Author for correspondence:
John J. Wiens
e-mail: wiensj@email.arizona.edu

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Do mutualistic interactions last longer than antagonistic interactions?

Yichao Zeng and John J. Wiens

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

YZ, 0000-0001-5004-8780; JJW, 0000-0003-4243-1127

Species interactions are crucial and ubiquitous across organisms. However, it remains unclear how long these interactions last over macroevolutionary timescales, and whether the nature of these interactions (mutualistic versus antagonistic) helps predict how long they persist. Here, we estimated the ages of diverse species interactions, based on phylogenies from 60 studies spanning the Tree of Life. We then tested if mutualistic interactions persist longer than antagonistic interactions. We found that the oldest mutualisms were significantly older than the oldest antagonisms across all organisms, and within plants, fungi, bacteria and protists. Surprisingly, this pattern was reversed in animals, with the oldest mutualisms significantly younger than the oldest antagonisms. We also found that many mutualisms were maintained for hundreds of millions of years (some greater than 1 billion years), providing strong evidence for the long-term stability of mutualisms and for niche conservatism in species interactions.

1. Introduction

Species interactions are widespread and crucial across organisms. Eukaryotic cells are powered by an ancient mutualism with bacteria (mitochondria), whereas plant cells are powered by two (mitochondria, chloroplasts). Most plants rely on animals for reproduction (i.e. pollination; [1]) and on mycorrhizal fungi in their roots to obtain water and nutrients [2]. Much of Earth's land surface (approx. 7%) is covered by lichen [3], a mutualism between fungi and algae. Coral reefs, the ocean's most species-rich habitat (approx. 25–33% of marine species), involve a mutualism between cnidarian animals and protists [4,5]. Most animals rely on other animals and/or plants for food. In response, both animals and plants have evolved a staggering diversity of behavioural, morphological and chemical adaptations to avoid being eaten [6,7]. Many animals host unique species of parasites, mutualists and commensals, including mites, nematodes, microsporidian fungi, protists and bacteria [8]. Interactions with parasites can decimate host populations or even drive entire species to extinction (e.g. chytrid fungus and frogs; [9]).

An interesting pattern suggested by these examples is that many mutualistic interactions span thousands of species and/or hundreds of millions of years (e.g. eukaryotes–mitochondria, plant–chloroplast, angiosperm–pollinator, corals, lichens). Thus, the interaction is not simply between a pair of species, but rather between members of two clades that interact over time, even as individual species in each clade arise and go extinct (i.e. the interaction is older than any pair of interacting species). By contrast, many antagonistic interactions (e.g. disease) appear more transitory, involving fewer species and shorter timescales. Although some studies have discussed how long species interactions last macroevolutionarily [10], none have directly tested whether the type of interaction helps predict how long they are maintained.

Here, we test the prediction that species interactions with beneficial fitness effects for both interactors (mutualisms) last longer than those with negative fitness effects for one interactor (antagonisms). This pattern might be expected based on the simple idea that there may be selection on both interactors to maintain a mutually beneficial interaction, whereas there should be strong

selection on one interactor to end an antagonistic interaction. However, there is also an extensive literature suggesting that mutualisms may be unstable, given conflicts of interest between interacting species and the potential for cheating ([11–16], but see [17,18]). Testing whether mutualisms are stable or not requires something to compare them to (e.g. antagonisms) and a common metric that can be used to compare across diverse interaction types (e.g. age).

Previous studies have not addressed how the macroevolutionary ages of interactions are related to the interaction type. Nevertheless, many past studies may be relevant. The most relevant study [19] tested if there was greater phylogenetic conservatism in interactions that were antagonistic versus mutualistic, among 116 clades (mostly genera) across living organisms. They found significant phylogenetic signal in 69% of the antagonistic interactions, and 59% of the mutualistic systems. Phylogenetic signal/conservatism was assessed based on whether closely related species shared similar hosts. However, they did not address how long interactions persisted over time, and the timescale was relatively shallow.

There is also an extensive literature on the stability of mutualisms over time [13,15–18,20]. In general, any trait or interaction may persist longer if it is associated with positive diversification rates (speciation > extinction) and lower transition rates to alternative states. The interdependence of species in obligate mutualisms may make such mutualists more vulnerable to extinction [10,21,22], potentially decreasing their long-term persistence. Theoretical work suggests that mutualists are susceptible to extinction and reversals to antagonisms [23]; yet, other theoretical research found that mutualisms may be stable, despite cheaters, because of competition between cheaters and mutualists [17]. Furthermore, empirical studies found that bacteria rarely return to parasitic or autonomous states once they become mutualists [24,25]. A review of eight phylogenetic studies across diverse organisms found that gains of mutualism were more common than losses [18]. In primates, a mutualistic lifestyle (frugivory) was more phylogenetically conserved than an antagonistic one [26]; however, these studies did not directly address how long these interactions persisted over time.

