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ORIGINAL ARTICLE

Geographically structured host specificity is caused by the range expansions and host shifts of a symbiotic fungus

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The inability to associate with local species may constrain the spread of mutualists arriving to new habitats, but the fates of introduced, microbial mutualists are largely unknown. The deadly poisonous ectomycorrhizal fungus *Amanita phalloides* (the death cap) is native to Europe and introduced to the East and West Coasts of North America. By cataloging host associations across the two continents, we record dramatic changes in specificity among the three ranges. On the East Coast, where the fungus is restricted in its distribution, it associates almost exclusively with pines, which are rarely hosts of *A. phalloides* in its native range. In California, where the fungus is widespread and locally abundant, it associates almost exclusively with oaks, mirroring the host associations observed in Europe. The most common host of the death cap in California is the endemic coast live oak (*Quercus agrifolia*), and the current distribution of *A. phalloides* appears constrained within the distribution of *Q. agrifolia*. In California, host shifts to native plants are also associated with a near doubling in the resources allocated to sexual reproduction and a prolonged fruiting period; mushrooms are twice as large as they are elsewhere and mushrooms are found throughout the year. Host and niche shifts are likely to shape the continuing range expansion of *A. phalloides* and other ectomycorrhizal fungi introduced across the world.

Introduction

As species experience range expansions, individuals encounter novel antagonists and mutualists (Richardson *et al.*, 2000; Torchin and Mitchell, 2004), and changes in the frequency, diversity and function of interactions may influence the persistence of populations in novel ranges (Mitchell *et al.*, 2006). The loss of predators or pathogens may facilitate the spread of a species (Torchin and Mitchell, 2004; Colautti *et al.*, 2004), whereas loss of compatible mutualists may constrain spread (Richardson *et al.*, 2000; Pringle *et al.*, 2009a, b).

Symbionts introduced to novel ranges may bypass the constraints imposed by the lack of hosts from a native range by forming associations with novel hosts (Richardson *et al.*, 2000; Pringle *et al.*, 2009a, b). We define these novel associations as host shifts; a symbiont in a new range forms a functional symbiosis with a species it never encountered in its original range. A vast literature on microbial pathogens suggests that host shifts are common during range expansions (Anderson *et al.*, 2004; Woolhouse *et al.*, 2005; Slippers *et al.*, 2005; Stukenbrock and McDonald, 2008). In contrast to pathogens, very little is know about the potential for microbial mutualists to shift hosts as they spread, even though they are commonly introduced to new ranges (van der Putten *et al.*, 2007; Vellinga *et al.*, 2009). Host shifts and range expansions may be widespread, but go unnoticed because most microbial mutualists are difficult to observe and the effects of mutualists on hosts are less apparent than pathogens (Litchman, 2010).

The extent to which a mutualist will shift to novel hosts and expand its range after introduction to a novel habitat depends on (1) the level of specificity evolved between the mutualist and its hosts in native ranges and (2) the distribution of compatible hosts within novel ranges (Vellinga *et al.*, 2009). If a mutualist is a generalist in its native range, it may shift to a diversity of novel species in the introduced range. If compatible hosts are widespread, the mutualist may also become widespread.

If the mutualist is specific to a few hosts in its native range, it may either fail to establish or be restricted within the geographic distribution of a limited number of compatible hosts. There is some evidence to support the hypothesis that species with the ability to establish and rapidly spread in new ranges are generalists (Vázquez, 2005; Vellinga et al., 2009), but tests of the role of specificity in determining the outcomes of range expansions are rare. Moreover, it is unclear how patterns of specificity will unfold across geographically disjunct range expansions, where the availability of hosts and diversity of endemic mutualist communities may differ.

Host shifts during range expansions may also cause changes in the timing, abundance and composition of resources exchanged between host and symbiont. Changes in the resources provided by hosts to obligate symbionts may affect the fitness and persistence of symbiont populations (Kiers et al., 2003, Kuikka et al., 2003, Markkola et al., 2004, Oono et al., 2009). Data on these phenomena are scarce, and as far as we are aware, niche shifts associated with the range expansions of microbial mutualists, and the functional implications of these niche shifts for the growth of the mutualist, have not been documented in the literature.

To explicitly test for a role of host specificity in constraining or facilitating the range expansion of a microbial mutualist, we focus on an ectomycorrhizal (EM) fungal species, *Amanita phalloides*. EM fungi form symbiotic associations with the roots of woody plant species, providing soil nutrients in exchange for carbon from host plants. EM fungi generally function as mutualists, but may occasionally function as parasites (Karst et al., 2008). Associations of EM fungi with hosts can be described using two different metrics at regional and local scales: (1) host specificity describes the richness of all potential hosts across a geographic range of a species of EM fungus ('host range' in Molina et al., 1992), whereas (2) host selectivity describes the frequency with which an EM fungal species associates with a particular host plant (or plants) in a specific local environment (similar to 'ecological specificity' in Molina et al., 1992). A given species of EM fungus might have low host specificity (and be a generalist) across the breadth of its distribution, but in a particular forest might be highly selective and associate with only a few of the available host species, either because of abiotic or biotic controls. We are currently unaware of other studies that have clearly documented geographic variation in host selectivity of an EM fungal species, but this phenomenon has been observed in the lichen symbiosis (for example, Yahr et al., 2006) as well as with antagonistic interactions between plants and insects (Fox and Morrow, 1981).

