

Association of Mandible Shape and Nesting Material in *Osmia* Panzer (Hymenoptera: Megachilidae): A Morphometric Analysis

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ABSTRACT We combined methods of geometric morphometrics and phylogenetically controlled comparisons to examine the relationship between the shape of mandibles and material used to construct the nest for 5 *Osmia* Panzer species. We measured leaf- and mud-using species from 2 subgenera: in the subgenus *Osmia*, *O. lignaria* Cresson (mud-user), *O. cornuta* Latreille (mud-user) and *O. ribifloris* Cockerell (leaf-user), and in the subgenus *Centrosmia*, *O. tanneri* Sandhouse (mud-user), and *O. bucephala* Cresson (leaf-user). Landmark-based geometric morphometrics and multivariate statistics provided a way to compare mandible shapes, distill major shape differences and visualize mandible regions that differed most among bee species. Mandible shape differed significantly among all species based on paired comparisons of Mahalanobis distances. Principal component analysis of the shape variables (relative warps analysis) revealed greater similarity between the 2 leaf-using bees, than among the mud-users. There were however, no consistent differences between these mud- and leaf-using species. Testing with two-way multivariate analysis of variance showed that the differences in shape between the leaf- and mud-using species depended on the subgenus. A broad proximate bite surface characterized leaf-using species. No such trait was common to mud-using species. Mandibles of leaf-using species may vary less because of selection on shape, or because of functional constraints for shearing and macerating leaf material; both constraints are absent in mud-using species. Mandibles of mud-using bees may respond more to selection imposed by other aspects of bee biology.

KEY WORDS *Osmia*, solitary bee, mandible, nest material, geometric morphometrics, morphology

TO INVESTIGATE ADAPTATION, it is necessary to characterize patterns of association between ecological factors and phenotypic traits. Evolutionary biologists have inferred adaptive significance from such associations in multispecies comparisons (Clutton-Brock and Harvey 1979). More recently, workers have begun to interpret comparative studies within a phylogenetic framework, trying to account for the nonindependence of related species and to infer the selective regime under which a trait evolved (Felsenstein 1985, Harvey and Pagel 1991). To demonstrate unequivocally that a trait is an adaptation, it is necessary to show that the trait is causally linked to enhanced performance that increases the relative fitness of the organisms possessing the trait within their population (Bock and von Wahlert 1965, Harvey and Pagel 1991). We investigated variation in mandible shape among 5 *Osmia* species (Hymenoptera: Apoidea) and its association with materials that bees use to construct their nests. To explore mandible shape we employ geometric morphometrics. The techniques, which incorporate data from the whole object, allowed us to visualize regions of the mandible that differ among bee species and so pose specific hypotheses about the evolution of mandible shape in bees. The differences among spe-

cies raise a number of questions that call for continued study of the relationship of mandible shape and nesting ecology within a more complete phylogenetic context.

Nesting solitary bees typically enclose individual brood in single cells that they have provisioned with pollen and nectar. These brood cells form the basic units for nest construction in burrowing and nonburrowing species (Stephen et al. 1969). Nest architectures of burrowing and nonburrowing species are diverse (e.g., Linsley 1958, Linsley and MacSwain 1959, Krombein 1967, Stephen et al. 1969, Thorp 1969, Rust et al. 1974, Parker 1975, Eickwort and Sakagami 1979, Frohlich and Parker 1983, Neff and Simpson 1992). The variety of materials used to construct or seal brood cells is equally diverse. Within the Megachilidae, different species collect plant hairs, leaves, flower petals, mud, pebbles, resins, or combinations of these materials (Linsley 1958, Stephen et al. 1969, Torchio 1989). During nesting, the mandibles are used by different species to excavate mud, shear and macerate leaves, grasp collected material, and plaster it into place at the nest (e.g., Frohlich 1982, Torchio 1989; N.M.W., unpublished data).

