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LETTER

Evolutionary dynamics of specialisation in herbivorous stick insects

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Abstract

Understanding the evolutionary dynamics underlying herbivorous insect mega-diversity requires investigating the ability of insects to shift and adapt to different host plants. Feeding experiments with nine related stick insect species revealed that insects retain the ability to use ancestral host plants after shifting to novel hosts, with host plant shifts generating fundamental feeding niche expansions. These expansions were, however, not accompanied by expansions of the realised feeding niches, as species on novel hosts are generally ecologically specialised. For shifts from angiosperm to chemically challenging conifer hosts, generalist fundamental feeding niches even evolved jointly with strong host plant specialisation, indicating that host plant specialisation is not driven by constraints imposed by plant chemistry. By coupling analyses of plant chemical compounds, fundamental and ecological feeding niches in multiple insect species, we provide novel insights into the evolutionary dynamics of host range expansion and contraction in herbivorous insects.

Keywords

Chaparral biome, host shift, plant secondary metabolites, plant-herbivore interaction, realised vs. fundamental niche, redwood, *Timema* stick insect.

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INTRODUCTION

Long-standing hypotheses suggest that the evolution of the tremendous diversity of insect herbivores (Lawton 1983; Farrell 1998; Novotny *et al.* 2006) relates to speciation driven by adaptation to novel host plants (Mitter *et al.* 1988; Schluter 2000; Futuyma & Agrawal 2009; Matsubayashi *et al.* 2010; Hardy & Otto 2014). Many studies have focused on identifying the genetic basis of adaptations to novel hosts (Via 1991; Feder *et al.* 2003; Nosil 2007; Soria-Carrasco *et al.* 2014; Simon *et al.* 2015), but what factors constrain the colonisation of novel hosts remains largely unknown (Mayhew 2007; Yoder & Nuismer 2010). Indeed, multiple factors, including plant species-specific chemical compounds, which reduce insect growth and survival, are expected to hamper the ability of insect herbivores to shift to novel hosts (Scriber 1984; Hartley & Jones 1997; War *et al.* 2013a,b; Portman *et al.* 2015).

Overcoming constraints imposed by plant chemical compounds should be especially difficult for insect species that are specialised on few related host plant species, which appears to be the case for the vast majority of herbivorous insects (e.g. Fox & Morrow 1981; Janzen 1988; Novotný & Basset 2005; Dyer *et al.* 2007). Indeed, surveys of insect occurrences on plants in natural populations suggest that approximately 76% of all herbivorous insects are mono- or oligophagous, feeding on plant species belonging to a single genus or family (Forister *et al.* 2014). In spite of the widespread specialisation, transitions from specialist to generalist habits have occurred repeatedly during the evolution of herbivorous insect clades (e.g. Funk & Bernays 2001; Nosil & Mooers 2005; Forister *et al.* 2012), questioning the idea that adaptation to plant chemical compounds generally hampers the colonisation of novel hosts. Resolving this paradox has thus far been difficult because the majority of comparative and empirical studies on herbivore specialisation (including the ones mentioned above) have only focused on the number of hosts used in natural population (i.e. the *realised* feeding niche; Colwell & Futuyma 1971; Nyffeler & Sterling 1994; Blüthgen *et al.* 2006; ~~Rasmann *et al.* 2014~~; Fordyce *et al.* 2016). Realised feeding niches depend on multiple factors, including insect adaptations to plant chemistry, insect preferences (e.g. Dethier 1954; Forister *et al.* 2013) as well as species interactions (notably predation and competition; e.g. Lewinsohn & Roslin 2008; Holt 2009; Ingram *et al.* 2012). However, little or no information is available on the range of plants allowing for survival, growth and reproduction of herbivorous insects (i.e. the *fundamental* feeding niche, Whittaker *et al.* 1973; Leibold 1995). Thus, the evolutionary dynamics of fundamental feeding niches are elusive and it even remains unknown whether the breadths of the fundamental and realied feeding niches generally change in parallel.

We hypothesise that the ability to use different plant species as hosts and consequently the breadth of the fundamental feeding niche is influenced by the evolutionary history of an insect lineage (see also Futuyma & Peterson 1985; Futuyma & McCafferty 1990). Specifically, if insect lineages can retain the ability to use their ancestral hosts as a food source after having shifted to a novel host, host shifts are expected to generate fundamental niche expansions (i.e. the lineage would become more generalist). By contrast, if insect lineages do not retain the ability to use their ancestral hosts, fundamental feeding niches will be independent of the evolutionary history of host plant use. More generally, colonisation of novel host plants would be facilitated if insect lineages retained plasticity in host use present in their ancestors.

We used *Timema,* a small genus of herbivorous stick insects from western North America (Vickery 1993) to study the evolutionary dynamics of fundamental and realised feeding niches. Different *Timema* species have colonised plants from phylogenetically distant families, ranging from one to eight families of host plants per *Timema* species (Table 1). In terms of realised feeding niche, the *Timema* genus thus comprises a range of specialist to generalist species, and a tendency towards increased ecological specialisation over evolutionary time was reported in a previous study (Crespi & Sandoval 2000). The genus originated about 30 million years ago (Riesch *et al.* 2017), in conjunction with the origin and spread of the chaparral biome to which most species are adapted (Sandoval *et al.* 1998). Ancestral *Timema* populations were most likely associated with angiosperms characterising the chaparral biome, specifically the genera *Ceanothus* (lilac) and *Adenostoma* (chamise) (Crespi & Sandoval 2000). Nonetheless, transitions from angiosperm to conifer hosts have occurred multiple times in the genus. Ten of the 23 known *Timema* species regularly use conifers from one or multiple families as hosts (Table 1). At least two conifer species (redwood, *Sequoia sempervirens* and white fir, *Abies concolor*) represent recent host shifts, as both redwood and white fir are hosts for monophyletic groups of closely related *Timema* species (Fig. 1).

