Geographic isolation drives speciation in Nearctic aphids

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Across herbivorous insect clades, species richness and host-use diversity tend to positively covary. This could be because host-use divergence drives speciation, or because it raises the ecological limits on species richness. To evaluate these hypotheses, we performed phylogenetic path model analyses of the species diversity of Nearctic aphids. Here, we show that variation in the species richness of aphid clades is caused mainly by host-use divergence, whereas variation in speciation rates is caused more by divergence in non-host-related niche variables. Aphid speciation is affected by both the evolution of host and non-host-related niche components, but the former is largely caused by the latter. Thus, our analyses suggest that host-use divergence can both raise the ecological limits on species richness and drive speciation, although in the latter case, host-use divergence tends to be a step along the causal path leading from non-host-related niche evolution to speciation.
ne out of every three species is an herbivorous insect. How did they become so diverse? Although biologists tend to think of speciation as being driven by geographic isolation between subpopulations, the diversification of herbivorous insects is more often thought to be driven by divergent selection on host-use, stemming from co-evolutionary antagonism with their host plants.

Two general features of the relationships between herbivorous insects and their host plants would seem to make speciation via host-use divergence especially likely. First, the relationships tend to be specific. Second, the relationships are complex. As herbivorous insects feed on their hosts, they compete with other herbivores and plant parasites, and contend with host defenses and natural enemies. Moreover, in many herbivorous insect species, host plants provide sites for mating and oviposition. With so much of their biology linked to specific host associations, divergence on host-use could very well cause reproductive isolation. Indeed, some of the most trumpeted examples of sympatric speciation feature herbivorous insects, much of the classic theory of co-evolution and co-divergence was inspired by patterns of herbivorous insect diversity, and as per predictions of this theory, comparative phylogeneticists have repeatedly found positive links between the species richness and host-use diversity of herbivorous insect clades.

Nevertheless, few cases of sympatric speciation via host-use divergence have been documented in detail, many predictions of the classical co-diversification theory have yet to be tested with rigor, and the positive associations between clade species richness and host-use diversity could have causes other than speciation via host-use divergence. Here, we consider one alternative causal hypothesis in particular (Fig. 1), namely, that speciation in herbivorous insects tends to be via divergence in non-host niche components linked to geographic isolation between subpopulations, and that subsequent host-use divergence raises ecological limits on species diversity. More concretely, host-use divergence determines the extent to which herbivorous insect species can coexist in a community—that is, the overall force of competitive exclusion—as well as the overall geographic extent of an herbivorous insect clade—essentially a composite of the geographic ranges of their host plants. If this is the case, we would still expect strong positive links between species richness and host-use diversity, but there would be less correspondence between rates of speciation and rates of host-use divergence. Of course, these hypotheses are not mutually exclusive; host-use divergence could both drive speciation and raise the limits on species richness.

So, how does host-use divergence tend to affect the species richness of herbivorous insects? Does it tend to drive speciation?

Or is it more important in boosting the limits on species richness? For insight, we performed a comparative analysis of Nearctic aphids for which we have exceptionally detailed views of host-use and geographic distributions. We inferred their phylogenetic relationships, characterized their niches, and used phylogenetic path models to estimate causal relationships between speciation and niche component evolution. We found that host-use divergence both drives speciation and raises the limits on species richness. But evolutionary divergence in host-use tends to be driven by divergence in non-host-related niche components, which have greater combined direct and indirect effects on speciation.

Results

We considered two sets of models. In one set, we looked at what causes variation in the species richness of aphid genera. In a second set, we looked at what causes variation in speciation rates across the branches of the aphid phylogeny. To reiterate, disparities in species richness across clades can be caused by differences in net speciation rates, or differences in the limits on species richness. Our first set of models can identify niche components that limit species richness; our second set can identify niche components that drive speciation. A few words about the characterization of non-host niche components will help with interpretation. In broad strokes, we correlated the spatial distribution of aphid species with that of environmental variables such as soil type, elevation and primary productivity. Such variables tend to be spatially auto-correlated, so divergence along non-host niche axes will tend to entail geographic divergence. But it is theoretically possible for non-host niche divergence to take place without obvious geographic isolation—for example, if environmental patches interdigitate—or for populations to be spatially isolated but occupy otherwise indistinguishable environments. So, our models explicitly distinguish between host-use and non-host niche components, and provide indirect evidence about the role of geographic isolation. See the “Methods” for further detail.