Given the critical role of mandibles in nesting, we might expect nesting habits and materials used in construction to impose selection on mandible shape. A shape effective for manipulating resin may differ from

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a shape that facilitates gathering small pebbles. Comparing species that share most aspects of nesting biology other than those of interest will minimize variation in shape introduced from other selective factors, which may confound the ability to detect correlation with the factor of interest. We chose sets of species that use mutually exclusive nest materials. Two species construct the partitions between their brood cells from masticated plant fiber and the others use mud to shape brood cells or to build partitions. We refer to these categories as leaf-using and mud-using. We also used only nonburrowing species to avoid influence of different nest substrates on mandible shape.

We chose species from 2 subgenera, which showed at least some variation in nesting material among species. We made comparisons at the subgeneric level because a preliminary survey of shape showed that at higher taxonomic scales (e.g., interfamily), differences in the shape of the bite surface were so great that homologous points for comparison could not be identified (Michener and Fraser 1978). Although we lack a well-resolved phylogeny for most of the genus *Osmia*, our subgenera are related more distantly to each other relative to the species within each subgenus. Assuming the independence of the pairs, we can test whether mandible shapes are consistent with a pattern of convergent evolution in bees that use similar nest materials by comparing the added component of shape variation caused by the effects of nesting material.

A number of studies have identified correlations between mandible shape and feeding ecology across different insects and other arthropods (Bernays 1991, McKeever et al. 1991, Leschen 1993, Samways et al. 1997), and some have found evidence for adaptation (Bernays 1991). Trophic foraging in bees differs from most other insects because most bee species do not use their mandibles extensively. Bees that chew into anthers to get pollen or actively rob nectar are exceptions and do show unique morphological features (Inouye 1983).

Studies of morphological variation in mandibles of other insects most often have characterized differences based on presence/absence or counts of structures, and qualitative features (e.g., rough versus smooth). In many cases, presence/absence characters will not exist or may not describe the most relevant differences. For continuous shape variation, studies often have measured linear distances between points or structures. Sets of linear distances face problems of intercorrelation, do not retain the geometry of the object of interest, and cannot separate differences in shape from differences in size. Shape and size properties may evolve in different ways and the ability to dissect the 2 can provide information on different adaptations.

To avoid these problems, we adopted the methods of geometric morphometrics. This approach allows statistical comparison of shapes using the coordinates of specific points (landmarks) whose geometric relationships are preserved throughout analysis. Specimens described by coordinate data can be aligned in

a consistent and mathematically defensible manner. Variation can be partitioned into shape and size components, which facilitates comparison of shape alone, shape and size together (Rohlf and Slice 1990), and different types of shape change (Bookstein 1990, Rohlf et al. 1996).

We make phylogenetically controlled comparisons to address the following two questions about the relationship between solitary bee mandible shape and material they use to construct nests. (1) Do species that use similar nesting materials have more similarly shaped mandibles than more closely related species that use different nesting materials? (2) Are there particular shape characteristics common to bees that use similar nest materials (plant fiber versus mud)?

Materials and Methods

Species Examined. We chose 5 bee species from 2 *Osmia* subgenera (Hymenoptera: Megachilidae). *Osmia lignaria propinqua* Cresson, *O. cornuta* Latreille (subgenus *Osmia*), and *O. tanneri* Sandhouse (subgenus *Centrosmia*) collect mud to construct brood cells, or partitions between cells (Levin 1966, Rust 1974, Parker 1975, Torchio 1989, Bosch 1994). *Osmia ribifloris* Cockerell (subgenus *Osmia*) and *O. bucephala* Cresson (subgenus *Centrosmia*) use masticated plant material to construct partitions between brood cells (Krombein 1967, Rust 1986). These latter two do not incorporate mud into their nests, although *O. bucephala*, which nest within tunnels in wood, use their mandibles to shave wood from the sides of the tunnel and incorporate these fibers into the cell partitions (Krombein 1967). These species provided two phylogenetically independent comparisons of mandible shape between mud-collecting bees and leaf-collecting bees. *Osmia lignaria*, *O. cornuta*, and *O. ribifloris* are closely related and form an unresolved polytomy in a recent phylogenetic analysis (R. W. Rust and J. Bosch, University of Nevada, Reno, and USDA Bee Biology and Systematics Laboratory, Logan, UT, unpublished data).