Taking advantage of this variability in host plant use in *Timema,* we tested whether (1) insect performance on host plants is constrained by plant phylogeny and chemistry, (2) the fundamental feeding niche breadth changes following a shift to a novel host, (3) insects retain the ability to use ancestral host plants following host shifts, and (4) fundamental and realised feeding niche sizes are correlated.

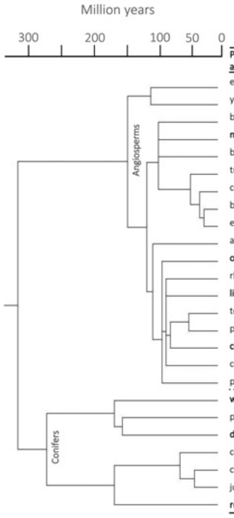
We first characterised the realised feeding niches of the 23 known *Timema* species. To this aim, we generated a complete list of host plants for each species, using information from previous studies and field surveys (Table 1). For nine of the 23 species, we also estimated the realised feeding niche breadth at the population level. We then estimated the breadth of the fundamental feeding niche of these nine *Timema* species. To this end, we measured juvenile insect performance on seven phylogenetically diverse plants from the *Timema* host plant species pool (Table 1). This sampling strategy allowed us to study the evolutionary dynamics of specialisation at the realised and fundamental niche levels. Finally, in order to explore potential mechanisms generating variable performances of insects on different plant species, we analysed phenolic and terpenic secondary metabolites, which are toxins and/or feeding deterrents for many herbivorous insects (Acamovic & Brooker 2005; Dearing *et al.* 2005; Fürstenberg-Hägg *et al.* 2013).

MATERIAL AND METHODS

Realised feeding niches

In order to characterise the breadth of the realised feeding niche at the species level, we established a list of all the known host plants for each of the 23 known *Timema* stick insects species from the literature (Vickery 1993; Vickery & Sandoval 1997, 1999, 2001; Law & Crespi 2002; Sandoval &

Table 1 *Timema* species and their recorded host plants in the wild.



HOST PLANTS				Northern clade								Santa Barbara clade		Southern clade						Others						
Plant abbreviation	Common name	Latin name	Plant family	*T. californicum*	*T. douglasi A*	*T. knulli*	*T. landelsensis*	*T. petita*	*T. poppensis*	*T. shepardi A*	*T. sp «Cuesta Ridge»*	*T. cristinae*	*T. monikensis A*	*T. bartmani*	*T. boharti*	*T. genevievae A*	*T. podura*	*T. tahoe A*	*T. chumash*	*T. sp «Limberpine»*	*T. coffmani*	*T. dorotheae*	*T. morongensis*	*T. nakipa*	*T. ritensis*	*T. nevadense*
ely	wheatgrass	*Elymus spp*	Poaceae												•											
yuc	yucca	*Yucca spp*	Asparagaceae												X		•									
buc	buckwheat	*Eriogonum fasciculatum*	Polygonaceae												•		•						X			
mz	**manzanita**	***Arctostaphylos spp***	**Ericaceae**	Ⓧ	•		X		•	X	X	•		•	•		X		•					X		
bal	mountain balm	*Eriodictyon spp*	Boraginaceae	•													•									
tri	American trixis	*Trixis californica*	Asteraceae														•									
coy	coyote brush	*Baccharis pilularis*	Asteraceae	•																						
bri	shrubby brickellbush	*Brickellia frutescens*	Asteraceae	•											•											
eri	eriophyllum	*Eriophyllum sp*	Asteraceae	•																						
ace	bigleaf maple	*Acer macrophyllum*	Aceraceae	•																						
oak	**oak**	***Quercus spp***	**Fagaceae**	Ⓧ	•	•	•		•	•	X	•	•	•	•	•	X		X					X	•	
rha	spiny redberry	*Rhamnus spp*	Rhamnaceae									•														
lil	**californian lilac**	***Ceanothus spp***	**Rhamnaceae**	X	•	Ⓧ	X	Ⓧ	•	X	X	Ⓧ	X		X	•	X		X			X		X		
toy	toyon	*Heteromeles arbutifolia*	Rosaceae	X						•		•							X							
pru	bitter-berry	*Prunus virginiana*	Rosaceae														•									
cha	**chamise**	***Adenostoma fasciculatum***	**Rosaceae**	X							X	Ⓧ	X		X	X	Ⓧ		X			•		X		
cer	mountain mahogany	*Cercocarpus betuloides*	Rosaceae	X						•		X	X		Ⓧ	•	X		Ⓧ							
pick	pickeringia	*Pickeringia montana*	Fabaceae									•														
wf	**white fir**	***Abies spp***	**Pinaceae**											Ⓧ			•	X								
pin	limber pine, knobcone pine	*Pinus spp*	Pinaceae	•		X								X	•		•			X						X
df	**douglas fir**	***Pseudotsuga menziesii***	**Pinaceae**	•	X	•			X	•																
ced	Insense cedar	*Calocedrus decurrens*	Cupressaceae											•			•									
cyp	sargent cypress	*Hesperocyparis sargentii*	Cupressaceae			•					X															
jun	Juniper	*Juniperus spp*	Cupressaceae																		X				X	X
rdw	**redwood**	***Sequoia sempervirens***	**Cupressaceae**		X	Ⓧ			Ⓧ																	
Number of typical host plants				6	2	3	2	1	2	2	5	3	3	2	4	1	5	1	5	1	1	1	1	4	1	2
Number of putative* host plants				13	5	6	3	1	5	6	5	8	4	5	10	4	13	1	6	1	1	2	1	4	2	2

