Article (refereed) - postprint

Stone, Graham N.; Lohse, Konrad; Nicholls, James A.; Fuentes-Utrilla, Pablo; Sinclair, Frazer; Schonrogge, Karsten; Csoka, Gyorgy; Melika, George; Nieves-Aldrey, Jose-Luis; Pujade-Villar, Juli; Tavakoli, Majide; Askew, Richard R.; Hickerson, Michael J.. 2012 Reconstructing community assembly in time and space reveals enemy escape in a Western Palearctic insect community. *Current Biology*, 22 (6). 532-537. [10.1016/j.cub.2012.01.059](10.1016/j.cub.2012.01.059)

Report

Reconstructing Community Assembly in Time and Space Reveals Enemy Escape in a Western Palearctic Insect Community

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Running title: Community assembly in time and space

Summary (198 words, 200 max)

How geographically widespread biological communities assemble remains a major question in ecology [1-4]. Do parallel population histories allow sustained interactions (such as host-parasite, or plant-pollinator) among species, or do discordant histories necessarily interrupt them? Though few empirical data exist, these issues are central to our understanding of multispecies evolutionary dynamics [3-6]. We use hierarchical approximate Bayesian analysis [7] of DNA sequence data for 12 herbivores and 19 parasitoids to reconstruct the assembly of an insect community spanning the Western Palaearctic, and assess the support for alternative host tracking and ecological sorting hypotheses [2,8,9]. We show that assembly occurred primarily by delayed host tracking from a shared eastern origin. Herbivores escaped their enemies for millennia before parasitoid pursuit restored initial associations, with generalist parasitoids no better able to track their hosts than specialists. In contrast, ecological sorting played only a minor role. Substantial turnover in host-parasitoid associations means that coevolution must have been diffuse [4-6], probably contributing to the parasitoid generalism seen in this and similar systems [10,11]. Reintegration of parasitoids after host escape shows these communities to have been unsaturated [12,13] throughout their history, arguing against major roles for parasitoid niche evolution or competition [3,4,6] during community assembly.

Results and Discussion (2253 words)

Community assembly, encompassed in the emerging field of metacommunity dynamics, is seen increasingly in terms of population interactions at a regional scale [1-4], with growing appreciation of the need to incorporate population history into models of multispecies interactions [3-4]. It is intuitively obvious that the extent to which species share concordant geographic distributions through time will influence the spatial and temporal scales of evolutionary interactions between them [4,5,14]. Long-term stability of species associations at the population level is compatible with strong coevolutionary interactions [2,5,14], while high turnover results in diffuse coevolution and selection for more 'general purpose' phenotypes [5,6,15]. Turnover in species associations also provides an indication of the extent to which processes such as competition and niche evolution structure communities [3,4]. However, with a few notable exceptions focused primarily on parasites of vertebrate hosts [e.g. 2,8,16] the population history of species interactions remains largely unknown. This applies particularly to the species-rich communities of insect herbivores and parasitoid enemies whose study underlies much of our understanding of foodweb structure, population dynamics and life history evolution [10,17,18]. These two trophic groups comprise more than 50% of all terrestrial animal species [19] and include many serious pests as well as providers of crucial ecosystem services such as pollination and biological control.

Here we reconstruct the assembly history of a geographically widespread insect community associated with deciduous oaks in the Western Palaearctic, in which many of the same species of herbivores and parasitoid enemies are sympatric across almost 5000 miles of longitude. Such widespread species associations are characteristic of many insect communities associated with temperate forest trees, and may result from at least three different multispecies histories. First, species may disperse together from a shared origin, such that biotic associations (and hence,

75 potentially, coevolution) are sustained at the population level [8]. Second, species may disperse at

different rates from a shared origin, such that interactions are disrupted in newly colonised

populations – either permanently, or until subsequent arrival of more slowly dispersing species.

This pattern is predicted in demographic models of host-parasite interactions during range

expansion [14], resulting in temporary 'enemy free space' for the hosts at the invasion front [10,17].