Mutualisms might also increase diversification rates [27–29], and thereby increase long-term persistence of mutualist lineages (by reducing extinction). Yet, the relationship between mutualism and diversification has been controversial [10,25,28–30]. A recent systematic review [31] found that mutualisms generally increase diversification, whereas antagonisms generally decrease diversification (in the victim clade), thus mutualisms might last longer than antagonisms. However, these effects on diversification do not directly address the long-term persistence of these interactions.

The evolution of parasite virulence may be relevant to the persistence of antagonistic interactions. An early view suggested that parasites should become commensalists to increase their long-term survival, if host survival is required for transmission [32]. Indeed, some empirical studies found reduced virulence in pathogen–host interactions [33]. However, this view was challenged by considering competition among pathogen strains, infectious period and transmission rate [34]. No studies have broadly inferred how long these antagonistic interactions persist, especially in comparison to mutualisms.

Here, we test if the type of species interaction has predictable effects on the persistence of these interactions over macroevolutionary timescales. We compile analyses of species interactions and time-calibrated phylogenies of interacting clades. We obtain estimates of the age of each interaction (i.e. how long each interaction has lasted, from its origin to the present day). We then test whether mutualistic interactions are significantly older than antagonistic interactions, comparing the oldest interactions in each category (i.e. whether mutualisms can last longer than antagonisms). Our results generally support the idea that mutualisms persist longer than antagonisms, with an important exception.

2. Material and methods

(a) Finding case studies

We used two approaches to find case studies. First, we conducted systematic searches for macroevolutionary studies of species interactions [31]. We conducted 11 searches using the Web of Science Core Collection on 25 September 2019. Each search used one of 11 pairs of keywords, with one word from each of two groups (connected with 'AND'). The first group was related to species interactions, including 'amensalism', 'commensalism', 'competition', 'herbivory', 'mutualism', 'neutralism', 'parasitism', 'pollination' and 'predation', and the general terms 'symbiosis OR ectosymbiosis OR endosymbiosis' and 'species interaction'. The second group focused on macroevolution (diversification OR macroevolution). The document type was 'Article'. These searches yielded 4162 papers. We searched relevant papers for those containing ancestral-state reconstructions of species interactions on time-calibrated phylogenies.

As a second approach, we systematically reviewed recent summaries of different types of antagonistic and mutualistic interactions [35–37]. We then searched for time-calibrated phylogenies of the relevant taxa using Google Scholar (September–November 2019); both approaches were systematic, and we did not 'cherry pick' studies to favour a particular outcome.

Our goal was to compare how long interactions of each type can last. Therefore, we assembled studies that addressed the oldest origin of a given interaction (e.g. oldest origin of insect pollination in angiosperms, not every change in pollination); this reduced sample sizes, but we nevertheless found 60 usable case studies. Details of each study are in the electronic supplementary material, appendix S1, studies are summarized in table S1 and listed in table S2.

(b) Inferring ages of interactions

For antagonistic interactions, we generally used the time when the interaction originated in a phylogeny of the antagonist (e.g. parasite). For a few clades without time-calibrated phylogenies, we used estimated ages of their host clades (electronic supplementary material, table S2). For mutualisms, we used the time when the mutualism originated, from the phylogeny of either mutualist clade (this should be identical for both). All age estimates are imperfect but this should not bias the results (i.e. making antagonisms older versus younger than mutualisms). Whenever possible, we used published ancestral-state reconstructions to infer the branch where the interaction originated (11 out of 60 studies; electronic supplementary material, table S2). We then used the crown age of the clade above that branch as the minimum age of the interaction. We required that ancestral-state reconstructions show a continuous interaction to the present day (even if the interaction was lost and/or changed partners in some species). Thus, we required an uninterrupted series of nodes sharing that interaction, from the interaction's origin to the present day, in some or all extant taxa.

When no ancestral reconstruction was available, we used the crown age of the clade in which the interaction was generally

present (electronic supplementary material, table S2), but absent in related clades. If these criteria were not met, we excluded the study. Thus, if an interaction was present in most clade members, we considered the trait present in the ancestor, especially if species lacking that interaction were far from the root. The ancestral state of a clade will be determined by those nodes closest to the root. Therefore, these inferences are not necessarily any less accurate than those based on explicit ancestral-state reconstructions. Some papers estimated these interaction ages, and we used their estimates. When a range was given, we used the midpoint. Details for each study are in the electronic supplementary material, appendix S1.

