Plant-pollinator specialization: Origin and measurement of curvature

TO DO

-table s2, sample sizes -reformat table s3 -pollinator diversification in intro -incorporate ailene edits -incorporate tiago edits -read Macleod 2002

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Abstract

The curvature of flowers and pollinator mouthparts (e.g. hummingbird bills) along the lateral plane is a widespread, convergent trait with important ecological and evolutionary implications. Pollination ecologists are concerned with flower-pollinator curvature because it appears to be a derived trait associated with specialization, competition, and species co-existence. In this review we summarize and evaluate the methods historically used to measure curvature and suggest a clarification of its definition by referring to the differential geometry literature. Intuitively, curvature is the degree to which a line is not straight, or more formally, the rate at which the unit derivative changes direction with respect to arc length. To apply this definition we suggest a protocol wherein a line is regressed against landmarks placed on a lateral image of an organism, then computing curvature at many points along the fitted line and taking the sum. This protocol is demonstrated here by studying the development of nectar spur curvature in *Epimedium* (Berberidaceae). By clarifying the definition of curvature, the language of comparitive morphology is made more precise. In this study we found *Epimedium koreanum* to have an order of magnitude greater curvature than the closely related *E. grandiflorum* and *E. violaceum*. This is to say that *E. koreanum* had greater total degrees of rotation along the arc of the nectar spur. The functions used to quantify floral curvature in this study are available as an open-source R package 'curvy'. The major advantages of this method are 1) precision of measurement is increased without introducing expensive field equipment or computing power, 2) precision of terminology within pollination ecology is improved by adopting the existing mathematical lexicon for studying line-curves.

1. The ecology of flower-pollinator curvature

> "We are beginning to understand why some hummingbird bills are long, whereas others are short, and why some hummingbird flowers are wide, whereas others are narrow. Now, why are bills of some hummingbirds and the tubes of the flowers they visit curved?" – Temeles (1996).

At the center of plant-pollinator diversification is a remarkable variety of floral form. The notion that plant communities experience selection to reduce interspecific mating ("floral isolation", Grant, 1949) points to the importance of floral diversity in initiating and reinforcing reproductive isolation (Armbruster and Muchhala, 2009). For example, in the rapid radiation of Andean *Centropogon* (Campanulaceae), competition for pollination led to the divergence of floral traits associated with bat and hummingbird pollination (Lagomarsino and Muchhala, 2019). In the case of South African *Lapeirousia* (Iridaceae), geographic variation in floral tube length has initiated reproductive isolation between morphs with short and long corolla tubes, despite sharing the same fly pollinator (Minnaar et al., 2019). Patterns of plant-pollinator evolution point to both contemporaneous and asymmetrical diversification (Cardinal and Danforth, 2013; Tripp and McDade, 2013). In either case, floral morphology is a key phenotype associated with the diversification of plants and pollinators (Kay and Sargent, 2009; Niet and Johnson, 2012; Ollerton, 2017).

Flower-pollinator curvature as viewed from the side (lateral plane), has been a trait of special interest since the post-Darwin era of pollination ecology. In making pollinator observations of the Cape flora, Scott-Elliott (1890) noticed that the flowers of *Leonotis ocymifolia* (Lamiaceae) visited by *Nectarinia* sunbirds were "curved with the same curvature as that of the bird's beak." (p. 272). Robertson (1889) insightfully notes that the curved nectar spurs of *Viola* spp. (Violaceae) "serves to limit the insect visits much more than the mere length of the spur." (p. 172). From these early observations curvature has been synonymous with specialization; we expect curvature to limit the range of functional taxa in a plant-pollinator mutualism and strengthen interactions between the existing participants. And these expectations have largely been supported: Stiles (1975) first posited that neotropical *Heliconia* partition hummingbird visitation by flower-bill curvature, and that specialization by curve-billed hummingbirds allow co-existence within the species-rich *Heliconia* clade. Subsequent research supports this hypothesis (Maglianesi et al., 2014): along the slopes of the Central Cordillera (Costa Rica), the degree of flower-hummingbird bill curvature is proportional to plant-pollinator interaction strength (Dehling et al., 2014) and extent of specialization (*d'*, (Blüthgen et al., 2006). More recently the scope of plant-pollinator research has expanded to address the biogeography of curvature. As predicted by Stiles (2004), Maglianesi (2015) and Sonne (2019) find curvature to be most prevalent in the lowland environments of the neotropics. Explanations for this pattern range from heightened competition at lower elevations to environmental filtering in the Andean highlands (Stiles, 2004; Graham et al., 2009). Because the neotropical subfamily Phaethornithinae comprises the majority of hummingbird species with curved bills, we might expect plant-hummingbird curvature to have a predictable global distribution. In the case of honeycreepers and honeyeaters and sunbirds ???

