Limited role of character displacement in the coexistence of congeneric *Anelosimus* spiders in a Madagascan montane forest

Ingi Agnarsson, Nicholas J. Gotelli, Diego Agostini and Matjaž Kuntner

I. Agnarsson (iagnarsson@gmail.com) and N. J. Gotelli, Univ. of Vermont, Dept of Biology, 109 Carrigan Drive, Burlington, VT 05405-0086, USA. – M. Kuntner and IA, Dept of Entomology, National Museum of Natural History, Smithsonian Inst., NHB-105, PO Box 37012, Washington, DC 20013-7012, USA. MK also at: Evolutionary Zoology Laboratory, Biological Inst. ZRC SAZU, Ljubljana, Slovenia, and Centre for Behavioural Ecology and Evolution, College of Life Sciences, Hubei Univ., Wuhan, Hubei, China. – D. Agostini, Univ. of Puerto Rico at Rio Piedras, San Juan, PR 00931, USA.

Evolutionary and ecological theory predicts that closely related and similar species should coexist infrequently because speciation is more likely to occur allopatrically than sympatrically, and because co-occurring species with similar traits may compete for limited resources, leading to competitive exclusion or character displacement. Here we study the unusual coexistence of 10 similar congeneric species of *Anelosimus* spiders within a small forest fragment in Madagascar. We asked if these species radiated in sympatry or allopatry, and if there was evidence for local-scale character displacement in body size and other species-level traits. We sampled ~ 350 colonies (6346 individuals) along a 2800 m transect. We identified colonies using morphology and DNA barcoding, and tested the monophyly of local and regional species assemblages with time-calibrated phylogenies. We used null model analysis and phylogenetic signal inference to test for patterns of segregation in body size, microhabitat, phenology, and seasonality of coexisting species. We found that all species belong to a Madagascan clade that radiated during the Pliocene, but that contemporary local assemblages are non-monophyletic. This is consistent with allopatric speciation during periods of global cooling and expansion of grasslands, and subsequent species assembly as forest fragments re-expanded and coalesced. We found no evidence for character displacement, except for overdispersion and even spacing in phenology: species were segregated by instars in a manner consistent with resource partitioning or maintenance of reproductive isolation. Overdispersion or even spacing in phenology may contribute to coexistence either through resource partitioning or mate recognition. However, there was no support for a scenario of resource partitioning and divergence of body size or other correlated morphological characters. These traits are better explained by evolutionary forces operating during speciation, rather than ecological forces operating during local community assembly.

Phylogenetic methods can provide insight into the assembly of ecological communities (Gillespie 2004). The coexistence of functionally convergent but distantly-related species is relatively common (Jacquemyn et al. 2014, Tran et al. 2014). However, theory predicts that multiple closely-related species with similar traits should coexist infrequently (Levin 1970, Schluter 2000), due to both historical processes operating at a regional scale, and evolutionary and ecological processes operating at a local scale. The historical process operating at the regional scale is that speciation in most animals is more likely to occur allopatrically than sympatrically (Mayr 1942, Futuyma 2009). Unless there is subsequent dispersal and re-mixing, allopatric speciation will generate disjunct geographic ranges of sister taxa (Lynch 1989, Razafindratsima et al. 2013), which will not co-occur locally.

At the local scale, in the absence of strong habitat filtering, co-occurring species with similar traits may compete for limited resources, leading either to ecological competitive exclusion (MacArthur and Levins 1967) or to evolutionary character displacement – closely related species in sympatry

diverging from one another in phenotypic traits (Brown and Wilson 1956, Schluter and McPhail 1993). Overdispersion or even spacing in a trait, or set of traits, provides indirect evidence for character displacement, which facilitates the partitioning of resources among species and/or the enhancement of reproductive isolation (Brown and Wilson 1956, Schluter and McPhail 1993, Pfennig and Murphy 2000, Montana et al. 2014).

On the other hand, habitat filtering and phylogenetic inertia may limit the divergent effects of resource competition and promote the coexistence of closely-related species with similar traits (Kochmer and Handel 1986, McKitrick 1993, Kellermann et al. 2012). Due to phylogenetic inertia and limited time for phenotypic divergence, closely-related species that co-occur locally often tend to resemble each other more than distantly-related species (Schliewen et al. 1994, Cavender-Bares et al. 2004, Grinsted et al. 2012).

It thus remains unclear how often character displacement explains species coexistence and adaptive radiations (Schluter and McPhail 1993, Schluter 2000, Pfennig and

Pfennig 2009). In fact, despite long-term interest and work on the patterns and mechanisms of character displacement, relatively few examples have held up to statistical analysis (Dayan and Simberloff 1994, 2005, Kluge and Kessler 2011, Bennett et al. 2013, Pellissier et al. 2013). Null model analyses of patterns of body size spacing also reveal few cases of non-random trait distributions of local assemblages that are consistent with the predictions of character displacement (Simberloff and Boecklen 1981). Studies combining ecological and phylogenetic evidence can provide insight into divergence times, speciation mode, and the existence of character displacement in local assemblages (Miles and Dunham 1996).

Spiders are good subjects to test for character displacement because 1) they are top predators that can trigger trophic cascades in many terrestrial food webs (Schmitz and Suttle 2001); 2) there is good evidence for food limitation in many spider assemblages (Wise 1995); 3) traits such as body size and web architecture impact the composition and size distribution of captured prey (Nyffeler 1999). Here, we studied local species coexistence of 10 sympatric species of the social spider genus *Anelosimus* sampled in a forest fragment in Madagascar. We used phylogenetic methods to test the monophyly and age of this assemblage, and a suite of null models to test for character displacement in body size, web size, phenology, microhabitat use, and seasonality.

We tested two major hypotheses that are not mutually exclusive: 1) that competition among close relatives has led to character displacement and statistical overdispersion or even spacing of species traits; 2) that speciation took place sympatrically, generating sister species that co-occur regionally and creating local assemblages comprised of monophyletic lineages.