When inferring interaction ages, the interaction was expected to be continuous between clades over macroevolutionary time. We inferred that a parasite clade continuously interacted with a host lineage over macroevolutionary time if the host species formed a clade. In some cases, the parasite clade was younger than their host clade, but the interaction nevertheless appeared to be continuous over time for the parasite. We did not require co-speciation between hosts and parasites. However, co-speciation was considered evidence of long-term interactions. Host-switching suggests instability, but host-switching among close relatives was not considered problematic. For example, there is not strict co-speciation between most plants and their insect pollinators [38,39], but pollination often involves one plant clade (angiosperms) and one insect clade (lepidopterans). Therefore, we included insect pollination of angiosperms as a long-term interaction between these clades. Interactions with unclear durations were excluded.

We did not include exclusively fossil-based studies. We only used interactions in which lineages and interactions persisted to the present, with ages based on time-calibrated phylogenies, to ensure that all results were comparable.

These reconstructions did not necessarily correct for transition-rate-heterogeneity [40] or state-dependent diversification [41,42]. Although this could lead to some errors, this should apply to both antagonisms and mutualisms. For example, both mutualisms and antagonisms (for the antagonist) can increase diversification [31]. Furthermore, many studies lacked the necessary data to apply methods that could address these issues (e.g. large-scale, species-level trees), especially at deeper timescales.

Similarly, we did not incorporate uncertainty in ancestral-state reconstructions or estimated clade ages. This information was not available for every study, and it is unclear how it could be incorporated. We analysed a single estimated age for each study. Most importantly, there is no clear mechanism by which uncertainty in reconstructions or clade ages should bias our conclusions (e.g. systematically make mutualisms older than antagonisms). Instead, this uncertainty should impact both interaction types, making it harder to find a significant pattern.

Finally, these estimated ages give only the current age of each interaction. Ideally, we would know how long each interaction lasts from beginning to end. The 'end' would be when all members of one or both clades went extinct or transitioned to another interaction type (or interacting clade). Of course, interactions occurring now will end in the future. Nevertheless, interactions that arose earlier and persisted to the present clearly lasted longer than those that arose more recently. Thus, we assume that interactions of different types have been originating over the evolutionary history of each group, and that more short-lived interaction types are less likely to persist from an ancient origin until the present in each group. The oldest interactions of more short-lived interaction types will, therefore, be younger than the oldest interactions of other types. Conversely, more stable types of interactions can persist from an ancient origin until the present, and so the oldest interactions of these types should be older. In summary, even without knowing the future, we know that interactions which persisted from their origin to the present have lasted for that time period, and these time periods can give us insights into how long different interactions (and interaction types) can last.

(c) Classifying interaction types

In each study, we classified interactions as predominantly mutualistic or antagonistic for the focal clade. Mutualisms benefit individuals of both interacting clades [36]. Benefits included nutrition (e.g. providing food, facilitating digestion), fertilization, dispersal, defence, shelter and cleaning. Antagonisms benefit one participant but harm the other (e.g. predation, parasitism, herbivory). Most antagonisms were related to feeding (getting nutrition while harming the provider). We did not include competition, because competition is not an antagonism nor mutualism (i.e. it is negative for both participants).

Species interactions can be variable and context-dependent within species in terms of costs and benefits [43]. We assumed the original studies characterized each species correctly. We also assumed that our characterization of interactions as positive or negative applied to most species in each clade. We see mischaracterizations of individual species as random error that would prevent us from finding significant effects of interaction types on ages.

(d) Statistical analyses

Our main question was whether mutualisms last longer than antagonisms. Therefore, we primarily focused on testing whether the oldest mutualisms are older than the oldest antagonisms. The mean ages of each interaction type may reflect the frequency at which interactions originate, with younger mean ages potentially reflecting many recent origins of these interactions, regardless of their long-term persistence. On the other hand, if one type of species interactions is more stable than another, it should be reflected in the oldest interactions of that type being older. Therefore, we focused on comparing the oldest ages among interaction types, not means or youngest ages.

We compared the upper 0.95th quantiles in ages of antagonisms and mutualisms to test how the oldest tails of the age distributions differ (i.e. which type lasts longer). The specific value of 0.95 is arbitrary, but is analogous to the standard cut-off for statistical significance. We performed general quantile comparison across two groups [44] based on a distribution-free quantile estimator [45]. The two groups were the two interaction types (antagonistic, mutualistic). This test was performed using the function 'qcomhd' in the R package *WRS2* [46]. *p*-values were determined through bootstrapping. A sample size of greater than or equal to 20 observations per group is recommended to ensure a Type I error probability close to 0.05 [44,46]. We, therefore, flagged results with smaller sample sizes. We compared all antagonistic to all mutualistic interactions, and performed separate tests for specific taxa, including animals, plants (including algae), fungi, protists and bacteria. To maximize power while controlling for multiple comparisons, we applied a sequential Bonferroni procedure [47] to each table of results.