80 Finally, novel enemy species may join the community during range expansion by shifts from other

hosts providing similar resources – a process of ecological sorting [2]. While enemy escape is well

documented over ecological timescales (e.g. [20] for oak gallwasp communities), the dynamics of

host-parasitoid associations over longer timescales and regional spatial scales are unknown.

85 Our target community comprises 31 sampled species (12 herbivorous gallwasps and 19 parasitoid

natural enemies; Table S1), all of which are specialist inhabitants of oak galls. The galls represent

natural microcosms whose host-parasitoid interactions [10,21,22] and phylogeographic history [e.g.

23-26] have been studied in considerable detail. As with many other taxa, the population histories

of gall communities are dominated by the Pleistocene glaciations (ca. 2.0 million to 12,000 years

90 before present). During glacial maxima, populations of component species were restricted to a

longitudinal series of southern glacial refugia centered on Iberia, the Balkans and Asia Minor. The

gallwasp herbivores diversified in Iran and Asia Minor ca. 10 million years ago (mya) [9], followed

by multiple parallel westwards migrations into Europe by extant species [23-26]. Here we use

population genetic data for a single highly informative locus (mitochondrial DNA) to reconstruct

95 the assembly of the oak gall community by inferring the number and relative age of longitudinal

colonisation events between neighbouring refuge pairs for sets of species in the gallwasp and

parasitoid guilds (here defined as specialist herbivore and parasitoid inhabitants of the gall

environment [10,11,20]). Our approach (see Experimental Procedures) uses coalescent simulations

and approximate Bayesian computation [7,27] to estimate pairwise population divergence times across multiple co-distributed species, while incorporating coalescent and demographic variation among them. This incorporation of single locus data for many species results in 'borrowing strength' [28] for parameter estimation analogous to multi-locus inferences of divergence in a single species pair [7,29]. With the assumption (supported in this system; [23-25]) that one refugial population is derived from its neighbor with little or no subsequent gene flow, these population divergence events correspond to multispecies pulses of longitudinal range expansion. Though we cannot know the strength of interactions between species in the past [7], we can ask to what extent herbivore and parasitoid populations spread across the Western Palaearctic together and so were potentially able to interact. While occupation of the same refuge is compatible with continuing interaction between species, restriction to different refugia definitively prevents it. We assess the support for two competing models of community assembly.

(i)   The **Host tracking hypothesis** [9] assumes a shared eastern origin and parallel westwards range expansion for gallwasps and parasitoids. It predicts earlier population divergence between Asia/Balkans than Balkans/Iberia in each guild, and (because under this model parasitoids cannot survive without gallwasps) divergence dates for parasitoids that parallel or follow those for gallwasps but do not predate them.

(ii)   The **Ecological sorting hypothesis** [2] allows parasitoid recruitment by host shifts from other communities during host range expansion. The two guilds may thus have contrasting geographic origins, and parasitoid divergence times between refugia can predate those for their hosts.

Our analyses reveal contrasting population histories across guilds, and between the two neighbouring refuge pairs. For the herbivores, we infer several multispecies dispersal pulses (mode

n=3) between Asia and the Balkans (Fig.1A, S1A, Table 1), but only a single pulse involving all gallwasp species between the Balkans and Iberia (Fig.1B, S1B, Table 1). The earliest herbivore divergence is between Asia and the Balkans, while the youngest between these refugia is contemporary with the single pulse between the Balkans and Iberia (Table 1, Fig.2). This pattern is consistent with an eastern origin for the gallwasp guild, with multiple pulses of westwards gallwasp range expansion into Europe, the most recent of which may well have been contemporary with continued expansion into Iberia. These overall patterns are highly robust to uncertainty in the number and age of population divergence pulses (Supplemental Experimental Procedures, Fig. S2 and Table S3).