Methods

Study system

Species of the worldwide spider genus *Anelosimus* range in behavior from solitary to social, and their behavior dictates ecological traits such as web type and size (Aviles 1997, Agnarsson 2006). Globally, *Anelosimus* species rarely overlap in their ranges with other congeners of similar social structure. For example, Serro do Japi in Brazil is remarkable in having up to six *Anelosimus* species living in close proximity (Guevara et al. 2011), but these span the entire range from solitary species that build small delicate webs to highly social species that build massive robust webs that can cover entire tree canopies. Hence, these species are not likely to capture similar prey or to use similar microhabitats for web construction. In Madagascar, however, initial sampling yielded six subsocial species building very similar webs within a single montane rainforest fragment (Fig. 1; Agnarsson and Kuntner 2005); more intensive sampling revealed the coexistence of 10 *Anelosimus* species in this habitat (Agnarsson et al. 2015a). This sympatric assemblage of species belongs to a monophyletic Madagascar radiation (Agnarsson et al. 2010, 2015a), but it is unclear whether or not they are each other's closest relatives at local scales, what the age of this radiation is, and if they show any evidence of character displacement and microhabitat preferences.

Transect

We established a ~ 2800 m transect along a trail in Périnet Special Reserve, a part of Mantadia-Andasibe National Park in eastern Madagascar, 3–23 April, 2008. We placed 14 stations at approximately 200 m intervals along the trail (Supplementary material Appendix 1, Table A1), with stations 7–11 occurring in closed forest and the remainder in open forest (Fig. 2). Because the trail loops back on itself, distances to the nearest station ranged from 50 to 200 m. We sampled one station per day by 1) a thorough two-hour search by two people during which we collected all *Anelosimus* colonies within a 10 m radius of the sampling point, and 2) a general sample collected by beating and sweeping for one hour each – in an attempt to capture males within each sampled area. In April, we also collected haphazardly along the trail parallel to stations 1–3, and 14 (Fig. 2). From 10 November to 30 November 2008, we repeated the collection at each station in an identical manner.

For each colony detected, we measured the height of its web in m from ground, and the width, depth, and length of the web (Fig. 1). Each colony was then collected entirely in a single one quart Ziplock bag. Within 24 h, colonies were dissected and all individuals within each colony were preserved in a separate labeled whirl-pak® with 95% ethanol. Beating and sweeping samples were combined into a single whirl-pak® per station. All specimens were examined in the laboratory under a Leica MZ16 microscope. Adults were identified to known species and to putatively novel morphospecies. All specimens from each colony were then sorted into size classes by eye, based on conspicuous differences and discontinuities in sizes representing putative instars (numbers of molts since emergence from egg sac). The numbers of individuals per colony and size class were counted, and one individual from each size class was measured to determine the instar based on the total body length and the length of patella-tibia 1. We estimated at what stage in the phenology each species was by calculating the weighted average instar number present in each colony. For example, if, for a given species, 20 individuals were of instar 4 and 60 were of instar 5, the weighted average instar $= (20 \times 4 + 60 \times 5)/80 = 4.75$. We also calculated the ratio of adults to juveniles per species per season, and noted the presence of egg sacs as more qualitative estimates of phenology.

We compared species relative abundances in open versus closed forest with a 2-way contingency table (species × habitat). We also compared species relative abundances in April versus November with a 2-way contingency table (species × season). We tested the effects of seasonality (April vs November) and species on web size and web height from the ground with generalized linear models (GLM) and least squares analyses in JMP ver. 10.0.0 (SAS Inst.). Difference in abundance and web size at different times of the year could further evidence differences in phenology among species. Web size data were normal after log transformation.

DNA barcoding

To verify morphological identifications, and to identify colonies containing only juveniles – which cannot be identified from morphology – we extracted DNA from one individual
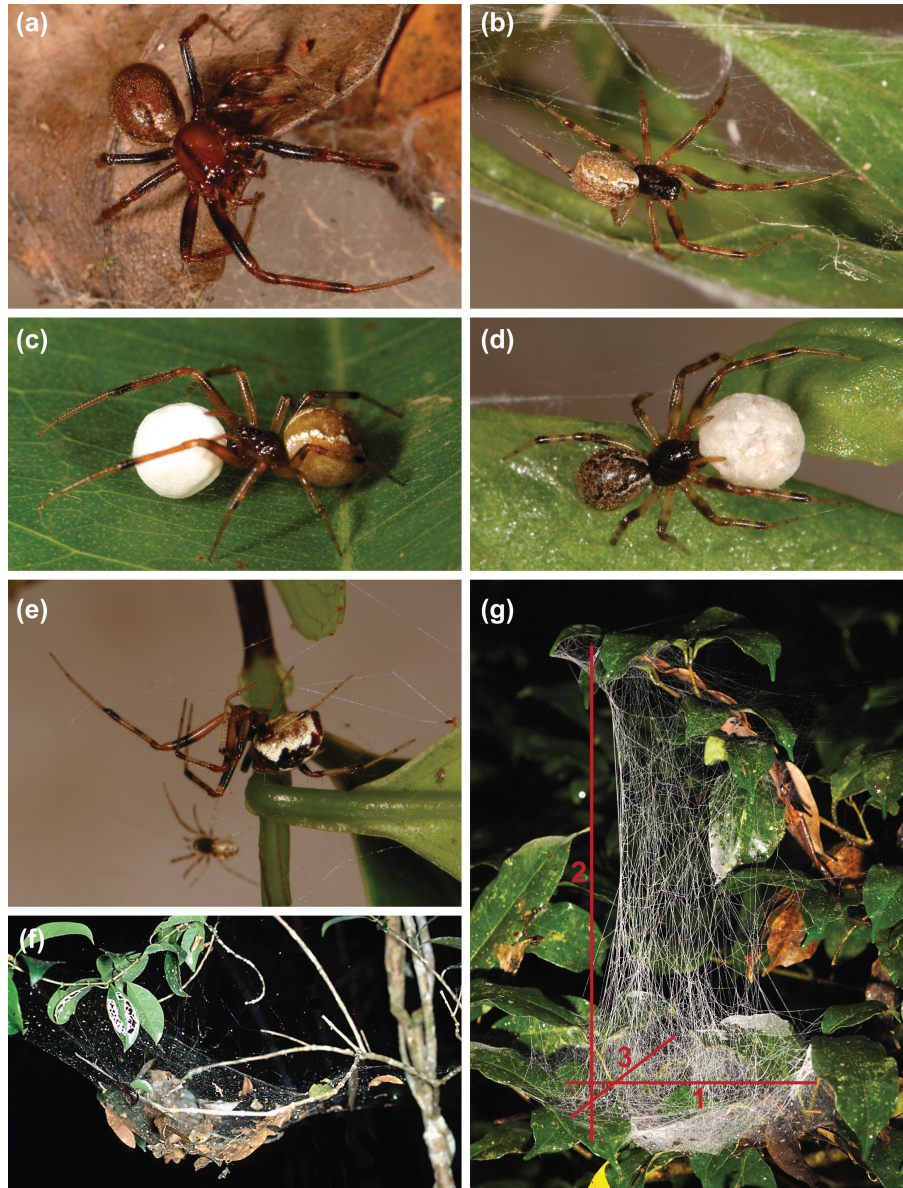
Figure 1. Some of the sympatric *Anelosimus* species and their colonies: (a) female *A. nazariani*; (b) female *A. wallacei*; (c–d) two *A. vondrona* females carrying egg-sacs; (e) *A. salut* female and juvenile; (f) web of *A. ata*; (g) measured web parameters (depicted a colony from north Madagascar): 1, web width; 2, web height; 3, web depth.