When we analysed all interactions together, we counted each interaction only once (*n* = 60), rather than counting both interacting clades separately. However, when we analysed each major group separately, we included some interactions in the separate analyses of both interacting groups. For example, lichens were included when analysing fungi separately and when analysing plants/algae separately, but were counted only once when analysing all taxa together. We only counted interactions that arose within the group, and not before. Note that our units of analysis here are species interactions (not clades), and that a single species can be involved in many different interactions of very different ages.

Our main analyses included all data (especially the oldest data points), but we also performed analyses in which we tested for and excluded outliers. Specifically, we performed Grubb's test (following recommendations [48]). We identified and removed one outlier at a time and repeated this until no outliers remained (i.e. normality can be assumed). We did not perform this test on groups with less than seven data points [49]. We used the 'grubbs.test' function
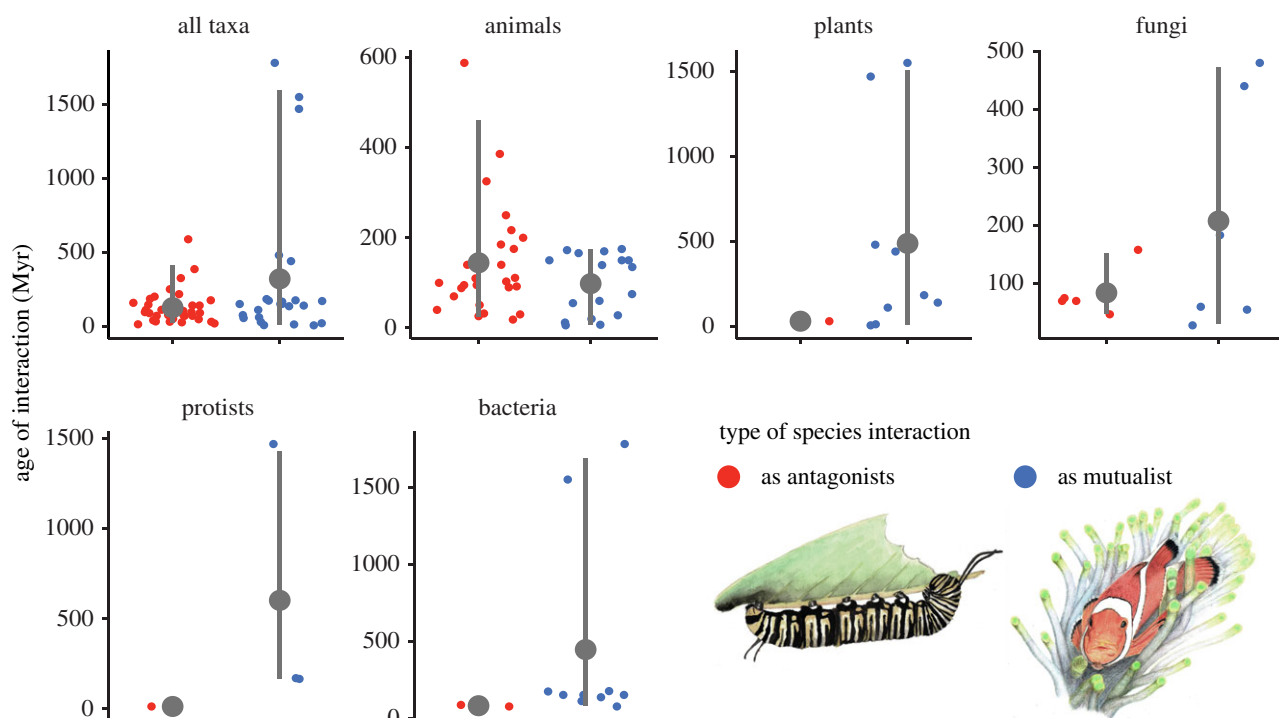
Figure 1. Comparison of the ages of antagonistic and mutualistic species interactions. Each red circle (left) is the estimated age of an antagonistic interaction, whereas blue circles (right) are mutualistic interactions. Upper and lower ends of grey bars indicate the estimated 0.95 and 0.05 quantiles, respectively. Our primary focus here is on comparing these upper 0.95 quantiles (the oldest interactions of each type in each group), and not the means (grey circles). These oldest interactions tell us how long these interactions can persist. Note that for each separately analysed group, the antagonist data points are cases where members of that group are the antagonists, not the victims. Data are in the electronic supplementary material, table S2. Illustrations by Yu Long, Peking University.

in the R package *outliers* [50]. However, excluding the oldest data points is clearly problematic, given our main question.

