Biological challenges to conclusions from molecular phylogenies: behaviour strongly favours orb web monophyly, contradicting molecular analyses

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This first-ever extensive review of the construction behaviour of orb webs, of webs secondarily derived from orbs, and of non-orbs shows that the evidence favouring monophyly over convergent evolution of orbs is stronger than previously appreciated. The two major orb-weaving groups, Uloboridae and Araneoidea, share 31 construction behaviour traits, 20 of which are likely to be both derived and to have feasible alternatives, making convergence an unlikely explanation. Convergence in two lineages seems unlikely, and convergence in five different lineages, as proposed in some recent molecular studies of phylogeny, is even less credible. A further set of seven shared responses in orb design to experimentally constrained spaces also supports orb monophyly. Finally, a 'control' case of confirmed convergence on similar 'pseudo-orbs' in a taxonomically distant group also supports this argument, as it shows a low frequency of behavioural similarities. I argue that the omission of behavioural data from recent molecular studies of orb web evolution represents a failure of the analytic techniques, not the data, and increases the risk of making mistakes. In general, phylogenetic studies that aim to understand the evolution of particular phenotypes can benefit from including careful study of the phenotypes themselves.

ADDITIONAL KEYWORDS: Araneidae – Araneoidea – Deinopidae – constructing phylogenies – non-orb evolution – Uloboridae.

INTRODUCTION

Orb web construction is a classic example of complex, un-learned, stereotyped behaviour in a small animal with a relatively simple nervous system. The question of whether orb webs evolved only once or multiple times has long been controversial. Early analyses used morphological and behavioural traits to support different phylogenetic hypotheses (summarized in Coddington, 1986a), but recently emphasis has been on molecules, including target genes, whole genomes, transcribed gene regions, introns, ultraconserved elements (UCEs), transcriptomic data and fast/slow evolving loci (e.g. Bond *et al.*, 2014; Fernández *et al.*, 2014; Dimitrov *et al.*, 2016; Garrison *et al.*, 2016; Wheeler, 2017; Coddington *et al.*, 2019; Kallal *et al.*, 2020; Kulkarni *et al.*, 2021). Historically, most discussions of orb monophyly focused on whether orbs evolved once or twice, converging in Uloboridae (linked in some analyses with the derived, net-casting Deinopidae) and Araneoidea (seven different families). Some molecular studies, however, have indicated three to six (Fernández *et al.*, 2018) or, more recently, three to five independent derivations of orbs (Kallal *et al.*, 2020): uloborids; deinopids; and three groups of araneoids: Tetragnathidae; Araneidae + Nephilidae (or Nephilinae) + Theridiosomatidae; and Anapidae + Mysmenidae + Symphytognathidae. Here I focus mostly on the two-group convergence hypothesis (uloborids and araneoids). My arguments against a convergence between these two groups contend even more strongly against the likelihood of a higher number of convergences.

The typical procedure in molecule-based analyses has been to omit morphological and behavioural information while generating phylogenies, and only

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later map these traits onto hypothesized trees. One reason for omitting morphological and behavioural traits is that the numbers of molecular characters are so large that their effects swamp trends in other data, at least if characters are given equal or even approximately equal weights. An additional factor is that collecting morphological and behavioural data for a large number of species requires more time and expertise than does generating molecular data. Furthermore, in the absence of objective weighting techniques, morphological and behavioural data do not easily lend themselves to biologically meaningful quantifications that would allow their effects to be evaluated in combination with the molecular traits.

It is important to realize, however, that this limitation is due to practical problems, not to shortcomings in the morphological and behavioural data themselves. In other words, the omission of phenotypic data from recent phylogenetic analyses is due to inadequacies in the analytical techniques being used, not to irrelevance of the data. This failure of the analytical techniques can have important consequences, because (as will be documented below) behavioural data sometimes generate clear implications regarding evolutionary derivations. In short, the lack of a mechanism that would include these other traits in formal analyses does not remedy the fact that ignoring relevant behavioural and morphological data increases the risks of making mistakes in evolutionary analyses.

The question of weighting behavioural characters in phylogenetic analyses is not simple, however. One problem in analysing orb evolution is that defining an 'orb web' as a single 'trait' (for a possible definition, see Coddington, 1986a) belies the multiple behavioural traits that underlie an orb's construction; these include different motor programmes, the order in which these motor processes are executed, and the behavioural programmes responsible for both sensing internal and environmental stimuli and for translating them into behavioural adjustments. There is strong evidence that many of these aspects of orb web construction behaviour are 'modular': they have been semi-independently acquired (and sometimes lost) over evolutionary time (Eberhard, 2018, 2020a). In addition, they are elicited independently by parasitic wasps that manipulate the spider's behaviour (Eberhard, 2001; Takasuka, 2019). Convergence on the same sets of components in such an array of independent or semi-independent traits is clearly less likely than convergence on a single trait. An additional complication is that different components may have different histories, and some traits may be shared due to inheritance from common ancestors rather than convergence. This study is the first-ever major review of published descriptions of behaviour that examines the consequences for understanding orb web evolution when this multiplicity of components is taken into account.

The point of departure concerns differences in the intrinsic likelihood of convergences on particular behavioural traits, first laid out in the context of orb evolution by Coddington (1986a). If a behavioural trait is used to perform a particular job in the construction of an orb, and there are no other alternative feasible behavioural traits that could result in this job being performed satisfactorily, then the likelihood of convergence on this particular behaviour in different lineages is greater than if, in contrast, the particular job can be performed by other, alternative behavioural traits (Seilacher, 1973). Using Coddington's terms, the likelihood of convergence on a behavioural trait is greater when there are 'fabricational constraints'. Conversely, convergence is less likely in a trait in which alternative behaviour could also accomplish the same job. In terms of orb webs, sharing a derived trait is more convincing evidence for orb web monophyly if the trait is not fabricationally constrained—if alternative ways of accomplishing the same job in orb construction are feasible.

An example of a fabricational constraint is the order of the construction of non-sticky lines and a sticky spiral. Uloborid and araneoid orb-weavers both build non-sticky lines (radii, frames, hub) first, and then add sticky spiral lines to this support web. This order of operations is functionally constrained—it has no feasible alternative: an aerial spiral of sticky lines can only be built if there are already other lines present to which they can be attached. In addition, if spiral sticky lines were laid first, the spider would presumably have to walk on them and thus disturb their spacing while building subsequent non-sticky lines. Coddington (1986a: p. 142) expressed the general idea that convergence is less likely when a behavioural trait does not have such a fabricational constraint (see also Solano-Brenes *et al.*, 2021) as follows: '… if many possible ways to construct and attach silk lines exist, the probability of identical or closely similar behaviors should be small in any one instance, and minute for several independent instances combined.'

Determining whether fabricational constraints exist for some orb web construction traits is not always simple. For example, it is not intuitively clear whether it is feasible for a spider to lay a regularly spaced spiral of sticky silk without using the site of the previous loop of sticky line as a guide (as do both uloborids and many araneoids—trait #21 in Table 1). One can imagine alternative ways of accomplishing the job of uniform spacing: the spider might measure the distance it moves inward along a radius after attaching a sticky line to some reference point (for example, the temporary spiral) and then move outward the same distance along the next radius; or it might

Table 1. The relative feasibilities of alternatives to traits involved in orb construction shared by uloborid and araneoid orb-weavers are summarized: when alternatives are feasible, convergence is a less likely explanation for similarities. Behavioural traits that are shared by at least some uloborid and araneoid orb-weavers are listed in column a (based largely on Eberhard, 2020a; see footnotes and text). Alternatives to these traits that have been observed are in columns b-e. Column f gives the degree of confidence that alternatives to the shared traits in the first column are feasible (and thus that convergence is less likely): 'C' = alternative traits certainly feasible because they occur in modern species (orb- or non-orb weavers); 'P' = alternative traits probably feasible, according to theoretical arguments in the text; '?' feasibility of alternative traits is uncertain; 'FC' = fabricational constraints (alternatives are apparently not feasible). Column g gives the overall validity, as discussed in the text, of using the shared trait to support orb monophyly. The stages of orb construction cited in traits #1 and 12 are: (A) explore and build early radii; (B) construct radii and frame lines; (C) construct hub spiral; (D) construct temporary spiral; (E) construct sticky spiral; (F) add a stabilimentum; (G) remove the center of the hub (some species then fill it in); (H) build thin, supplementary radii after finishing the sticky spiral; (I) break and lengthen radii near the hub; and (J) remove and replace the entire hub. The letters in column G represent the following families: T = Titanoecidae (Szlep, 1966); P = Psechridae (Eberhard, 1987); D = Dictynidae (Eberhard, 2019, 2020b, 2021); F = Filistatidae (Eberhard, 2020a); Z = Zoropsidae (Eberhard et al., 1993). Additional references for shared traits are given in the text, and the complete species names and references for alternatives follow the footnotes. Where the primary literature is large and dispersed, and for publications in Japanese, I cited only secondary sources. All 'None' entries are subject to the proviso that only a small fraction of the many species has been observed for these traits. The 'allies' of *Poecilopachys* are *Pasilobus*, *Paraplectana* and *Cyrtarachne*; the 'ally' of *Cyrtophora* is *Mecynogea*; the 'allies' of *Trichonephila* are *Phonognatha* and the Nephilinae of Kuntner *et al.* (2008)