For the parasitoids, our data strongly support a single multispecies dispersal pulse between Asia and the Balkans for all 16 species (Figs.1C, S1C, Table 1), and multiple pulses (mode n=3) between the Balkans and Iberia (Figs. 1D, S1D, Table 1). The single Asia/Balkans pulse is the same age, or older than, two of the Balkans/Iberia pulses (incorporating 14 parasitoid species) (Table 1, Fig.2) – suggesting an eastern origin for most members of this guild. However, the earliest Balkan/Iberian pulse (mode n=3 parasitoid species) significantly predates the single Asia/Balkan divergence for this guild (Table 1) – rejecting an eastern origin for these taxa. Again, these patterns are strongly robust to uncertainty in the number and age of population divergence pulses (Supplemental Experimental Procedures, Fig. S2 and Table S3).

Patterns across guilds imply a dominant role for host tracking

Support for multiple divergence pulses in both trophic groups implies regional turnover in host and parasitoid species outside Asia. Concordant range expansion by the entire community – the most stringent form of the Host Tracking hypothesis – can clearly be rejected. Since most parasitoid

divergences are older in the east than west, and either contemporary with, or younger, than those for

gallwasps, community assembly is best explained by a combination of simultaneous and delayed

host tracking from a shared eastern origin. The first pulse of gallwasp dispersal between Asia and

150 the Balkans significantly predates the single parasitoid pulse between the same refugia (Table 1;

confidence limits for the two events do not overlap). This implies escape of these gallwasp lineages

(strictly, mitochondrial matrilines) from all 16 Asian parasitoid species. Similarly, gallwasp

dispersal from the Balkans to Iberia almost certainly involved escape from the 8 species in the

youngest parasitoid divergence between these two refugia. Support for the alternative Ecological

155 sorting hypothesis comes from the earliest Balkans/Iberia divergence for three parasitoid species,

which predates any Asia/Balkans divergence for either trophic level, implying that these parasitoids

were present in Iberia and the Balkans before the arrival of oak gallwasps. This is incompatible with

either an eastern origin or host tracking for this subset of parasitoids, instead implying recruitment

to westwards-dispersing gallwasps by host-shifts from ecologically similar hosts in central Europe

160 or Iberia.

The timescale of community assembly and enemy escape

While our assessment of community assembly models depends solely on the relative ages

of co-divergence pulses, molecular clock calibration (see Experimental Procedures) suggests that

165 Western Palaearctic gallwasp communities assembled over the last 3.5 million years (Table 1,

Fig.2). Initial gallwasp expansion into Europe from Asia is placed in the Late Pliocene - Early

Pleistocene, 1.3-4.2 million years ago, with expansion into Iberia around 400,000 years ago. All

gallwasp dispersal pulses and parasitoid divergence between Asia and the Balkans predate the

current interglacial. In contrast, the most recent parasitoid divergence pulsses between the Balkans

170 and Iberia may have occurred in the Holocene (Table 1). Gallwasps in the first dispersal pulse to

enter the Balkans escaped Asian parasitoids for at least 450,000 years (the minimal difference between confidence limits for the ages of the two pulses, Table 1).

Community assembly is a regional process

175   Deciduous oaks (*Quercus* section *Quercus*) are keystone taxa across the Western Palaearctic, supporting more associated insects than any other forest trees [30]. Oaks are thought to have expanded into Europe from Asia 5-7 mya [10], and our analyses suggest that most current interactions within one associated insect community – gallwasps and their parasitoids - also evolved in Asia. Expansion into Europe involved a diversity of population histories, with substantial

180   turnover in regional species associations. Community assembly across the Western Palaearctic through the Pleistocene involved a westwards-moving wave of increasing regional (a) diversity, with an associated rise and fall in between-refuge (b) diversity, but little change in global (g) diversity [12]. This pattern underlines the importance of a regional historical perspective when considering diversity patterns within communities [1,13,31].