A. phalloides is an EM fungus native to Europe and introduced to North America (Pringle and Vellinga 2006; Pringle et al., 2009a, b). This mushroom-forming basidiomycete is deadly poisonous, and since its introduction to North America, it has been responsible for numerous poisonings including several fatalities (Beug et al., 2006). It has established on both the East and West Coasts of North America, with different patterns of distribution and abundance within each of these ranges (Wolfe et al., 2010). On the West Coast, *A. phalloides* is widespread in California, and is less frequently found in disturbed habitats throughout the Pacific Northwest (Oregon, Washington and British Columbia). On the East Coast, *A. phalloides* is less abundant and is generally restricted to forestry plantations and trees planted in disturbed habitats. Anecdotal reports suggest that in Europe *A. phalloides* most frequently associates with *Quercus* spp. or other species of the Fagaceae, but also infrequently associates with Pinaceae (Courtecuisse and Duhem, 1994). However, to date, there have been no systematic surveys of associations between *A. phalloides* and its hosts in either Europe or North America.

To understand whether changes in host specificity or host selectivity are associated with the range expansions of *A. phalloides*, we first compared the host associations of the fungus in its native and introduced ranges. We then measured host selectivity within individual forests, and tested whether host selectivity will constrain the spread of *A. phalloides* in California. We also asked whether shifts to novel hosts cause changes in autecology or niche, as measured by stable isotopes of carbon and nitrogen, reproductive output and phenology.

In the aggregate, our data document a mutualism in flux; the different approaches to probing specificity, selectivity and changes in autecology provide a synergistic portrait of a species reaching new ranges, selectively associating with endemic plants and behaving differently in association with its novel hosts. Moreover, and to the best of our knowledge, our study provides the first data on the potential role of hosts in shaping the outcomes of invasions by microbial mutualists at an intercontinental scale.

Materials and methods

Host specificity across Europe and North America
To document the host associations of *A. phalloides* in its native and introduced ranges, we used data collected from herbarium specimens (positively identified as *A. phalloides*) or collection reports that clearly noted which species or genus of tree was found in association with an *A. phalloides* mushroom (Supplementary Table S1). Most data are from specimens collected or observed within the past 20 years, and were taken from sources that covered the entire range of each of the three ranges included in this study (Europe, East Coast of North America and the West Coast of North America). If an individual data source from a particular region had a very large number of records over the past

20 years (for example, the Fungal Records Database of Britain and Ireland), we only used the past 5 years worth of available data to avoid including a disproportionate number of records from any country or region.

Because of the large geographic scale of this study, herbarium records are the only available proxy for host associations. Herbarium records have limitations for determining host associations. Hosts of EM fungi are usually identified by noting the species of tree closest to where the mushroom was collected. The hosts that are apparent to a collector of a mushroom might not be the hosts forming EM fungal root tips belowground. Quantitative estimates of potential discrepancies between perceived aboveground host associations and belowground host associations are lacking. However, any bias or limitation in herbarium records should be similar across the multiple biogeographic regions that we target; differences among biogeographic regions are likely to reflect differences in biology.

We used χ^2-tests to test for significant differences in the frequency of host genera among three major regions: Europe, the East Coast of North America and the West Coast of North America. We also calculated generic richness and Shannon's diversity index for host genera of the three regions, to make qualitative comparisons of host composition. We focused on generic diversity because many host identifications were only made to the level of genus. We then quantified the number of host shifts experienced by A. phalloides by determining the number of endemic North American plant species found to associate with the fungus. In this analysis, we restricted our data set to only those records where hosts were identified to species.

Stable isotope measurements of niche shifts

Associations between host shifts and changes in the nutritional dynamics of A. phalloides were determined by quantifying the stable isotope composition of nitrogen and carbon in mushroom tissue across each of the ranges of A. phalloides (Supplementary Table S2). We specifically measured these isotopes because changes in nitrogen and carbon within mushroom tissues can reflect differences in host associations (Högberg et al., 1999; Taylor et al., 2003), although other variables can also influence these kinds of data (see Discussion). Homogenized samples of dried gill tissue were analyzed for carbon and nitrogen composition using standard protocols (Hobbie et al., 2001; see Supplementary Methods). To determine the statistical significance of differences in stable isotope data from the three regions (Europe, East Coast and California), we used Kruskal–Wallis nonparametric tests. For post hoc comparisons, we used Mann-Whitney U-tests. A Bonferroni correction was used to correct for multiple comparisons.

Mushroom biomass and phenology

We obtained mushroom biomass and phenology data from the same set of mushrooms that we used to infer host associations. Phenology data were supplemented with additional records from Mushroom Observer (http://www.mushroomobserver.org), additional herbarium specimens that did not contain host information, and our own observations in the field. We only used Mushroom Observer records where it was clear from photographic evidence that the mushroom collected was A. phalloides.

To obtain biomass data, individual mushrooms that were fully intact and fully mature (each cap was completely open, with all gills fully exposed) were weighed after being dried for 24 h at 37 °C. Here, we define a population as a set of mushrooms occurring within a forest stand, not larger than 50 m × 50 m that are clearly clustered together and separated from another set of mushrooms by at least 1 km. Individual genets of A. phalloides are small (generally less than 0.5 m), and often individual mushrooms collected near to one another are unique genetic individuals (Pringle, unpublished data). For each population, we calculated mean biomass per mushroom, and used these population-level data for our statistical analyses. Phenology data were collected by determining the time of collection of a mushroom for a unique population. The date collected was converted to day of year (1 to 365) to standardize collection dates across years. To test for statistical significance of differences in mushroom biomass and phenology between the four biogeographic regions (Europe, East Coast, California and the Pacific Northwest), we used the same statistical analysis used with the stable isotope data.