(a) Shared trait	(b) Alternative in Uloboridae	(c) Alternative in Araneidae + Nephilidae + Theridiosomatidae	(d) Alternative in Tetragnathidae	(e) Alternative in Anapidae + Mysmenidae + Symphytognathidae[1]	(f) Feasibility of alternative	(g) Trait significant support for monophyly?
STAGES						
(1) Stages are A, B, C, D, E	B and C combined early then C alone; all species lack G except one species with an orb-like cone below the orb	B and C mixed only near end of B in many araneids; D is absent in the araneids *Poecilopachys*, *Pasilobus*, and in some theridiosomatids	None	D is absent in anapids, in one symphytognathid, and in one but not another mysmenid. Three additional stages (H, I, J) occur in all three families (see text)	C	Yes
(2) Non-sticky spiral built from inside working outward	None	None	None	None	P	Yes
(3) Sticky lines built from outside working inward	None	None	None	None	P	No(?)
(4) Non-sticky spiral removed before web finished	None (though removal sometimes incomplete)	None except left intact in *Trichonephila* and allies; *Scoloderus*	None	–[2]	C	Yes
(5) Sticky lines do not zig-zag between non-sticky lines (instead form spiral)	None	None	None	None	P	Yes
(6) Sticky lines not laid along non-sticky lines (instead hang free)	Several exceptions occur on both radii and frame lines near edge of orb in several genera, and also in derived webs in *Hyptiotes* and *Polenecia*	None except *Eustala*	None	None except *Conoculus* and perhaps the anapid *Comaroma* and the mysmenid *Trogloneta*[3]	C	Yes

Table 1. Continued

(a) Shared trait	(b) Alternative in Uloboridae	(c) Alternative in Araneidae + Nephilidae + Theridiosomatidae	(d) Alternative in Tetragnathidae	(e) Alternative in Anapidae + Mysmenidae + Symphytognathidae[1]	(f) Feasiblity of alternative	(g) Trait significant support for monophyly?
(7) Sticky lines are never doubled or piled up	None	None except the araneid *Eustala*	None	None	C	Yes
(8) Frame lines form part of the orb	Frames lacking a few short radii in several genera	None except frames lacking in *Cyrtarachne*; parts of the orb of *Meta*	None except frames lacking in *Tetragnatha lauta* and *Tetragnatha* sp.	Frames lacking in some anapids	C	Yes
(9) Process of building a new frame includes construction of a new radius	None except occasionally in *Philoponella* and the derived webs of *Hyptiotes*	None except several types of exception in *Trichonephila*	None except when exit radius was removed while the spider moved out to the frame line in *Leucauge*	None	C	Yes
(10) During radius construction the spider returns to the hub along the new line just laid	None	None	None	None	P	Yes(?)
(11) Sticky lines never laid before any non-sticky lines present	None	None	None	None	FC	No(?)
ORDER OF STAGES						
(12) The order is consistently A-B-C-D-E-F-G	None (lack stage F)	None (some lack stage F), but one *Theridiosoma* removes, immediately rebuilds, and immediately destroys the hub	None	Stages H, I, J (similar to B and C) are repeated following E in all three families	C	Yes
(13) Removal of temporary spiral always occurs during sticky spiral construction	None	None	None	None	P(?)[4]	Yes
(14) Silk stabilimenta are always constructed after finishing the sticky spiral and the hub modification	None	None, but detritus stabilimenta that were built previously are often included in *Cyclosa* and *Allocyclosa*	No silk stabilimenta (detritus stabilimentum of *Dolichognatha* is reused in successive orbs)	No silk stabilimenta	C	No
UNIFORMITY OF STAGES						
(15) Temporary spiral and radius stages are not mixed	None	None except in *Cyrtophora* and allies, and *Trichonephila* and allies	None	None	C	Yes

Table 1. Continued

(a) Shared trait	(b) Alternative in Uloboridae	(c) Alternative in Araneidae + Nephilidae + Theridiosomatidae	(d) Alternative in Tetragnathidae	(e) Alternative in Anapidae + Mysmenidae + Symphytognathidae[1]	(f) Feasiblity of alternative	(g) Trait significant support for monophyly?
(16) Sticky spiral and radius stages are not mixed	None	None	None	None	C	Yes
(17) Radii neither reinforced nor discarded after being constructed	None (but both occur in Deinopidae)	None	None	None	C	Yes
(18) Temporary spiral construction continuous without interruptions	None except *Hyptiotes*	None	None	-[2]	C	Yes
(19) No repeating of combination of non-sticky and sticky spiral	None	None except in the modified orbs of *Wendilgarda*	None	None	C	Yes
(20) Hub (intersection of radii) is formed before any frames are built	None except in modified orbs of *Hyptiotes*	None (no frame lines in *Oglunius*)	None	(no data)	C	Yes
CUES GUIDING STICKY SPIRAL CONSTRUCTION						
(21) Site of inner loop guides sticky spiral placement	None except in the cone built below the orb in one *Uloborus*	None except no contact with inner loop in some theridiosomatids and in the araneids *Poecilopachys, Cyrtarachne* and (probably) *Pasilobus*	None	None except no contact in the mysmenid *Mysmena* and the anapid *Conoculus* in the orb plane; also no contact with the inner loop on radii above the orb plane in some anapids	C	Yes
(22) Distance from outer loop of temporary spiral guides sticky spiral placement	None[1]	None[1]	None[1]	(no data)	P	Yes
(23) Tensions on radii do not affect sticky spiral spacing	None[1]	None[1]	None[1]	(no data)	P	Yes
(24) Amount of silk in silk glands affects sticky spiral spacing	None[1]	None[1]	None[1]	(no data)	P[5]	No(?)
(25) Hub or outer loop of temporary spiral directly guides placement of next loop of temporary spiral[6]	None except *Hyptiotes* lost contact with the outer loop	None except *Theridiosoma* lost contact with the hub while building the circular loop of temporary spiral	None[7]	-[2]	C	Yes

Table 1. Continued

(a) Shared trait	(b) Alternative in Uloboridae	(c) Alternative in Araneidae + Nephilidae + Theridiosomatidae	(d) Alternative in Tetragnathidae	(e) Alternative in Anapidae + Mysmenidae + Symphytognathidae[1]	(f) Feasibility of alternative	(g) Trait significant support for monophyly?
DETAILS OF MOVEMENTS						
(26) Spider always lays line when moving, except just before and just after end of sticky spiral[8]	None	None except in *Poecilopachys* and allies	None	None	C?[9]	No
(27) Legs hold the line to which the spider is attaching a line[8]	None	None	None	None	FC(?)[10]	No(?)
(28) Spider walks only along non-sticky lines while building the web	None	None	None	None except *Conoculus*, which climbs sticky lines from water attachment	C(?)[11]	No
(29) Temporary spiral is attached once to each radius it crosses	None	None except *Trichonephila* and allies	None	None[2]	C?[10]	No
(30) Break and reel non-sticky lines at some stage during construction	None	None	None	None	P	?
(31) The spider holds non-sticky draglines with leg IV as it moves	None	None	None	None	P	Yes(?)

[1]Data are available for only one or two species/family.
[2]No temporary spiral present in species whose behaviour has been observed.
[3]Homologies of this web with orbs are not certain (Lopardo *et al.*, 2011).
[4]Removal per se supports monophyly although its timing may be a fabricational constraint—see text.
[5]Modulating construction behaviour on the basis of available silk is selectively advantageous, so convergence is less unlikely (see text).
[6]'Direct' guidance implies that either the spider does not lose contact with the guiding line; 'indirect' guidance would be if the spider only used the guiding line as a reference point, for instance moving a predetermined distance from the guiding line.
[7]No experimental evidence. Spider did not maintain contact during temporary spiral construction.
[8]Contrast with the related group Oecobiidae (Solano-Brenes *et al.*, 2021).
[9]Spiders in many other groups routinely produce draglines wherever they go, so the trait shared by uloborid and araneoid orb-weavers is probably a symplesiomorphy.
[10]Probably plesiomorphic (see text).
[11]May be plesiomorphic (see text).
References (in addition to Eberhard, 2020a) are the following (numbering refers to the row numbers in the table):
(1) Theridiosomatidae (Eberhard, 1982, 2020a, Coddington, 1986c); Anapidae and allies (Eberhard, 1987a, Shinkai & Shinkai, 1988, Hiramatsu & Shinkai, 1993, Lopardo *et al.*, 2011; *Poecilopachys australasia* (Griffith & Pidgeon, 1833) (Clyne, 1973); *Pasilobus* sp. (Robinson & Robinson, 1975).
(4) Uloborids (Eberhard & Opell, in press); *Trichonephila* spp. (Wiehle, 1931, Shinkai, 1982, Hesselberg & Vollrath, 2012); *Scoloderus tuberculifer* (O. Pickard-Cambridge, 1889) (Eberhard, 1975).
(6) *Hyptiotes* spp. (Marples & Marples, 1937, Eberhard & Opell, in press); *Polenecia producta* (Peters, 1995); *Eustala* sp. (Eberhard, 1985); *Comaroma simoni* Bertkau, 1889 (Kropf, 1990); *Conculus lyugadinus* Kishida, 1940 (Shinkai & Shinkai, 1988); *Trogloneta granulum* (Hajer, 2000, Hajer & Řeháková, 2003).
(7) *Eustala* sp. (Eberhard, 1985).