per colony and sequenced the COI gene. We extracted DNA using the QIAGEN DNeasy Tissue Kit (Qiagen, Valencia, CA) from two legs (adults) or entire specimens (juveniles). We amplified COI with the LCO1490 (Folmer et al. 1994), and C1-N-2776 (Hedin and Maddison 2001) primer pair using standard protocols as described in Agnarsson et al. (2007). Sequences were interpreted from chromatograms using Phred and Phrap (Green and Ewing 2002, Green 2009) via the Chromaseq module in the evolutionary analysis program Mesquite 3.02 (Maddison and Maddison 2012) with default parameters. Sequences were then proof-read with reference to chromatograms. Alignments were done in MAFFT (Katoh 2013) through the online portal EMBL-EBI. DNA barcodes were then analyzed to identify putative species using neighbor joining in MEGA ver. 5 (Tamura et al. 2011). Morphological identifications

were verified by examining the distribution of identified adults among DNA clusters. Colonies consisting only of juveniles were identified to species based on the clustering with the adults.

Phylogenetic analyses

To test the monophyly and placement of Madagascan *Anelosimus*, and the relationship among Madagascan species, we added 2–3 specimens of each putative species to the global *Anelosimus* phylogeny presented in Agnarsson (2012). Further, to test the monophyly of the species assemblages in each forest and thus test the allopatry hypothesis, we added specimens of species currently being described (Agnarsson et al. 2015a) from three other Madagascan for-
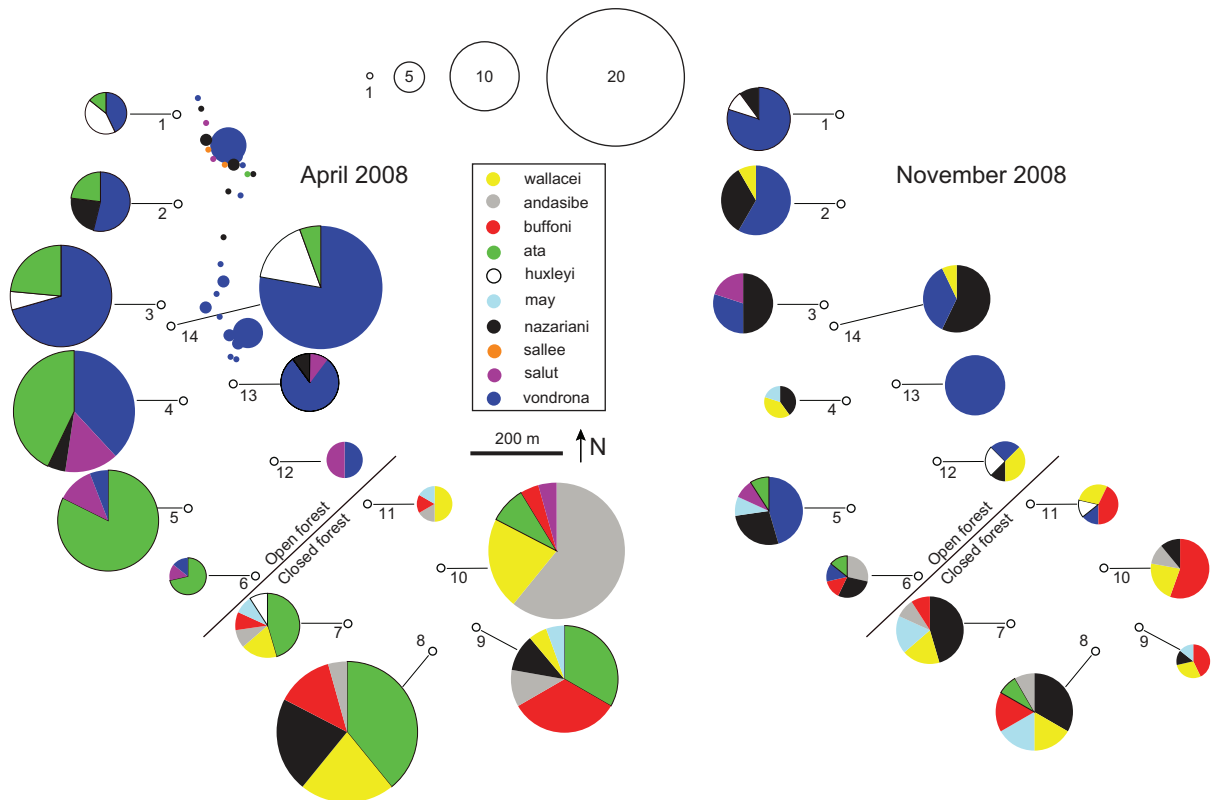
Figure 2. Relative abundances of colonies of the ten species sampled in the transect, stations 1–14 (April and November 2008), and outside of it (April 2008). Pie charts are proportional to the number of colonies of each species encountered at each station, scale is indicated by open circles above. Line demarcates a rough boundary between closed canopy and open canopy forests.

ests: the Ambohitantely Reserve (a total of eight specimens representing four putative species), Montagne d'Ambre (a single available specimen), and Ranamofana (multiple specimens of a new species). We extracted DNA as described above, and, in addition to COI, amplified two additional mitochondrial genes (16S, ND1) and two nuclear genes (28S, ITS2) using primers and protocols as described elsewhere (Agnarsson et al. 2007). Processing and alignment of sequences was done as for the DNA barcodes, and the data were added to the matrix of Agnarsson (2012). The appropriate substitution model for Bayesian phylogenetic analysis was chosen for each partition using jModeltest 0.1.1 (Posada 2008): 28S, COI, 16S, = GTR + I + Γ; ITS2 = GTR + Γ; ND1 = HYK + Γ. We then analyzed the concatenated matrix in MrBayes (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) for 10 000 000 generations, with all base frequencies estimated from the data and parameter estimates unlinked ('unlink statefreq = (all) revmat = (all) shape = (all) pinvar = (all)'). After verifying thoroughness of analyses through examination of stationarity of the results in Tracer 1.5 (Rambaut and Drummond 2007), the first 2 000 000 generations were discarded as 'burnin'.