-Curvature and niche partitioning: -evidence that curvature is correlated with a shift from insectivory to nectivory in hawaiin honeycreepers (Carothers, 1982). -honeyeaters take longer to feed and intake less nectar on experimentally curved flowers (Collins, 2008) -aussie honeyeaters with curved bills tend to be small nectivores and aerial insectivores rather than stout-billed ground foragers. -see: wolf 1972 (Science), 1975 (Ecology)

Pollinator specialization has major effects on macroevolutionary and biogeographic patterns (Kay and Sargent, 2009; Armbruster and Muchhala, 2009; Vamosi et al., 2018), and curvature is a component, but widespread feature of specialist systems. Therefore, to synthesize our knowledge of curved plant-pollinator systems, curvature is a concept that needs an exact definition and method of measurement. In the following section we summarize the approaches to measuring curvature within the field of bird pollination, identify strengths and shortcomings, and offer a solution with the aim of improving the precision with which curvature is measured within the field of pollination ecology. Although this review is motivated by the problem of measuring curvature in plant-hummingbird systems, the solution is general to any biological form modelled as a line curve: this case is hopefully made in the demonstration to follow.

2. Summary of the literature

We searched the scientific literature for studies focusing on or considering the curvature of flowers and their pollinators - a trait commonly measured as a proxy for specialization. We make the distinction between measuring curvature (e.g. of petals) in the lateral plane versus the curvature of surfaces. While lateral images are analysed for line-curvature, images of specimens in the transverse plane can be used to analyse surface (Gaussian) curvature (Nath et al., 2003; Coen and Rebocho, 2016). The methods used in the latter are relatively more complex, and perhaps because of this, comparitively well-defined. At present, surface curvature has yet to be considered in the context of pollination. However, because line and surface curvature are related mathematical concepts, it will benefit pollination research to clarify the simplest case (lines), with the goal of generating interest in related ideas including the curvature of surfaces.

The literature was sourced by querying Web of Science and Google Scholar for a topic search of (curv*) AND (pollinat*) AND (flower OR corolla OR *bird OR *bee OR moth OR *fly). The initial search returned over 300 studies that were then screened for those that measured flowers and/or animal mouthparts (e.g. bird bills, moth tongues). We sorted studies based on the criteria that 1) the study focused on pollination, including qualitative measures of curvature and 2) the study measured flower or animal (mouthpart) curvature for other reasons, but measurements must be quantitative. 44 pollination studies were found using some form of curvature metric (Table 1). An additional 11 publications discussing curvature, but not related to pollination are included in Table S1. There were numerous studies of plant-pollinator morphology that did not address curvature - these were omitted.

The first dedicated discussion of lateral curvature in plant-pollinator interactions begins with Hainsworth (1973, in reference to *Helicona* and Hermit hummingbirds). Curvature in pollination ecology is first empirically studied by Feinsinger (1978), though methods for measuring curvature of bird bills outside of a pollination context can be found much earlier (Baldwin et al., 1931). We identified six common approaches to measuring curvature. First, there are qualitative descriptions, e.g. "very curved", "less curved", but these are generally out of use. Second, the *arc:chord* method wherein curvature is a ratio of two lines: a straight line (chord) from tip to base (of the flower or bill) and a line that traverses a path along the arc of the flower/bill (Figure 1). Third, the *mandibular index* method which defines curvature as a ratio of two lines: a straight line from base to tip and a perpendicular line that measures the width of the flower/bill. This method is another form of the *arc:chord* method because for a given chord length, the length of the perpendicular line will be proportional to the arc length. Fourth, the *angle of deflection* method which considers curvature as the angle between the base of the flower/bill and its tip. This is another form of the *inverse radius* method which approximates the entire length of the flower/bill as a segment of a circle. These methods are interchangeable because the radius of a circle can be calculated from the length and angle of a line that passes through it (Bell, 1956; Temeles et al., 2009), see: Figure S1. Sixth, geometric morphometrics, which quantifies shape as a configuration of homologous points (landmarks) existing on a coordinate plane (Figure 2).

The strength of methods 2 and 3 are their portability and accessibility. These measurements can be taken in the field, or soon after from photographs. The methods are intuitive and in the simplest case, require only a ruler, string, and protractor. However, these methods have some conceptual flaws (discussed in Berns and Adams, 2010), principally that there are many shapes that could produce the same curvature value. For the *inverse radius* method, a curve is approximated with the segment of a circle. This method is insufficient for any flower and bill shapes that deviate from having constant curvature (*e.g.* nectar spurs of *Delphinium*). Similarly, the *angle of deflection* is not sensitive to local features along the length of the flower/bill - only the start and end points are considered in the calculation.