To confirm that mushroom biomass is correlated with reproductive output in A. phalloides, we assessed the correlation between total mushroom biomass and potential reproductive output (biomass of spore producing tissue) using 34 mushrooms from across three populations within Point Reyes National Seashore (Heart's Desire, Drake's Landing and Horse's Trail). We dried the gill tissue (spore bearing structures) separately from the pileus, stipe, bulb and annulus tissue. We specifically collected mature mushrooms of different sizes so that we could capture the range of mushroom biomass variation.

Host selectivity within Californian forests

To assess host selectivity at a local scale within mixed forests, we sampled EM root tips from under A. phalloides mushrooms at three sites on the Point Reyes Peninsula. The three sites, Heart's Desire, Drake's Landing and Horse's Trail, are separated from each other by at least 2.5 km. Each site has a different composition of EM hosts, as described in Supplementary Material.

EM root tips were extracted from soil samples taken directly under mushrooms of A. phalloides at all three sites and also from randomly sampled soil

cores at Heart's Desire and Drake's Landing (as part of another study within these same plots; Wolfe *et al.*, 2010). We also collected aboveground tissues (leaves/needles) of potential EM hosts at each site. The host tissue present in each root tip colonized by *A. phalloides* was identified by comparing root tissue DNA sequences with aboveground tissue sequences (see Supplementary Methods).

Previous work from similar forests (Kennedy *et al.*, 2003) and our own preliminary observations suggested that conifer roots are abundant in soil cores collected at these sites, whereas angiosperm tree roots are less frequently encountered. We confirmed this observation by surveying the abundance of tree roots in the three plots where we also sampled root tips (see Supplementary Methods). The total number of roots that were successfully amplified by PCR at each site varied: Heart's Desire = 30, Drake's Landing = 25 and Horse's Trail = 29.

To test for host selectivity of *A. phalloides* at these three sites, we used a statistical approach described by Gilbert *et al.* (2008). We calculated the binomial probabilities of finding equivalent or greater numbers of root tips of *A. phalloides* associated with a particular host given the available abundance of host roots. We focused our statistical analyses on *Quercus agrifolia*, because mushroom observations suggested that it was the most frequent host of *A. phalloides* at Point Reyes (see results below). Binomial probabilities were calculated in JMP version 5.0.1a (SAS Institute Inc., Cary, NC, USA).

Testing for constraints on the distribution of A. phalloides *in California*

To determine whether the distribution of *A. phalloides* in California is constrained within the geographic ranges of its available hosts, we used a Monte Carlo randomization approach modified from Brown *et al.* (2009). We used a subset of the *A. phalloides* occurrence data reported in Wolfe *et al.* (2010); this subset comprised 75 recent, confirmed occurrences of *A. phalloides* associating with native hosts throughout California. Host distributions were obtained from United States Geological Survey (2006). We assessed overlap between *A. phalloides* and those species of trees that we determined to be hosts of *A. phalloides* in California (see Results); these hosts included *Q. agrifolia*, *Q. kelloggii*, *Q. wislizenii*, *Lithocarpus densiflorus*, *Corylus cornuta* var. *californica*, *Pseudotsuga menziesii* and *Pinus muricata*. We first determined the number of occurrences of *A. phalloides* inside the range of each host species, from the 75 total occurrences. To create a null distribution, we randomly chose 75 samples without replacement from a pool of 500 random points located throughout the range of *A. phalloides* in California, delineated by a minimum convex polygon around observed occurrence data. For these 75 random samples, we next determined whether they occurred within the range of the particular host species. The Monte Carlo randomization tested whether the probability of *A. phalloides* occurring within a potential host range was significantly greater than the probability of randomly placed sampling points across the current range of *A. phalloides*. The Monte Carlo simulation was run with 1000 replicates for each host species.

Results

Host specificity of A. phalloides *is geographically structured*

Surveys of host associations among more than 200 populations of *A. phalloides* across native and introduced ranges show that *A. phalloides* is not a generalist, and instead exhibits geographically structured host specificity (Figure 1). In Europe, *A. phalloides* associates most frequently with oaks and other species of the Fagaceae. On the East Coast of North America, where *A. phalloides* is restricted in its distribution as is rarely found in native forests, it associates most frequently with pines, which are rarely hosts of the fungus in Europe. In contrast, on the West Coast of North America, where *A. phalloides* is widespread and commonly found in native forests, host associations are more similar to the host associations documented for European populations, with oaks as the most frequent hosts. In California, *A. phalloides* associates almost exclusively with the endemic coast live oak (*Q. agrifolia*); 81% of all California records identified to species list *Q. agrifolia* as the host (Supplementary Table S1).

The frequencies of associations between *A. phalloides* and its four most commonly encountered host genera (*Quercus*, *Pinus*, *Fagus* and *Corylus*) differ significantly across Europe and the East and West Coasts of North America ($\chi^2 = 115.31$, $P < 0.001$), and in North America the fungus has shifted to associate with 11 novel hosts. A novel host is defined as a plant endemic to North America, for example, eastern white pine (*P. strobus*) and California's hazel (*C. cornuta* ssp. *californica*). The richness and diversity of host genera is lower in *A. phalloides'* introduced ranges, as compared with Europe (Supplementary Table S3), although the Pacific Northwest is an exception. In this region, *A. phalloides* associates with a diversity of non-native angiosperm species that have been planted in urban parks and yards, for example, a filbert native to Europe (*C. maxima*; Figure 1, Supplementary Table S1).

