of the Smithsonian Institution as part of his dissertation research (Beecher 1977b). In several instances, Beecher’s notes (pers. comm.) list only generic names, and it is not clear whether such entries are for only a single species or multiple species within a genus. Thus, n = 76 may be a conservative estimate of the number of species included in our analysis. Sample sizes for Beecher’s taxa are not listed, but he did record intrataxonomic (either intraspecific or intrageneric) variation in some cases (see the entries for Tadarida, Mormopterus, and Eumops in Table 1). Data for the remaining 32 species in our sample were collected by MJR at the Field Museum of Natural History. These species were scored using a four-state system: (1) unfused with smooth symphyseal plates, (2) partially fused with simple rugosities (simple partial fusion), (3) partially fused with complex, interlocking rugosities (complex partial fusion), and (4) fully ossified with no intraspecific variation (Fig. 1; see also Scapino 1981). The information available in Beecher’s notes allowed us to reconcile his morphological descriptions with our scoring system. Prior to analysis, the first three character states were collapsed into a single state, which we refer to hereafter as unfused; thus, species were ultimately coded as follows: unfused, partially fused, or variably partially or fully fused = 0; full fusion, with no intraspecific variation = 1.

PHYLOGENETIC ANALYSIS

Previous phylogenetic analyses of bats have been able to resolve the majority of relationships within Noctilionoidea, whereas relatively large datasets have been unable to resolve most of the intertribal relationships within Vespertilionoidea (Hoofer and Van Den Bussche 2003; Lack and Van Den Bussche 2010; Roehrs et al. 2010). To account for this uncertainty, we obtained from GenBank an approximately 2.6 kilobase fragment spanning the 12S rRNA, tRNA^VAL, and 16S rRNA genes of the mitochondrial DNA (mtDNA) genome for all chiropteran taxa examined in this study. GenBank accession numbers are given in Table S1. Sequences were aligned using the Geneious aligner (Drummond et al. 2010) and edited in MacClade (Maddison and Maddison 2002). Any position for which positional homology was questionable was excluded from further analysis, resulting in 1,988 analyzed positions (898 excluded positions).

We conducted a Bayesian phylogenetic analysis with BEAST (Drummond and Rambaut 2007) using a relaxed lognormal clock, a Yule speciation tree prior, and a GTR + I + I model of nucleotide substitution, as suggested by MrModeltest (Nylander 2004). Two internal fossil calibrations were used (with lognormal priors) to obtain branch lengths in absolute time. The first was a prior minimum age of 30 million years ago (Ma) for the Phyllostomidae. The oldest probable stem phyllostomids are found in the Whitneyan (30–32 Ma; Czaplewski et al. 2008). Therefore, the offset for the lognormal prior was set at 30 Ma with the upper bound for the 95% quantiles set to the Eocene–Oligocene boundary (34 Ma). The second calibration was a minimum of 37 Ma for the split between Vespertilionidae and Molossidae, because verified vespertilionid and molossid fossils have been found from middle Eocene deposits (McKenna and Bell 1997). The upper bound of the 95% quantiles was set at 48 Ma to encapsulate the entire middle Eocene in the prior.

An initial analysis of 50,000,000 generations with 10% burn-in was run to tune operators. The final analysis consisted of two separate runs of 50,000,000 generations, each with 10% burn-in. Results of these final two runs were combined using LogCombiner—a component of the BEAST package—to obtain the maximum clade credibility topology. All runs were checked for sufficient mixing, stable convergence on a unimodal posterior, and effective sample sizes (Drummond et al. 2002) greater than 200 for all parameters using TRACER (Drummond and Rambaut 2003). The maximum clade credibility tree in Newick format, with branch lengths in millions of years, is presented in Appendix S1. We also obtained 1,000 trees from the posterior sample of the BEAST analysis to incorporate uncertainty in vespertilionoid phylogenetic relationships into our analysis. This analytical step was
Table 1. Samples and data used in this study. Taxa that lack a sample size, indicated by a dash (—), were provided by R. Beecher; in many cases, only genus names are available for this portion of the dataset. Fusion scores are as follows: 1 = unfused, with smooth surfaces; 2 = partially fused, with simple rugosities; 3 = partially fused, with complex, interlocking rugosities; 4 = fully fused.