An additional problem is that terminology is inconsistent between authors. For example, the *arc:chord* method is also called the *maxillary index*, while the *angle of deflection* method is sometimes refered to as the *angle of declension* method. In the application of the *mandibular index* one study adjusted for bill length while a subsequent study did not (Table 1). Many studies create their own terminology for the concept of arc length: the length of a curve between two points. Most studies define their own terms for measuring curvature without reference to previous studies that have done the same. This creates uncertainty about how to compare and convert metrics used between studies. We believe these problems could be remedied by referring to the mathematical literature for the derivation and defintion of curvature and related concepts.

Starting in 2010, geometric morphometrics (GM) emerges in the pollination literature. GM comprises a set of protocols for quantifying and comparing shapes. This approach has steadily gained in popularity due to its mathematical rigour, reproducibility, and the appealing visual representations of shape comparisons (*e.g.* illustrations of geographic variation in flower shape Gómez et al. (2009)). We briefly outline the reasoning of a GM protocol to introduce relevant concepts, but recommend the concise and authoritative introduction by Webster and Sheets (2010). A GM protocol for a 2-D object begins by placing the specimens on an *xy* grid and assigning landmarks to locations on the specimen that are topologically or biologically homologous. A landmark is defined so that its location can be reproduced within and between samples. The set of landmarks representing the shape of an organism is a 'landmark configuration'. In a comparative study, the samples are overlayed so that their shape information is isolated from their orientation, location, and size. This is done using a least-squares type protocol, most commonly the Generalized Procrustes Analysis (GPA). GPA-adjusted landmark configurations hereafter exist in a multidimensional shape space defined by the number of landmarks and spatial dimensions implemented. Each landmark configuration contains unique information about the specimen's shape, and as such, occupies a unique position in the corresponding shape space. These

Figure 1: Figure 1. Overview of most commonly used curvature metrics. 1. arc:chord ratio. 2. mandibular index 3. inverse radius. 4. angle of deflection

configurations are then "projected" onto a simpler Euclidian space, similar to the reduction of a spherical Earth onto a two-dimensional map (Webster and Sheets, 2010). From here, familiar statistical procedures (*e.g.* PCA) can be performed to quantify variation in landmark configurations (shape) between samples.

This is giant leap forward for morphological studies because GM is a complete protocol for measuring, quantifying, and comparing shapes with high precision, as well as the covariation of these shapes with ecological variables of interest. Because GM has a traceable mathematical lineage (Bookstein, 1997), its vernacular is well-defined and used consistently between practitioners. The limitation of GM in quantifying curvature is that this method is concerned with analyzing configurations of landmarks, *i.e.* the entirety of a shape summarized as a set of xy coordinates. Once the specimen has been reduced to a landmark configuration, it exists as a point in shape space. Parsing segments of landmark configurations for separate analyses (*e.g.* for curvature) is not currently part of the geometric morphometrics toolkit. Therefore, studies that have used this technique to analyse biological forms are able to compare shapes in their entirety, but are ultimately limited to making descriptive statements about how segments of shapes appear to have different curvatures (*e.g.* Berns and Adams, 2013).

Comment on Macleod 2002: Geometric morphometrics and geological shape-classification systems

3. What is curvature?

Reviewing the literature leads us to ask, "what is curvature?". Within pollination ecology there are at least four metrics in use, with few references to their origins or the the meaning of the associated units. Therefore, we propose starting from first principles and turn to the field of geometry. There, we again find several definitions resulting from a history of independent derivations (reviewed in Coolidge, 1952; Bardini and Gianella, 2016). Nonetheless these definitions share a conceptual theme; curvature is a local property that can measured point-wise on a line. This concept is fundamentally different from those reviewed above where curvature is single property of an entire shape. Here we follow the conventions of Casey (1996) and Rutter (2000) and present a definition of curvature that is tractable for analyzing biological shapes.

Figure 2: Figure 2. Overview of a geometric morphometrics protocol. 1. Landmarks and semi-landmarks are assigned to a specimen. Each landmark is assigned an xy coordinate. 2. For each specimen a configuration of landmarks exists as a single point in a non-Euclidian shape space (abstracted here as a sphere segment). Red points represent landmark configurations from other specimens. 3. Shape data is projected onto a Euclidian plane – a tangent space approximation. This allows statistical analyses of shape variation (e.g. principal components analysis).