Let us look first at what causes the variation in the species richness of aphid genera. We considered two alternative causal hypotheses, one in which the diversity of host-use niches and non-host-related niches could each directly affect the species richness of aphid genera, and a second in which the diversity of non-host-related niches could only affect aphid species richness indirectly, through its effects on host-use niche. We found that the latter was a better fit to the data: non-host-related niche diversity positively affects host-use diversity, but does not directly affect the species richness of aphid genera. The direct effect of host-use diversity on the species richness of aphid genera was 0.53 standard mean differences (SMD) (±0.07 se), the direct effect of non-host-related niche diversity on host-use diversity was 0.68 SMD (±0.07 se), and the indirect effect of non-host-related niche diversity on aphid species richness—calculated as the product of path coefficients—was 0.36 SMD (±0.07 se) (Fig. 2a). Variation in the extant diversity of aphid clades is caused more by host-use diversity than by non-host-related niche diversity.

Let us now turn to the causes of variation in speciation rates. We first considered each special form of a general model in which (1) the rates of host-use evolution (changes in location in the host-use component space) and non-host-related niche evolution each could directly affect aphid speciation rates, (2) the rates of non-host-related niche evolution and host-use evolution were correlated—that is, each could potentially cause variation in the other, and (3) host-use evolution could potentially affect speciation rates indirectly via its effects on non-host-related niche
The direct effect of host-use evolution on speciation was 0.25 SMD (±0.04 se). In summary, as in models that ignore variation in host-use breadth, increasing the rate of host-use evolution increases the net speciation rate, but the rate of host-use evolution itself is determined by the rate of non-host-related niche evolution. By and large, speciation starts with non-host-related niche divergence.

**Discussion**

These analyses of Nearctic aphids, although taxonomically constrained, constitute the most comprehensive tests to date of the classical hypothesis that herbivorous insect speciation is driven primarily by host-use divergence. We pitted this hypothesis against an alternative, to wit, that following speciation by other means, host-use divergence raises ecological limits on the species richness of aphid clades and communities. We found that although there is evidence for both processes, the latter appears more important: whereas the disparities in species richness across aphid genera are explained more by host-use divergence than non-host-related niche divergence, the opposite is true of the disparities in speciation rates across aphid lineages. Thus, it appears that speciation in aphids tends to start with divergence in non-host-related niche components that would be expected to diverge with geographic isolation, and host-use divergence primarily shapes aphid species diversity by raising the ecological limits on species richness. That being said, we did find evidence of speciation via host-use divergence per se. Thus, returning to our initial question—of why herbivorous insects are so species rich—our analyses suggest that it is because speciation can be caused by both geographic isolation and host-use divergence, which can also increase the conservation of species diversity resulting from either process.

This is not the first indication that the importance of speciation via host-use divergence in herbivorous insects may have been somewhat overstated. In particular, previous researchers have noted that the phylogenetic pattern of host-use in some aphid subcaldes24-26, and some non-aphid clades27, suggest that less than half of all speciation events are linked to a major shift in host use. Our analysis builds on this work, by considering more complex models of herbivorous insect niches, and performing more explicit tests of competing causal hypotheses about the ecology of herbivorous insect speciation. Although we did not directly model the phylogenetic evolution of geographic ranges, or use such models to demonstrate a direct link between geographic range evolution and speciation, we did show that
speciation is driven by divergence of non-host-related components of aphid niches, such as net primary productivity and soil type, that are spatially auto-correlated and thus likely diverge through geographic isolation19,20. Moreover, our models of the non-host-related components of aphid niches rest on correlations between aphid species occurrence in geographic and environmental space; hence, there is a fundamental concordance between non-host-related niche divergence and geographic isolation.

The degree to which our inferences apply to other groups of herbivorous insects may be limited by the fact that in many ways aphids are unusual28. For example, many aphid species have unusually complex life cycles and high reproductive rates39,40. Further study of other groups of herbivorous insects should give us a better sense the generality of our results. Nevertheless, what we found in aphids is consistent with what one might expect for most non-herbivorous-insect groups: speciation tends to be a geographic process, with niche divergence in isolated populations increasing the odds of species co-existence when populations converge.