We estimated node ages using a relaxed molecular clock approach under a Bayesian framework as implemented in the program BEAST ver. 1.8 (Drummond et al. 2012). This method estimates node ages while accounting for uncertainties in node calibration and for phylogenetic uncertainty (Rutschmann 2006, Drummond et al. 2012). We combined fossil evidence with informed priors on the mitochondrial

substitution rate to calibrate the relaxed clock and to estimate divergence times and constrained monophyly of *Anelosimus* to set the outgroup and also reduced the number of model partitions, linking all mitochondrial data in one partition and all nuclear data in another partition. Relaxed lognormal clocks were used for each partition. For the mitochondrial partition an informed prior was based on spider-specific substitution rates estimates for mitochondrial genes across several spider groups. Thus the rate parameter (ucld.mean) was set to 0.0112 (SD = 0.001) (Bidegaray-Batista and Arnedo 2011, Kuntner et al. 2013). For the nuclear genes, initial mean substitution rates were set at an order of magnitude lower than the reported mitochondrial rate (Kuntner et al. 2013) and assigned uniform flat priors.

There is no fossil record of *Anelosimus* or the subfamily Anelosiminae. However, its sister subfamily Theridiinae is known to occur in Dominican amber (~15 mya), but not in Baltic amber (~4 0 mya). We used this information to set the minimum age of the split between Anelosiminae and Theridiinae to 15 my. This calibration point was assigned an exponential distribution, with starting value corresponding to the minimum bound. We also used BEAST to obtain a Bayesian ultrametric phylogeny for comparative analyses. We ran 10 million generations; the correct mixing of each MCMC chain and the burnin was visualized with TRACER. Trees were summarized using the BEAST accompanying program TreeAnnotator. An analysis of lineages through time was done in Mesquite (Maddison and Maddison 2012).

Evolutionary analyses

To test for phylogenetic signal – whether traits showed greater or lesser similarity than expected by random and thus test the phylogenetic inertia and habitat filtering hypotheses – we calculated Pagel's λ and Blomberg's K on a BEAST phylogeny pruned to contain one terminal per species, using the Phytools (Revell 2012) and Ape (Paradis et al. 2004) packages in R. Pagel's λ is a tree transformation parameter that measures the fit of a trait to a tree and the effect of gradually eliminating phylogenetic structure (Pagel 1999). Blomberg's K measures the scaled ratio of variance among species over variance in the contrasts (Blomberg et al. 2003) with the null expectation estimated through simulation. In both cases a value close to 0 implies absence of phylogenetic signal in a trait, in effect that an unresolved star phylogeny represents no worse a fit to the data than the observed phylogeny. Pagel's λ values approaching 1 (maximum) would indicate a complete match between the trait and the phylogeny. Blomberg's K differs in being a ratio and can thus be > 1. Significant values of Blomberg's $K < 1$ suggest that, although there is a phylogenetic signal in the trait, divergence among species is greater than expected by Brownian motion evolution. Significant values of Blomberg's $K > 1$ suggest that related taxa are more similar in their traits than expected by Brownian motion evolution.

We tested for signal in female body size across the global phylogeny in general, as well as specifically within the Madagascan clade. We also tested for phylogenetic signal within the Périnet assemblage in habitat preference (% occurrence in open vs closed) and phenology (weighted average instar in webs in November). In addition, we estimated phylogenetic signal for size data on the original phylogeny as well as on a phylogeny to which the newly-discovered *A. lamarcki* was added (Agnarsson et al. 2015a). These analyses were run across a set of 100 trees from the post-burn-in distribution of the Bayesian analysis to account for phylogenetic uncertainty. Results given are averages.

Trait distribution analyses

We tested for non-random distributions of morphological and demographic traits of the 10 coexisting *Anelosimus* species. For adult females of each species, we measured the average body length and the length of the first tibia + patella. We also calculated an average habitat index for each species based on its relative abundances in open versus closed forest. During the November survey, we measured species web size and an index of seasonality based on changes in relative abundances of species in the March versus November survey transects. Finally, we calculated an index of phenology, which is the average weighted instar of each species in the November transect.

For each trait X, we ordered the average trait values X_i from smallest to largest for the $i = 1$ to 10 species: $\{X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}\}$. Unusual spacing of X could be expressed either as the difference between trait values of adjacent species $(X_{i+1} - X_i)$ or as the ratio of trait values of adjacent species (X_{i+1}/X_i). Constancy in trait differences would correspond to a maximally even spacing of points along the line segment, whereas constancy in trait ratios would imply a constant proportional spacing of adjacent species in the sequence (Simberloff and Boecklen 1981). For morphological measurements of *Anelosimus*, analyses of trait ratios and trait differences gave qualitatively similar results. However, analyses of trait ratios are not sensible for variables such as phenology or habitat use, so we present here only analyses of trait differences for all variables.

For each trait, we calculated both the minimum trait difference D_{min} between all pairs of adjacent species, and the variance in trait difference between all pairs of adjacent species D_{var}. D_{min} can be interpreted as a measure of over-dispersion, whereas D_{var} can be interpreted as a measure of even spacing. If there is an ecological limit to the similarity of coexisting species (MacArthur and Levins 1967), then D_{min} should be larger than would be expected by chance, which is a pattern of over-dispersion. If the trait distribution is unusually even, differences in traits between pairs of species will be similar, so that D_{var} would be smaller than expected by chance (Poole and Rathcke 1979). In the extreme case, if there is identical spacing between all adjacent species, then $D_{var} = 0$.