Our main analyses compared the oldest ages of each type, but we also compared the central tendencies (but see strong caveat above). We performed a Shapiro–Wilk normality test on the combined age data using the R package *stats* [51]. For most comparisons, data were not normally distributed. Therefore, we $\log_{10}$-transformed the ages and performed a Welch's *t*-test using *stats*. We also used nonparametric tests for untransformed ages in all comparisons (Wilcoxon rank-sum test) because some were still not normally distributed after log transformation. All tests were two-sided, with a significance level of 0.05.

Our tests were not phylogenetically corrected, for several reasons [31]. First, a phylogenetic correction assumes that species are not independent data points because they can share a trait owing to common ancestry. However, this assumes that the trait is homologous among species (i.e. shared owing to common ancestry). Here, a phylogenetic correction would treat all mutualistic interactions as homologous, and all antagonistic interactions as homologous. This assumption is clearly incorrect, and potentially misleading. Furthermore, we focused on traits that arose separately within each clade, and these separate origins should be phylogenetically independent. Therefore, a phylogenetic correction should be unnecessary. Moreover, a phylogenetic correction would not be straightforward here. Most phylogenetic methods correct statistical analyses using branch lengths, but here the branch lengths were the primary data. Nevertheless, we analysed major taxa separately, which helped identify clade-specific effects.

3. Results

We obtained estimated ages for 60 species interactions, including 36 antagonistic interactions and 24 mutualistic interactions. These are briefly summarized in the electronic supplementary

material table S1, listed in full in table S2, with details on each in appendix S1. We label the sample size for antagonisms as nA and for mutualisms as nM.

We primarily compared the oldest interactions of each type (0.95 quantile comparison). When all clades were combined, the oldest mutualisms were significantly older than the oldest antagonisms (figure 1 and table 1; $nA = 36$, $nM = 24$, $p = 0.026$). Consistent with this overall pattern, the oldest mutualistic interactions were also significantly older than the oldest antagonistic interactions in plants, fungi, protists and bacteria (table 1; plants: $nA = 1$, $nM = 9$, $p < 0.001$; fungi: $nA = 5$, $nM = 6$, $p = 0.031$; protists: $nA = 1$, $nM = 3$, $p < 0.001$; bacteria: $nA = 2$, $nM = 10$, $p < 0.001$; with plants, fungi, protists and bacteria as the antagonists in their respective antagonistic interactions). However, in these group-specific tests, Type I error might be larger than suggested by *p*-values of less than 0.05 (table 1), given limited sample sizes (see Methods). Nevertheless, the patterns within these four taxa were consistent with the overall pattern across all five groups (figure 1).

By contrast to this overall pattern, the oldest interactions in which animals were mutualists were significantly younger than the oldest interactions in which they were antagonists (figure 1 and table 1; $nA = 27$, $nM = 17$, $p = 0.001$). We also performed separate tests for interactions involving insects, which were involved in many of these interactions ($n = 33$). Antagonisms were older in insects, including insect–host interactions and insect–plant interactions (electronic supplementary material, table S3). Furthermore, antagonistic insect–host interactions (insects as antagonists) were older than mutualistic ones, based on comparisons of upper and lower quantiles and central tendencies ($nA = 20$, $nM = 2$; table 1; electronic supplementary material, tables S4 and S8).

Table 1. Comparisons of the oldest ages between antagonisms and mutualisms. (The 0.95th quantile (i.e. oldest tail) of each distribution is compared. Positive differences (Myr; million years) indicate that mutualisms are older than antagonisms. Significant results are in bold. *p*-values with asterisks indicate Type I error rates that may be greater than 0.05, given sample sizes in one or more categories less than 20. All results are significant based on a sequential Bonferroni test [47].)

	0.95 quantile (Myr)		
	antagonistic	mutualistic	difference (Myr) and *p*-value
all interactions	412.25 (*n* = 36)	1591.79 (*n* = 24)	1179.64 (**p = 0.035**)
plants as antagonists or mutualists	30.00 (*n* = 1)	1507.71 (*n* = 9)	1477.71 *(**p < 0.001**)
fungi as antagonists or mutualists	152.67 (*n* = 5)	473.05 (*n* = 6)	320.38 *(**p = 0.023**)
bacteria as antagonists or mutualists	84.86 (*n* = 2)	1692.02 (*n* = 10)	1607.16 *(**p < 0.001**)
protists as antagonists or mutualists	12.82 (*n* = 1)	1431.11 (*n* = 3)	1418.29 *(**p < 0.001**)
animals as antagonists or mutualists	460.02 (*n* = 27)	173.72 (*n* = 17)	−286.30 *(**p = 0.001**)

Despite these patterns in the ages of the oldest interactions, we found few significant differences in the central tendencies of ages between antagonisms and mutualisms (electronic supplementary material, table S4). When comparing youngest ages, results were mixed (electronic supplementary material, table S5). However, these central tendencies and youngest ages were not our focus here.