Changes in resource use, reproduction, and phenology

Stable isotopes of carbon and nitrogen from mushrooms collected across the native and introduced ranges correlate host shifts with changes in resource use. On the West Coast, we restricted sampling to California, because *A. phalloides* is widespread in California and because this state houses the
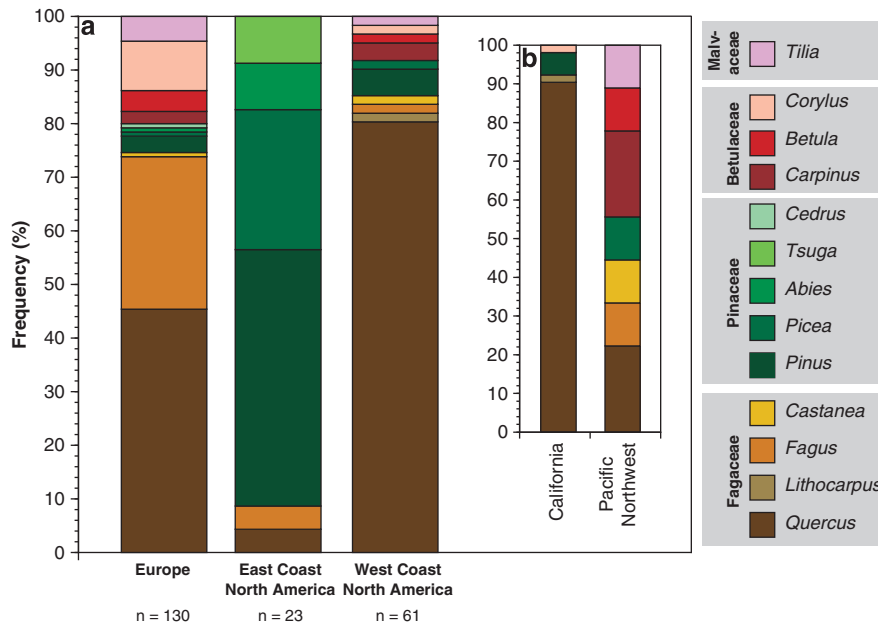
Figure 1 (a) Frequency of host associations across the native (European) and introduced (North American) ranges of *A. phalloides*. (b) West Coast data are divided into California and the Pacific Northwest to show that in California the dominant hosts are *Quercus* spp., whereas in the Pacific Northwest dominant hosts are from a mix of introduced angiosperm genera (see text for details). n = number of populations from each region.
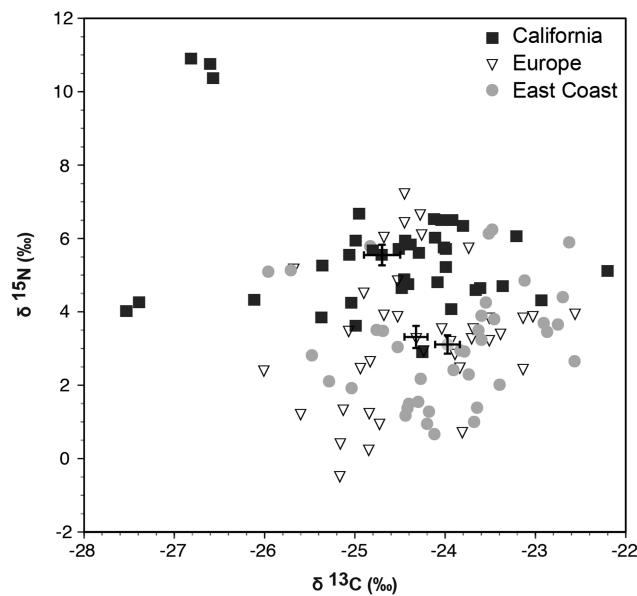

Figure 2 $\delta^{13}C$ ‰ and $\delta^{15}N$ ‰ of mushrooms collected in Europe, the East Coast of North America and California. Each data point represents a single mushroom. Values with standard error bars represent mean values for all mushrooms from each region (± 1 s.e.).

greatest density of North American populations (Wolfe *et al.*, 2010). Stable isotope signatures differed significantly among the three biogeographic regions for both $\delta^{13}C$ ‰ ($P = 0.016$) and $\delta^{15}N$ ‰ ($P < 0.001$; Figure 2). Mushrooms from California were more variable for both isotopes, with some mushrooms falling well outside of the range of $\delta^{13}C$ and $\delta^{15}N$ observed for both Europe and the East Coast. East Coast mushrooms were significantly less depleted in ^{13}C compared with mushrooms from Europe (Mann–Whitney U test, $P = 0.049$) and

California ($P = 0.0061$). The mean $\delta^{13}C$ value of mushrooms from California was more negative than European mushrooms, but this difference was not significant ($P = 0.3632$). Californian mushrooms were significantly enriched in ^{15}N compared with both East Coast ($P < 0.001$) and European ($P < 0.001$) mushrooms. There was no difference in $\delta^{15}N$ between East Coast and European mushrooms ($P = 0.5353$).

A total of 5 mushrooms from California, including the only 3 mushrooms sampled from one site (Albion) and 2 of 14 mushrooms sampled from

sites in Tomales Bay State Park, had stable isotope values that were well outside the range of all other observed values (Figure 2). We removed these samples from our data set and repeated statistical analyses. The differences in $\delta^{13}C$ were no longer statistically significant ($P = 0.085$), but differences in $\delta^{15}N$ were still significant ($P < 0.001$). Interestingly, the three sporocarps from Albion, among the most depleted in ^{13}C and the most enriched in ^{15}N, were the only mushroom collections associated with *L. densiflorus*.