Methods

Aphid phylogeny. Despite their economic importance as agricultural pests—in particular vectors of plant diseases—prior to this study the aphid phylogeny was poorly resolved28. A few genera have been studied in detail31,32, but previous estimates of supra-generic relationships have been based on DNA sequence data only from a few genetic loci and species33–35. Such data have proven insufficient to infer deep phylogenetic relationships with any confidence. To improve on that situation, we obtained a large DNA sequence data set with Ultra-Conserved Element (UCE) target-enriched genomic sequencing of 454 aphid samples, covering 403 taxa collected across Canada and the United States. Specimen data are given in Supplementary Data 1. Samples cover each of the three aphid families (Aphididae, Adelgidae, and Phylloxeridae) along with 14 of 24 of the nominal subfamilies of Aphiidea. Two psyllid species were included as outgroups for rooting.

DNA isolation. DNA was extracted using either Omega E.Z.N.A. Tissue DNA Kits (Norton, CA) following manufacturer guidelines or Quiagen DNeasy 96 Blood and Tissue Kits (Toronto, ON) with modifications following56 and elution into 2 × 20–25 μl volumes to increase DNA yield. The abdomen of each specimen was punctured by a single pin prick and the whole body used for overnight lysis of tissues. Following DNA isolation, intact specimen cuticles were retained as voucher specimens and deposited in the Canadian National Collection of Insects, Arachnids and Nematodes. DNA concentration was quantified using the double-stranded high sensitivity assay for the Qubit 3.0 Fluorometer (Invitrogen), and where appropriate, 10 μl of DNA of up to four specimens from a single sample was pooled.

Library preparation, target enrichment, and sequencing. DNA libraries were prepared, enriched and sequenced at either Arbor Biosciences (Ann Arbor, Michigan) or Agriculture and Agri-Food Canada’s (AAFC) Molecular Technologies Laboratory (MTL, Ottawa, ON). At Arbor Biosciences, libraries were prepared with Illumina Nextera kits with iTru Y-yolk adapters57 following standard protocols, and sequencing was on the Illumina NovaSeq5 v4 platform. At MTL, libraries were prepared using NEBNext Ultra II FF DNA library prep kits (New England BioLabs, Ipswich, MA), also with iTru Y-yolk adapters. Library preparation steps used half reaction volumes (except where noted), but otherwise followed manufacturer guidelines with a few optimizations. DNA was sheared by enzymatic digestion (Norcross, GA) following manufacturer guidelines or Qiagen DNeasy 96 Blood and DNA isolation kit. DNA was extracted using either Omega E.Z.N.A. Tissue DNA Kits (Toronto, ON) with modifications following56 and elution into 2 × 20–25 μl volumes to increase DNA yield. The abdomen of each specimen was punctured by a single pin prick and the whole body used for overnight lysis of tissues. Following DNA isolation, intact specimen cuticles were retained as voucher specimens and deposited in the Canadian National Collection of Insects, Arachnids and Nematodes. DNA concentration was quantified using the double-stranded high sensitivity assay for the Qubit 3.0 Fluorometer (Invitrogen), and where appropriate, 10 μl of DNA of up to four specimens from a single sample was pooled.

Post-PCR libraries were enriched for 2731 Ultra-Conserved Elements (UCEs) using 40,207 baits (UCE Hemiptera myBaits - Hyb Capture Kit, Arbor Biosciences) designed by Faircloth58. To decrease sequencing costs, 8–10 libraries were multiplexed at equimolar ratios for a total of 50–500 ng per enrichment pool following fluorometric quantification. Enrichment reactions followed manufacturer’s protocol except the amount of baits was decreased to 2 μl per reaction before a 24 hr hybridization at 65 °C. Post-capture library pools were amplified with an on-bead approach using Illumina PCR primer cocktail (20 μM) and a 1 min PCR extension step. PCR products were assessed for quality via Qubit and 4200 TapeStation High Sensitivity D1000 assays, as well as qPCR with touchdown primer pairs on a Roche 480. Then pooled (n = 41–72) at equimolar ratios and loaded at 8–10 pM with phiX1 in four runs on an Illumina MiSeq using 600 (v3) cycle kits.