To create a simple null distribution, we drew 8 random values from a uniform distribution bounded at its minimum by X_1 and at its maximum by X_{10}. Because the results of null model tests for morphometric patterns are potentially sensitive to the choice of this distribution (Cole 1981), we conducted a parallel set of analyses in which we simulated 10 random values chosen from a gamma distribution. Maximum likelihood estimation with the fitdr() function in R (R Core Team) was used to obtain shape and scale parameters of the gamma distribution from the sample data. These parameters yielded size distributions that were visually similar to those of the original data. However, analyses based on the gamma distribution gave qualitatively similar results to those based on the uniform distribution, so we are only presenting the latter.

For each trait analysis, we generated 1000 random assemblages by random sampling from a uniform distribution to specify trait values and then created the histogram of D_{min} or D_{var}. We next compared the observed D_{min} or D_{var} to this null distribution, and estimated the one-tailed probability of the observed data, given this null hypothesis (Manly 2006). Analyses were run in EcoSimR ver. 0.1.0 (Gotelli et al. 2015), a set of functions and script files for null model analysis in R (R Core Team). The specific R functions we wrote for morphometric analyses are now incorporated in the R package EcoSimR, which is available from the CRAN repository ($<$ http://cran.r-project.org $>$).

Data available from the Dryad Digital Repository: $<$ http://dx.doi.org/10.5061/dryad.g02n5 $>$ (Agnarsson et al. 2015b).

Results

Transect

The transect sampling yielded 204 colonies and 5149 individuals (150 females, no males, and 5099 juveniles) in April 2008, and 147 colonies and 1221 individuals
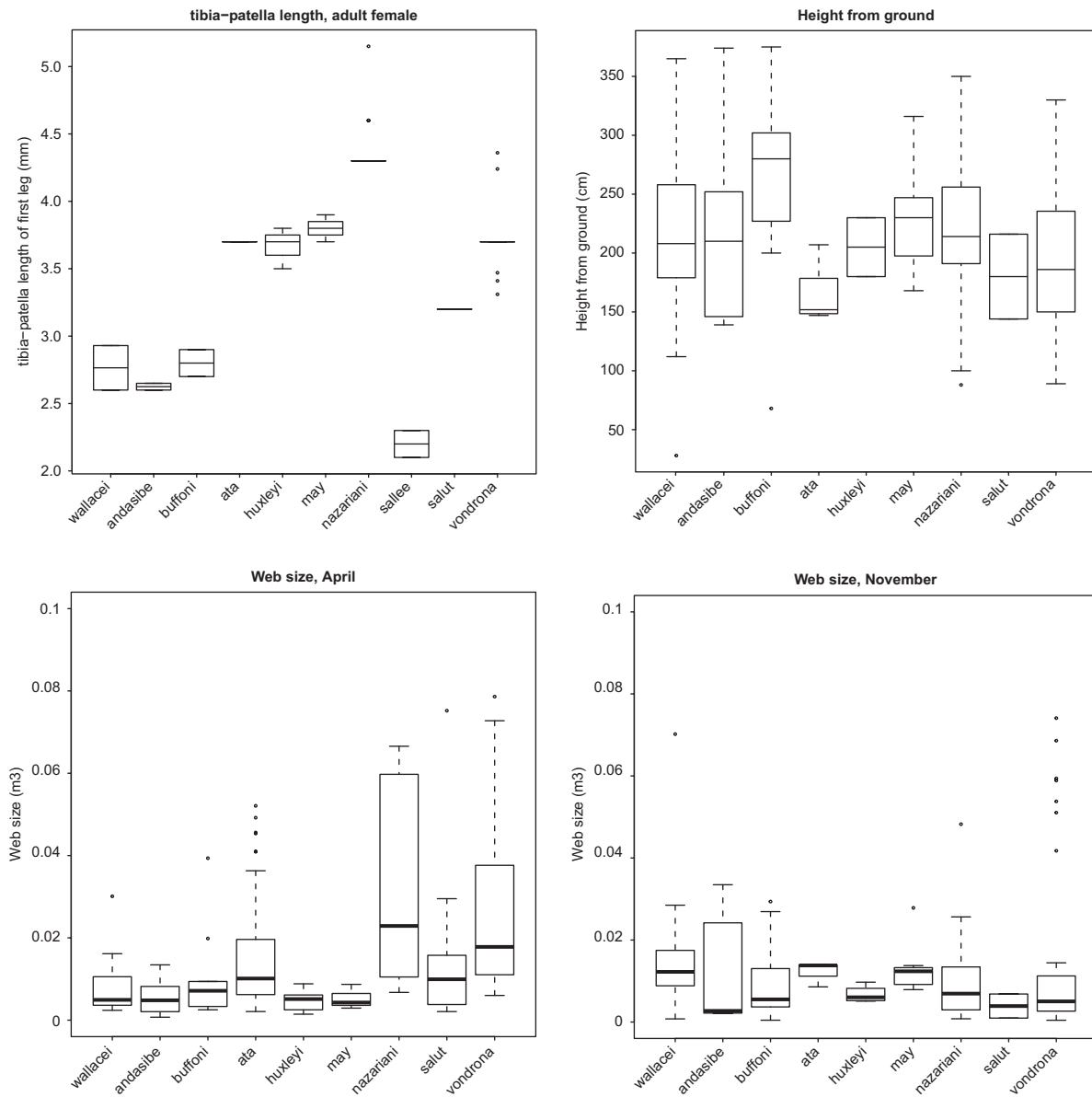
Figure 3. Box plots comparing female size (patella-tibia I), web placement (height from ground), and web sizes (April and November) of the coexisting *Anelosimus* species.