Table 1. Continued

(8) *Cyrtarachne* sp. (W. Eberhard, unpub. data); *Meta menardi, M. bourneti* (Hesselberg *et al.*, 2019); *Tetragnatha* sp. (Eberhard, 2020a); anapids (Eberhard, 1987a, Kropf, 1990).
(9) *Philoponella vicina* (O. Pickard-Canbridge, 1899) (Eberhard, 1990); *Hyptiotes cavatus* (W. Eberhard, unpub. data); *Trichonephila clavipes* and *Leucauge mariana* (Eberhard, 1990).
(9) Uloborids (Eberhard & Opell, in press); *Trichonephila clavipes* (Eberhard, 1990); *Leucauge mariana* (Eberhard, 1990).
(12) Uloborids (Eberhard & Opell, in press); *Theridiosoma epeiroides* Bösenberg & Strand, 1906 (Shinkai & Shinkai, 1985); anapids and allies (Eberhard, 1987a; Lopardo *et al.*, 2011).
(14) *Cyclosa* spp. (Rovner, 1976. W. Eberhard, unpub. data); *Allocyclosa bifurca* (McCook, 1887) (W. Eberhard, unpub. data); *Dolichognatha* sp. (W. Eberhard, unpub. data).
(15) *Cyrtophora citricola* (Kullmann, 1958); *Trichonephila* and allies (Wiehle, 1931, Shinkai, 1982, Kuntner *et al.*, 2008, Hesselberg & Vollrath, 2012).
(17) *Poecilopachys australasia* (Clyne, 1973); *Pasilobus* sp. (Robinson & Robinson, 1975); *Paraplectana* references in Stowe (1986); *Cyrtarachne* sp. (W. Eberhard, unpub. data); *Eustala* sp. (Eberhard, 1985).
(18) *Hyptiotes paradoxus* (C.L.Koch, 1834) (Marples & Marples, 1937); *H. cavatus* (W. Eberhard, unpub. data).
(19) *Wendilgarda* spp. (Eberhard, 1989, 2000).
(20) *Hyptiotes cavatus* (W. Eberhard, unpub. data).
(21) *Uloborus conus* (Lubin, 1986); theridiosomatids (Eberhard, 1982, Shinkai & Shinkai, 1985); *Poecilopachys australasia* (Clyne, 1973); *Cyrtarachne* sp. (Eberhard, 1982); *Pasilobus* sp. (Robinson & Robinson, 1975) (deduced from spaces being much larger than size of spider); *Conculus lyugadinus* (Shinkai & Shinkai, 1988); anapids and relatives (Eberhard, 1987a, Lopardo *et al.*, 2011).
(24) *Zosis geniculata* (Eberhard & Barrantes, 2015); *Leucauge mariana* and *Micrathena sexspinosa* (Eberhard, 1990).
(25) *Hyptiotes cavatus* (Eberhard & Opell, in press); *Theridiosoma gemmosum* (L. Koch, 1877) (W. Eberhard, unpub. data).
(26) *Poecilopachys australasia* (Clyne 1973, see summary in Stowe, 1986).
(27) (Eberhard, 1982).
(28) *Conoculus lyugadenis* (Shinkai & Shinkai, 1988).
(29) *Trichonephila* spp. (Wiehle, 1931, Shinkai, 1982, Kuntner *et al.*, 2008, Hesselberg & Vollarth, 2012).

sense the presence of the previous loop via vibrations of the radius. It is not obvious, however, whether a spider would be capable of such measurements. In addition, some of the alternative traits mentioned in Coddington's discussion of fabricational constraints are not as feasible as he supposed (Eberhard, 1990).

Doubts as to the feasibility of alternatives can sometimes be eliminated, however, when comparative studies of behaviour show that species in other lineages possess alternative traits to accomplish the same functions. For instance, some orb-weavers and secondarily derived non-orb-weavers guide sticky spiral attachments on the basis of measurements of distances moved along the radius (see summary in Eberhard, 2020a), demonstrating that this is a feasible alternative. The existence of alternate behavioural traits demonstrates their feasibility in several respects: it is mechanically possible to build an orb this way ('mechanical feasibility'); it can be selectively advantageous for a spider to build an orb this way ('selective feasibility'); and a spider's nervous system is capable of executing the tasks required to perform construction in this way ('neurological feasibility').

Orb construction behaviour is special in two ways that favour these analyses. In the first place, behavioural data are relatively abundant. There are descriptions of at least some details of the construction of orbs and related webs for between 150 and 200 different species, and they have been summarized recently (Eberhard, 2020a). Secondly, a number of secondarily modified web designs derived from orbs exist, and the construction behaviour has been described for many of these derived web forms (summaries in Eberhard, 2018, 2020a). These derived webs are especially useful in studies of behavioural phylogeny, because the species are, on the basis of morphological traits, undisputed members of groups that build typical orb webs. Thus the differences in their behaviour are clearly secondarily derived from ancestral orb construction behaviour; and the details of this ancestral behaviour can be deduced, because many details of orb construction are uniform at higher taxonomic levels (Eberhard, 1982, 2018, 2020a; Kuntner *et al.*, 2008). Additional information on trait polarities is also available from a recent summary of observations of non-orb weaving species (Eberhard, 2020a), facilitating checking for possible plesiomorphies using out-group comparisons.

Finally, there is a 'control' group, the psechrid genus *Fecenia*, that builds 'pseudo-orbs' that are widely accepted to represent a convergence on the typical orb design (see summary in Eberhard, 2020a). Although many behavioural details are still unknown, web designs permit determination of several shared and non-shared aspects of construction.

This paper assembles for the first time the many different shared web construction behaviour traits of uloborid and araneoid orb-weavers, evaluates their usefulness as evidence favouring orb web monophyly, and then discusses their implications for building phylogenetic trees and mapping behaviour onto these trees.

MATERIAL AND METHODS

I compiled observations of the construction behaviour in orb-weavers and groups that have secondarily modified orbs in order to test the hypothesis that the traits shared between uloborid and araneoid orb-weavers are the results of fabricational constraints in order to test the validity of using these similarities to support the orb web monophyly hypothesis. I included only construction from scratch, even though the evolutionary origins of orbs may have also included behaviour to repair or add to existing webs. I also omitted take-down behaviour (Carico, 1986; Eberhard, 2020a) due to lack of data. I discussed the mechanical feasibility of alternatives in cases in which no alternatives are known. Those cases in which similarity is not due to fabricational constraints or to symplesiomorphies were judged to constitute stronger evidence of monophyly. I present the data separately in Table 1 for all five groups (except Deinopidae) that

Kallal *et al.* (2020) mentioned as possibly representing independent derivations of orbs, but focus on possible convergence between uloborids and araneoids (in effect, I compared the uloborid column b with the sum of the three columns c-e). Although there are good reasons to consider deinopid webs as derived from orbs (Coddington, 1986b), I omitted deinopids from this analysis because of uncertainties regarding the homologies of some traits; resolution of the deinopid question is not crucial to resolution of the single vs. double or multiple derivation of orb webs.

RESULTS

BEHAVIOUR TRAITS SHARED BY ULOBORID AND ARANEOID ORB-WEAVERS AND POSSIBLE ALTERNATIVES

Table 1 lists web construction behaviour traits shared by uloborid and araneoid orb-weavers. These traits are described and discussed below (numbered as in the table) to explain their classification regarding their degree of support for the monophyly hypothesis (column g of Table 1). Figure 1 illustrates several of the web structures involved.