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accomplished by taking the combined tree file (100,000 trees prior to burn-in), discarding the first 50% as burn-in (50,000 phylogenies), and using LogCombiner to resample this tree file at a lower frequency.

Character Evolution

To test the hypothesis that fusion is irreversible in these two chiropteran clades, we initially examined patterns of character-state evolution using Mesquite’s (Maddison and Maddison 2011)
parsimony and maximum likelihood options to map character states onto the maximum clade credibility tree, with each superfamily being examined separately. Maximum-likelihood reconstructions were performed using Mesquite’s AsymmMk model (also referred to as Mk2), which estimates separate rates for each character-state transition—that is, unfused to fused, and fused to unfused. We estimated support for likelihood reconstructions at each node using a decision threshold of $T = 2$ (Mesquite’s default). This statistic is the difference between the log-likelihoods for each character state at the node in question. If this difference exceeds the threshold, then the state with the higher likelihood is considered better supported; otherwise, the reconstruction is considered ambiguous.

Ancestral-state reconstructions based on parsimony and the AsymmMk maximum-likelihood model can be problematic because they assume that net diversification rate (i.e., speciation rate minus extinction rate) is independent of the state of the character under study (Cunningham 1999; Goldberg and Igić 2008). If this assumption does not hold, then reconstructions of ancestral states for a given character will be biased, leading to incorrect rejections of irreversibility (Cunningham 1999; Goldberg and Igić 2008). For example, if organisms with fused symphyses diversify more rapidly than those with unfused symphyses, then fusion will eventually be much more common in a phylogeny, and species with unfused symphyses will appear to have evolved from an ancestor with a fused symphysis even if the transition did not in fact occur.

This issue can be overcome by using methods that incorporate information regarding the effect of character states on diversification rates (Goldberg and Igić 2008). Following recent studies (Goldberg and Igić 2008; Lynch and Wagner 2010; Kohlsdorf et al. 2010; Wiens 2011), we used one such approach—Maddison et al.’s (2007; see also FitzJohn et al. 2009) binary state speciation and extinction (BiSSE) model—to further evaluate the irreversibility hypothesis in vespertilionoids and noctilionoids. BiSSE computes the likelihood for a model with up to six estimated parameters: a speciation rate under each character state ($\lambda_0$ and $\lambda_1$), an extinction rate under each character state ($\mu_0$ and $\mu_1$), and transition rates from each character state to the other ($q_{01}$ and $q_{10}$) (Maddison et al. 2007). Different models of evolution can be constructed by fixing one or more of these parameters, and these models can be compared to each other to determine which provides the best fit.

We compared four such models. The first of these was the un constrained model, in which all six parameters were estimated using maximum likelihood. Support for this model indicates that fusion is reversible and that net diversification rates for the two character states differ. The second model was the equal-diversification model, in which the net diversification rates for the two character states were set equal to each other—that is, $\lambda_F = \lambda_U$ and $\mu_F = \mu_U$, so that $\lambda_F - \mu_F = \lambda_U - \mu_U$, where the subscripts F and U denote fused and unfused joints, respectively. Support for this model indicates that fusion is reversible and that character state does not affect diversification rate. The third model was the irreversible model, in which the transition rate from a fused joint to an unfused joint ($q_{FU}$) was set equal to zero. Support for this model indicates that reversals have not occurred and that net diversification rates for the two character states differ. The final model was the irreversible, equal-diversification model, which prohibits reversal (as in the irreversible model) and equalizes net diversification rates for the two character states (as in the equal-diversification model). Support for this model indicates that reversals have not occurred and that net diversification rates for the two character states do not differ.