Plants labeled with an 'X' correspond to a common host for a given *Timema* species, where experimental evidence confirms that the plant is used as a food source. Plants labelled with '.' correspond to rare/anecdotal observations where it is unclear whether these plants are used as a food source (or solely for resting). Columns highlighted in grey indicate the *Timema* species used in the present study, sampling locations are specified in Table S1. Plants used for feeding experiments are written in bold. The plants on which the corresponding *Timema* populations were collected for this study are encircled. Note two of the *Timema* species are undescribed: *Timema* 'Limberpine', mentioned first by Sandoval & Crespi (2008), and *Timema* 'Cuesta ridge' from Riesch *et al.* (2017). The phylogenetic distances between the plant genera are estimated with information from the public database TIMETREE (http://timetree.org/; Hedges *et al.* 2015; Kumar *et al.* 2017).

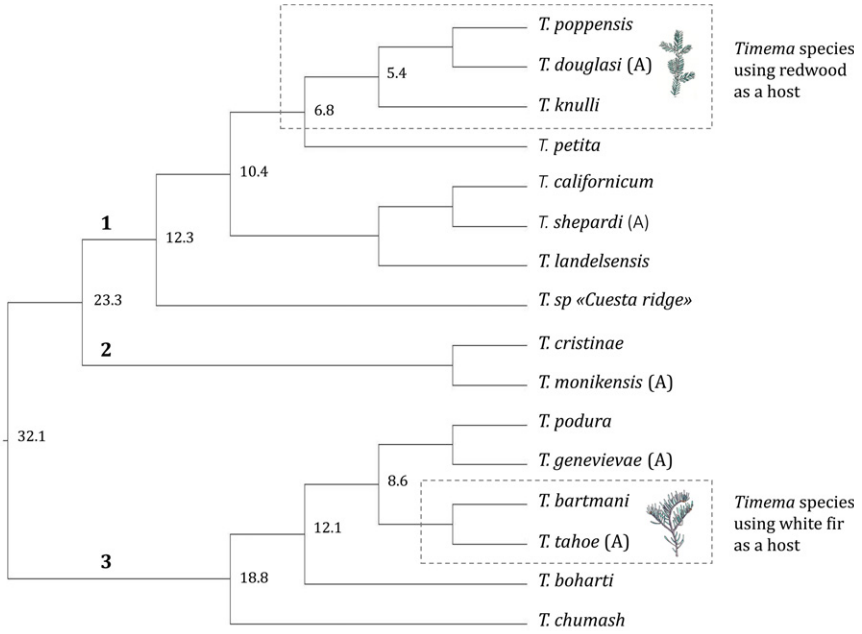


Figure 1 *Timema* phylogeny highlighting the species using the novel host plants redwood and white fir. Phylogeny redrawn from Riesch *et al.* 2017; with asexual lineages (A) added from Schwander *et al.* 2011. The phylogenetic position for the missing *Timema* species (see Table 1) is not known. Bold numbers 1, 2 and 3 correspond to the three described *Timema* clades, respectively, Northern, Santa Barbara, and Southern clade. Node ages (Mya) are from Riesch *et al.* 2017.

Crespi 2008), and completed the list with personal observations (Table 1). We distinguished between plants for which we found evidence that *Timema* feed on them (hereafter 'typical host plants'), and plants for which it was unclear whether they are used as a food source, or solely for resting, as only observation data were available (hereafter 'putative host plants'; see Table 1). We also characterised the realised feeding niche at the population level for a subset of 22 populations from nine species (1–6 populations per species; Table S1). To this end, we chose locations where a minimum of three plants from the *Timema* host plants pool (Table 1) were present. We surveyed these plants and determined the relative frequency of stick insects on each plant in each of the 22 locations (Table S1).

Fundamental feeding niches

To measure insect performance on different hosts and their fundamental feeding niche breadths, we chose seven plants known to be commonly used by several *Timema* species, while trying to cover the phylogenetic diversity of all potential host plants (Fig. 1; Table 1). Stick insects were collected from 12 populations belonging to nine *Timema* species throughout California (Table S1) using sweep nets. Between 10 and 80 females per host plant were used to measure survival and weight gain over 10 days, for a total of 70–220 females per population (1330 insects in total; see Fig. S1 for details on the experimental setup). The variation in numbers of insects per population was generated by the natural variation in the availability of females in different populations, as well as by the high mortality on certain plants that prevented us from obtaining weight gain estimates for all *Timema* populations. Whenever possible, we used more females for combinations generating high mortality.

We only used fourth-instar juvenile females in order to minimise age-related effects, and avoid the spurious effects of extreme mortality when manipulating younger instars. Experimental limitations due to our inability to raise *Timema* hatchlings did not allow us to study in detail the degree of specialisation over the full life-cycle. Indeed, under laboratory conditions, up to 80% of hatchlings die even if reared on the host plant of their parents. We, however, performed multiple tests using early nymphal stages in order to exclude the possibility that the diet during early development would influence the ability to use different plants during late nymphal or adult stages (see Karowe, 1989). For example, not a single early nymph (out of over 500) survived on plants causing high (> 95%) adult mortality (data not shown; see also Nosil *et al.* 2006 for experimental evidence that host preference in adults is not affected by juvenile diet).