1. *The same stages of construction occur in both uloborid and araneoid orb-weavers*. The shared stages (see summary in Eberhard, 2020a) are: (A) the spider explores, building a few early radii that meet at or
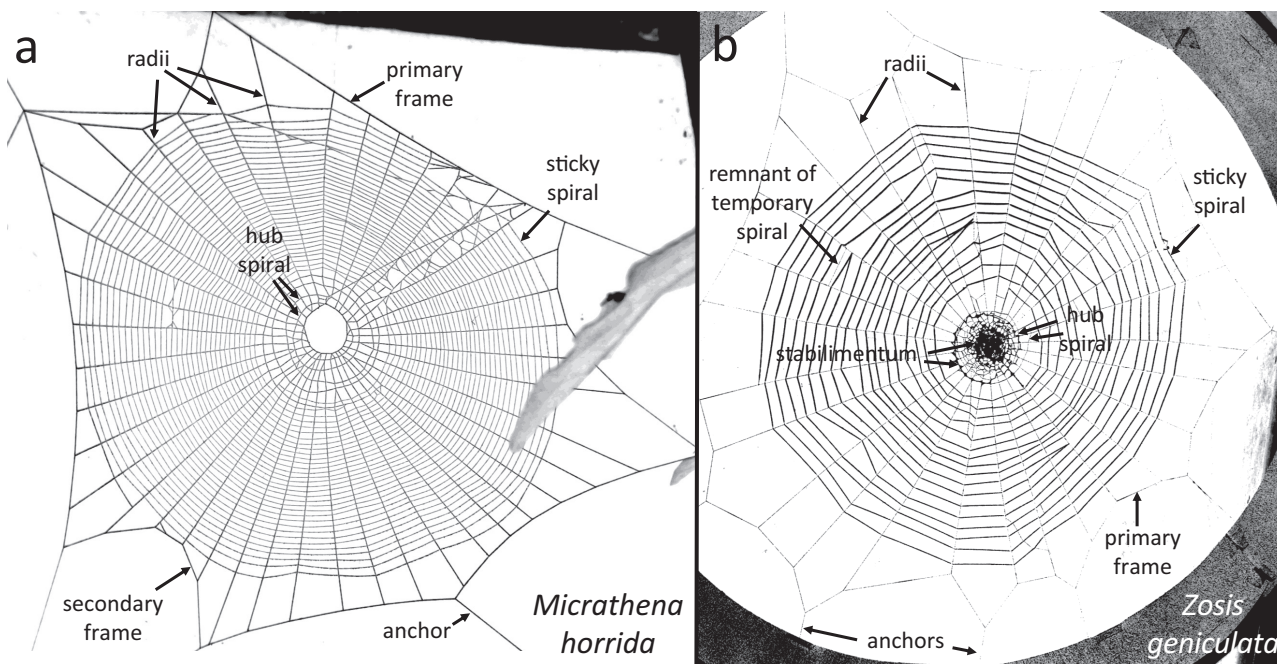

Figure 1. Representative orb webs of the araneid *Micrathena horrida* (Taczanowski, 1873) in the field (a), and the uloborid, *Zosis geniculata* (Olivier, 1789) in captivity (b), with corresponding lines labelled. The spider failed to remove the segment of temporary spiral in (b) when building the sticky spiral.

near the future hub; (B) additional radii are built, both during the process of constructing frames and after the frames are completed; (C) a hub spiral is built during and following radius construction, working outward as the spider circles around the centre; (D) a widely spaced non-sticky temporary spiral is attached to each radius as the spider spirals away from the hub; (E) the spider attaches sticky lines to the radii in an approximate spiral, moving from the outer edge of the web toward the hub; (F) a dense mat of white silk (the stabilimentum) is added to radii or other web lines (homologies for silk stabilimenta are uncertain, however—see #14). The temporary spiral is absent in anapids and in one mysmenid, but present in another mysmenid and a symphytognathid (Eberhard, 1987a; Shinkai & Shinkai, 1988). It is secondarily reduced in some theridiosomatids (Coddington, 1986c), and absent in others (*Ogulnius*—W. Eberhard, unpub. data) and in some webs of the uloborid *Polenecia* (Peters, 1995). One late stage in araneoids does not occur in uloborids: (G) the central portion of the hub is removed and then (in many species) filled in. Three further stages not shared with Uloboridae also occur following completion of the sticky spiral in the araneoid families Anapidae, Symphytognathidae and Mysmenidae: (H) further, thinner supplementary radii; (I) break and extend (loosen) the primary radii (some species also break the supplementary radii); and (J) build a new hub (Eberhard, 1987a; Shinkai & Shinkai, 1988; Hiramatsu & Shinkai, 1993; Lopardo *et al.*, 2011).

The variety of alternative traits implies that the shared traits A–E constitute significant evidence favouring orb monophyly.

2. *A non-sticky spiral is built from the inside working outward.* No exceptions are known in orb weavers, but the reverse direction of work is physically feasible. In a radially organized web, the spider could use frame lines as the outermost original bridges between radii and subsequent loops of non-sticky spiral as later bridges. The circular rather than spiral non-sticky lines in some theridiosomatid orbs represent another alternative (see summary in Eberhard, 2020a). The feasibility of alternative traits implies that the shared traits in uloborid and araneoid orb-weavers constitute significant evidence favouring orb monophyly.

3. *A sticky spiral is built from the outside working inward.* No exceptions are known, although adding lines in the reverse direction is surely physically feasible, as illustrated in the highly ordered 'rectangular orb' of the synotaxid *Synotaxus* in which sticky lines are built working outward from near the spider's retreat (Eberhard, 1977;

Eberhard *et al.*, 2008). Several non-orb cribellate groups add sticky lines starting at the edge and working inward, however (Eberhard, 1987b, 2020a; Solano-Brenes *et al.*, 2021), giving a tentative indication that the uloborid-araneoid similarity may be a symplesiomorphy and thus that it is not clear evidence favouring monophyly.

4. *The non-sticky temporary spiral is removed before web finished.* Typically, both uloborid and araneoid orb-weavers (and also a deinopid—Coddington, 1986b) break the segments of temporary spirals during sticky spiral construction. Orbs with an intact temporary spiral are feasible, however. The temporary spiral lines are left intact in two distantly related groups of araneoids, *Trichonephila* (= *Nephila*) and its close relatives (Kuntner *et al.*, 2008), and *Scoloderus* (Eberhard, 1975; Stowe, 1986). Removal of non-sticky lines while laying sticky lines is unknown in non-orb spiders (Eberhard, 2020a), so temporary spiral removal is likely a derived trait of orb-weavers. Its shared presence in uloborid and araneoid orb-weavers thus constitutes significant evidence favouring orb monophyly.

5. *With few exceptions*, *all sticky lines form a spiral*, *rather than zig-zagging between radii.* Many other alternative patterns are feasible. Zig-zag patterns of sticky lines between pairs of non-sticky lines, between a non-sticky line and the substrate, and across a sheet of non-sticky lines are widespread in non-orb cribellates, including Dictynidae, Austrochilidae, Psechridae, Eresidae, Stiphidiidae, Desidae and Phyxelidae, and in the araneoid *Synotaxus* (see summaries in Eberhard, 2020a, 2021a). Another alternative, arcs of sticky lines, occurs in Titanoecidae, Dictynidae and Psechridae (Szlep, 1966; Eberhard, 1987, 2019; see summary in Eberhard, 2020a). The shared uniform spiral pattern is thus significant evidence favouring orb monophyly.

6. *Sticky lines are not laid along non-sticky lines*, *and often hang free.* Alternatives are feasible, as sticky lines laid along non-sticky lines occur in the outer loops of several species of orb-weaving uloborids (Eberhard & Opell, in press), in the derived webs of the uloborids *Polenecia producta* (Simon, 1873) (Peters, 1995) and *Hyptiotes* (Marples & Marples, 1937; Eberhard & Opell, in press), and in the araneid genera *Eustala*, *Paraplectana* and *Cyrtarachne* (Eberhard, 1985; Stowe, 1986). Sticky lines laid along non-sticky lines are widespread in non-orb cribellate families, including Gradungulidae, Filistatidae, Psechridae, Desidae, Eresidae, Titanoecidae and Dictynidae (Eberhard, 1987b, 2019, 2020a, b, 2021a; Ramírez & Michalik, 2019). The

consistently free-hanging sticky lines of uloborid and araneoid orb-weavers are thus significant evidence favouring orb monophyly.

7. *Sticky lines are never doubled or accumulated in masses on radii.* Doubled sticky lines are mechanically feasible, and occur at some sites in the modified orbs of *Eustala* sp. (Eberhard, 1985). An alternative trait, piling up loose accumulations of sticky silk on non-sticky lines, occurs in some non-orb cribellates, including some dictynids (Eberhard, 2020b, 2021a), the gradungulid *Progradungula otwayensis* Milledge, 1997 (Ramírez & Michalik, 2019) and the filistatid *Kukulcania hibernalis* (Hentz, 1842) (Griswold *et al.*, 2005; Eberhard, 2020a). This shared absence in uloborid and araneoid orb-weavers thus constitutes significant evidence in favour of orb monophyly.

8. *Frame lines support radii at the web's edge, and are attached to other frame lines or to anchor lines that are attached to the substrate.* It is feasible to build orbs lacking frame lines, as they are secondarily absent in webs with very low numbers of radii in the araneid *Cyrtarachne bufo* (Bösenberg & Strand, 1908) (Suginaga, 1963 in Stowe, 1986), the tetragnathids *Tetragnatha lauta* Yaginuma, 1959 (Shinkai, 1988) and *Tetragnatha* sp. (Eberhard, 2020a), and the orbs or portions of orbs built in smaller spaces in some anapids (Shinkai & Shinkai, 1988; Eberhard, 1987a, 2020a), *Meta menardi* (Latreille, 1804) and *Meta bourneti* Simon, 1922 (Hesselberg *et al.*, 2019). Frames were also omitted by a uloborid and a tetragnathid when spiders were obliged to build in especially small spaces (Table 2) (Eberhard & Barrantes, 2015). This similarity between uloborid and araneoid orb-weavers is thus not due to a fabricational constraint, and the shared presence of frames in uloborid and araneoid orb-weavers constitutes significant evidence favouring orb monophyly.