We applied FitzJohn et al.’s (2009) correction for incomplete sampling of taxonomic diversity. Our samples consisted of 32 of 177 noctilionoid species and 44 of 515 vespertilionoid species, representing 72 of the 130 genera recognized by Simmons (2005) in these two superfamilies. All BiSSE computations were conducted using the program diversitree (FitzJohn 2011) in R (R Development Core Team 2011). We compared models using Akaike’s information criterion with adjustment for small sample size (AICc; Burnham and Anderson 2002). Using this approach, the relevant statistic is $\Delta$AICc, which is the difference between the AICc values for a given model and the model with the lowest AICc. The model with the lowest AICc value ($\Delta$AICc = 0.0) is the best supported. For models with nonzero $\Delta$AICc values, we used the following rule of thumb (Burnham and Anderson 2002, p. 70): $\Delta$AICc = 0–2 indicates substantial support for the model, $\Delta$AICc = 4–7 indicates considerably less support, $\Delta$AICc > 10 indicates essentially no support.

Results

VESPERTILIONOIDA

The parsimony-based character-state reconstructions indicate the presence of at least seven reversals in the vespertilionoid maximum clade credibility tree (Fig. 2). The unfused symphysis of Corynorhinus rafinesquii may also represent a reversal, but this case is uncertain due to ambiguity in reconstructions at two deeper nodes. In contrast, there are no unequivocal instances of reversal when character states are reconstructed using AsymmMk maximum likelihood (Fig. 2). Under this approach, the reconstructions at nearly all of the internal nodes are ambiguous; one state is judged better than the other (i.e., the likelihood decision threshold is exceeded) at only two of these nodes: in the ancestor of Promops and Molossus (fused), and in the ancestor of Natalus and Chilonautal (unfused; note that the latter two genera have been collapsed into the family Natalidae in Fig. 2). The highest
relative likelihood of fusion at an ancestral node that has at least one descendent with an unfused symphysis is 0.696 (the ancestor of *Nyctalus* and *Pipistrellus*). For comparison, the relative likelihoods of the states that characterize the *Promops–Molossus* and *Natalus–Chilonatalus* ancestors are 0.887 and 0.934, respectively, and the highest relative likelihood of a state at a node with an ambiguous reconstruction is 0.830 (fusion in the ancestor of *Nyctophilus* and *Chalinolobus*).

The results of the BiSSE analysis of the Vespertilionoidea are presented in Table 2. The equal-diversification model receives the highest support, but the unconstrained model has a very low ΔAIC value, indicating that these two models are essentially indistinguishable from each other. We are therefore unable to choose between character-independent and character-dependent diversification, and thus the latter remains a possibility, along with its potentially confounding effects. (The parameter estimates for the unconstrained model indicate that lineages characterized by fusion diversify at a rate that is nearly 3.5 times greater than that for lineages without fusion.) Given this ambiguity, the BiSSE model is clearly more appropriate than parsimony and likelihood reconstructions for evaluating the reversibility of symphyseal fusion in vespertilionoids. The BiSSE results nevertheless confirm the signal obtained using parsimony reconstructions: the two irreversible models have ΔAIC values that are greater than four (Table 2), indicating that support for the models that allow reversal is considerably greater. This result is robust to uncertainty regarding the phylogenetic relationships within our vespertilionid sample. Figure 3 shows the distribution of ΔAIC values for the irreversible model as compared to the unconstrained model computed for the 1000 topologies from the posterior sample of the Bayesian phylogenetic analysis. The irreversible model never has a lower AIC value than the unconstrained