Sequence assembly, alignment, and analysis. Raw Illumina reads were deposited in the NCBI SRA repository under BioProject PRJNA819460. Reads were assembled using the Snakemake32 pipeline developed for analysis of targeted enrichment data (https://github.com/AAFC-BIC/snakemake-target-enrichment-data) and described in detail elsewhere36. In brief, reads were trimmed using BBduk (https://sourceforge.net/projects/bbmap/) and assembled using four approaches: Abyss44 with and without prior merging of paired-ends using BBMerge46, SPAdes45, and rnaSPAdes46. Phyloseq40 was used to identify and filter UCE loci from the assemblies with probe sequences as input and default parameters for minimum identity and coverage. Only the longest fragment for each locus across all assemblies was used in further analyses. For each locus, we performed a multiple sequence alignment with MAFFT40, and trimmed ambiguous regions with trimAl53. We then used a Python script (Supplementary Data 2) to construct trimmed locus alignments, yielding a supermatrix of ~400,000 sites, spanning more than 10,000 loci.

Phylogenetic relationships were estimated with maximum likelihood optimization with RAxML54, with each locus evolving under a separate general time-reversible (GTR) nucleotide substitution model with gamma-distributed rate heterogeneity. The search began with a starting point derived from 10 independent, non-parametric bootstrap data sets. Then each fifth optimal bootstrap tree was used as a starting point for optimization of the observed DNA data. With this approach, we estimated a phylogeny with strong statistical support (Supplementary Data 3; 417 of 452 nodes with non-parametric bootstrap values >90%). The implications of our results were compared with what might be expected with more data. Here, it is worth saying, that we found that most of the currently recognized supra-generic taxa are monophyletic.

To further extend the taxonomic breadth of our view of aphid phylogeny, we used the UCE-based tree estimate as a back-bone constraint in a FastTree28 phylogenetic analysis of a global GTR nucleotide substitution model and the 403 sequences of 401 species that had been obtained for DNA barcoding, including 77 species not represented in the UCE data set (accession data given in Supplementary Data 4, and the tree in Supplementary Data 5).

Aphid Niches. To characterize the host-use niches of Nearctic aphid species, we combined data from published catalogs8,52 provided in Supplementary Data 6 with data from aphid specimen collections (details given below). (Note that because aphids are sessile and colonial, the associated host plants is relatively unbiased by incidental and ecologically trivial observations). We used these data to make a qualitative incidence matrix of the use by aphid species of host plant families. For a more efficient coding of host use, we used the R package vegan54 to calculate Jaccard distances from the host-use incidence matrix and to simultaneously analyze. We used the first two coordinate principal components in subsequent analyses, accounting for 34% of the variation in host-use in our models of the species richness of aphid genera, and 40% of the variation in our models of speciation rates. These coordinate provide an address for each aphid species in a multivariate space, with the coordinates that could affect the suitability of habitats for aphids and their host plants. Climate data were obtained from Worldclim2. From MODIS, we took data on elevation, net solar radiation, net primary production (as measured by carbon dioxide output), vegetation and leaf area indices, and land cover types. We used soil data from the Unified North American Soil Map56. We took and atmospheric data from the Consortium for Spatial Information58. We calculated ranges and mean values. To avoid bias from outliers, ranges spanned the 25 and 75 quantile values rather than the minimum to maximum. We then
used a principle components analysis to reduce the dimensionality of the environmental niche characterizations, using the first two principle components in path analyses, representing 34% of the variation in the species richness models (Supplementary Data 11), and 31% in the speciation rate models (Supplementary Data 12; Note the values of this tables are phylogenetically-independent contrasts, with a row for each internal nodes in the aphid phylogeny).

**Phylogenetic path models.** To understand how ecological divergence affects aphid species divergence, we conducted phylogenetic confirmatory path analyses. In brief, with path models we can see if the correlations or statistical dependencies among a set of variables are consistent with those predicted by a causal hypothesis, and with phylogenetic path models we can do that in a way that accounts for the non-independence of variable observations due to shared ancestry. Here, we first considered the ecological causes of disparities in the species richness of aphid genera, and we then considered the ecological causes of variation in aphid speciation rates.