9. *The process of building a new frame line includes adding a new radius.* This similarity was first mentioned by Coddington (1986b). It is

Table 2. The strong similarity between the uloborid *Zosis geniculata* and the araneoid *Leucauge argyra* in changes in behaviour when they built orbs in very constrained spaces (after Barrantes & Eberhard, 2012 and Eberhard & Barrantes, 2015.). Those changes that can be attributed to simple physical limitations imposed by smaller spaces, and that thus cannot be confidently considered to be due to decisions by the spiders, are marked with '*'. The seven variables that, according to the conservative criteria of Barrantes & Eberhard (2012), are likely to reflect independent decisions by the spiders are preceded by letters A-G. Other variables whose cause-and-effect relations with respect to cues and responses that Barrantes & Eberhard (2012) thought may be more complex are not labelled. See the text for further discussion

		Z. geniculata	*L. argyra*
Radii, frames, anchor lines			
A	Number of frame lines	Smaller	Smaller
A	Proportion of radii attached directly to the substrate	Greater	Greater
A	Proportion of frame lines supporting only a single radius	Greater	Greater
A	Number of radii/frame lines	Smaller	Smaller
A	Proportion of radii that end on 'V' frame	Greater	Greater
B	Number of radii	Smaller	Smaller
*	Length of radii	Smaller	Smaller
Relative areas			
*	Capture area	Smaller	Smaller
C	Hub area	Smaller	Smaller
D	Symmetry of web form	?	Greater
E	Area of free zone	Smaller?	Smaller
	Free zone area/total area	Greater	Greater
	Hub area/total area	Greater	Greater
Hub			
C	Number of loops in hub spiral	?	No change
C	Space between hub loops	?	
Sticky spiral			
F	Space between adjacent loops of sticky spiral on longest radius	Smaller	Smaller
	Consistency of sticky spiral spacing on longest radius	No change	No change
G	Distance from outer loop of sticky spiral to end of radius	Smaller	Smaller
	Number of loops of sticky spiral	Smaller	Smaller
E	Distance from outer loop of hub to inner loop of sticky spiral (free zone)	Smaller	Smaller

geometrically feasible to build a new frame line without building a new radius, for instance by attaching a line near the outer end of a radius, walking to the hub and to the outer end of an adjacent radius and attaching this new line there, then returning to the hub along either the second or the first radius (see Eberhard, 1990: fig. 26). Minor variants occur occasionally in the uloborid *Philoponella*, and in the modified orb of the uloborid *Hyptiotes*. This shared trait in uloborid and araneoid orb-weavers constitutes significant evidence favouring orb monophyly.

10. *During radius construction the spider returns to the hub along the newly laid radius, rather than along the exit radius or some other radius.* I know of no exceptions to this pattern, but it is easy to imagine reasonable alternatives. For instance, the spider could simply retrace the path it had followed in laying the new radius (the distance between the attachments of the new radius and the exit radius to the frame is typically short, less than one body length). Although returning more directly to the hub could result in a slight conservation of energy, this saving would be relatively small. Thus, this shared trait in uloborid and araneoid orb-weavers constitutes tentative evidence favouring orb monophyly.

11. *Sticky spiral lines are never laid before any non-sticky lines are present.* As discussed above, this similarity is probably due to a fabricational constraint: when an aerial sticky line is laid in a spiral, it cannot be built unless a supporting array of other lines is already present (some other patterns of sticky lines would not depend on non-sticky lines being present, however). At least if the sticky lines are in a spiral and are supported by non-sticky lines, this shared trait does not constitute significant evidence favouring orb monophyly.

12. *There is uniformity in the order in which the stages are performed.* Uloborid and araneoid orb-weavers typically perform stages A-G in the same order. Uloborid and araneoid orb-weavers never return to making a spiral of any sort once they have started to build the stabilimentum. Exploration is never resumed after radius and frame construction has begun, or after any subsequent stage, though it would clearly be feasible. Uloborids and most araneoids never return to radius or frame construction after sticky spiral construction has begun. Nevertheless, alternative orders are clearly possible. Radii are built after the sticky spiral is finished in mysmenids and symphytognathids (Eberhard, 1990; Lopardo *et al.*, 2011). The entire hub is removed and then rebuilt after the sticky spiral is finished in anapids and their allies (Eberhard, 1987a, 2020a). In non-orb-weaving

cribellates (and non-orb spiders in general), the sequences of construction stages are also variable (Eberhard, 2020a) The shared uniform order of stages in uloborid and araneoid orb-weavers thus constitutes significant evidence favouring orb monophyly. Conservatively I have counted this uniformity as a single shared trait; I recognize, however, some transitions as separate traits (#15, 16, 19, 20 below) because clear examples of alternatives are known.

13. *Removal of the temporary spiral always occurs as a part of sticky spiral construction, and never occurs separately either before or after.* There are no known exceptions to this pattern in orb weavers, nor to the consistent lack in non-orb-weavers of removal of newly built lines during web construction. Nevertheless, the pattern of removal may result from fabricational constraints. One of the likely functions of the temporary spiral is to guide sticky spiral construction (Zschokke, 1993; Eberhard, 2020a), so destruction prior to building the sticky spiral would be selectively disadvantageous. In addition, destruction following sticky spiral construction would probably be difficult to accomplish without substantially disturbing the pattern of uniform spacing of the sticky spiral. In sum, removal itself constitutes significant evidence favouring orb monophyly (counted as trait #4), but its timing does not.

14. *Silk stabilimenta are always constructed after sticky spiral and hub modification are complete* (Herberstein *et al.*, 2000). It is clearly feasible for stabilimenta to be built any time after the radii or frame lines on which they are placed have been built. Indeed, the detritus stabilimenta of araneids such as *Cyclosa* and *Allocyclosa* are often present on a line (usually on a radius) from a previous orb before the orb is even initiated (Rovner, 1976; Eberhard, 2020a, W. Eberhard, unpub. data). Nevertheless, stabilimenta have evolved convergently in several lines of Araneidae, perhaps after the origin of orbs (Herberstein *et al.*, 2000; Blackledge *et al.*, 2011), and it is not clear whether any of them are homologous with uloborid silk stabilimenta. In sum, shared production and timing of silk stabilimenta do not give convincing support for orb monophyly.

15. *Construction of radii and the temporary spiral is not mixed.* There is no obvious mechanical need for this consistency, and such mixing occurs secondarily in the araneoids *Cyrtophora citricola* (Forsskål, 1775) and *Trichonephila* (and its allies), which continue to build radii during temporary spiral construction (Kullmann, 1958; Kuntner *et al.*, 2008; see summary in Eberhard, 2020a). This shared trait in uloborid and araneoid orb-weavers

constitutes significant evidence favouring orb monophyly.

16. *Once sticky spiral construction has begun, it is never interrupted to build further radii*. There is no obvious mechanical need for this consistency, and the feasibility of alternative sequences is illustrated in the secondarily derived webs of the theridiosomatid *Wendilgarda*, which builds its webs in sections that include both non-sticky radii and sticky lines, and by the supplemental radii of anapids and allies that are added following completion of the sticky spiral (Eberhard, 1987a; Lopardo *et al.*, 2011). In non-orb-weavers, mixing construction of sticky and non-sticky lines is so widespread that it is probably the norm rather than the exception (see summary in Eberhard, 2020a, 2021a). In sum, this shared trait in uloborid and araneoid orb-weavers constitutes significant evidence favouring orb monophyly.

17. *New radii are neither reinforced nor cut and discarded*. No exceptions are known in uloborids or araneoids. Nevertheless, both modifications are feasible and have been observed in the derived cribellate deinopid *Deinopus* sp., which consistently reinforces the central radius by adding lines to its central portion, and also cuts the midline radius below the hub before initiating the temporary spiral (Coddington, 1986b). These two traits, conservatively lumped here as one, constitute significant evidence favouring orb monophyly.

18. *Temporary spiral construction is continuous, and not interrupted by returning to the hub*. Pauses to return to the hub during building are mechanically feasible, and occur routinely in the derived orb weaving uloborid *Hyptiotes*, which returned to the hub after building each loop of temporary spiral [Marples & Marples, 1937; W. Eberhard, unpub. data on *H. cavatus* (Hentz, 1847)]. Interruptions to return to the retreat or to a central point in the web are widespread in non-orb cribellate species (Eberhard, 2020a). The shared lack of interruptions in uloborid and araneoid orb-weavers thus constitutes significant evidence favouring orb monophyly.