**Figure 2.** Maximum clade credibility tree for the Vespertilionoidea showing parsimony-based (left) and likelihood-based (right) ancestral-state reconstructions: white circle = unfused, partially fused, or variable; black circle = fully fused; black-and-white circle = equivocal ancestral-state reconstruction (pie charts show the relative likelihood for each character state). The gray rectangles indicate positions where reversals are inferred to have occurred. Branch lengths are not to scale, but ages of selected internal nodes are given in millions of years (Ma). Not all 44 taxa included in the analysis are shown; in some cases, clades that are homogeneous with respect to symphyseal morphology have been collapsed.
Comparison of BiSSE models. The parameters are as follows: $\lambda_U$ and $\lambda_F$ are the speciation rates for lineages with unfused and fused symphyses, respectively; $\mu_U$ and $\mu_F$ are the extinction rates; and $q_U$ and $q_F$ are the unfused-to-fused and fused-to-unfused transition rates, respectively. Dashes (−) indicate that the parameter was fixed (i.e., for equal diversification, $\lambda_U = \lambda_F$ and $\mu_U = \mu_F$; for irreversible, $q_U = 0$). The final three columns give the log-likelihoods ($\ln L$) for each model and the difference between each model’s AICc value and minimum observed AICc value (ΔAICc). The model with the minimum ΔAICc (in bold) is the best supported.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\lambda_U$</th>
<th>$\lambda_F$</th>
<th>$\mu_U$</th>
<th>$\mu_F$</th>
<th>$q_U$</th>
<th>$q_F$</th>
<th>$\ln L$</th>
<th>ΔAICc</th>
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<tr>
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<td>2.3 × 10⁻⁵</td>
<td>1.5 × 10⁻⁵</td>
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<td>8.4 × 10⁻⁸</td>
<td>−</td>
<td>0.0517</td>
<td>0.0529</td>
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<td>0.0221</td>
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<td>0.0928</td>
<td>−</td>
<td>−190.07</td>
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<td>0.0267</td>
<td>−</td>
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<tr>
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<td>0.0256</td>
<td>−</td>
<td>−120.57</td>
<td>0.0607</td>
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</table>

Figure 3. Histogram showing the distribution of BiSSE-derived ΔAICc values (irreversible AICc minus unconstrained AICc) for the 1000 topologies from the posterior sample of the Bayesian phylogenetic analysis. The white arrow indicates the position of the maximum clade credibility tree.

The distribution of character states in noctilionoids presents a striking contrast to that exhibited by vespertilionoids. Whereas the fused and unfused symphyses are equally common in the latter clade, 28 of the 32 noctilionoids in our sample are characterized by fusion. Such a distinction might be expected if diversification of the Noctilionoidea began much later in comparison to vespertilionoids, but this is not the case (compare divergence dates in Figs. 2 and 4). For example, the least inclusive clade that contains both Sphaeronycteris and Erophylla is over 20 million years old and contains 24 taxa, all with complete symphyseal ossification (Fig. 4). On the other hand, vespertilionoid clades of similar age are much more morphologically diverse (e.g., the least inclusive clade containing Nycticeius and Scottoecus; Fig. 2).

Parsimony reconstructions suggest that the unfused symphyses inferred in the last common ancestor of Desmodus rotundus and Diaemus youngi is an instance of reversal (Fig. 4). The other two noctilionoids with unfused symphyses—Macrotus waterhousii and Furipterus horrens—may also be descended from ancestors with fused symphyses, but the parsimony reconstructions at the relevant internal nodes are equivocal. Uncertainty in the likelihood reconstructions does not allow identification of any instances of reversal (Fig. 4).

The BiSSE results for this clade indicate that the irreversible model is the best supported, with the irreversible, equal-diversification model being nearly as well supported (Table 2). Thus, as in the case of the Vespertilionoidea, neither character-dependent nor character-independent diversification provides a better fit to our dataset. Moreover, although the ΔAICc values for the two models that allow reversal (the unconstrained and equal-diversification models) are greater than two, they are less than four, the threshold at which support for a model is regarded as considerably less (Burnham and Anderson 2002). In other words, these results do not provide strong support for irreversibility over reversibility in this superfamily. Our data therefore do not allow us to claim with confidence that reversals have not occurred in this clade. Note that because the phylogenetic relationships depicted in the noctilionoid maximum clade credibility are robust (i.e., there is little variation in these relationships from tree to tree in the posterior sample), we do not present a distribution of ΔAICc values as we did for the Vespertilionoidea.
Discussion

PATTERNS OF SYMPHYSEAL EVOLUTION IN BATS

Analysis of symphyseal evolution in vespertilionoid chiropterans indicates that reversals from complete ossification do occur and may be common in some clades. On the other hand, while the results of our examination of the Noctilionoidea are inconclusive, it appears that reversals, if they have occurred in this group, are far less common than they are in vespertilionoids, hinting at an important difference in symphyseal evolution between these two superfamilies. It is important to emphasize that we have sampled only a fraction of living noctilionoids (approximately 18%), and that further sampling of this group may blur this apparent distinction. However, it is worth noting that in response to one reviewer’s comments, we more than doubled the size of our noctilionoid sample from 15 to 32 species, and of these additional taxa, only a single one (M. waterhousii) has an unfused symphysis. Thus, it appears that symphyseal evolution in noctilionoids resembles that in anthropoid primates to some extent—that is, stasis in symphyseal form over millions of years despite a high level of ecological diversity, including frugivory, insectivory, nectarivory, and carnivory (e.g., Norberg and Rayner 1987).