Data Capture. We selected 15 female specimens of each species, each of which was either collected as it emerged from the cocoon or appeared newly emerged based on wing wear and general appearance. We avoided older individuals because mandible shape can be substantially affected by wear (Kokko et al. 1993). *Osmia* mandibles are slightly spoon-shaped and lie in a relatively horizontal plane with respect to the head when closed. As a result, there is an upper surface whose shape can be captured when the mandibles are spread (Fig. 1).

We used a reflex microscope (Maclarnon 1989) to record the two-dimensional coordinates of 8 landmarks around the perimeter of the right mandible from each specimen (Fig. 1). Points included the acetabulum (landmark 1), apex (8), pollex teeth (4 and 6), and crevices between the teeth (Michener and Fraser 1978). These points captured the shape of the bite surface (landmarks 2–8) and its relation to the point of attachment to the head capsule (landmark 1).

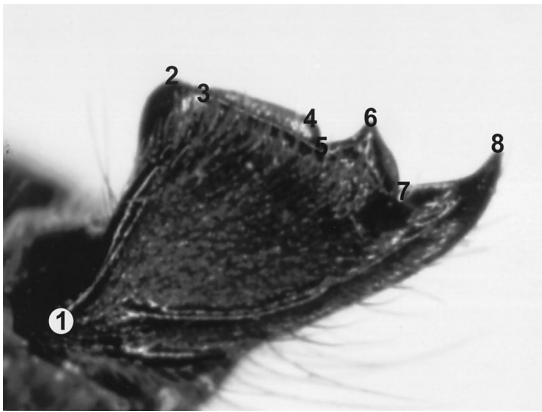


Fig. 1. *O. ribifloris* right mandible (landmarks 1–8 = 1.1 mm.). Point 1 is the acetabulum, points 2–4 the proximate bite surface, 6 a pollex tooth and 8 the apex.

We consider the landmarks to be homologous across species and they conform to landmark types accepted for geometric morphometrics (Bookstein 1991).

We aligned coordinate data from all specimens by least squares fit using generalized orthogonal Procrustes analysis (Rohlf and Slice 1990). The analysis with a least squares fit simultaneously minimizes the squared distances between homologous landmarks across all specimens. This procedure removed shape differences between all specimens that were due to size, as well as to orientation and placement of the specimen during data capture. The remaining variation among specimens corresponded to true shape differences. From these aligned data, we calculated a consensus configuration of landmarks (mean specimen) for all specimens across all species (Rohlf and Slice 1990) using GRF-ND (Slice 1993). We also

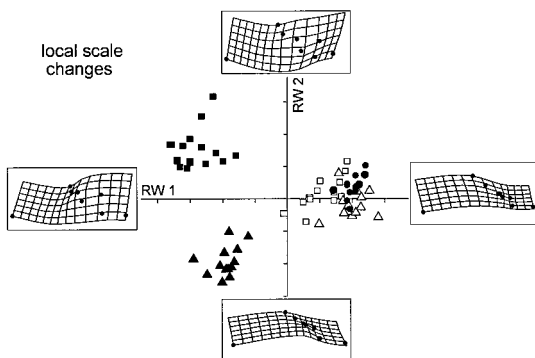


Fig. 2. Relative warps (RW) plot of the 5 species with only local shape differences (nonaffine) included. ■, *O. lignaria*; ●, *O. cornuta*; ▲, *O. ribifloris*; □, *O. tanneri*; △, *O. bucephala*. Transformation grids at the ends of the axes show shape deformations along the axes. A grid at the origin would be a regular grid of rectangles.

aligned specimens for each species separately and computed separate consensus configurations.

Visualizing Shape Changes and Constructing Shape Variables for Analyses. Shape differences between the consensus for all species and each specimen were calculated using the thin-plate spline function (Bookstein 1991). The function translates points of the consensus configuration to corresponding points on any of the individual specimens through linear combinations of variables. We used the thin-plate spline to map shape deformation between the mean from each species to the overall consensus by constructing transformation grids from the overall consensus to the mean mandible shape for each species (calculations and plots constructed using TPSSPLINE; Rohlf 1997b). We used the transformation grids to visualize differences in shape among species.