19. *Spiders do not build the orb in repeated 'sticky spiral + non-sticky line' sections*. There is no obvious mechanical reason for this 'all-at-once' pattern in construction. Both the theridiosomatid *Wendilgarda* and the non-orb 'rectangular orb' araneoid *Synotaxus*, and the 'pseudo-orb' cribellate *Fecenia*, build a series of sections, each of which contains both sticky and non-sticky lines (Eberhard, 1977, 1989; Zschokke & Vollrath, 1995). Thus, this pattern in uloborid and araneoid

orb-weavers is not necessary in order to produce webs that are very highly ordered geometrically. In sum, this similarity between uloborid and araneoid orb weavers favours the orb monophyly hypothesis.

20. *No frame lines are built prior to the preliminary formation of the hub where the radial lines intersect*. This sequence does not seem mechanically necessary, and an alternative has been observed: the hub of the modified orb of *Hyptiotes* was not formed until after the first frame line was built (W. Eberhard, unpub. data on *H. cavatus*). This shared trait in uloborid and araneoid orb-weavers thus constitutes significant evidence favouring orb monophyly.

21. *The site of the inner loop of the sticky spiral guides subsequent sticky spiral placement*. Use of the site where the previous inner loop of sticky spiral is attached to the radius as a cue to guide sticky spiral placement has been demonstrated in both uloborids and araneoids on the basis of exploratory movements of certain legs that immediately precede each attachment of the sticky spiral (see summaries in Eberhard, 1982, 2020a), and the finding that experimental removal of lines causes displacement of subsequent lines in both groups (see summary in Eberhard, 2020a). The legs used and the orientation of the spider's body vary in different araneoids, but intra-specific variation in some, such as the araneid *Micrathena duodecimspinosa* (O. Pickard-Cambridge, 1890) (Eberhard, 2020a), suggests they may be taken as a single trait. The use of other cues (probably distances moved toward and away from the temporary spiral or the hub) is feasible, as confirmed by observations of building behaviour in the araneid *Poecilopachys australasia* (Clyne, 1973), several theridiosomatids, the anapid *Anapisona simoni* Gertsch, 1941 and the mysmenid *Mysmena* sp. (see summary in Eberhard, 2020a). Thus, this shared cue constitutes significant evidence favouring orb monophyly.

22. *The distance from the outer loop of the temporary spiral to the inner loop of the sticky spiral guides sticky spiral spacing*. Experimental manipulations of webs during sticky spiral construction in araneoids (Hingston, 1920; Eberhard, 2020a), and responses in unmanipulated webs to changes in the distance between the outer loop of the temporary spiral and the inner loop of the sticky spiral in araneoids and the uloborid *Zosis geniculata* (Olivier, 1789) (Eberhard & Hesselberg, 2012; Eberhard & Barrantes, 2015) show that sticky spiral spacing decreases when this distance decreases. Given the use of the site of the inner

loop to guide sticky spiral placement (#21), the shared use of this somewhat redundant second cue constitutes significance favouring orb monophyly.

23. *Tension-related cues do not influence sticky spiral placement.* Experimental manipulation of radial tensions demonstrated that sticky spiral spacing was not altered in the uloborid *Zosis geniculata* and in the araneoids *Micrathena duodecimspinosa* (Araneidae) and *Leucauge mariana* (Taczanowski, 1881) (Tetragnathidae) (Eberhard & Hesselberg, 2012; Eberhard & Barrantes, 2015). These results indicate that neither tension, nor several tension-related cues such as resonant vibrations or radius extensibility guide sticky spiral spacing behaviour. This similarity between uloborids and araneoids is striking in light of the fact that spiders in both groups sense and respond to tension differences in other contexts, such as web repair (Tew *et al.*, 2015; Eberhard, 2021b; W. Eberhard, unpub. data on *Uloborus diversus* Marx, 1898) and that several variables related to radius tension could provide useful information to guide sticky spiral placement (see summary in Eberhard, 2020a). The shared lack of use of tension-related cues thus constitutes significant evidence favouring orb monophyly.

24. *The amount of sticky spiral silk in the spider's glands influences web design.* Experimental manipulation of the amount of sticky spiral silk (aggregate and flagelliform silk) in the silk glands of two araneoids showed that when the spider had more silk available, it placed the sticky spiral loops closer together and increased the size of the orb (Eberhard, 1988). A similar effect on sticky spiral spacing occurred in natural experiments with the uloborid *Z. geniculata* (Eberhard & Barrantes, 2015). Modulation of web size and design on the basis of silk reserves is probably selectively advantageous and may be an ancient trait that was present in non-orb ancestors (though no experimental data are available). So, conservatively, this shared trait in uloborid and araneoid orb-weavers does not constitute reliable evidence favouring orb monophyly (nevertheless, it would seem that modulating the specific design trait of the distance between sticky spiral lines is less likely to be ancestral for orb-weavers).

25. *During temporary spiral construction, the spider is guided by maintaining contact with the previous, outer loop.* The use of this cue is suggested by experiments with a tetragnathid and several araneids (Eberhard, 1987c, 2020a), along with observations of other species that never lose contact with the previous loop during temporary spiral construction. It is feasible not to use this cue. The uloborid *Hyptiotes* lost contact with the previous loop during temporary spiral construction (Marples & Marples, 1937; Eberhard & Opell, in press). Similar loss of contact is strongly suggested by the small size of the spider compared with the spaces between loops of temporary spiral in theridiosomatid webs (Coddington, 1986c; Eberhard, 2020a). This shared trait thus constitutes significant evidence favouring orb monophyly.

26. *The spider continually produces a new line as it moves during web construction, with the single exception (in both groups) of moving from the end of the temporary spiral to the beginning of the sticky spiral.* Alternatives are feasible, as a few exceptions occur in araneoid orb-weavers (*Poecilopachys* and allies) that build secondarily modified webs and repeatedly interrupt silk line production when they interrupt sticky spiral construction and move to other sites in the web (Clyne, 1973; Stowe, 1986). Continuous dragline production is widespread in non-orb weavers, however, and may be plesiomorphic. In addition, it is possible (though I know of no direct data) that initiating production of sticky lines may be impeded at the spinnerets when a non-sticky line is being produced. Thus, this shared trait of uloborid and araneoid orb weavers does not constitute reliable evidence favouring orb monophyly.

27. *In the process of attaching a newly produced line to an earlier line, the spider holds the earlier line with one or more legs and often presses it against its spinnerets.* Observations of this detail in cribellate non-orb-weavers are scarce, but the alternative is feasible, and the dictynid *Dictyna meditata* sometimes did not hold the earlier line when attaching its dragline (Eberhard, 2021a). Nevertheless, holding the line firmly and precisely against the spinnerets may be necessary in the complex process of making a strong attachment with piriform silk (Wolff *et al.*, 2019), and strong attachments are crucial in radius-to-frame and frame-to-anchor attachments in orbs. Conservatively, this shared trait does not constitute reliable evidence favouring orb monophyly.

28. *The spider avoids walking along sticky lines during web construction.* This might be a fabricational constraint, because the great extensibility of araneoid sticky lines probably makes walking on them likely to result in both less secure footing for the spider and also disruption of the regular spacing between sticky lines due to their resulting adhesion to other lines. This shared trait does not provide reliable evidence favouring orb monophyly.

29. *The temporary spiral line is attached at a single point to each radius that it crosses.* Single point attachments are the rule in uloborids and araneoids, but double attachments, which

may function to increase the tensions on radii (Hesselberg & Vollrath, 2012; Eberhard, 2020a), occur in *Trichonephila* and *Cytophora* and their allies (and are facultatively omitted in barrier webs next to *Trichonephila* orbs) (see summary in Eberhard, 2020a). The necessary behavioural dexterity is present in uloborid orb-weavers, which make double attachments of the hub spiral to radii (Eberhard & Opell, in press). Nevertheless, single attachments between lines are widespread in non-orb spiders (see review in Eberhard, 2020a). This shared trait may be plesiomorphic, and is thus unreliable as an indicator of orb monophyly.

30. '*Break-and-reel' behaviour occurs at some point during construction.* This behaviour involves rapid, complex and precisely coordinated activities of legs, palps and spinnerets to attach the dragline to other lines and to the loose ends of other lines (Eberhard, 2020a). It may be a synapomorphy linking deinopids, uloborids and araneoids (Eberhard, 1982, 2020a, 2021a; Coddington, 1986b). Break-and-reel behaviour is used to accomplish multiple functions, including shifting attachment sites, adjusting tensions, replacing one line with another, and mitigating the build-up of silk in general (Eberhard, 1990). Nevertheless, orb construction is not strictly dependent on break-and-reel behaviour; this fact, and the complex coordination needed to execute this behaviour (Eberhard, 2020a), favour orb monophyly. On balance, the implication of this shared trait for the orb monophyly hypothesis is unclear.

31. *The spider holds its non-sticky drag line with one leg IV while moving in the web and making attachments.* This trait appears to occur uniformly during web construction in uloborids, araneoids and deinopids (Eberhard, 2020a), and is absent in cribellate non-orb weavers (Eberhard & Hazzi, 2013, 2017; Eberhard, 2020a, b, 2021a). Nevertheless, current data on non-orb weavers are very sparse, so this shared trait is only a weak, tentative indicator of orb monophyly.