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European regional communities have never been saturated

          A striking feature of the oak gallwasp system is that despite inferred variation in range expansion histories, all three refugia now contain very similar sets of species occupying characteristic trophic niches [10,21,32]. In each trophic level, species left behind early in the

190   longitudinal range expansion process have successfully reintegrated after separations lasting from tens to hundreds of millenia. This in turn implies very limited niche evolution during the assembly process. In particular, there is no evidence of resource monopolisation by early colonists to the exclusion of later arrivals [3,4,6]. More generally, our results are highly relevant to the long-standing debate on saturation in biological communities [12,13]. Inferred turnover in Balkan and

195   Iberian species pools (including recruitment of 3 new species) implies that European gallwasp communities have remained unsaturated throughout their assembly history.

        One obvious question is whether observed variation in species dispersal histories can be explained by variation in life history traits [33,34]. Demographic models of host-parasite range

200   expansion [14] lead to the expectation that generalist parasitoids (which attack a wider range of hosts and so maintain larger effective population sizes [10,21]) should track their hosts more closely than specialists, and hence predominate in earlier parasitoid divergence pulses. Oak gall parasitoids vary widely in host range, from extreme generalists (such as *Eurytoma brunniventris*, *Ormyrus pomaceus* and *Eupelmus urozonus*) to specialists (such as *Aprostocetus cerricola* and *Megastigmus*

205   *stigmatizans*) (see Table S1) [21,22,32]. However, our data reveal no impact of host range on longitudinal colonization history. Generalist and specialist parasitoids alike were part of a single multispecies pulse between Asia and the Balkans. Post-hoc allocation of parasitoids to divergence pulses between the Balkans and Iberia (Supplemental Experimental Procedures; Table S1) also shows no significant difference in host range between species allocated to the two more recent (i.e.

210   host tracking) parasitoid pulses (mean host range for parasitoids in the youngest pulse = 55.7, n=7; for the older pulse mean = 40.6, n=5; Mann-Whitney U=10, NS). This suggests that impacts of host range on colonization over ecological timescales may not extend to larger temporal and spatial scales.

215   Species turnover through time and space has important implications for our understanding of co-evolution in this and ecologically similar communities. Gallwasp defence against parasitoids is provided primarily by gall structures, which though formed of oak tissues are under gallwasp control [10,35]. Selection has favoured evolution of gall morphologies that reduce parasitoid attack,

leading to reciprocal arms race coevolution of parasitoid countermeasures [10]. However, contrary to expectations of host resource partitioning among parasitoids [10,17], many parasitoids in this community [10,21,22] (and in others centred on endophytic insects [11]) attack multiple hosts and coevolution is diffuse. Our results suggest that this pattern could in part be driven by regional species turnover. Gall phenotypes must have evolved in response to selection imposed by a changing cast of parasitoids, and likewise the broad host ranges of many gallwasp parasitoids may be a response to high turnover in available hosts [15]. Such turnover selects against the resource partitioning and parasitoid host specificity otherwise expected to evolve among competing generalist enemies [5,36]. We hypothesise that a similar community history may explain the dominance of generalist parasitoids in other foodwebs [11].

Future meta-analyses integrating genome-scale data with ecological information such as trophic links and species distributions will provide an increasingly resolved picture of the evolutionary and ecological dynamics underpinning community structure. A future aim for the oak gallwasp system will be to incorporate appropriate data for the oak food plants, and hence to reconstruct assembly across three trophic levels, though as yet no equivalently informative sequence data for regional oak taxa exist. This study shows that, as long as lineages are sampled from many species, and inference is not critically dependent on exact reconstruction of individual species histories, model-based analyses of even a single genetic locus can robustly reconstruct key aspects of community assembly. Such integrated approaches offer a glimpse of the 'ghost of communities past' and can bridge the often-observed divide between community ecology and evolutionary biology [1,3,4,6,37].

Experimental Procedures

Generation of sequence datasets

We generated datasets for 31 species comprising the major regional gallwasp lineages and representatives of all six associated chalcid parasitoid families, including the major agents of parasitoid-induced gallwasp mortality [10,21] (see Table S1). A total of 22 species were sampled from all three refugial regions, Iberia (Spain), the Balkans (Hungary) and Asia (Iran), while 9 were sampled from two neighbouring regions comprising their full geographic range. We generated 433-745 base pairs of mitochondrial sequence data for multiple individuals of each species (a total of 1169 sequences), either for cytochrome b (*cytb*, 18 species) or cytochrome c oxidase subunit 1 (*CO1*, 13 species) (Table S1). The two loci show very similar mutation models and per site substitution rates, assumed to be the same in all analyses ([38], and see Supplemental Experimental Procedures and Table S2).