To create shape variables for statistical analyses, we transformed parameters of the thin-plate spline function to partial warp scores (Rohlf 1993, Rohlf et al. 1996). Spline parameters, as well as other shape variables can be used for analyses; however, partial warp scores have several useful attributes. Partial warps partition shape differences across different geometric scales (Bookstein 1991) and because they are non-redundant and geometrically orthogonal, they are amenable to standard multivariate analyses (Rohlf et al. 1996).

Statistical Comparisons. We used partial warp variables to compare all specimens and to test our ecological and evolutionary hypotheses with standard multivariate techniques.

We decomposed shape differences into those localized to 1 region of the mandible (nonaffine) and those at infinite scale (affine; Bookstein 1990, Rohlf et al. 1996). The most tangible morphological interpretation of affine differences among specimens is shearing and stretching of the whole object. We performed separate principal component analyses on the localized shape differences and then on total shape differences (localized plus infinite scale). We used the partial warp scores for local scale parameters (Bookstein 1991, Rohlf 1996) and linearized Procrustes method to estimate infinite scale parameters (Bookstein 1996). The principal component analyses were calculated using relative warps analysis (Bookstein 1991; Rohlf 1993, 1998). Relative warps analysis distills the dominant trends in shape variation among specimens and can help separate groups of specimens with different morphology. In each analysis, we weighted all geometric scales of shape change equally (Rohlf 1996). We used transformation grids to illustrate areas of the mandible that showed the greatest difference between extremes of the first 2 relative warp axes (Rohlf 1993). Although the principal component approach is invaluable for qualitative assessment, it does not allow statistical testing of shape differences.

To test differences in mandible shape among species, we performed a canonical vectors analysis (CVA; Marcus 1993) using NTSYS (Rohlf 1997a), from which we obtained Mahalanobis distances (D^2) between the mandible shapes of each species. We then used Ho-

tellings multivariate *t*-test to test for significant shape differences between all pairs of species based on D^2 (Marcus 1993). We also tested the effect of nesting material (ecology) and phylogenetic relationship on mandible shape using a two-way multivariate analysis of variance (MANOVA) with *Osmia* subgenus and nest material as independent factors. To explore differences in the bite surface of the mandible, we used landmark coordinate data from GLS aligned configurations to calculate the width of the proximate bite surface relative to the total bite surface (landmarks 2–8). We then tested the difference in this proportional width between the mud-using and leaf-using species with two-way ANOVA including phylogeny as the 2nd factor.

Results

Mandible Shape Differences Among Species. Relative warps (RW) ordination using only the shape components at the local geometric scale did not clearly separate mud-using and leaf-using species. Mandible shapes of the 3 mud-using species were distinct from each other, but the 2 leaf-users clustered together along with *O. cornuta*, a mud-user (Fig. 2). The first 2 relative warps axes explained 56.7 and 19.8% of the shape variation. The grids at each end of the axes illustrate the extremes of deformation along the first 2 relative warps axes. Moving from the negative to positive direction, axis 1 shows relative broadening of the proximate bite surface (landmarks 2–4), reduction in the distal teeth (landmarks 6 and 8) and expansion of the bite surface away from the acetabulum (landmark 1). Axis 2 shows primarily a shortening of the apical tooth and compression of the bite surface toward the acetabulum (Fig. 2). When the stretching and shearing components were included, the 3 mud-using species were again separated, as were the 2 leaf-using species. The 2 subgenera were only weakly separated and the distance between species within a subgenus (e.g., *O. lignaria* versus *O. ribifloris*, or *O. tanneri* versus *O. bucephela*) was greater than that between subgenera. The stretching and shearing components explained an additional 7% of the variation in the first 2 relative warp axes (60.7 and 22.8%). Moving from negative to positive, axis 1 now shows a stretching of the bite surface away from the acetabulum and axis 2 shows substantial narrowing of the proximate bite surface (Fig. 3). As seen with local-scale changes (Fig. 2), the relative warps analysis did not clearly partition species based on nesting material. Interestingly, the mandible shapes of the mud-using species *O. cornuta* were contiguous with those of the related leaf-user *O. ribifloris*. Between the 2 subgenera and within the subgenus *Osmia*, the positions of the mud-using species relative to the leaf-using species were very different (Figs. 2 and 3).