The fact that these two superfamilies present such contrasting patterns suggests that vespertilionoids and noctilionoids may represent a model system that can be used to understand why fusion is ubiquitous in anthropoids. In this context, it is notable that although many studies have investigated the functional significance of increased symphyseal ossification, particularly in primates (Beecher 1977a,b, 1979; Hylander 1979a,b, 1984; Hylander et al. 1987, 1992, 1998, 2000, 2011; Ravosa 1991, 1996, 1999; Hylander and Johnson 1994; Ravosa and Hylander 1994; Ravosa and Hogue 2004; Vinyard et al. 2005, 2006, 2007; Scott et al. 2012; for other mammals, see Scapino 1981; Freeman 1995; Hogue and Ravosa 2001; Ravosa and Hogue 2004; Williams et al. 2008; Davis et al. 2010; Scott et al. 2012), very few attempts have been made to explain why selection might favor an unfused symphysis. Researchers who have examined this question have suggested that a mobile joint may be important for certain types of occlusion (Scapino 1965, 1981; Crompton and Hiiemae 1970; Hylander 1979a; Freeman 1995; Hogue and Ravosa 2001; Ravosa and Hogue 2004; Williams et al. 2008, Davis et al. 2010; Scott et al. 2012), but no study has evaluated differences in chewing performance among taxa that vary in symphyseal form. The Chiroptera may represent a natural experiment that can be used to test hypotheses regarding these issues. Importantly, our results provide the first clear demonstration (to our knowledge) of decreased ossification of the symphyseal joint in a mammalian clade, indicating that either (1) a mobile symphyseal joint is selectively advantageous in some contexts o
(2) an ossified symphysis will become unfused via neutral processes in the absence of selection to maintain fusion. Establishing which of these factors accounts for reversal in vespertilionoids, or if both contribute, is critical.

It is important to recognize that the determinants of symphyseal fusion in bats are still poorly understood. Differences in jaw-muscle activity patterns between *Myotis lucifugus* (Kallen and Gans 1972), which has an unfused joint, and *Pteropus giganteus* (De Guehlre and De Vree 1988), which has a fused joint, suggest that variation in symphyseal form may be related to masticatory stresses along the symphysis (Ravosa and Hogue 2004). However, complete ossification is observed in all dietary categories represented in our sample, including frugivores, insectivores, nectarivores, carnivores, and sanguivores (based on dietary characterizations presented in Norberg and Rayner 1987; Schondube et al. 2001; Van Cakenberghe et al. 2002). Although the experimental and comparative studies conducted on primates provide strong support for the hypothesis that increased symphyseal ossification in this clade functions to resist elevated joint stresses, which are a concomitant of processing mechanically challenging foods (e.g., Hylander et al. 1992, 2000; Hylander and Johnson 1994), other factors have been implicated in the case of chiropterans. For example, Freeman (1995) linked fusion to the use of the tongue in food acquisition in nectarivorous bats, and Davis et al. (2010) argued that complete ossification in the vampire bat *Diphylla* is related to the positioning of the incisor teeth in relation to the symphyseal joint. Thus, if bats are to be used as a model system for testing hypotheses about the selective advantage of an unfused symphysis, it is necessary to firmly establish which factors lead to fusion in this clade.