Evaluation of phylogenetic constraints regarding host use

We tested whether closely related *Timema* species had similar performances ('Survival' as the proportion of surviving individuals and 'Growth' as the proportion of weight gained during 10 days; both coded as continuous traits) on the different plants. Branches from the most recent *Timema* phylogeny (Riesch *et al.* 2017) were pruned to create a phylogeny of the 12 populations from the nine species sampled for this study (Fig. 1). We used Mesquite 2.75 (Maddison & Maddison 2017) to reconstruct the ancestral states of the *Timema* performances on each of the seven plants [Mesquite module 'Continuous-character Model Evaluation for phylogenetic signal testing' (Felsenstein 1981; Lee *et al.* 2006)]. Maximum parsimony with unordered, equal-weighted characters, and a cost of any state change = 1 was used to minimise the total number of character-state changes over the tree. We compared the number of character-state changes inferred on the observed *Timema* phylogeny to the number of changes inferred on 1000 trees for which the characters were randomised across the tips in Mesquite. The null hypothesis that the character is randomly distributed on the phylogeny was rejected if the observed number of state changes fell outside of the upper or lower 2.5 percentiles of the random distribution (Maddison & Slatkin 1991).

Estimations of the degree of specialisation

To quantify the breadth of *Timema* feeding niches, we calculated the Tau specialisation index (τ) (Yanai *et al.* 2004), as follows:

$$\tau = \frac{\sum_{i=1}^{n}(1-\hat{x}_i)}{n-1}; \hat{x}_i = \frac{x_i}{\max\limits_{1\le i\le n}(x_i)}$$

where n corresponds to the number of plants, x_i represents the frequency of occurrence (for the realized niche) or the weight gain (for the fundamental niche) on plant i, and max (x_i) the maximum occurrence or weight gain for the focal population. The index ranges from 0 (generalist) to 1 (pure specialist). Note that we used 1-Tau in Figs 2 and 3 (where specialist = 0 and generalist = 1) for ease of interpretation. We chose this measure to estimate the degree of specialisation because of its robustness to small sample sizes and because our data were quantitative and continuous (Kryuchkova-Mostacci & Robinson-rechavi 2016). Because this index needs positive values to be calculated, we transformed weight gain percentages, which are negative when individuals lose weight, to relative weights of insects at the end of the experiments (i.e. an insect that lost 30% of its weight would be assigned the value 0.7, while one that gained 30% would be assigned 1.3).

We then tested whether broad fundamental feeding niches translate into broad realised niches at the species or population level. To this end, we correlated the fundamental specialisation indices Tau with the realised feeding niche breadths at the species and population levels, measured, respectively, by the number of host plants used by each species (Table 1) and the Tau indices based on the frequency of different host plants used within populations (Table S1). We used Phylogenetic generalised least squares (PGLS) analyses to account for phylogenetic non-independence among *Timema* species, using a Brownian motion model for trait evolution. The λ parameter, which predicts the pattern of covariance among species for a given trait, was estimated by maximum likelihood to avoid inflating Type I errors. These analyses were conducted using the *ape* (Paradis *et al.* 2004) and *Caper* (Orme *et al.* 2013) R packages (R Core Team 2017).

Plant chemical profile characterisation

We extracted and quantified compounds in the phenolic and terpene classes of secondary metabolites from leaves of the seven plant species included in our experiments (see Table 1), using methods adapted from Pratt *et al.* (2014) and Moreira *et al.* (2015). For each plant species, we extracted compounds from five independent replicates for both phenols and terpenes (see detailed methods for plant chemical analyses in Appendix S1).

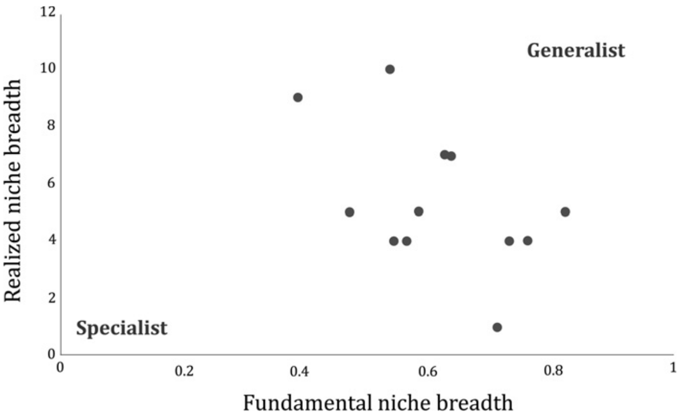


Figure 2 The size of the fundamental feeding niche does not constrain the realised feeding niche in *Timema*. Each point corresponds to a *Timema* population. For each population, the realised feeding niche breadth is estimated by the number of plant families used by the species, and the breadth of the fundamental feeding niche is estimated using the Tau index (based on insect weight gain).

We ordinated the chemical diversity data found across plant species by conducting a distance-based redundancy analysis (dbRDA) (Legendre & Anderson 1999) using the *vegan* R package (Oksanen *et al.* 2018). This allowed us to quantify the extent to which plants vary in chemical compositions and the fraction of this variation explained by plant phylogeny.

For the subset of chemical compounds that are present in multiple plants, we evaluated whether insect performances were negatively (or positively) correlated with the amount of a given compound. We conducted Spearman correlation tests (separately for each *Timema* species) between insect weight gain and each of the chemical compounds. These tests provided us for each *Timema* species with a list of compounds significantly correlated with insect performance. We then tested whether these lists were more similar between different *Timema* populations than expected by chance, using hypergeometric tests with the *phyper* function in R (Johnson *et al.* 2005). Thus, we were not interested in the specific lists of significant chemical compounds per *Timema* population (which comprise many false positives due to multiple testing), but we were interested to see if the same compounds affect the performance of multiple *Timema* populations.