The statistical results paralleled those from the relative warps ordination. Canonical vectors analysis on total shape variation showed significant differences among species (Wilks $\Lambda = 6.94 \times 10^{-6}$; $P < 0.0001$). In pairwise comparisons, *O. lignaria* differed signifi-

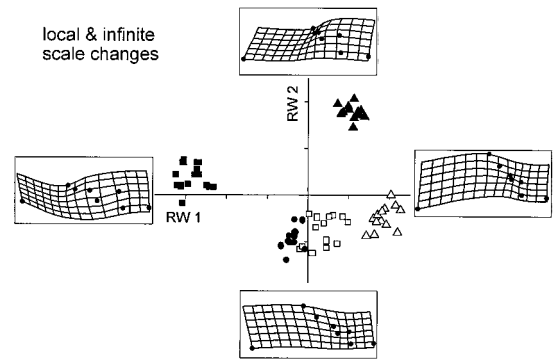


Fig. 3. Relative warps (RW) plot of the 5 species with local and infinite scale shape components included. ■, *O. lignaria*; ●, *O. cornuta*; ▲, *O. ribifloris*; □, *O. tanneri*; △, *O. bucephela*. Transformation grids at the ends of the axes show shape deformations along the axes. A grid at the origin would be a regular grid of rectangles.

cantly from all other species (Table 1, Mahalanobis distances). The only other significant difference was between *O. tanneri*, the mud-using *Centrosmia*, and *O. ribifloris*, the leaf-using *Osmia*. Nesting ecology and phylogenetic relatedness were poor predictors of shape differences. *O. cornuta* was closest to *O. ribifloris* ($D^2 = 7.98$), less than half the distance to either of the other mud-using species. In this analysis too, the mandible shapes of leaf-using species were relatively close together, those of the mud-using species were distant.

The thin-plate splines to consensus (mean) specimens for each species further illustrate differences among species and nesting ecologies (Fig. 4). Leaf-users share a broad proximate bite surface (Fig. 4, *O. ribifloris* and *O. bucephela*), but such general similarities do not exist among the mud-using bees. *O. lignaria* has relatively long pollex teeth and intermediate proximate bite surface; *O. tanneri* shows a substantially narrowed proximate bite surface, but less prominent teeth; *O. cornuta* has a broader proximate bite surface and overall shape more similar to the leaf-using species. The similarity between *O. cornuta* and *O. ribi-*

Table 1. Mahalanobis distances between mandible shapes

Species	D^2	<i>F</i>	<i>P</i> <
LIG vs RIB	21.196	7.133	0.01
LIG vs TAN	20.228	6.363	0.01
LIG vs COR	19.212	5.145	0.05
LIG vs BUC	24.646	7.753	0.01
RIB vs TAN	12.784	4.021	0.05
RIB vs COR	7.982	2.138	>0.50
RIB vs BUC	9.977	3.138	0.15
COR vs TAN	14.250	3.538	0.25
COR vs BUC	13.791	3.712	0.25
TAN vs BUC	12.650	3.424	0.25

α adjusted for multiple comparisons.

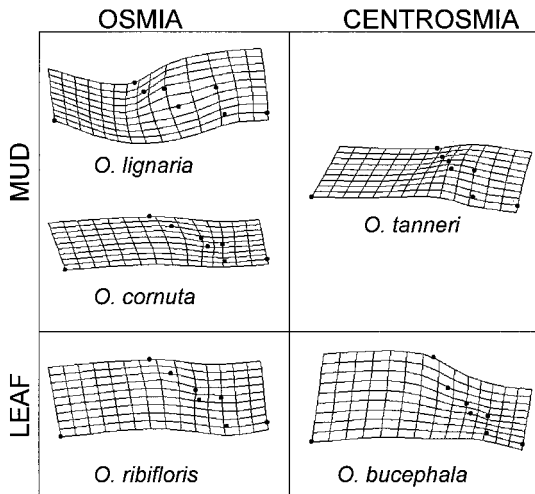


Fig. 4. Thin-plate spline visualizations of mean mandible shapes for *O. lignaria*, *O. cornuta*, *O. ribifloris*, *O. tanneri*, *O. bucephala*. Transformation grids show the areas of greatest shape change from the overall consensus. The overall consensus would be a regular grid of rectangles.

floris is especially evident; the main difference is that *O. cornuta*'s mandible is relatively narrower overall.