SIMILAR RESPONSES TO CONSTRAINED SPACES

Table 2 summarizes additional behavioural data on shared responses when uloborid and araneoid orb-weavers were forced to build orbs in especially small spaces. Of 19 orb variables measured, the same 16 were clearly affected in the uloborid *Zosis geniculata* and the tetragnathid *Leucauge argyra* (Walckenaer, 1841) (one other showed no change in either species, and there was uncertainty in the other two). In addition, all of changes were in the same direction (Table 2) (Eberhard & Barrantes, 2015).

Because orbs are geometrically highly regular, and some of the traits that were measured were probably not independent of each other, a second, conservative analysis was limited to comparing seven variables that were most surely independent (letters in bold in Table 2). The same independent web traits were affected in both species, and all were modified in the same way (Eberhard & Barrantes, 2015). These stimulus-response similarities support orb monophyly.

FEW SIMILARITIES IN A LIKELY CONVERGENCE

Spiders in the psechrid genus *Fecenia* build planar, approximately vertical, 'pseudo-orb' webs that resemble orbs in having radially organized non-sticky lines, distinct non-sticky frame and anchor lines, and roughly spiral sticky lines (Zschokke & Vollrath, 1995; Bayer, 2011; Agnarsson *et al.*, 2012; Eberhard, 2020a). Psechridae is a small family (the only other genus, *Psechrus*, builds open-meshed horizontal sheet webs) that is distant from uloborids and araneoids in all recent phylogenies; a careful check of its affinities confirmed that the resemblance to orbs is due to convergence (Agnarsson *et al.*, 2012; Blackledge *et al.*, 2011) (if, as some phylogenies suggest, the 'retrolateral tibial apophysis (RTA)' clade to which Psechridae belongs secondarily lost orb webs, then this convergence represents an independent reacquisition of orbs). *Fecenia* thus offers the opportunity to check the degree of behavioural similarity in a confirmed convergence on an orb-like web. If, as suggested in the previous analyses, the resemblances between uloborid and araneoid orb-weaver behaviour are due to monophyly rather than to convergence, *Fecenia* is likely to show fewer similarities with orb-weavers. If, on the other hand, the similarities between uloborid and araneoid orb-weavers are due to fabricational constraints, then *Fecenia* should also show a similar degree of similarity.

Web photos, along with data on the paths that spiders followed while building their webs (Robinson & Lubin, 1979; Zschokke & Vollrath, 1995; Bayer, 2011) (accepting the assumption of Zschokke and Vollrath that spiders moving especially slowly were producing cribellate silk, and that their paths during web construction represented lines being added), show that four of the traits in Table 1 shared by uloborid and araneoid orb-weavers are not shared in *Fecenia* (data are summarized in Eberhard, 2020a). Two stages of those listed in trait #1 were lacking, the temporary spiral (D) (Eberhard, 2020a) and the hub spiral (C) [see especially the web of *Fecenia cylindrata* Thorell, 1895 figured by Bayer (2011) (in other photos the hub was largely obscured by other lines)]. In addition, the radius construction stage was not 'pure' (trait #15), because many other non-radial lines that were

continuous with radii in *Fecenia protensa* Thorell, 1891 were relatively long but had neither radial nor spiral forms (Zschokke & Vollrath, 1995) (see Eberhard, 2020a on the probable identity of this species). The overall sequence of construction was different, as the web was built in sections that included both non-sticky and sticky lines (trait #19). A final possible, though less clearly documented, difference was that the radial pattern of the 'radii' was only approximate in one species (*F. cylindrata*), whose radial lines were more highly inter-connected in some sectors of the 'pseudo-orb' than in others, and did not tend to split more often at larger distances from the hub.

There are also several similarities. Sticky lines were built in approximate spirals rather than zig-zags (trait #5), and were not laid along non-sticky lines (trait #6). Sticky lines were also laid from the outside moving inward (trait #3), but this trait occurs widely in cribellates and may represent a symplesiomorphy rather than a convergence. Non-sticky lines were built prior to adding sticky lines (trait #11); but, as noted above, this is probably a fabricational constraint. In sum, of the traits that were shared in uloborid and araneoid orb-weavers and that could be compared with confidence in *Fecenia*, four (and perhaps five) were different and four were similar, but two of the similarities have other probable explanations. Convergence on orbs by *Fecenia* has thus involved a substantially higher frequency of differences (at least four of six informative traits) than in uloborid and araneoid orb-weavers (three of 34 traits overall; three of 23 informative traits that could favour monophyly) (see below). This favours the orb monophyly hypothesis.

DISCUSSION

Of 31 web construction traits shared by uloborid and araneoid orb-weavers, 20 clearly supported orb monophyly, two (#10, 31) only tentatively supported it, four (#3, 11, 24, 28) tentatively failed to support it, four (#14, 26, 27, 29) clearly did not support it, and one (#30) was inconclusive (Table 1). The precise numbers are not especially significant, since the evidence is stronger for some traits than for others; some possibly independent traits were lumped together (e.g. #12, 17), and there may be additional traits.

In contrast, there are only three additional behavioural traits known to consistently differ between these two groups (or at least between large segments of them) (data are summarized by Eberhard, 2020a and Eberhard & Opell, in press): araneoids often remove the centre of the hub following completion of the sticky spiral, but uloborids never do; uloborids build a small circular proto-hub early in radius construction,

but no araneoid is known to make a proto-hub; and uloborids consistently use the 'leading' radius as an exit while building radii, while araneoids usually use the uppermost of the two possible exit radii. Overall, 31 of 34 traits are similar, and 20 of the 31 similarities clearly support monophyly.

IMPLICATIONS

The data in Tables 1 and 2 have important implications for understanding orb web evolution and for studies of the evolution of complex traits in general.

CONSTRUCTING PHYLOGENIES

General patterns that favour monophyly

Many behavioural traits support a monophyletic over a polyphyletic origin for orb webs. That such a great number of behavioural traits that could feasibly differ but are nevertheless identical in the two main groups of orb-weavers is strong evidence favouring monophyly. Convergence on a single trait can be biologically plausible, but convergence on a suite of independent traits as large as that in Table 1 seems unrealistic.

Data in Table 2 give further support for the monophyly hypothesis. At least seven different independent aspects of orbs were altered in both uloborid and araneoid orb-weavers when spiders were confronted with especially small spaces in which to build, and the polarities of these changes were the same in all seven. Again, convergence on so many responses seems improbable.

Finally, comparison with a confirmed case of convergence on an orb-like web in the psechrid *Fecenia* revealed relatively frequent differences with other orb-weavers (four of six informative comparisons). The contrast with the three differences in 34 comparisons between uloborid and araneoid orb-weavers is again in accord with the orb monophyly hypothesis.

In summary, behavioural data provide strong support for a single rather than a double origin of orb webs.

The three to five origins hypothesis

Evaluating monophyly against the three to five origins hypothesis (Kulkarni *et al.*, 2021) is more complicated than evaluating it against diphyly. Data are lacking for some traits in some groups; and the numbers of species observed for each of the putatively independent lineages are smaller, reducing confidence in conclusions drawn from the data. In addition, some of the differences between groups could be considered independently derived apomorphies. For instance, relaxing the radii and replacing the entire hub after the sticky spiral is finished

would represent new derived traits in the proposed Anapidae + Symphytognathidae + Mysmenidae lineage of orbs, and only the other three groups would be similar in this aspect of trait #1 in Table 1. Despite these complications, a general point is clear: the likelihood of three to five convergent origins on the numerous behavioural details of orb construction behaviour that are shared among the groups in Table 1 is much smaller than the likelihood of only one or two origins.

Possible solutions?

This study does not propose a solution to the technical question of how to weight the support from behavioural data and combine it with molecular data to produce an improved phylogeny. Nor does it imply that molecular data are not relevant. It seems inevitable that some (perhaps all) of the behavioural traits mentioned here are influenced by multiple genes that determine likely nervous system properties that affect motor, sensory and analytical activities. Take, for instance, use of cues from the inner loop to guide sticky spiral spacing (trait #21 in Table 1). It requires exploratory behaviour by a specific leg (often of the first pair of legs—leg I); performance of this exploration at specific moments during sticky spiral construction; and translation of stimuli perceived during these leg movements into the leg and body movements that result in turning, grasping the radius with legs III and IV, and attaching the sticky line at a certain point relative to the point where leg I contacts the inner loop.

Despite the lack of a technical solution, it seems undeniable that ignoring the complexity of the behaviours involved increases the risk of making mistakes about their evolution. The omission of behavioural data in recent analyses is due to a limitation of current techniques, not to a lack of validity in the data themselves. As a practical matter, it might be reasonable, at a minimum, to mention additional traits in future molecular studies of web evolution that the technique was not able to analyse, and to explain whether these other traits reinforce or weaken the phylogenetic hypotheses derived from the molecular data. In view of the fact that current phylogenetic reconstruction techniques have provided such strikingly different results (e.g. from one to six different origins of orbs) (Fernández *et al.*, 2014; Coddington *et al.*, 2019; Kallal *et al.*, 2020), a certain amount of caution regarding conflicting hypotheses seems appropriate.