Changes in host associations are also associated with changes in reproductive allocation and phenology. In California, mushrooms are almost twice as large as they are anywhere else ($P < 0.001$, Figure 3a). The timing of reproduction has also changed in California ($P < 0.001$), and mushrooms appear throughout the year. In contrast, mushrooms appear only in late summer or fall in Europe, the East Coast and Pacific Northwest (Figure 3b).

A. phalloides *selectively associates with coast live oak in Californian forests*

Molecular techniques used to identify the plant in EM root tips colonized by *A. phalloides* confirm that *A. phalloides* selectively associates with a subset of potential hosts within mixed forest communities. Root tips are the physical site of a symbiosis between an EM fungus and host. Across three sites within the Point Reyes Peninsula (Marin County, CA, USA), *A. phalloides* selectively associated with *Q. agrifolia*, even though the available root community was dominated by conifers, particularly *P. muricata* and *P. menziesii* (Figure 4). *Q. agrifolia* was identified as the host in 56% of the 48 EM root tip samples colonized by *A. phalloides*. In contrast, only 9% of the 70 available roots sampled across the three sites were *Quercus* roots; *P. muricata* and *P. menziesii* made up 88% of the rest. The binomial probabilities of finding these observed or greater numbers of *A. phalloides* root tips associated with *Q. agrifolia*, given the frequency of *Q. agrifolia* roots, were very low at each site (Heart's Desire: $P < 0.001$; Drake's Landing: $P < 0.001$; and Horse's Trail: $P < 0.001$), confirming that *A. phalloides* shows selectivity for *Q. agrifolia*.

A. phalloides *appears constrained within the range of coast live oak in California*

The coast live oak is the dominant host of *A. phalloides* across California, and selectivity may constrain the death cap to spread within and track the range of *Q. agrifolia*. To determine whether the distribution of *A. phalloides* in California is constrained within the geographic ranges of its available hosts, we used a Monte Carlo randomization approach to test whether the probability of *A. phalloides* occurring within a potential host range was significantly greater than the probability
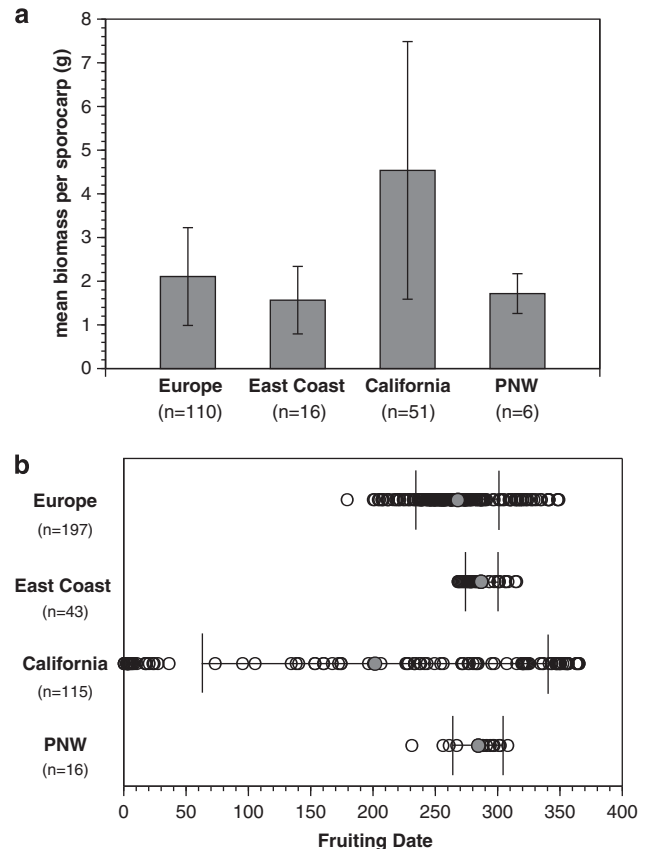
Figure 3 (**a**) Mean mushroom biomass and (**b**) timing of reproduction of *A. phalloides* across four biogeographic regions; n = number of populations from each region. Error bars represent ± 1 s.d. from the mean. In **b**, individual gray circles represent the mean value. Sampling intensity varies across different regions, but is proportional to the number of known populations in each region (Wolfe *et al.*, 2010). PNW, Pacific Northwest region of North America. Fruiting date indicates the day of the year (1 to 365).