Estimating the number and age of multispecies co- divergence pulses

We used an approximate, likelihood-free framework [39] incorporated in the software msBayes [7,29] to estimate the number and relative ages of multispecies population divergence times between pairs of neighbouring refugia. msBayes uses hierarchical approximate Bayesian computation (HABC) [27] to jointly analyse sequence data for multiple codistributed population pairs under a divergence model (see Supplemental Experimental Procedures). Our approach incorporates coalescent variance, known and unknown species differences in demographic parameters and variation in DNA fragment length (see Supplemental Experimental Procedures and Table S1; for other applications of this approach, see [40,41]). Species are free to have independent effective population sizes and divergence times that are modelled through global prior distributions. The degree of synchrony [7,42] in divergence time between species is captured by two

hyperparameters; Ψ, which quantifies the number of divergence times; and $Var(\tau)/E(\tau)$, the overall variability in divergence times (see Supplementary Information). Estimation of hyper-parameters and comparison of relative ages of divergence pulses between trophic groups is independent of any molecular clock calibration.

270   For each pair of refugia and trophic group we initially ran a separate msBayes analysis with an unconstrained, discrete uniform prior on Ψ (see Fig.1), assuming all Ψ values from 1 (synchronous divergence) to the maximum number of pulses (Ψ= number of pairs) to be equally likely (for further details on parameterisation and priors see Supplemental Experimental Procedures). Fit of the data to different values of Ψ was assessed using Bayes Factors (BF)

275   following the Jeffries scale [43]. Where Ψ >1 was strongly supported for a given trophic group and refuge pair, we performed additional msBayes analyses conditional on all values of Ψ with posterior probabilities of > 0.05 to estimate the relative ages of the Ψ divergence pulses conditional on the posterior probability of different Ψ values (Fig. 2). Our approach thus specifically incorporates uncertainty in the number of divergence pulses between each refuge pair. Subsequently we infer the

280   number of taxa involved in each pulse for the most strongly supported value of Ψ.

        The mitochondrial loci used in our analyses show clock-like evolution in oak gall community members [23-26], and in the absence of taxon-specific calibrations we use Brower's estimate of 2.3% pairwise sequence divergence per million years [44] for insect mitochondrial DNA to provide an order of magnitude timing of events, i.e. to either the Holocene or the Pleistocene.

285   Our confidence in this approximate calibration is supported by the good agreement with independently derived calibrations for nuclear loci in an oak gall parasitoid [26].

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Figure Legends

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Figure 1. Numbers of inferred multispecies population divergence pulses between refugia for the gallwasp and parasitoid trophic levels. (A)-(D) Prior (pale bars) and posterior (dark bars) probability distributions for Ψ, the number of divergence pulses between pairs of refugia for gallwasps (A, B) and parasitoids (C, D). The Ψ value given on each figure is the most strongly

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supported value, with Bayes factors relative to alternatives in parentheses (see Experimental Procedures). Additional support for single *versus* multiple divergence pulses for each trophic group and pair of refugia is presented in Figure S1.

Figure 2. The ages of multispecies population divergence pulses between refugia for the gallwasp

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(red) and parasitoid (blue) trophic levels. (A) Divergence between Asia and the Balkans. (B) Divergence between the Balkans and Iberia. Each panel shows the posterior probability distribution for the modal age of inferred multi-species pulses, with the shading intensity of each distribution conditional on the posterior probability of the associated value of Ψ (Fig. 1) inferring each pulse. The plots thus incorporate uncertainty in the inferred number of divergence pulses. The underlying

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divergence time distributions for Ψ = 2-5 where Bayes factors strongly support Ψ>1 are shown in Figure S2. For ease of interpretation, divergence times have been converted into years assuming 2.3% pairwise divergence per million years (see Experimental Procedures).