(54 females, 9 males, and 1158 juveniles) in November 2008 (summarized in Fig. 2, see also Supplementary material Appendix 1). These belonged to 10 species, all of which were collected at least twice. Lack of singletons (species represented by only a single individual) implies that we have likely sampled all the species in the area detectable by our sampling methods (Gotelli et al. 2012). Species composition differed significantly between open and closed forest ($\chi^2 = 43.3$, DF = 8, p < 0.001), and between the April and November surveys ($\chi^2 = 46.3$, DF = 9, p < 0.001; Table 2). All *Anelosimus* species were found in both open and closed forest, except the rare *A. sallee* only collected twice in open forest. Abundances differ among most species in open and closed forest ($X^2 < 0.05$) with *A. andasibe*, *A. wallacei*, and *A. buffoni* occuring with significantly greater abundance in closed forest, *A. may*, *A. salut*, *A. vondrona*, and *A. huxleyi* in open forest, while two species *A. ata* and

A. nazariani occurred at similar abundances in open and closed forest ($X^2 > 0.05$). Abundances in closed forest are relatively more even (Hurlberts PIE, Average Diversity = 0.822) while two species (*A. vondrona* and *A. ata*) dominate in the open forest making abundances less even (Hurlberts PIE, Average Diversity = 0.67). In other words, the probability that two individuals, drawn at random, represent different species is higher in the closed forest. The GLM analysis detected significant effects of both season and species on web height from ground (season: $\chi^2 = 11.26$, DF = 1, p = 0.0008; species: $\chi^2 = 31.29$, DF = 8, p = 0.0001). Two of the nine compared species exhibited significant differences in web height from ground between the seasons (*A. andasibe* 3: $\chi^2 = 3.86$, p = 0.0495; *A. vondrona*: $\chi^2 = 21.16$, p < 0.0001, Fig. 3). Although least squares analysis generated comparable results in the overall effects on web height from ground (transect: $F = 11.1$, DF = 1,

p = 0.001; species: $F = 3.98$; DF = 8, p = 0.0002), the only species with significant difference between the seasons was *A. vondrona* ($t = -4.6$, p < 0.0001). Similarly, all analyses found that both phenology and species significantly explained the spider web sizes (GLM transect $\chi^2 = 23.61$, DF = 1, p < 0.0001; GLM species $\chi^2 = 25.19$, DF = 8, p = 0.0014; least squares transect $F = 23.74$, DF = 1, p < 0.0001; least squares species $F = 3.17$, DF = 8, p = 0.0018) and that webs were differently sized between the seasons in two out of nine species (Fig. 3), in *A. andasibe* 2 (GLM $\chi^2 = 11.297$, p = 0.0008; least squares $t = -3.34$, p = 0.0009) and in *A. vondrona* (GLM $\chi^2 = 10.70$, p = 0.0011; least squares $t = 3.25$, p = 0.0013). Body size variances differed significantly by species (ANOVA $F = 93.2$, DF = 7, p < 0.001, Supplementary material Appendix 1, Fig. A1).

DNA barcoding

DNA barcoding placed specimens from all sampled colonies in 10 clades (Fig. 4), representing all 10 of the known species. All adults previously identified based on morphology were unambiguously placed within the clade containing other adult conspecifics. All juveniles were unambiguously
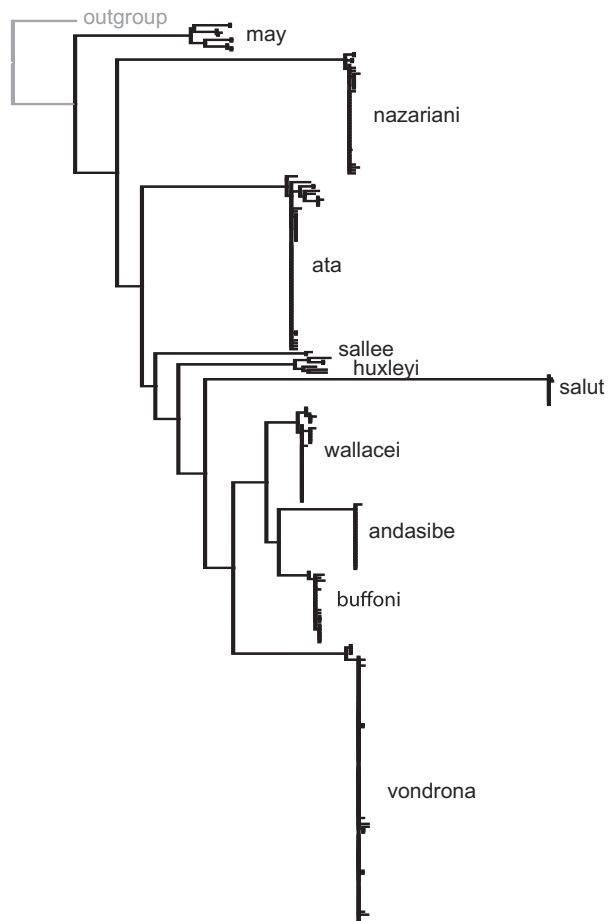

Figure 4. Clustering of DNA barcoded individuals from the transect. All individuals were unambiguously assigned to one cluster and all adults identified based on morphology clustered with conspecifics suggesting the barcodes are efficient at species identification.

placed within one of the ten clades, with high posterior probability of support, which allowed us to identify them with confidence. All species also are clearly diagnosable based on patterns of COI substitutions (Agnarsson et al. 2015a).

Phylogenetic analyses

Both the Bayesian and BEAST analyses agree that the Madagascar subsocial *Anelosimus* are a monophyletic recent radiation approximately 5.5 my old (Fig. 5), and sister to an American radiation of similar age. As indicated by dating and lineages through time analyses, most diversification occurred relatively early in this radiation, in the Pliocene between 2–5 my (Fig. 5, Supplementary material Appendix 1, Fig. A3). The Périnet species assemblage is not monophyletic. Among these samples, even the three very closely related species in the *A. andasibe* species complex (*A. andasibe*, *A. buffoni*, and *A. wallacei*) previously thought to be monophyletic were rendered paraphyletic by the discovery and phylogenetic placement of a new species from Ranamofana forest, *A. lamarcki* (Fig. 5, Agnarsson et al. 2015a).

Evolutionary analyses

For the global *Anelosimus* phylogeny, both female adult body size and first patella + tibia length showed a significant phylogenetic signal in which phylogeny predicts body size (lambda size 0.61, p < 0.001, leg size 0.71, p < 0.001; Bloomberg's K size 0.44, p = 0.005, leg size 0.72, p = 0.001). This signal is especially strong in leg size. However, within the Madagascan radiation, the pattern was not significant, even for leg size (lambda 0.006, p = 1, Blomberg's K 0.41 p = 0.81, Supplementary material Appendix 1, Fig. A1). Neither phenology nor habitat association showed any phylogenetic signal within Périnet; such data were not available for other species of the Madagascan radiation.

Trait distribution analyses

Five of the six trait variables measured (female body length, female first patella + tibia length, web size, habitat quality, and seasonality) exhibited no evidence for even spacing or overdispersion of trait values (Table 1). For female body length, female first patella + tibia length, and seasonality, some species had identical trait values, which is almost impossible when sampling from a continuous distribution. Thus, these traits were in the left-hand tail of the probability distribution (p = 1.000) and exhibited significant aggregation for D_{min}. In contrast, the phenology index was significantly over-dispersed for D_{min}, which was larger than expected (p = 0.033) and significantly evenly-spaced for D_{var}, which was smaller than expected (p = 0.020).