For the species-richness models, the species richness of each aphid genus was obtained from Aphid Species File. We excluded from the richness counts any species that does not occur in the Neartic or which has been recently introduced. We also excluded from the species richness values any species not recovered in a clade with the majority of its nominal congeners. Conversely, we did count non-congeneric species that were recovered nested within a nominal genus clade. As a rule, where aphyd phylogeny and classification were in conflict, we gave priority to phylogeny. Likewise, when characterizing composite niches of aphid genera, we used the species included in the richness tallies. We made a version of our aphid phylogeny with just one tip branch per genus (Supplementary Data 13). We had a total of 140 genera represented. Phylogenetic path models were specified and fit with the R package phytools, and compared with C-statistic information criteria (AICc scores). To be clear, although model, we analyzed variation in the species richness of aphid genera because the genetic classification is a ready-made scheme for dividing the extant aphid species richness into a set of mutually-exclusive clades.

For the speciation-rate models, we removed from the phylogeny any species for which we lacked sufficient spatial data (fewer than ten records); this left 371 species. We then calculated a branch-specific index of speciation rates—the Diversification Rate (DR) statistic of Jetz et al., using R code from Rabosky and Goldblum. This is the inverse of a weighted sum of branch lengths connecting a tip branch to the root of a phylogeny, with each successively more rootward branch down-weighted by a factor of 0.5. Our aim was to causally link variation in this speciation rate index to variation in the rates of niche component evolution. Thus, for each niche component, we calculated a Trait Rate (TR) statistic that is analogous to the DR statistic (see Supplementary Data 8). We reconstructed the phylogenetically ancestral states of each niche component with maximum likelihood optimization of a Brownian Motion model. Then, we summed between-node differences in ancestral trait values along the branch path from each tip to the root. This sum for each tip was the TR statistic. For the calculations, we used the R packages phytools and phytotools. To control for the phylogenetic non-independence, we then calculated phylogenetic independent contrasts (PICs) for the DR statistic and the TR statistic.

Because most of our aphid niche variables exhibited considerable skew and kurtosis, prior to path modeling, variables were normalized using the R package bestNormalize. We then specified and fit confirmatory path models using the R package lavaan. Model fit was assessed through AICc scores and p-values, where a model p-value below an alpha-level of 0.05 was interpreted as indicating a missing relationship. In one set of models, we looked at how speciation rates were affected by rates of environmental niche evolution and host-use evolution in a multivariate host-use space. In another set of models, we considered the rate of host-use breadth evolution in addition to the rate of host-use in that multivariate space. Model scripts are provided in Supplementary Data 14 and 15. An outline of our analysis workflow is provided in Supplementary Data 16.

**Data availability**
The DNA sequence data used for phylogenetics are in an NCBI SRA repository under BioProject PRJNA819460; the rest of the data are provided as supplementary information.