Figure 1

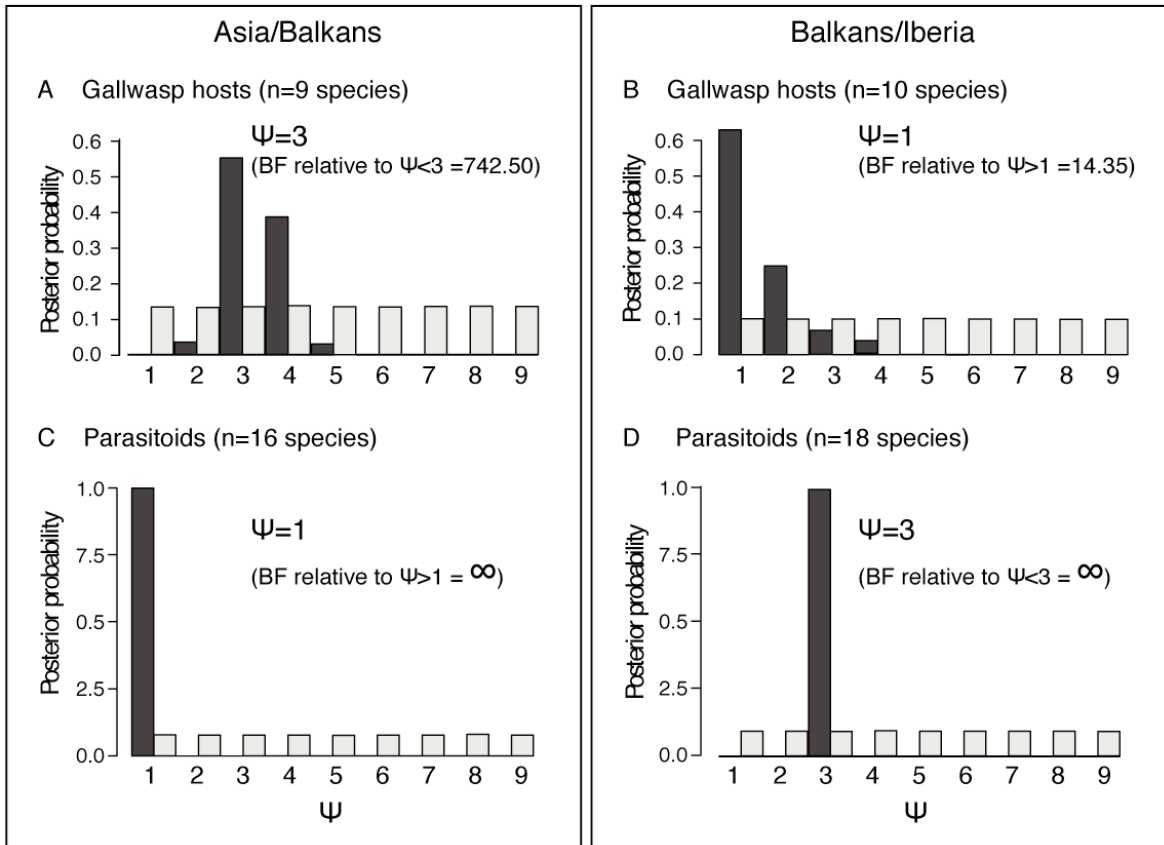**Asia/Balkans**

A Gallwasp hosts (n=9 species)

Ψ=3
(BF relative to Ψ<3 =742.50)

C Parasitoids (n=16 species)

Ψ=1

(BF relative to Ψ>1 = ∞)

Balkans/Iberia

B Gallwasp hosts (n=10 species)

Ψ=1
(BF relative to Ψ>1 =14.35)

D Parasitoids (n=18 species)

Ψ=3

(BF relative to Ψ<3 = ∞)

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Figure 2