Discussion

The assembly of multiple *Anelosimus* species into diverse communities of related and morphologically similar species (Fig. 2) contrasts with many island community assembly

Table 1. Null model tests of overdispersion and even spacing for 6 traits of 10 coexisting *Anelosimus* species. D_{min} = minimum trait difference between a pair of species (overdispersion). D_{var} = variance in trait differences measured between pairs of all consecutively ordered trait values (even spacing). The tabled values indicate the observed indices (Obs(D_{min}), Obs(D_{var})), the expected values, estimated as the average of the 1000 simulated assemblages (Exp(D_{min}), Exp(D_{var})), and the one-tailed probability value for each test (P(D_{min}), P(D_{var})). Low p-values for D_{min} indicate that the observed D_{min} was larger than expected (overdispersion), and low p-values for D_{var} indicate that the observed D_{var} was smaller than expected (even spacing). See the text for details on the trait measurements of female body length, female patella + tibia length, web size, seasonality, and phenology.

Trait	Obs(D_{min})	Exp(D_{min})	P(D_{min})
Female body length	0	0.047	1.000
Female patella + tibia length	0	0.027	1.000
Web size	87	194.02	0.664
Habitat quality	0.010	0.012	0.538
Seasonality	0	0.014	1.000
Phenology	0.046	0.014	0.020

Trait	Obs(D_{var})	Exp(D_{var})	P(D_{var})
Female body length	0.428	0148	0.993
Female patella + tibia length	0.070	0.053	0.768
Web size	1.969×10^3	2.164×10^3	0.500
Habitat quality	0.015	0.010	0.874
Seasonality	0.015	0.010	0.825
Phenology	0.004	0.015	0.033

patterns, such as in Hawaiian *Tetragnatha* spiders (Gillespie 2004). There, persistent habitat patches (islands up to 5 million yr old) are occupied by mini-radiations of closely-related species that have evolved into distinct (and repeatable) ecomorphs with little or no coexistence among similar relatives. In contrast, local *Anelosimus* assemblages consist of polyphyletic and paraphyletic sets of species that are phenologically segregated, but overlap considerably in body size, morphology, habitat preference, and web architecture. Below we explore some possible explanation for the rather unusual species coexistence of *Anelosimus*.

All species except the rare *A. sallee* were found in both open and closed forest and thus all species potentially interact. Although these *Anelosimus* species differ in many traits such as body size, web placement, relative abundance in open vs closed forest and in different seasons, there was no evidence that these differences were a consequence of species interactions. The only result consistent with character displacement is that the average weighted instar present in colonies of each species is evenly spaced and the minimum difference between species is larger than expected by chance (over-dispersed). In other words, each species is likely to mate, lay egg sacs, emerge, and develop to adulthood, at different times of the year, as also evidenced by the different life stages present of each species during the sampled time-slices (Table 2). However, displacement in development time is a demographic trait that does not necessarily have a heritable component (Reale et al. 2003, Nussey et al. 2005), although it could contribute to species coexistence by leading to segregation of body size distributions.

However, character displacement in response to resource competition is not the only explanation for this pattern. Traits that reinforce reproductive isolation among closely related species can be expected to evolve rapidly (Eberhard 1985, 1986) and staggered spacing in the timing of spiderling maturation is potentially one such trait. Interestingly, the one morphological trait that differs most clearly among these *Anelosimus* species – the size and shape of the male pedipalps – may reinforce reproductive isolation because the male pedipalps function as secondary sperm transfer organs that are hypothesized to have a close evolutionary relationship with the female genitalia (Eberhard 1985). Unfortunately, males remain unknown for some of the species, so that the generality of this observation cannot yet be established.

If even spacing or over-dispersion of traits results from species interactions (Rabosky et al. 2011), then species must have coexisted for sufficient time for trait displacement to occur. We cannot precisely estimate how long these species have coexisted, but we can evaluate the evidence for or against sympatric speciation, which would imply lengthy coexistence since species origin, versus allopatric speciation, which would imply more recent coexistence through subsequent community assembly.

To infer sympatric speciation, stringent conditions must be fulfilled: groups hypothesized to arise via sympatric speciation must form monophyletic endemic species flocks, and the biogeographic history of the groups must make the existence of an allopatric phase unlikely (Coyne and Orr 2004).

Table 2. Phenological data on 10 coexisting species of *Anelosimus* from the Périnet transect demonstrating differences among species in size, habitat, and phenology. Column one is total body length based on taxonomic descriptions and observed variation. Column two is the relative abundance of each species in open versus closed forest. Columns 3–8 give seasonal changes in relative abundances, and number of adults and eggs, all highlighting phenological differences among species.