of randomly placed sampling points across the current range of *A. phalloides*. The distribution of *A. phalloides* in California was most significantly associated with the distribution of *Q. agrifolia* (Figure 5, Supplementary Table S4), and 68% of *A. phalloides* occurrences are recorded within the geographic range of *Q. agrifolia* ($P < 0.001$). Significant associations were also found for *C. cornuta* var. *californica* and *P. muricata*, but not for the other *Quercus* species that are hosts of *A. phalloides* in California. The statistical significance of the overlap between *A. phalloides* and *P. muricata* may be an artifact caused by the highly restricted and spatially aggregated range of *P. muricata* and the high density of *A. phalloides* in one small part of the range of *P. muricata* (the Point Reyes Peninsula). In fact, at Point Reyes, *A. phalloides* actually occurs most frequently in association with *Q. agrifolia*, as described above. Moreover, only 9% of *A. phalloides* records occurred within the range of *P. muricata*.
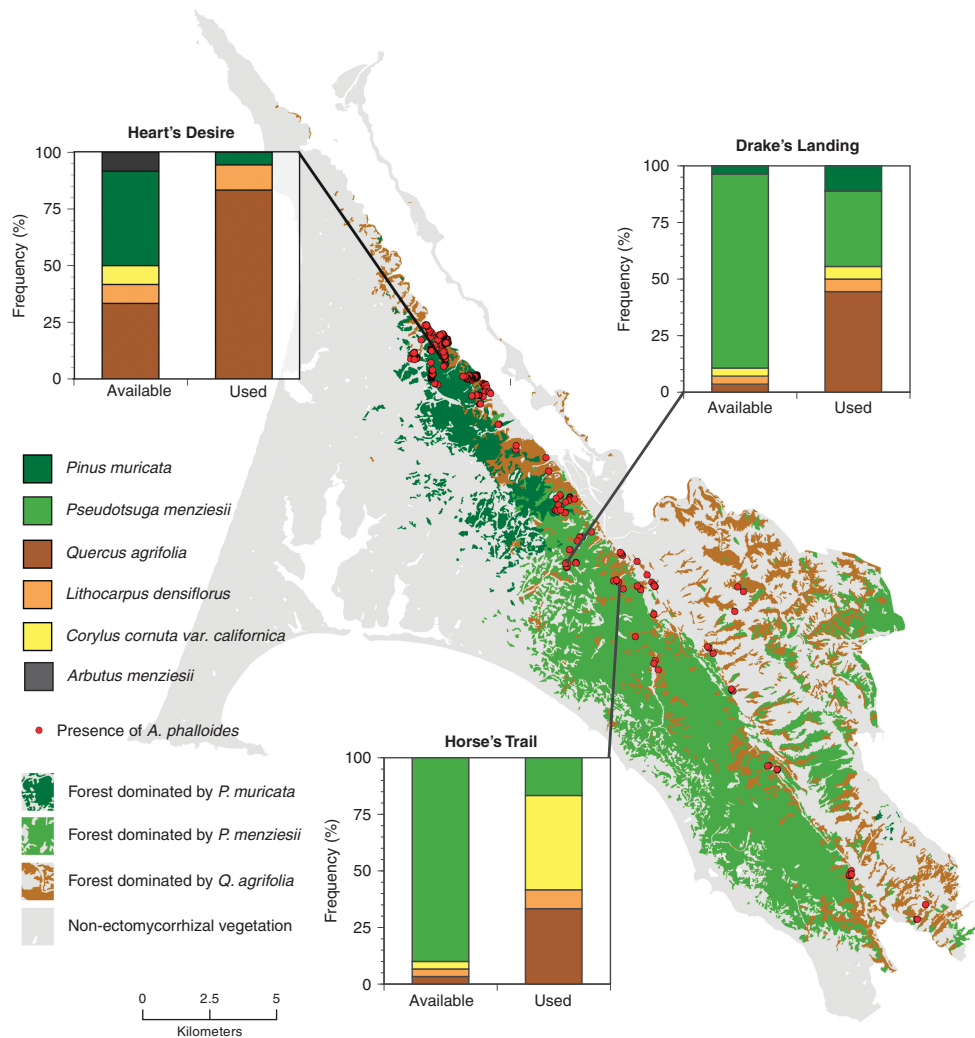
Figure 4 Host selectivity of *A. phalloides* at three sites on the Point Reyes Peninsula in Marin County, California, USA. Map shows peninsula shaded with distributions of the dominant EM host species (*Q. agrifolia*, *P. muricata* and *Pseudotsuga menziesii*). Graphs show the available root community and the percent of the total number of *A. phalloides* root tips where each species was detected as a host.

Discussion

Host specificity and selectivity of A. phalloides *in North America*

Shifts by introduced microbial mutualists to endemic hosts have rarely been demonstrated. Introduced microbial mutualists may become abundant in novel ranges, but they generally persist with their introduced hosts and rarely establish functional symbioses with novel hosts (Diez, 2005; Vellinga *et al.*, 2009; Dickie *et al.*, 2010; Jairus *et al.* 2011). Our work with *A. phalloides* provides a sharp contrast to these previous studies and demonstrates that this European fungal symbiont can shift to associate with multiple hosts that are endemic to North America. Host shifts are not uniform across ranges, causing a strong geographic structure in patterns of host specificity across North America. On the East Coast, the species associates almost exclusively with conifers, although these are rarely hosts of *A. phalloides* in its native range. On the

West Coast, in California, *A. phalloides* associates most frequently with oaks, similar to the pattern of host associations observed in its native range. In the Pacific Northwest, *A. phalloides* is primarily associated with European hosts planted in parks and yards, and is not yet known to associate with native hosts.