**Code availability**
Our codes are provided as supplementary information.

**References**
1. Rosskopf, Y. et al. Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist. www.catalogueoflife.org/annual-checklist/2019 (2019).
2. Barraclough, T. G. & Vogler, A. P. Detecting the geographical pattern of speciation from species-level phylogenies. Am. Nat. 155, 419–434 (2000).
3. Coyne, J. & Orr, H. Speciation (2004).
4. Ehrlich, P. R. & Raven, P. H. Butterflies and plants: a study in coevolution. Evolution 18, 586–608 (1964).
5. Futuyma, D. J. & Agrawal, A. A. Macroevolution and the biological diversity of plants and herbivores. Proc. Natl Acad. Sci. USA 106, 18054–18061 (2009).
6. Hardy, N. B., Kacewinski, C., Bird, G. & Normark, B. B. What we don’t know about diet-breadth evolution in herbivorous insects. Annu. Rev. Ecol. Evol. Syst. 51, 103–122 (2020).
7. Forstner, J. L. et al. The global distribution of diet breadth in insect herbivores. Proc. Natl Acad. Sci. USA 112, 442–7 (2015).
8. Sezer, M. & Butlin, R. K. The genetic basis of oviposition preference differences between sympatric host races of the brown planthopper (Nilaparvata lugens). Proc. R. Soc. B Biol. Sci. 265, 2399–2405 (1998).
9. Berlocher, S. H. Host race or species? Allozyme characterization of the ‘flowering dogwood fly’, a member of the Rhagoletis pomonella complex. Heredity 83, 652–662 (1999).
10. Filchak, K. E., Roethele, J. B. & Feder, J. L. Natural selection and sympatric divergence in the apple maggot Rhagoletis pomonella. Nature 407, 739–742 (2000).
11. Drès, M. & Mallet, J. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. B Biol. Sci. 357, 471–492 (2002).
12. Pashley Prowell, D., McMichael, M. & Ois Silvain, J. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Am. Entomol. Soc. Am. 97, 1034–1044 (2004).
13. Wheat, C. W. et al. The genetic basis of a plant-insect coevolutionary key innovation. Proc. Natl Acad. Sci. USA 104, 20427–20431 (2007).
14. Nij, N., Nylin, S. & Wahlberg, N. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. BMC Biol. 6, 1–10 (2006).
15. Fordyce, J. A. Host shifts and evolutionary radiations of butterflies. In Proceedings of the Royal Society B: Biological Sciences Vol. 277, 3735–3743 (Royal Society, 2010).
16. Rabosky, D. L. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. Ecol. Lett. 12, 735–743 (2009).
17. Hardy, N. B. & Cook, L. G. Testing for ecological limitation of diversification: a case study using parasitic plants. Am. Nat. 180, 438–449 (2012).
18. Nosil, P. Ecological Speciation. Ecological Speciation (Oxford University Press, 2015).
19. Legendre, P. Spatial autocorrelation: trouble or new paradigm? Ecology 74, 1659–1673 (1993).
20. Dormann, C. F. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. Glob. Ecol. Biogeogr. 16, 129–138 (2007).
21. Hardy, N. B. & Otto, S. P. Specialization and generalization in the diversification of phytophagous insects: tests of the musical chairs and oscillation hypotheses. Proc. R. Soc. B Biol. Sci. 281, 1–10 (2014).
22. Nylin, S., Slove, J. & Janz, N. Host plant utilization, host range oscillations and diversification in nymphalid butterflies: a phylogenetic investigation. Evolution 68, 105–124 (2014).
23. Peterson, D. A., Hardy, N. B. & Normark, B. B. Micro- and macroevolutionary trade-offs in plant-feeding insects. Am. Nat. 188, 640–650 (2016).
24. Jouselin, E. et al. Is ecological speciation a major trend in aphids? Insights from a molecular phylogeny of the conifer-feeding genus Canara. Front. Zool. 10, 1–18 (2013).
25. Meseguer, A. S., Coeur d’acier, A., Gerson, G. & Jouselin, E. Unravelling the historical biogeography and diversification dynamics of a highly diverse conifer-feeding aphid genus. J. Biogeogr. 42, 1482–1492 (2015).
26. Lee, Y., Kanturski, M., Footitt, R. G., Kim, S. & Lee, S. Molecular phylogeny and evolution of Calaphidinae (Hemiptera: Aphididae). Cladistics. https://doi.org/10.1111/CLA.20487 (2021).
27. Winkler, I. & Mitter, C. In Specialization, Speciation, and Radiation: the Evolutionary Biology of Herbivorous insects (ed. Tilmon, K.) 240–263 (University of California Press, 2009).
28. Hardy, N. B. In Insect Biodiversity 591–625 (John Wiley & Sons, Ltd, 2018).
29. Moran, N. A. The evolution of aphid life cycles. Annu. Rev. Entomol. Vol. 37, 331–348 (1992).
30. Hardy, N. B., Peterson, D. A. & von Dohlen, C. D. The evolution of life cycle complexity in aphids: ecological optimization or historical constraint? Evolution 69, 1423–1432 (2015).
31. Stern, D. L. A phylogenetic analysis of soldier evolution in the aphid family (Hemiptera: Pemphigidae). Proc. R. Soc. B Biol. Sci. 256, 203–209 (1994).
32. Yang, Z. X., Chen, X. M., Havill, N. P., Feng, Y. & Chen, H. Phylogeny of Rhus gall aphids (Hemiptera: Pemphigidae) based on combined molecular analysis.
33. Von Dohlen, C. D. & Moran, N. A. Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. *Biol. J. Linn. Soc.* 71, 689–717 (2000).

34. Ortiz-Rivas, B. & Martínez-Torres, D. Combination of molecular data support the existence of three main lineages in the phylogeny of aphids (Hemiptera: Aphididae) and the basal position of the subfamily Lachninae. *Mol. Phylogenet. Evol.* 55, 305–317 (2010).