RESULTS

Insect performances on different plants

The performance (survival and weight gain during 10 days) of *Timema* individuals was strongly dependent on the plant species tested. For 10 of the 12 *Timema* populations, both survival and weight gain varied significantly among individuals reared on different plant species, while for the two remaining populations, only weight gain varied significantly (Table S2,

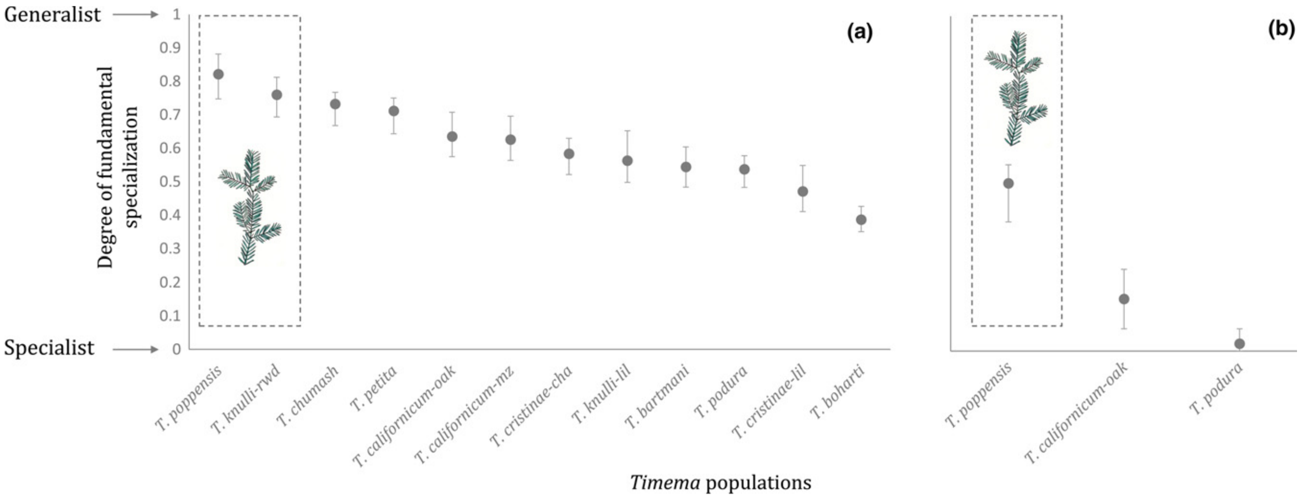


Figure 3 Breadth of the fundamental feeding niche of herbivorous stick insects. Niche breadth is quantified via the specificity index Tau (with 95% CI), based on insect weight gain on different plants (other measurements of specialisation generate the same outcome, see Figs S3 and S4). The insect populations are listed from the least to the most specialist. Two independent analyses of specificity are presented. In the first one (a), the degree of specialisation of 12 populations is based on their performance on seven plants from the *Timema* host plant pool. In the second one (b), the degree of specialisation of a subset of populations is based on their performance on three novel plants not used by *Timema* stick insects in natural populations (sugar sumac, coyote bush and sage bush). The dotted rectangles highlight populations native to redwood.

Fig. S2). Insect survival and weight gain were also significantly correlated (Spearman rank correlation, $r = 0.66$, $P < 0.0001$), even though the most extreme situation (i.e. when all *Timema* of a given population died on a specific host plant before 10 days) could not be included in the analysis.

Generally, we found that insect performance was not maximal on the host plant they were collected on (henceforth referred to as the native host plant) (Table S2, Fig. S2). Indeed, for only five out of the 12 populations, individuals survived best on their native host plant, while for only six out of 12 populations they gained the most weight. In some cases, the performance of insects increased dramatically when individuals were reared on plant species they do not use as host in the field. For example, 100% of *T. bartmani* survived for 10 days on lilac, while only 35.4% of them survived on their native host plant, white fir (Table S2).

We also observed that some host plants are a consistently better food source than others. For instance, lilac was almost always the best food source, even for *Timema* species that never use lilac in natural conditions. Specifically, relative survival on lilac was high for all populations (between 76.9 and 100%, Table S2), and individuals from nine of the 12 *Timema* populations gained more weight when reared on lilac than when reared on any other plant species (Fig. S2). Lilac is the native host for only three of these nine populations (*T. cristinae*–lil, *T. knulli*-lil and *T. petita*), the six remaining ones were collected on manzanita (*T. californicum*-mz), chamise (*T. cristinae*-cha), oak (*T. californicum*–oak), mountain mahogany (*T. boharti* and *T. chumash*) or redwood (*T. knulli*-rdw). Only *T. podura*, *T. poppensis* and *T. bartmani* individuals had the highest weight gain when fed with their native host plant, with lilac ranking second.

Redwood was on the opposite end of the host plant quality spectrum, as it was only exploitable by *Timema* individuals originally collected on it. Relative survival on redwood for individuals from the two native redwood populations was high (75.0 and 86.7% for *T. poppensis* and *T. knulli*-rdw, respectively; Table S2), while survival was low for all other *Timema* populations (ranging from 0 to 55.6%; Table S2). Similarly, *T. poppensis* and *T. knulli*-rdw were the only species that gained significant weight when fed with redwood for 10 days (mean weight gain was 45.3 and 67.7% for the two species, respectively; Fig. S2). For the 10 other populations, if individuals are able to survive for 10 days on redwood, they typically lost weight (80% of surviving individuals) or only gained very little (20% of surviving individuals gained weight, with a maximum gain of 9.9%; Fig. S2). For the *T. bartmani*, *T. boharti*, *T. podura* and *T. cristinae*-cha populations, not a single individual survived for 10 days on redwood.