**WHY MIGHT SYMPHYSAL FUSION BE CONSTRAINED IN SOME CLADES?**

Given the pattern-based approach adopted here, our analysis cannot identify the evolutionary mechanism responsible for stasis of symphyseal fusion in the Anthropoidea (and possibly some noctilionoids) (Wagner 1995). As noted in the introduction, the invariance of symphyseal form in anthropoids in the face of remarkable ecological diversity suggests that stabilizing selection related to environmental factors (i.e., external selection) is not the primary mechanism driving this phenomenon, thus implicating the action of some sort of evolutionary constraint. In a brief discussion of symphyseal fusion in anthropoids, Lockwood (2007) raised two possibilities. The first is developmental canalization (e.g., Hallgrímsson et al. 2002; Siegal and Bergman 2002). According to this hypothesis, the developmental program of the anthropoid symphyseal joint was buffered from the action of external selection pressures very early in the history of this clade. Such buffering may have occurred because of the adaptive importance of symphyseal fusion; for example, the symphyseal joint of the last common ancestor of crown anthropoids may have been subjected to extended periods of intense diet-related stabilizing selection that eventually resulted in a highly conservative system.

The observation that fusion occurs very early in ontogeny in anthropoids—prior to weaning (Schultz 1956, 1969, 1973; Ravosa 1999)—may be particularly important in this context. Strepsirrhine primates that exhibit complete ossification (i.e., subfossil lemurs and Eocene adapids) are all “late-fusers” (Ravosa and Simons 1994; Ravosa 1996, 1999), meaning that fusion is not achieved until well after weaning has occurred. This difference in timing may reflect a distinction between anthropoids and other primates characterized by symphyseal fusion in the degree to which joint ossification is developmentally buffered. It may also signal a difference in the adaptive importance of fusion. For example, the mechanical challenge posed by the diets of some species may require that the symphysis be fused prior to the time when an individual adopts adult feeding behaviors (i.e., postweaning; Ravosa 1999), whereas complete ossification (as opposed to advanced partial fusion) may not be as critical in species in which fusion occurs relatively late in development. Thus, taxa that require early-fusing symphyses may be under strong selection for developmental canalization of this feature. These ideas are difficult to test, but an initial step in evaluating them would be to examine the developmental timing of fusion in chiropterans. Of particular interest is whether bat lineages in which reversion has occurred are characterized by late-fusing or early-fusing symphyses. A comparison between vespertilionoids, in which reversal has occurred are characterized by late-fusing or early-fusing symphyses, and other primates characterized by symphyseal fusion in the degree to which joint ossification is developmentally buffered. It may also signal a difference in the adaptive importance of fusion. For example, the mechanical challenge posed by the diets of some species may require that the symphysis be fused prior to the time when an individual adopts adult feeding behaviors (i.e., postweaning; Ravosa 1999), whereas complete ossification (as opposed to advanced partial fusion) may not be as critical in species in which fusion occurs relatively late in development. Thus, taxa that require early-fusing symphyses may be under strong selection for developmental canalization of this feature. These ideas are difficult to test, but an initial step in evaluating them would be to examine the developmental timing of fusion in chiropterans. Of particular interest is whether bat lineages in which reversion has occurred are characterized by late-fusing or early-fusing symphyses. A comparison between vespertilionoids, in which reversal appears to be relatively common, and noctilionoids, in which reversal appears to be less common (or absent, suggesting the existence of a potentially anthropoid-like constraint), might be especially illuminating.

The second possibility suggested by Lockwood (2007) derives from a concept articulated by Wagner and Schwenk (2000)—the evolutionarily stable configuration (ESC). An ESC is a character complex that remains stable through the action of internal selection to maintain functionality of a tightly integrated system, despite changes in ecology or environmental conditions. From this perspective, anthropoid symphyseal form is constrained from varying by the configuration of the other components of its functional system (i.e., the masticatory apparatus). In discussing this idea, Lockwood (2007) invoked Ravosa et al.’s (2000) argument that complete ossification of the symphysis in anthropoids is a functional outcome of changes in skull architecture (related to increased encephalization) that altered chewing kinematics such that the balancing-side deep maseter came to play a key role in effecting transverse jaw movements at the end of the chewing cycle. Experimental evidence indicates that the horizontal component of the force generated by this muscle bends the balancing-side mandibular corpus laterally, whereas the horizontal component of...
the bite force bends the working-side corpus in the opposite direction, generating a loading regime at the symphysis—referred to as lateral transverse bending, or wishboning—that an unfused joint is ill-equipped to resist (Hylander 1984; Hylander et al. 1987, 1998, 2000, 2011; Ravosa 1991, 1999; Hylander and Johnson 1994; Ravosa and Hylander 1994; Ravosa and Hogue 2004; Vinyard et al. 2005, 2006, 2007). According to this scenario, then, the anthropoid symphysis will remain fused as long as the other elements of its functional system are configured in a way that leads to wishboning of the mandible during mastication.