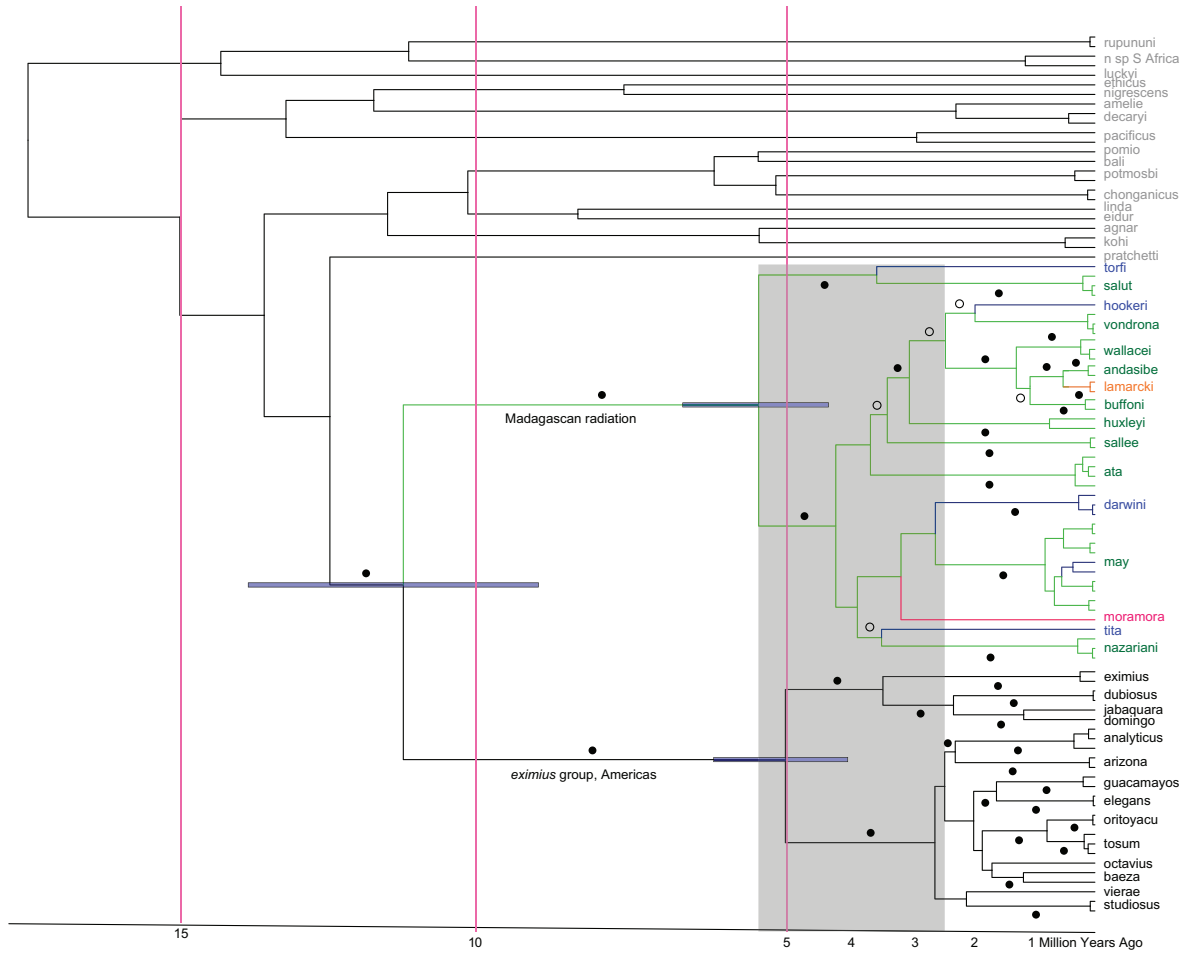| Species	Female length (taxonomic)	% total colonies found in closed forest	Δ relative abundance April vs November	% individuals adult in April	% nests containing adults in April	% individuals adult in November	% nests containing adults in November	% colonies with egg sacs in November
salut	3.6	0.14	0.57	4.6	83.3	100	100	10
nazariani	7.15	0.37	0.76	4.1	83.8	65	72	8.3
may	5.25	0.8	0.42	66.7	66.7	1.5	57	0
ata	5.15	0.38	0.94	1.9	69.5	3.4	50	13.6
buffoni	4.1	0.96	0.57	8.5	90	1.7	26.7	10
vondrona	5.25	0.01	0.08	3	77.2	4.9	21.4	15.1
andasibe	4	0.91	0.55	14.3	88.2	2.7	20	23.53
wallacei	4.1	0.78	0.42	6.2	93.3	0.1	17.6	25
huxleyi	5.25	0.2	0.58	5.8	100	0	0	20
sallee	3.5	0			9	100		0

Figure 5. A BEAST chronogram of world representatives of *Anelosimus* suggests that the Madagascar radiation is recent. Early speciation events correspond to a Pliocene period when grasslands were expanding causing forest fragmentation. The Madagascan radiation is monophyletic and sister to the American *eximius* group. Other *Anelosimus* used here as outgroups are identified with a grey font. Terminals from Madagascar are color coded based on locality, Périnet in green, Ambohitantely in blue, Montagne d'Ambre in red and Ranamofana in orange. Note non-monophyly of the focal group of study, the coexisting species from Périnet. Grey bar indicates key period of diversification within the Madagascan radiation, corresponding to a time when forests were being replaced by grasslands. Bars on branches indicate 95% confidence limits on age estimates. Solid dots indicate strong support (100% posterior probability), open dots moderate support (85–99%) based on MrBayes results.

Our system does not fulfill these conditions and instead is consistent with the more typical allopatric speciation model. Speciation within *Anelosimus* occurred in the Pliocene, probably during cycles of global cooling and warming resulting in forest fragmentation and re-expansion that persisted through the Pliocene and Pleistocene (Vences et al. 2009) (Fig. 5). Under this scenario, the current species assembly of multiple similar congeners that coexist locally resulted from secondary contact and sympatry during forest re-coalescence. Differences among species in size, habitat preferences, and seasonality may thus have evolved in the absence of any interactions among these species. Yet the clear boundary between open and closed forest and conspicuous changes species abundances across this boundary are suggestive of species interactions and competition influencing species assembly. In support of this argument, we see less evenness in species abundances in open forest where *A. vondrona* and *A. ata* dominate, and movement into open forest habitat when abundances of one of these dominant species changes. For example, *A. wallacei*, *A. andasibe*, and *A. buffoni* were not

documented in open forest in April, but all three occurred in open forest in November, when abundances of *A. ata* had dropped by 95% (Fig. 2).

In conclusion, our study integrates phylogenetic and ecological approaches in the study of local species coexistence and possible character displacement. Studying the coexistence of 10 related and similar species of subsocial spiders, we asked if these species originated in sympatry or allopatry and if species traits showed evidence of classical character displacement. Collectively, our results suggest that body size and other morphological traits in *Anelosimus* are best explained by evolution in allopatry, followed by community assembly during Pliocene expansion of forest fragments, rather than by interspecific competition and the evolution of morphological differences within local assemblages. This is an unusual assembly pattern that permits multi-species congeneric coexistence. *Anelosimus* contrasts with assembly patterns seen, for example, in *Tetragnatha* spiders in Hawaii where mini-radiations have evolved into distinct ecomorphs with limited coexistence among similar relatives (Gillespie

2004). Competition among species may help explain different patterns of species abundances in open vs closes forests. Differences in *Anelosimus* phenology could have emerged relatively rapidly after species assembly and may reflect resource partitioning, phenotypic plasticity in development, or the maintenance of reproductive isolation.

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Supplementary material (Appendix ECOG-01930 at <www.ecography.org/appendix/ecog-01930>). Appendix 1.