Both the evolutionary history of interactions between *A. phalloides* and its hosts in the native range, and the distribution of compatible hosts in novel ranges, may explain the geographically structured host specificity that we observed in North America. Different symbiont lineages may adapt to specialize on subsets of hosts (Thompson, 1994). Cryptic genetic species specific to particular hosts have been observed within other morphological species of fungi (Sato *et al.*, 2007). However, genetic surveys of *A. phalloides* in both Europe and North America provide no evidence of cryptic species within *A. phalloides* (Pringle *et al.*, 2009a). Nonetheless, on the West Coast, *A. phalloides* may have
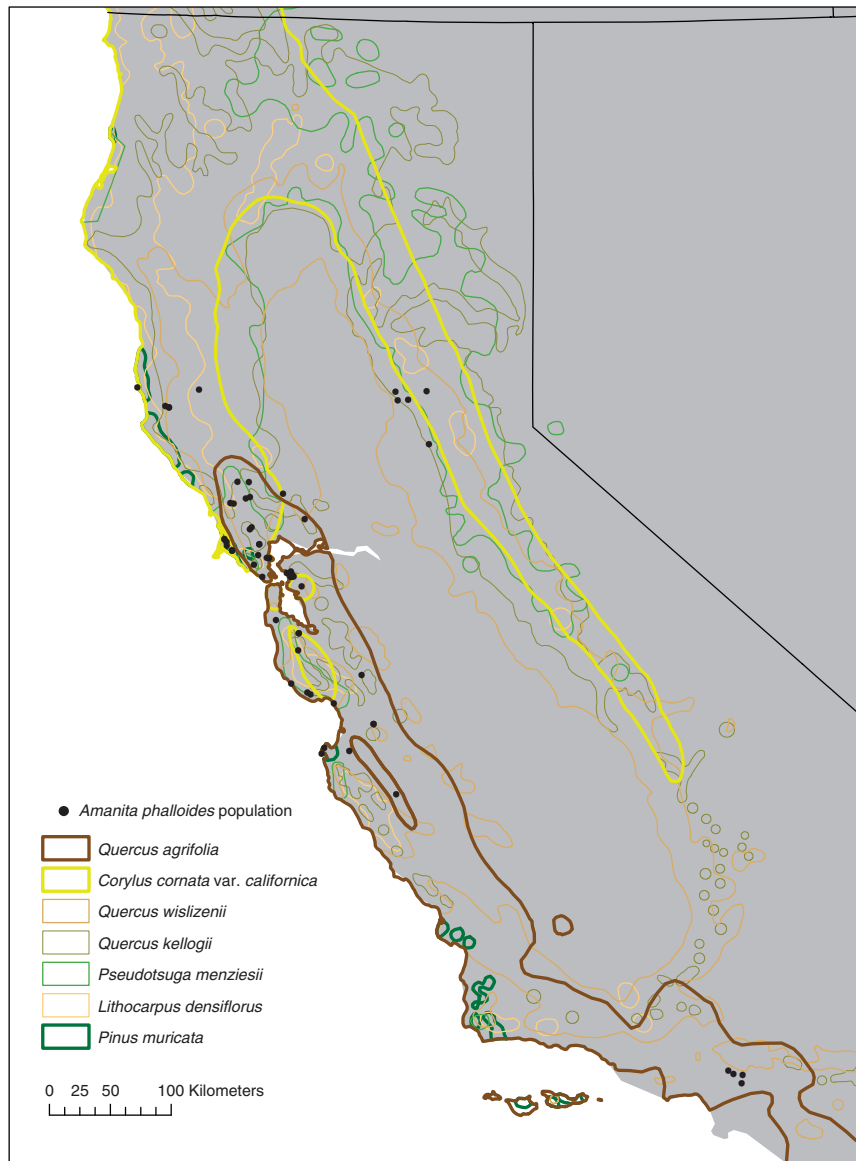
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Figure 5 Distribution of *A. phalloides* throughout California and the geographic ranges of associated host plants. A significant association between the distribution of *A. phalloides* and a host range is marked by the thicker borders around *Q. agrifolia*, *C. cornuta* var. *californica*, and *P. muricata*. The distribution of *A. phalloides* is largely within the distribution of its most frequent host, *Q. agrifolia*. See Supplementary Table S4 for statistics.

been introduced with cork oak imported from Europe (Saylor, 1984), and on the East Coast, *A. phalloides* may have been introduced on European conifer seedlings (Tanghe and Simons, 1973; Tanghe, 1983). If these different introductions included different genotypes of *A. phalloides* adapted to either oaks or conifers, observed host specificities could be the result of different genetic bottlenecks. Although this hypothesis remains untested, ongoing sequencing of *Amanita* genomes may soon provide the tools needed to identify the genetic basis of specificity.

The availability and geographic distribution of compatible species in the Fagaceae, the most frequent hosts of *A. phalloides* in Europe, could also have a role in creating geographically

structured host specificity. If *A. phalloides* were specific to a particular phylogenetic lineage within the Fagaceae, and if these lineages were more common on one coast of North America, then observed patterns of host specificity would be the result of differences in the availability of compatible hosts. However, in Europe, *A. phalloides* most commonly associates with *Q. robur*, which is closely related to both the most common oak in the Northeast of North America, *Q. rubra*, rarely a host of *A. phalloides*, and to *Q. agrifolia*, the most common host of *A. phalloides* on the West Coast (Manos *et al.*, 2001). Moreover, other North American species of Fagaceae and Betulaceae, including *Fagus grandifolia*, *Corylus americana* and *Betula lenta*, are closely related to European species of Fagaceae and

Betulaceae and are often found within forests on the East Coast where *A. phalloides* is found. Yet, we rarely observe *A. phalloides* associating with these hosts on the East Coast.

Differences in the diversity of available and colonized roots clearly demonstrate that in the mixed forests of Point Reyes *A. phalloides* is selecting *Q. agrifolia* as a primary host. The most abundant roots available for colonization at two of the three sites were *P. menziesii*, but at both sites *A. phalloides* most frequently associated with *Q. agrifolia*. Ecological processes are likely to influence the host associations of *A. phalloides* at these local scales, and competition for the resources provided by a single host may be an especially potent force when multiple symbiont species associate with the host (Saari *et al.*, 2005; Morris *et al.*, 2008). A symbiont may be able to form associations with a wide range of species, but only colonize a host at a particular site when it can establish and persist in the face of competition from other symbionts. Data from the West Coast suggest that EM fungal communities around plants of the Fagaceae are lower in species richness as compared with co-occurring Pinaceae (Massicotte *et al.*, 1999; Kennedy *et al.*, 2003; but see Smith *et al.*, 2009) and these data provide tentative support for our hypothesis.