We also performed analyses in which outliers were tested for and removed (electronic supplementary material, table S6), although this removed the oldest interactions. The results were generally similar in individual groups, because few outliers were identified (electronic supplementary material, tables S7–S8). However, when all data were combined, eight data points (all greater than 300 Myr old, five non-animal mutualisms, three animal antagonisms) were considered outliers (13.3% of the data) and the difference in oldest ages between mutualisms and antagonisms was not significant (electronic supplementary material, table S7). The oldest ages of antagonisms in animals remained significantly older than the oldest mutualisms after removing the three oldest antagonisms as outliers (electronic supplementary material, table S7).

Finally, we performed a set of *post hoc* analyses that combed all non-animal groups (electronic supplementary material, table S9), given the striking differences between animals and other groups (figure 1). Mutualisms were significantly older (both oldest ages and central tendencies), and after removing outliers.

4. Discussion

In this study, we test whether mutualistic species interactions tend to last longer than antagonistic interactions over macro-evolutionary timescales, based on 60 case studies spanning the Tree of Life. Our results across all clades show that the oldest mutualisms are significantly older than the oldest antagonisms. Furthermore, most major groups show this trend, including bacteria, protists, fungi and plants (figure 1). Intriguingly, animals show the opposite pattern, with the oldest antagonisms older than the oldest mutualisms. To our knowledge, the idea that mutualisms have longer macro-evolutionary persistence than antagonisms has not been tested before, and the finding that animals show a reversed pattern is particularly surprising.

(a) Are these patterns real or artefactual?

We recognize that some readers may be skeptical of these results, for several reasons described below. However, none would explain why one interaction type is significantly older than the other. First, it might appear that our results are explained by outliers in each group (figure 1), and so do not reflect a strong pattern. However, there are two exceptionally ancient mutualisms each in plants, fungi and bacteria, rather than a single extreme value in each one (figure 1). Moreover, in protists, even though sample sizes are small, all mutualisms are older than the antagonisms (figure 1). Thus, none of the patterns in these five comparisons are explained by a single outlier (but note that some mutualisms are shared between these groups). Furthermore, although all groups show many younger data points and few older points (for both interaction types), this asymmetry is actually the expected pattern. All time-calibrated phylogenies of extant species have many younger clades and very few older clades, and so the branches on which species interactions evolved should show a similar pattern (i.e. a few very old interactions and many young interactions). Yet, that does not make the pattern we found inevitable either. For example, we could have found instead that the oldest interactions in all five groups were antagonisms, or that in each group one of the oldest interactions was a mutualism and the other was an antagonism. We also performed formal analyses to identify and remove outliers (electronic supplementary material, table S6). In each group, this generally had little impact on the results. When all groups were combined, the results were no longer significant, but 13% of the data were removed as outliers (which seems excessive). Moreover, these combined data mix two conflicting patterns: older antagonisms in animals versus older mutualisms in the other four groups (figure 1). Both patterns remain significant when analysed separately and after excluding outliers (electronic supplementary material, tables S7 and S9).

Second, we focused on sampling the oldest interactions in each group, not every interaction in every group. Thus, our results are not a comprehensive survey of interaction ages, and are biased towards older interactions. Yet, our goal was specifically to compare the oldest ages between interaction types. Furthermore, even though sample sizes were limited in some groups, the oldest interactions should be far more limited in number (see above). Again the oldest interactions are

the most relevant to our question. Similarly, the central tendencies in age distributions are not our focus here, since they may not reflect how long interactions can last (see Methods and below). We also note that we sampled relatively few microbial antagonisms (which were all relatively young). However, we included several bacterial studies overall ($n = 12$), and this disparity represents the few studies we found that showed long-term macroevolutionary antagonistic interactions involving these taxa (despite many antagonistic bacteria). Our results also hinge on which ancient interactions have been found and studied. Yet, most of the oldest interactions described here are very widespread (e.g. mitochondria, chloroplasts, mycorrhizae, lichen, coral, insect pollination; electronic supplementary material, table S2), not rare and geographically localized.

Third, we do not know exactly when each interaction evolved on each phylogeny. We localized the origin of each interaction to a specific branch, but it is unclear when exactly the interaction evolved on that branch. We used the youngest age (crown age) for each branch, which provides a minimum age. Therefore, the ages may be systematically underestimated. However, the main comparison should not be biased, because ages were calculated identically for both mutualistic and antagonistic interactions. There are also various reasons why the reconstructions may be incorrect (i.e. the interaction did not actually arise on the inferred branch). There may also be errors in the phylogenies and in the estimated clade ages, which could both impact the estimated interaction ages; but again, these errors should impact both interaction types, rather than biasing one type to be older than another.