We observed the same pattern for *T. knulli*, the only *Timema* species using both redwood and lilac under natural conditions (Table 1). All individuals collected on redwood were able to live and grow on all tested plants (Table S2, Fig. S2). By contrast, practically all individuals of the same species collected on lilac died or lost significant weight on redwood (Table S2, Fig. S2).

Degree of fundamental and realised specialisation

The fundamental and realised feeding niche breadths were not correlated, neither at the species level, nor at the population level. At the species level, we found no significant correlation when considering the total number of host plant genera per *Timema* species [correlation corrected with Phylogenetic Generalised Least Squares (PGLS); $r = -0.10$, $P = 0.25$), the number of plant families (PGLS; $r = -0.47$, $P = 0.17$; Fig. 2) or when considering only the typical plant genera (PGLS; $r = -0.06$, $P = 0.56$)]. The lack of correlation is unlikely caused by a lack of power as the general pattern is suggestive of a negative correlation between realised and fundamental niches rather than the expected positive correlation (Fig. 2). At the population level, we also found no correlation between Tau indices estimating the fundamental feeding niche and Tau indices estimating the realised niche (Pearson's correlation, $r = 0.02$, $P = 0.91$).

The fundamental specialisation indices showed that the two *Timema* species from redwood were the most generalist (Fig. 3a). The *T. knulli* population collected on redwood was also significantly more generalist (Tau = 0.24, 95% CI 0.19–0.30) than the population of the same species collected on lilac (Tau = 0.44, 95% CI 0.34–0.50). Hence, *Timema* native to redwood had a broader potential feeding niche than populations living on other host plants. In order to verify that this tendency was not only generated by the performance of the insects on redwood, we recalculated the Tau indices across six plants, excluding data from redwood. *T. poppensis*. *T. knulli*-rdw remained the most generalist species when the Tau indices were calculated without data from redwood (Fig. S5), and the Tau indices with and without redwood were strongly correlated (Pearson's correlation; $r = 0.96$, $P < 0.0001$), indicating that the pattern was not solely driven by redwood.

These results suggest that the fundamental feeding niches of *T. poppensis* and *T. knulli*-rdw have expanded after they adapted to redwood. To corroborate these findings, we reared individuals from three *Timema* species (*T. poppensis*, *T. californicum*-oak and *T. podura*) on plants not used as hosts by natural *Timema* populations [*Rhus ovata* (sugar sumac), *Baccharis pilularis* (coyote bush) and *Artemisia californica* (sage bush)]. Again, *T. poppensis* native to redwood performed better on these novel host plants than the two other insect species (Fig. 3b).

Effect of plant chemical composition on *Timema* performances

To explore potential mechanisms generating variation in food quality among host plants, we studied phenolic and terpenic secondary metabolites. We found a total of 521 different chemical compounds (28 phenols and 493 terpenes) across the seven plant species tested, with 88% of the variance explained by differences between plant species (dbRDA: $F_{6,28} = 58.5$, $P < 0.001$). Most of this variance among plants (77.7%) is explained by the phylogenetic relatedness (dbRDA: $F_{5,28} = 35.5$, $P < 0.001$). We also found that the total ~~volume~~ of compounds varied widely among plant species (volume measured as μg ~~G~~allic ~~A~~cid ~~E~~quivalent per g ~~D~~ry ~~M~~atter; average: 564 μg g^{-1}; range 298–1192), with a smaller ~~volume~~ in angiosperms (average: 310 μg g^{-1}; range 298–331) than conifers (average: 902 μg g^{-1}; range 650–1192; Welch Two Sample *t*-test; $t_2 = -3.75$; $P = 0.063$).

The dbRDA differentiated four plant groups, containing: (1) lilac, (2) oak, chamise and manzanita, (3) redwood and douglas fir and (4) white fir (Fig. S6). Most of the isolated terpenes and phenols were specific to a single plant or a subset of plants (Fig. S7). Specifically, 45.9% of the 521 compounds were detected only in a single plant, and only 1.5% of the compounds occurred in all seven plants (Fig. S7). To test whether the performances of multiple *Timema* species were related to similar plant chemistries, we used the 162 compounds (31%) that occurred in at least three plant species. Among these, 84 (65 after FDR = 0.05 correction) were significantly correlated with insect weight gain in at least one *Timema* population. No single compound was found to be significantly correlated with the performance of *Timema* collected from both angiosperms and conifers (Fig. 4). By contrast, 26 compounds (30.5%) were significantly correlated with the weight gain of insects from six of the nine populations living on angiosperms. One additional compound was further correlated with the weight gain of individuals of both populations collected from redwood (*T. poppensis* and *T. knulli-rdw*; Fig. 4). As phenols and terpenes are known to play an important role in plant defense against herbivorous insects, they were expected to negatively affect insect performances. However, 59.2% of the compounds showed a positive effect (*r* varying between 0.77 and 0.99; Fig. 4), suggesting that some phenols and terpenes may favour rather than constrain *Timema* performance. The number of compounds significantly correlated with insect performance and shared among several populations significantly exceeded the amount of sharing expected by chance (Hypergeometric tests, *P* varying between 1e-06 and 1e-18).