The late-firing balancing-side deep masseter (referred to hereafter as the late deep masseter) has been recorded in all non-human anthropoid primates examined so far, including macaques, baboons, owl monkeys, marmosets, and tamarins (Hylander et al. 1987 1998, 2000, 2011; Hylander and Johnson 1994; Vinyard et al. 2006, 2007). Although this sample represents only a small fraction of extant anthropoid taxonomic diversity, the fact that species as distantly related and as ecologically and morphologically dissimilar as baboons and marmosets possess the late deep masseter suggests that this muscle-recruitment pattern probably characterizes most crown anthropoids. The late deep masseter is also observed in other mammals with fusion or advanced degrees of partial fusion (Herring and Scapino 1973; Wejs et al. 1989; Williams et al. 2007; Hylander et al. 2011).

The scenario described above is complicated by two observations. The first is that humans do not appear to possess a late deep masseter (Van Eijden et al. 1993). The second is that preliminary data for the New World monkey Cebus apella suggest that wishboning, if it does occur, is not the dominant loading regime in the symphysis of this taxon (Vinyard et al. 2011). Persistence of fusion in the absence of the phenomenon that appears to cause it is more consistent with the idea that the anthropoid symphysis is developmentally canalized, but these apparent exceptions do not necessarily refute the argument that a fused symphysis is an integral component of an anthropoid masticatory ESC. For example, it may be that the human muscle-recruitment pattern evolved relatively recently and that natural selection (or drift) has not had sufficient time to produce an unfused symphysis. It is also possible that wishboning is not the only loading regime that requires a fused symphysis (Ravosa and Hogue 2004). According to Wagner and Schwenk (2000, p. 160), the components of an ESC are not necessarily invariant (although some may be); rather, an ESC “comprises a set of phenotypes that vary within certain functional parameters, rather than a single, fixed form” (emphasis added). Thus, perhaps the human and Cebus patterns simply represent intra-ESC variation. Testing the hypothesis that stasis in symphysal fusion in anthropoids is due to membership in an ESC will require a better understanding of within- and between-clade variation in patterns of muscle recruitment and mandibular strain in the Anthropoidea and Strepsirrhini. Similar analyses in other taxa would further inform our understanding of constraints on the evolution of the mammalian feeding apparatus.

**Conclusions**

In summary, our results are consistent with the idea that the morphology of the mandibular symphysis is evolutionarily constrained in some mammalian lineages. Although reversals from full fusion in speciose clades such as the Anthropoidea and perhaps the Noctilionoidea have not occurred or are rare, re-evolution of an unfused joint appears to have been common over the course of vespertilionoid evolution. We have identified a number of avenues of future research that are critical in terms of further testing the constraint hypothesis and identifying its exact nature. These include the following: (1) establishing why selection might favor an unfused symphysis, (2) determining whether the developmental timing of the onset of fusion influences symphysal evolution, and (3) circumscribing the range of biomechanical environments in which fusion occurs. Comparative analysis of the two chiropteran superfamilies examined here will undoubtedly provide greater insight into these issues, particularly the first two. In vivo studies of muscle-recruitment patterns and mandibular bone strain during mastication on a greater number of mammalian taxa will also be necessary in terms of providing an appropriate functional context in which to evaluate the evolution of constraint.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Supporting Information

The following supporting information is available for this article:

Appendix S1. Maximum clade credibility tree for Chiroptera in Newick format (with branch lengths in millions of years before present).

Table S1. GenBank accession numbers.

Supporting Information may be found in the online version of this article.

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