Fourth, it can also be difficult to infer that two clades have been continuously interacting for long periods of time. For example, a clade that underwent many recent host switches among closely related host species might mimic a long-term interaction that was present in the ancestor of those host species. But again, these errors should not bias our results to favour one type of interaction being significantly older than the other. In summary, all these sources of error (and limited sample sizes) should make it harder to find the significant results that we found, and so are not justifications for dimissing our results as artefactual.

Finally, readers may reasonably be concerned that many comparisons of the central tendencies were not significant. Nevertheless, all the patterns that we found in the oldest ages were paralleled by differences in the means (i.e. mutualisms older in all groups except animals; figure 1). For both mutualisms and antagonisms we expect most data points to reflect relatively recent origins of new interactions (and for very few to be extremely old), and this is exactly what we found (figure 1). Therefore, the primary way that we expect differences in the persistence of interaction types to significantly impact these age distributions is in the oldest interactions of each type (i.e. the oldest tails). Nevertheless, when we combined all non-animal groups (electronic supplementary material, table S9), the central tendencies of mutualisms and antagonisms were indeed significantly different, with mutualisms older. This pattern might be obscured by the lower sample sizes in these non-animal groups (when analysed separately or when combined with animals). Furthermore, insects (the majority of animals) showed significantly older antagonisms than mutualisms when analysing their central tendencies (electronic supplementary material, table S4). Thus, our two main patterns remain largely supported.

(b) Why do mutualisms often last longer?

Assuming that the overall pattern of older mutualisms is correct (outside of animals), what might explain this pattern? In general, there are two obvious ways for species interactions to end: by extinction of one interacting lineage and/or transition to a different state (e.g. changing from antagonism to mutualism, or ceasing interactions with the other clade). Given this, one way that different interaction types might differ in longevity is through their impacts on diversification rates. If a mutualism increases diversification (i.e. speciation minus extinction), it might be less likely to be lost through either clade extinction or through transition to a different state, if many extant species retain the mutualism. Conversely, if antagonisms decrease diversification rates to the point where extinction exceeds speciation, then antagonisms may lead to extinction of the negatively impacted clade. The idea that mutualisms increase diversification has been controversial [10,29]. However, recent analyses found that mutualisms are associated with higher diversification rates overall [31]. Thus, in a given clade, species with a given mutualism have higher diversification rates than species that do not. Various processes may underlie this pattern (e.g. mutualists may have increased range sizes, increased ecological opportunity, greater potential for divergent selection among species associated with different mutualistic partners, and reduced extinction; [29,31]). More broadly, interactions with benefits for individuals of a clade generally seem to increase diversification rates, whereas antagonisms with costs for individuals of the host/prey clade generally decrease their diversification rates [31], but with some exceptions [52,53]. Thus, impacts on diversification rates might help explain patterns in interaction ages, but more work is needed to explicitly link ages and diversification rates. Simulations do suggest that reduced extinction rates associated with a given state will increase the ages of clades sharing that state [54].

Hypothetically, factors unrelated to mutualism or antagonism might help explain these patterns. For example, the data points that most strongly drive the overall pattern of older mutualisms involve organelles within eukaryotic cells (mitochondria, chloroplasts, secondary endosymbiosis of algae in protists), each approximately 1.5 billion years old (figure 1). The oldest mutualisms in animals also include single-celled participants (one bacterial, one protist, both approx. 170 Myr old), but neither is very old relative to other interactions in animals. The oldest mutualisms involving plants include lichens and mycorrhizae (480 and 440 Myr old, respectively), but neither has participants that are only single-celled or intracellular. Although the oldest interactions across life involve single-celled organisms, the oldest organisms are also unicellular. Moreover, the results for animals show that intimate, endosymbiotic interactions with single-celled organisms do not guarantee that these interactions will last longer than antagonisms with multicellular organisms. In summary, we suggest that the observed differences in the ages of different interaction types may be related to the interactions themselves (and not some other factor), but more work on other factors related to interaction ages would be valuable.

There is debate about whether organelles of endosymbiotic origins (e.g. mitochondria and plastids) are presently endosymbionts [55–58]. Importantly, the oldest mutualisms involve these organelles (electronic supplementary material, table S1). We follow the mutualism literature [35] in

considering these organelles to be nutritional mutualists. There is no debate that these organelles are derived from free-living organisms and retain their own DNA, but some authors consider them to not be endosymbionts because some of their proteins are encoded by their host's nuclear DNA [55,56]. However, this distinction is debatable [59], especially because similar processes occur in other bacterial endosymbionts that are not organelles [60,61]. An important question is whether endosymbiotic organelles began as mutualists or whether endosymbiosis created a fitness cost for the free-living bacteria [62] or their hosts [63]. However, regardless of the interaction at the time of its origin, it is generally agreed that the relationship subsequently became mutualistic [35].