MAPPING TRAITS ONTO A TREE

Setting aside the uncertainties regarding techniques used to generate branching patterns in orb-weaver phylogenies, there are also problems with the subsequent process of mapping complex traits onto the resulting trees. The realization that 'orb web' is not a single trait, but rather a swarm of more than 30 behavioural traits, calls into question procedures that have been commonly used to map characters onto trees. The problems can be appreciated by outlining (in simple terms) the commonly used 'maximum likelihood' (ML) technique of ancestral state reconstruction (Joy *et al.*, 2016). Use of this method on a given tree treats the character states at internal nodes of the tree as parameters, and attempts to find the parameter values that maximize the probability of the observed character states on that tree with a hypothesized a model of evolution. It combines assumptions regarding transformation models (probabilities of change between character states, such as a Markov process with independent state transitions at constant rates over time) (Joy *et al.*, 2016), edge distribution (branch lengths, time and rate of change along branches), and the distribution of rates of change among characters. The edge parameters are estimated from observed data; others are chosen to maximize the likelihood of a tree or trees. An important assumption for the analysis of character data is that these data are independent and identically distributed.

This technique, while reasonable for some types of molecular character state changes, seems unrealistic for different behavioural phenotypes of the type being discussed here. In mapping orb web traits onto trees, recent analyses of the origins of orb webs have lumped all of the orb traits (Table 1) into a single character ('orb web') (e.g. Fernandez *et al.*, 2018; Coddington *et al.*, 2019; Kallal *et al.*, 2020). But this is not realistic, because the different behavioural component traits have different properties and vary to some extent independently of one another. Some are under fabricational constraints and are linked to simple intrinsic characteristics of orbs. In contrast, most have multiple feasible states (above) and are likely to have different transition likelihoods. Similar doubts regarding biological realism arise concerning the assumption in a discrete-time Markov chain that the value of the next variable depends only on the value of the current variable and not on any variables in the past. Although I cannot quantify the transition probabilities of the orb web construction traits in Table 1 precisely, they are very likely to differ, and to change according to the presence of other traits. For instance, acquisition of sticky lines laid along a non-sticky line (trait #6) nearly certainly requires a simpler (and thus more likely) modification of behaviour (a second attachment to a non-sticky line) than would the transition from random placement of non-sticky lines to building a non-sticky spiral from inside working out. Similarly, building a spiral requires abilities to sense appropriate cues and to respond to them in appropriate ways so that the spider follows a spiral path. Furthermore, transitions involving the loss of a

neurologically or genetically complex behaviour are probably generally more likely than are transitions that involve acquisition of a new behaviour pattern, unless the behaviour is transferred from a different context or is recurrent, following an earlier deletion (West-Eberhard, 2003; Eberhard, 2018).

In addition, a multiple origin hypothesis must explain the many combinations of traits that are identical in the putatively different lineages in which the orbs are supposed to have arisen if they are to fit the observed distributions of behavioural traits in modern day orb-weaving spiders. If orb webs are taken to be only a single trait, the probability of evolutionary convergence in two different lineages is much greater than if they are taken to be a swarm of 23 informative traits that are shared among orb-weavers. As the numbers of coincident convergences increase, the likelihood of convergences in all of those traits decreases precipitously. In sum, the techniques used in recent studies to map the character 'orb web' onto phylogenies that were generated from molecular data are open to serious doubt [as indeed was mentioned, for other reasons, by some of these same authors (Dimitrov & Hormiga, 2021; Kulkarni *et al.*, 2021)].

NON-ORB EVOLUTION

Attempts to trace the evolution of non-orb spider webs also must confront the problems that stem from the complexity of behavioural traits (Eberhard, 2021a). Disregard of the multiple subunits of non-orb web characters, resulting from the use of overly simplified labels for different web types and the neglect of the behaviour patterns used to produce these webs, has unfortunately characterized recent publications. These problems have been confounded by imprecise definitions of some web types and, in some cases, by mistakes in describing existing webs (Eberhard, 2021a). Expansion of the numbers of the web types considered in recent studies (e.g. Kallal *et al.*, 2020; Kulkarni *et al.*, 2021) is a step in the right direction but using behavioural information to inform estimates of the transition probabilities among web types will be needed to produce more biologically realistic analyses.

LIMITATIONS OF THE PRESENT ANALYSIS

Several possible biases in the data can affect the strength of the preceding conclusions. In the first place, many if not all descriptions of construction behaviour (e.g. Eberhard, 1982) are typological. In one unusual study in which large samples were accumulated of several types of behaviour (Eberhard, 1990), one [*Trichonephila clavipes* (Linneaus, 1767)] performed a substantial number of variations (frame construction was especially variable, with an estimated > 50

variations). Sample sizes are usually not large with respect to either the number of taxa or the number of behavioural traits. The sensory limitations of the observers (e.g. lack of observations of chemical or tension differences between lines) may also lead to underestimates of variables and responses. The effects of these limitations, however, would likely limit the numbers of feasible alternative traits in Table 1, not to reduce the number of shared traits between uloborid and araneoid orb-weavers. In sum, these limitations likely produce underestimates rather than overestimates of the support for monophyly.

A second shortcoming of the analysis is that the polarities of some behavioural traits are uncertain; perhaps some traits shared by uloborid and araneid orb-weavers that were counted as shared derived traits are in fact shared plesiomorphies. Data from non-orb cribellate groups are sparse for most of the traits in Table 1 (Eberhard, 2020a), and are of limited usefulness in checking for plesiomorphies (possible exceptions are noted in the discussions of traits #3, 4, 6, 12, 13, 16, 26, 27 and 29–31). I opted for conservative schemes, but simplicity admittedly does not guarantee being correct. Take, for instance, my assumption that consistently refraining from laying sticky spiral lines along non-sticky lines is a shared apomorphy of uloborids and araneoids, despite the fact that sticky lines are routinely laid along non-sticky lines in the modified orbs of some uloborids (*Hyptiotes*, *Polenecia*), and also occur occasionally in the orbs of several others (Eberhard & Opell, in press). Some cribellate non-orb-weavers in distantly related families sometimes do lay sticky lines along non-sticky lines, and sometimes do not (Eberhard, 2020a, 2021a), so they offer no clear guidance. I based my decision on the current understanding of uloborid relationships (Coddington, 1990), which indicates that the webs of *Hyptiotes* and *Polenecia* are secondarily derived from orbs; however, more extensive modern studies of uloborid phylogeny are lacking. Similar problems of interpretation occur in other traits (for instance, the presence of temporary spirals) due to current uncertainties regarding the relationships among araneoid families (different versions are given, for example, in each of the following: Coddington, 1990; Scharff & Coddington, 1997; Dimitrov *et al.*, 2016; Wheeler *et al.*, 2017; Kallal *et al.*, 2020).

Classifying behaviour into discrete traits was also sometimes difficult; I tried to be conservative and lumped some traits that were distinguished separately in other studies (e.g. Eberhard, 2018). I also omitted additional traits shared by uloborids and araneoids that involve finer details, such as which legs hold which lines when attachments are made because they are too poorly documented in non-orb spiders to make reasonable decisions regarding polarity. The effect of such omissions and lumping likely produce

underestimates rather than overestimates of the number of traits requiring convergences under the multiple origin hypotheses.

I emphasized determination of the 'feasibility' (mechanical, selective and neurological) of alternative character states. However, determining that two states are both feasible is not equivalent to showing that they are equally probable. Perhaps, for instance, one alternative is more advantageous than the other because it requires less energy to perform or less investment in silk. Or perhaps one alternative is 'easier' to evolve because it requires less change from the ancestral state. Such balances are presently unknown. These possibilities will only have important consequences for the basic conclusions regarding monophyly here, however, if the state shared in uloborid and araneid orb weavers is more probable than the alternative, and if most of the many traits show this same balance.

GENERAL CONCLUSIONS: UNDERSTANDING PHENOTYPES IS IMPORTANT

Just like behavioural phenotypes, morphological phenotypes can also be the result of multiple, partially independent subunits resulting from developmental decisions (switch points) during ontogeny; and each of these decisions is likely to be influenced by multiple factors, both environmental and internal (West Eberhard, 2003). Using simple labels to distinguish complex behavioural and morphological phenotypes in studies of phylogeny runs the risk of being misleading. Simple labels for complex traits are tempting: they facilitate both writing and comprehension. However, as shown with the label 'orb web', they can seriously distort reality.

The general point is that understanding the evolution of phenotypes, behavioural or otherwise, is likely to be improved not only by producing phylogenies, but also by better understanding of the phenotypes themselves. Phylogenetic information is crucial to substantiate the polarity of evolutionary transitions in phenotypes. However, biologists should not blindly accept a phylogeny as correct when phenotypic data that strongly challenge its conclusions have been ignored.

DATA AVAILABILITY

Data in this paper were already published elsewhere.

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