Two-way MANOVA of phylogeny and nesting material showed that the effect of nesting material on mandible shape depended on the subgenus considered (significant subgenus \times nest material interaction Wilks $\Lambda = 0.093$; $P < 0.001$). The effect of nest material on the width of the proximate bite surface also depended on the subgenus (Table 2). Widths of the proximate bite surfaces (Fig. 1, landmarks 2–4) of 2 mud-using species (*O. lignaria* and *O. tanneri*) were proportionately narrower than their leaf using congeners; however, the 3rd mud-using species, *O. cornuta*, did not differ significantly from either leaf-user (Fig. 5, Tukey–Kramer, $\alpha = 0.05$).

Discussion

Phylogeny of *Osmia*. Phylogenetically controlled comparison is grounded on the validity of the independent relationship of the species considered. The exact taxonomic placement of species within *Osmia* has been contended over the past 50 yr. No complete phylogeny exists. Placement of *O. lignaria*, *O. cornuta*,

Table 2. Two-way ANOVA, response variable was proximate bite as a proportion of total bite surface

Source	MS	F-ratio	df	P value
Subgenus	0.057	7.876	1	0.007
Nest material	0.635	88.285	1	< 0.0001
Subgenus \times nest material	0.162	22.535	1	< 0.0001
Error	0.007		61	

ANOVA performed on arcsine transformed proportions.

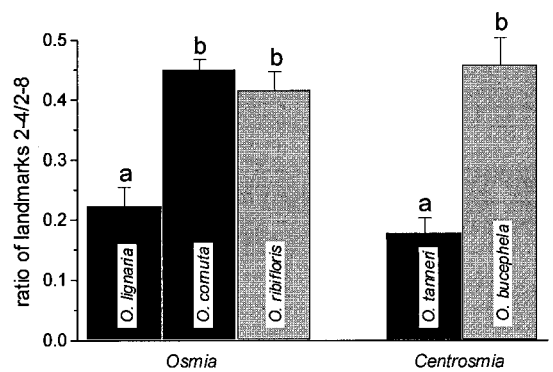


Fig. 5. Mean proportional proximate bite surface of mud and leaf-users, for both subgenera. Bars indicate standard deviations. Letters above the bars indicate significant differences using Tukey–Kramer honestly significant difference (HSD) method $\alpha = 0.05$.

and *O. ribifloris* within the subgenus *Osmia* has remained consistent across different studies of the genus (Sandhouse 1939, Sinha 1956, Rust 1974, Krombein et al. 1979). The relationship is also supported by the recent molecular phylogeny (R. W. Rust and J. Bosch, University of Nevada, Reno, and USDA Bee Biology and Systematics Laboratory, Logan, UT, unpublished data). This recent treatment also places *O. ribifloris* as the closest leaf-using species to *O. lignaria* and *O. cornuta* with strong support. Various authors have moved either, or both, *O. tanneri* and *O. bucephala* into different subgenera (Sandhouse 1939, Sinha 1956, Krombein et al. 1979), and some experts have questioned their current placement (Parker 1975; T. Griswold, USDA Bee Biology and Systematics Laboratory, Logan, UT, personal communication) For this study, we deferred to the most recently published account (Krombein et al. 1979). We feel that the pairings are defensible at the scale that our comparison was made. *O. lignaria*, *O. cornuta*, and *O. ribifloris* likely share a more recent common ancestor than do *O. tanneri* and *O. bucephala*.

Differences Between Mud and Leaf Users. Despite their functional significance in collecting and processing nest material and in constructing the nest, the variation in mandible shape was not satisfactorily explained by differences in the nest material used.