DISCUSSION

By studying the evolutionary dynamics of realised and fundamental feeding niches of multiple insect herbivores species in a phylogenetic framework, we developed novel insights into the mechanisms underlying feeding niche contractions and expansions. We analysed the fundamental and realised feeding niches of *Timema* stick insects, which comprise a range of ecologically specialist to generalist species. We showed that insects expanded their fundamental feeding niches after shifting to new hosts. These fundamental niche size expansions occurred via two mechanisms. First, fundamental feeding niches expanded gradually because the species that shifted to novel hosts retained the ability to use plant groups used by their ancestors, even though the latest host shifts in *Timema* occurred 3–12 million years ago (Fig. 1). Second, fundamental feeding niches expanded through major effect changes following adaptation to specific hosts. This seems to have been the case when *Timema* adapted to the chemically challenging redwood host plant, as insects adapted to redwood are able metabolise chemically diverse plants, including plants currently not used as hosts by any species of the *Timema* genus. However, our results do not allow us to formally exclude that niche expansion occurred via adaptation to another (currently unused) host prior to the switch to redwood. Nonetheless, in combination, these mechanisms can explain how generalist insect herbivores (as measured from the realised feeding niche) can evolve from specialists, a pattern detected repeatedly at the macroevolutionary scale (Scheffer & Wiegmann 2000; Schluter 2000; Janz *et al.* 2001, 2006; Termonia *et al.* 2001; Stireman 2005). Furthermore, fundamental feeding niche expansions following host shifts should facilitate future host shifts in the same lineage, which could generate frequent host turnovers via positive feedback loops of host adaptation and range expansion.

Our observation that host shifts generate fundamental feeding niche expansions because ancestral feeding capacities are

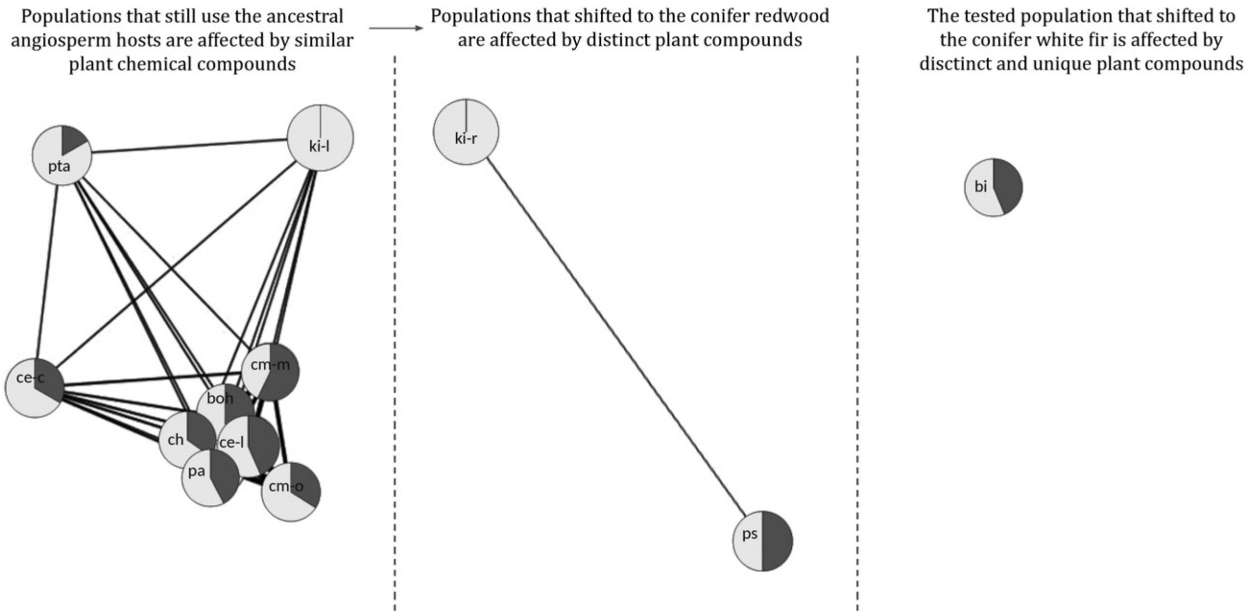


Figure 4 Similar plant chemical compounds affect the performance of insects native to angiosperm hosts, but different sets of compounds affect performances of insects native to conifers. Network built with Cytoscape 3.5.1 (Shannon *et al.* 2003). Circles in the network correspond to the 12 studied *Timema* populations. The length of the edges connecting two populations is negatively proportional to the number of shared compounds affecting insect weight gain (the more populations are affected by similar compounds the closer they are). The dashed lines separate groups of populations that are not affected by overlapping chemical compounds. *Timema* population name abbreviations are: bi: *T. bartmani* from white fir; boh: *T. boharti* from mahogany; cm-m: *T. californicum* from manzanita; cm-o: *T. californicum* from oak; ce-c: *T. cristinae* from chamise; ce-l: *T. cristinae* from lilac; ch: *T. chumash* from mahogany; ki-l: *T. knulli* from lilac; ki-r: *T. knulli* from redwood; pa: *T. podura* from chamise; ps: *T. poppensis* from redwood, pta: *T. petita* from lilac.

maintained, is in accordance with predictions of the *Oscillation Hypothesis* (Janz & Nylin 2008). The *Oscillation Hypothesis* suggests that host shifts begin with a host range expansion (Nylin & Janz 2009, Agosta *et al.* 2010), with the loss of ancestral feeding abilities following as a second step. Insect clades would therefore feature successive phases of host range expansions and contractions. Thus, according to the *Oscillation Hypothesis*, generalist phenotypes such as those of *Timema* populations from redwood would correspond to an evolutionarily transient phase, which is occasionally punctuating the overall tendency of lineages to increase specialisation (Bernays 1989, 2001; Agrawal 2000).