(c) Why are animals different?

An important exception to our finding that mutualisms tend to persist longer occurs in animals, in which the oldest mutualisms were younger than the oldest antagonisms (figure 1). One potential explanation is that the 'reversed' pattern in animals arose because animals originated as antagonists. Unlike many species in other groups (e.g. plants, fungi, bacteria, protists), most extant animals are heterotrophs that feed on other organisms, and appear to be descended from a heterotrophic, antagonist ancestor [64]. Thus, their earliest mutualisms may have evolved much later than their earliest antagonisms. More broadly, understanding why animals differ may shed light on why mutualisms appear to last longer in general. For example, interactions with positive fitness benefits for the focal clade (like mutualisms) generally seem to increase that clade's diversification rates [31]. However, this association seems to break down in animals, in which only about half of the studied clades show these increases.

In animals, all sampled interactions in which animals are antagonists might be descended from their origins as antagonists that fed on other organisms (electronic supplementary material, table S1). Therefore, one might argue that all their antagonist data points should be collapsed into a single data point associated with the origin of animals. We do not agree, as this would treat (for example) parasites and herbivores as equivalent. Nevertheless, this would still support our overall conclusion: that the oldest antagonism for animals is far older than the oldest mutualism.

(d) Niche conservatism and the stability of mutualisms

Our results are relevant to two controversial questions in ecology and evolution: the stability of mutualisms and the timescale of niche conservatism. Our results (figure 1) provide numerous examples of the long-term maintenance of mutualisms, including mutualisms that persisted for greater than 1 billion years involving bacteria, plants and protists, and two that are greater than 400 Myr old in fungi. Furthermore, all groups contain mutualisms that are hundreds of millions of years old. Even animals have numerous mutualisms that are greater than 100 Myr old (figure 1).

There has been considerable debate about the long-term stability of mutualisms [10–20]. Our results strongly support the idea that (at least some) mutualisms can be maintained over remarkably deep timescales. Reviews of phylogenetic and evolutionary studies suggested that mutualisms often 'breakdown', with one mutualistic partner becoming autonomous or parasitic [13,16]. However, the presence of some transitions out of mutualism is not inconsistent with their long-term

maintenance [18]. Indeed, these transitions are generally inferred when some clade members shifted from mutualism to another state, but the mutualism is maintained in other species. In summary, our results show numerous examples in all major groups of organisms in which clade-level mutualisms are maintained for hundreds of millions of years.

Finally, there has also been much debate about the maintenance (conservatism) of ecological niches over evolutionary timescales [65–69]. This literature initially focused on the climatic niche, and even strong proponents of climatic niche conservatism [65,68] suggested that this pattern occurs over limited timescales (e.g. among closely related species but not among genera in a family). Our analyses provide numerous examples in which a crucial aspect of the niche (species interactions) is maintained for hundreds of millions of years (or even greater than 1 billion years). Thus, our results provide some of the most extreme examples of niche conservatism to date.

The antiquity of these interactions is also intriguing because there is a tendency in the ecological literature to suggest that local-scale species interactions are primarily important at shorter timescales, whereas climate and other large-scale factors are important over longer timescales [70]. This may be based on the idea that local-scale species interactions are more transitory and short-lived. Here, we show numerous examples of the maintenance of local-scale species interactions (between clades) over hundreds of millions of years, in every major group of organisms across the Tree of Life.

5. Conclusion

In this study, we show, to our knowledge for the first time that the oldest mutualisms are generally older than the oldest antagonisms, with the intriguing exception of animals. Understanding the common mechanisms behind these shared patterns across diverse organisms (and the exceptions) should be an exciting area for future empirical and theoretical studies. Our results also suggest many other interesting areas for future research. For example, how long can a parasite continue to infect the same clade over macroevolutionary timescales, and what determines the longevity of this relationship? How is the longevity of both positive and negative interactions impacted by factors such as the degree of specialization of the participants, the level of association between them (e.g. endosymbionts versus less intimate interactions), and the type of transmission (e.g. vertical versus horizontal)? We note that the type of data that we use here (i.e. ages of interactions) could be used to address these and many other ecological and evolutionary questions. Finally, our results highlight that many species interactions between clades (especially mutualisms) can persist for hundreds of millions of years. These results may offer some of the strongest evidence to date for the stability of mutualisms and the conservatism of ecological niches.

8

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