Leaf-Users. There was some evidence for convergence in leaf-using species. Their mandible shapes clustered together much more than those of the mud-users in all of the analyses. The thin-plate spline figures (Fig. 4) revealed that in both subgenera, leaf-using species showed a broader proximate bite surface of the mandible than mud-using species. We do not know the leaf-harvesting behavior for *O. ribifloris* or *O. bucephala*. *O. bucephala* sometimes shaves wood fibers from inside its nest and incorporates these into partitions between cells, which may contribute to the stouter basal region of its mandible compared with *O. ribifloris* (Fig. 4, *O. ribifloris* and *O. bucephala*). Tor-

chio (1989) found considerable difference in leaf-cutting behavior between 2 species of *Cephalosmia*. *O. montana* females, which have a greatly enlarged apical tooth, used it to finely perforate the boarder of a small leaf piece and then tore the piece free. They macerated the piece for transport to the nest. *O. californica* cut and macerated the edge of the leaf without specifically using the apical tooth. Maceration was common to both species. Neither leaf-using species in our study exhibits the enlarged apex. We suggest the wider bite surface may help in shearing leaf material during collection and in maceration. Within the Megachilidae, there is variation in using macerated leaf material and whole leaf pieces in cell construction. Comparing mandibles of bees that use intact leaf pieces, such as *Megachile*, rather than macerated pieces might yield more information about potential functional roles of different characteristics of the leaf-users' mandibles.

Other insect taxa show convergent mandible shape correlated with use of different leaf materials. The grass-generalist Acrididae (Orthoptera) show multiple independent convergences to chisel shaped incisor cusps and ridged molar regions for grinding (Chapman 1964, Bernays 1991).

Mud-Users. In all analyses, the mandibles of mud-users were much more variable than the mandibles of leaf-users and did not differ from leaf-users in a consistent way. *O. lignaria* and *O. tanneri* had significantly narrower proximate bite surfaces than their leaf-using congeners, although they differ in other regions of the mandible (Fig. 4). *O. cornuta* showed no evidence of a relatively narrower proximate bite surface. The shape of its mandible was nearly identical to that of its leaf-user congener, *O. ribifloris*, in most respects (Fig. 4, *O. cornuta* and *O. ribifloris*).

These data suggest that mud-collection may impose fewer functional constraints; that is, it may be possible to effectively collect and manipulate mud with a variety of mandibles. Mud-using species may compensate for different shapes with specific behavior. Under such an argument, mandible shape may have been more responsive to selection from aspects of the species' biology that we did not measure. We know functional details of mud use only for *O. lignaria* (Torchio 1989), and therefore comparison with the other species is not possible. *O. tanneri* constructs nests that have more variable architecture than *O. lignaria* or *O. cornuta*; nests have been reported in twigs, sheltered open surfaces and in the ground (Parker 1975, Tepedino 1979). The mandible shapes of *O. lignaria* and *O. cornuta*, however, are at least as distant (Table 1), even though they construct very similar nests (Torchio 1989, Bosch 1994) and are putative sister species. Factors not related to nest construction may select on mandible shape. For example, 1 study showed that *O. lignaria* can suffer 25% nest usurpation by conspecifics (Tepedino and Torchio 1994). *O. lignaria*'s prominent teeth may be advantageous for grappling with other females within nest holes.

In summary, the existing data do not provide strong evidence for adaptive differences in mandible shape. First, the similarity of the mandibles of a leaf-user, *O.*

ribifloris, and a mud-user, *O. cornuta*, indicate that mandible shape does not always dictate nest material. An analogous pattern has been found for some trophically diverse clades of Staphylinid beetles: little variation among mandibles was apparent despite their importance in feeding (Leschen 1993). Second, the divergent mandible shapes of mud-using *O. cornuta*, *O. lignaria*, and *O. tanneri*, strongly suggest that nesting material does not necessarily impose consistent selection across species with similar ecologies.

Our initial look at the effect of nesting ecology on mandible shape raises the question of whether a more complete examination would bring to light patterns of adaptation in mandible shape. We plan to examine additional *Osmia* species as well as other Megachilidae that use diverse nesting materials and process them in unique ways. New geometric morphometric methods that integrate landmarks and curved outlines (Sampson et al. 1996, Bookstein 1998) could prove useful in further exploration of mandible shape variation in the Megachilidae. These techniques have not been fully developed. Photographs of mandibles suggest that leaf-users have relatively broader mandibles, but the curved posterior margin has no discreet points that could be used as homologous landmarks among species. Incorporating this curved surface with new techniques could improve our ability to separate taxa on the basis of ecological factors.

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