35. Nováková, E. et al. Reconstructing the phylogeny of aphids (Hemiptera: Aphididae) using DNA of the obligate symbiont Buchnera aphidicola. *Mol. Phylogenet. Evol.* 68, 42–54 (2013).

36. Cruaud, A. et al. Optimized DNA extraction and library preparation for metagenomic arthropods: application to target enrichment in chelid waps used for biocontrol. *Mol. Ecol. Resour.* 19, 702–710 (2019).

37. Glenn, T. C. et al. Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTRU & iNext). *PeerJ* 2019, e7755 (2019).

38. Faircloth, B. C. Identifying conserved genomic elements and designing universal bait sets to enrich them. *Methods Ecol. Evol.* 8, 1103–1112 (2017).

39. Köster, J. & Rahmann, S. Snakemake—a scalable bioinformatics workflow engine. *Bioinformatics* 28, 2520–2522 (2012).

40. Brunke, A. J. et al. The limits of Quedini at last (Staphylinidae: Staphylininae): a rove beetle mega-radiation resolved by comprehensive sampling and anchored phylogenies. *Entomol. Scien.* 46, 396–421 (2021).

41. Jackman, S. D. et al. ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. *Genome Res.* 27, 768–777 (2017).

42. Bushnell, B., Rood, J. & Singer, E. BBMerge—accurate paired shotgun read merging via overlap. *PLoS ONE* 12, e0185056 (2017).

43. Nurk, S. et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J. Comput. Biol.* 46, 794–817 (2013).

44. Bushmanova, E., Antipov, D., Lapidus, A. & Prijbelski, A. D. rnaSPAdes: a de novo transcriptome assembler and its application to RNA-Seq data. *Gigascience* 8, 1–13 (2019).

45. Faircloth, B. C. PHYLUCe is a software package for the analysis of conserved genomic loci. *Bioinformatics* 32, 786–788 (2016).

46. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780 (2013).

47. Capella-Gutierrez, S., Silla-Martinez, J. M. & Gabaldon, T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972–1973 (2009).

48. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313 (2014).

49. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2—accurate maximum-likelihood trees for large alignments. *PLoS ONE* 5, e9490 (2010).

50. Blackman, R. L. & Eastop, V. F. Aphids on the world's crops: an identification and information guide. *Aphids world'sCrop, anIdentif. Inf. Guid.* (2000).

51. Holman, J. Host Plant Catalog of Aphids (Springer, 2009).

52. Hardy, N. B., Petkovic, D. A., Ross, L. & Rosenstein, J. A. Does a plant-eating insect’s diet govern the evolution of insecticide resistance? Comparative tests of the pre-adaptation hypothesis. *Evol. Appl.* 11, 739–747 (2018).

53. Oksanes, J. et al. Vegan: Community Ecology Package. R package version 2.5–7 (2020).

54. Chamberlain, S., Ram, K., Barve, V. & Mcglinn, D. Package ‘rggft’: Interface to the Global ‘Biodiversity’ Information Facility ‘API’ (2017).

55. Stockwell, D. R. B. & Peterson, A. T. Effects of sample size on accuracy of species distribution models. *Ecol. Modell.* 148, 1–13 (2002).

56. Fick, S. E. & Hijmans, R. J. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37, 4302–4315 (2017).

57. Liu, S. et al. The unified North American soil map and its implication on the soil organic carbon stock in North America. *Biogecosciences* 10, 2915–2930 (2013).

58. Fabret, C. Aphid Species File v5.0. Available at: Favret, C. Aphid Species File. Version 5.0/5.0 (2020).

59. van der Bilj, W. phylopath: easy phylogenetic path analysis in R. *PeerJ* 2018, e4718 (2018).

60. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* 491, 444–448 (2012).

61. Shipley, B. *Cause and Correlation in Biology: A User’s Guide to Path Analysis, Structural Equations and Causal Inference with R* (2016).

62. Hardenberg, Avon & Gonzalez-Voyer, A. Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution* 67, 378–387 (2013).

63. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223 (2012).

64. Rosseel, Y. lava: an R Package for Structural Equation Modeling and More Version 0.5–12 (BETA) (2012).

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