Changes in niche and reproductive output of
A. phalloides *in California*
Changes in the stable isotope signatures of *A. phalloides* mushrooms collected from Europe and the East and West Coasts of North America suggest different dynamics of nutrient acquisition and loss across the different ranges; this is the first intercontinental comparison of isotopes taken from a mycorrhizal fungus. The carbon used by EM fungi to build biomass is taken directly from associated plants (Smith and Read, 1997) and the $\delta^{13}C$ of mushrooms should reflect the $\delta^{13}C$ of host plants. The less depleted $\delta^{13}C$ of the East Coast mushrooms compared with the Californian mushrooms provides independent support for a host shift to conifers, because evergreen conifers tend to be less depleted in ^{13}C than evergreen and deciduous angiosperms (Diefendorf *et al.*, 2010). Data of EM fungi within other forest ecosystems also document significant differences in stable isotope signatures based on host associations (Högberg *et al.*, 1999; Taylor *et al.*, 2003). The $\delta^{15}N$ values of EM fungal mushrooms are influenced by the source of N used by the fungus, the dynamics of N transfer from the EM fungus to the host and internal physiological processes independent of N transfer to a host (Hobbie and Colpaert, 2003; Hobbie and Hobbie, 2006); current data can only confirm these processes as different in Californian populations of *A. phalloides*. However, changes in carbon and nitrogen isotope dynamics are correlated with *A. phalloides'* associations with *Q. agrifolia* and may suggest the mutualism of

the fungus and this plant functions differently from the mutualisms of *A. phalloides* in Europe or the East Coast. Although stable isotopes of terrestrial biomes are also influenced by additional factors that vary between biogeographic regions, including differences in climate (Marshall *et al.*, 2007), large-scale meta-analysis and modelling approaches show that broad patterns of the $\delta^{15}N$ and $\delta^{13}C$ of plants and soils are similar across the three regions that we compared (Amundson *et al.*, 2003; Suits *et al.*, 2005; Diefendorf *et al.*, 2010).

Novel patterns of host use are associated with striking changes in the reproductive output of *A. phalloides* in California. The mushroom, or sporocarp, is the spore-bearing structure of a basidiomycete fungus. Mushroom size is highly correlated with investment in sexual reproduction in other mushroom-forming fungi (Guidot *et al.*, 2002; Schmit, 2002) and in *A. phalloides* (Supplementary Figure 1). Mushrooms of Californian *A. phalloides* are often more than twice as large as European mushrooms. Because larger mushrooms produce more spores, the increase in mushroom biomass increases the potential for the dispersal of spores capable of establishing new populations. Moreover, mushrooms in California can appear at any time of year, whereas in the other ranges *A. phalloides* is restricted to fruiting during specific times of the year. Shifts in the quantity of carbon taken from a host can alter the production of mushrooms by EM fungi (Kuikka *et al.*, 2003; Andrew and Lilleskov, 2009), and it is likely that changes in the dynamics of carbon exchange among *A. phalloides* and its Californian hosts are driving shifts in the reproductive output and phenology of this fungus. Shifts in the temporal allocation of carbon may also explain changes in reproductive output. The most frequent host in California, *Q. agrifolia*, is an evergreen oak. Because this host photosynthesizes throughout the entire year, opportunities to obtain carbon from this host extend throughout the year and may lead to increased opportunities for mushroom production by *A. phalloides* in California. Although the quantity and timing of carbon supply from hosts is likely the major limiting factor in the production of mushrooms by *A. phalloides*, we cannot rule out differences in abiotic conditions among these regions also having a role.

Constraints on spread of A. phalloides
Within California, the geographic distribution of a preferred host appears to shape range expansion of *A. phalloides*. The current distribution of the fungus in California is largely within the range of its most frequent host, *Q. agrifolia*, and *A. phalloides* selectively associates with this host in mixed forests. When populations of *A. phalloides* are found outside of the natural range of *Q. agrifolia*, they are often growing in association with

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planted *Q. agrifolia* trees used for landscaping (Wolfe, personal observation). On the East Coast, host specificity does not shape the distribution of *A. phalloides*. Although shifts to novel species of Pinaceae are common, the fungus has not spread within the distributions of these plants, and novel interactions are restricted to disturbed or managed habitats (Wolfe *et al.*, 2010).

A comparison of the fungus in its three ranges challenges simple hypotheses linking host specificity or its lack with constraints on spread (Richardson *et al.*, 2000; Vázquez, 2005; Pringle *et al.*, 2009b). A catalog of the fungus's associations across the globe defines it as a generalist, but within individual ranges the fungus preferentially associates with subsets of plants. Our California data are a direct contrast to available evidence taken from pollination mutualisms (Vázquez, 2005), and suggest that specificity in local habitats can influence the success of introduced mutualist species, even when species otherwise appear as generalists. Moreover, the ability to shift hosts does not guarantee spread, and a generalist capable of associating with local symbionts may not invade across the ranges of these species. However, when an introduced mutualist does shift hosts and also spread, the range of the novel hosts will be a critical control on the spread of the mutualist.

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