While several ecological factors, such as competition, predation or limited dispersal (e.g. Futuyma & Moreno 1988; Agosta 2006; Agosta & Klemens 2008) can drive ecological specialisation, plant secondary chemistry has been brought forward as a key component driving insect performance and host plant specialisation for herbivorous insects. In the present study, however, adaptation to a particular host plant chemistry does not explain ecological specialisation in *Timema*. Indeed, the performance of *Timema* individuals was typically not maximised on their native host plant, as previously shown in feeding experiments with chamise and lilac for insect populations adapted to these two. We also found that *Timema* living on the redwood host plant featured the broadest fundamental feeding niches of the genus, yet also the smallest realised one. In combination with the complete lack of correlation between fundamental and realised feeding niches in *Timema,* and the lack of phylogenetic constraint on fundamental niche size, these results suggest that plant secondary chemistry has little impact on insect hostplant specialisation. Accordingly, even though we may not have identified all the chemical compounds involved in defense, our analyses also revealed only minor effects of phenols and terpenes on insect performance.

Although we did not investigate the mechanisms driving host specialisation in *Timema*, previous work in one species (*T. cristinae*) has shown that predation and plant preference (independently of plant quality) are key factors determining the distribution of insects on potential hosts (Sandoval 1994; Nosil *et al.* 2003; Sandoval & Nosil 2005). There is also accumulating evidence from herbivorous insects in general that preferences for host plant species are often not linked to the quality of plants as a food source, suggesting that insect preferences evolve more rapidly than insect physiologies (Thompson 1988; Valladares & Lawton 1991; Fritz *et al.* 2000; Keeler & Chew 2008). Such preference-driven host plant selection in natural populations could help explain the lack of correlation between realised and fundamental niche size in *Timema*. Independently of the specific mechanisms driving host plant specialisation in *Timema*, our results indicate that insect herbivores are more constrained by the biotic pressures of their environment than by their intrinsic physiological ability to metabolise particular plant species.

In the case of redwood, host plant chemistry might, however, indirectly mediate host plant use by relaxing insect-insect competition or pathogen pressure. Redwood is a host for only few herbivore species (Furniss 1977; Grace & Yamamoto 1994), suggesting that competition on this host plant is low. In addition, laboratory experiments have shown that its wood inhibits the growth of bacteria (Scheffer 1966; Taha & Shakour 2016), and fungi (Shrimpton & Whitney 1968; Espinosa-Garcia *et al.* 1991), which may reduce pathogen pressure for insects. Finally, fires being very common and an essential component of the Californian ecosystems (Minnich 1983), can favour redwood-insect associations. Thanks to their thick bark, redwoods can easily withstand high levels of burning (Jacobs *et al.* 1985; Ramage *et al.* 2010). *Timema* on redwood may thus survive fires while they would perish on more profitable hosts such as lilac or chamise. Using redwood may thus be overall beneficial even if it represents a non-optimal food source.

Our results suggest that the specific ability to use redwood is a major feeding innovation that may allow for range expansions in species that shifted to this host. Our feeding experiments showed that redwood is toxic to all *Timema* populations except for the native ones, while populations collected on redwood were able to survive and grow on all other tested host plants, including plants currently not used as hosts in the genus. Only three *Timema* species are known to use redwood in nature: *T. poppensis* and *T. knulli* (used in this study) and *T. douglasi*, an asexual species very closely related to *T. poppensis* (Table 1). According to the most recent *Timema* phylogeny (Riesch *et al.* 2017), the last common ancestor of these three species occurred approximately 6.8 million years ago (Fig. 1), suggesting that the colonisation of redwood happened around that time. The *Timema* genus appears to have originated in Southern California or Northern Mexico and expanded northward (Law & Crespi 2002), with several range expansion events for the species currently occurring at the northern end of the distribution such as *T. poppensis* and *T. douglasi* (the exact distribution of *T. knulli* is not known). Therefore, the incorporation of redwood in their diet was likely to paramount importance for these herbivores to be able to expand their range northward. Indeed, the geographic distribution of redwood spreads over 750 km along the Pacific coast of the United States (Farjon 2005), while reaching further north than most other *Timema* host plants.

In conclusion, our study provides new insights into the consequences of host shifts for the breadth of the fundamental feeding niche. These consequences are highly relevant as they influence the probability for additional host shifts and potential host-associated diversification. Specifically, we showed that the ability to use ancestral hosts is maintained following major host shifts for at least 10 million years (as when moving from angiosperms to conifers), and that adaptations to chemically challenging hosts are not necessarily associated with decreased performance on alternative hosts. To the contrary, our results suggest that adaptations to chemically challenging hosts allowed insects to metabolise a broad range of phylogenetically unrelated plants, including plants that have not been used as hosts in natural populations. More generally, the joint analysis of fundamental and realised feeding niches in multiple-related species provides unique insights into the mechanisms driving the evolutionary dynamics of host range in herbivorous insects.

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AUTHORSHIP

CL and TS designed the study. CL, SR and TS collected data and CL analysed the results. All authors contributed to the manuscript.

DATA ACCESSIBILITY STATEMENT

The data supporting the results are archived in the private public repository Dryad; https://doi.org/10.5061/dryad.f12d430.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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