

Review

Parasitoid speciation and diversification

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Parasitoid wasps may well be the most species-rich animal group on Earth, and host–parasitoid interactions may thereby be one of the most common types of species interactions. Understanding the major mechanisms underlying diversification in parasitoids should be a high priority, not the least in order to predict consequences from high extinction rates currently observed. The two major hypotheses explaining host-associated diversification are the escape-and-radiate hypothesis and the oscillation hypothesis, where the former assumes that key innovations are major drivers of radiation bursts, whereas the latter rather assumes that diversification depends on processes acting on the standing genetic variation that influences host use. This paper reviews the recent literature on parasitoid speciation in light of these major hypotheses to identify potential key innovations and host use variability underlying diversification. The paper also calls upon recent theoretical advances from a similar system, plant–butterfly interactions, to provide shortcuts in the development of theories explaining the high diversity of parasitoid wasps.

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Current Opinion in Insect Science 2024, 66:101281

This review comes from a themed issue on **Ecology**

Edited by **Renee M Borges, Radhika Venkatesan and Saskya van Nouhuys**

Available online 9 October 2024

<https://doi.org/10.1016/j.cois.2024.101281>

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Introduction

Coleoptera were long considered the most species-rich animal order, but this view has recently been challenged by calculations suggesting that Hymenoptera may contain three times as many species as Coleoptera [1]. Recent years have

also revealed substantial cryptic speciation among particularly parasitoid Hymenoptera (e.g. [2,3]), often identified only through molecular markers, but species descriptions are likely also lagging due to the small size and the limited morphological variability of many parasitoid species, the difficulty in rearing larvae and thereby to describe host use, and the lack of taxonomic expertise. The high species diversity occurs despite, or perhaps because of, the complexity of the parasitoid lifestyle. Many parasitoids not only have to find and devour their prey but they often also take over the host through manipulations of the host physiology.

Recent years have seen substantial improvements in the understanding of Hymenopteran phylogenies and higher-order evolutionary history as a consequence of using molecular tools [4–8]. These studies show among other things that the parasitoid lifestyle evolved once in Hymenoptera but also that this innovation did not initially cause high diversification until their primary host groups began diversifying [5]. In fact, it has been suggested that the parasitoid lifestyle is not the main cause for high diversification but rather the high inbreeding rates due to common sib-mating, coupled with the haplodiploid sexual determination system that causes rapid local adaptation [9]. Interestingly, a key innovation leading to diversification instead seems to be the reversal to plant feeding in groups such as Eurytomidae and Cynipidae [5].

The expansion of molecular tools has similarly allowed an increasing number of full genome sequencing of parasitoid wasps (recent examples: [10,11–19]), which provide key resources for the exploration of evolutionary processes in host–parasitoid interactions, including speciation genomics [20]. Nevertheless, we have limited knowledge about the factors driving population differentiation and diversification in parasitoid wasps and the possible role of coevolution in the diversification process of parasitoids. One reason for this lack of information may be that few studies have explored variation within species or between sibling species. To meet this challenge, and to trigger additional studies, we examine diversification hypotheses in relation to parasitoid Hymenoptera and how network analyses can be used to differentiate between hypotheses. We first briefly overview previous research on parasitoid speciation with a particular focus on host-associated diversification and thereafter discuss the roles of key innovations versus processes acting on standing genetic variation for diversification rates. While our focus is on parasitoid Hymenoptera, we also use theoretical developments from

other coevolutionary systems, such as plant–herbivore interactions, showing how different diversification processes leave unique imprints in species networks that can be detected using recently developed analyses. Even though the lifestyle of parasitoids is quite particular, at least for those endoparasitoid species overtaking their hosts' physiology, there are many similarities to plant–herbivore systems such as host use typically being restricted to one or a few closely related species [21].

Host race formation and population divergence

Speciation obviously involves multiple stages that end in some type of reproductive isolation. We will here not review the various stages involved in the speciation process but rather focus on the underlying causes for diversification, which in parasitoids is likely to involve mostly host use changes. Host-associated diversification in parasitoids differs from herbivores because host expansions in parasitoids may not only occur to closely related host species but may also follow the host lifestyle or even the phylogeny of the host plant consumed by their herbivore host [22]. For instance, Leppänen et al. [23] showed that parasitoids attacking leafminers may switch to unrelated leafminer hosts, but that leafminers are not attacked by parasitoids on free-living species.

A small number of studies have examined the host-associated diversification process for parasitoids in more detail. The most well-documented case arguably involves parasitoids attacking *Rhagoletis* fruit flies [24,25], and particularly *Diachasma alboeum* (Braconidae), but other examples have been documented from a range of families, including *Nasonia vitripennis* (Pteromalidae; [26], but see Ref. [27]), *Asecodes parviclava* (Eulophidae) [28], *Aphelinus certus* (Aphelinidae) [29], and *Leptopilina japonica* (Figitidae) [30]. These cases show ecological differentiation where females preferentially lay eggs in the original host species, but population differentiation may also involve phenological changes matching the host phenology [31]. This far, even though some studies show genetic variation connected to host use, few studies have connected phenotypic differences and genomic changes (but see Ref. [32]), which limits our capacity to understand the ecological adaptations to different host species. An attempt in this direction was the phylogenetic analysis of parasitoids and pollinators associated with figs, which suggested that interspecific competition, leading to host-associated differentiation, may be a major driver of diversification in this system [33].

The rate of population divergence leading to speciation is certainly affected by multiple processes, including sexual selection, symbiont-mediated changes, learning of host cues etc [34]. For instance, the role of sexual selection in speciation has been a hot topic for decades,

where the most commonly discussed mechanism has been pre-mating mate choice that evolves in combination with changes in some secondary sexual trait (for recent reviews on the topic, see Refs. [35,36]). In parasitoid wasps, secondary sexual traits are often cryptic to the human eye but may involve sexual dimorphism in body size or color [34]. For instance, male wings in some species have a different, and often species-specific, hue compared with female wings, which may provide a template for sexual selection [2,37]. There is no doubt that sexual selection has a role in the speciation process of many organisms, but in most cases by increasing population divergence that arose due to some ecological process, such as host use changes [35]. Additional mechanisms underlying sexual selection in parasitoid wasps have been discussed, but their role in speciation and diversification is yet unclear.

Key innovations and adaptive radiations

A central problem in discussions about speciation is whether innovations affecting the genome preceded the diversification in ecological traits or whether speciation was rather a consequence of some ecological shift, for example, host shifts [25]. The adaptive radiation hypothesis assumes that diversification follows some kind of key innovation that opens up a new resource base for the consumers on which to diversify [38]. In herbivores, these key innovations often involve adaptations allowing individuals to detoxify specific chemical compounds. For instance, adaptations to detoxify the mustard bomb allowed butterflies to radiate on a large set of plants in Brassicaceae [39]. In parasitoid species, corresponding key innovations have been less explored but could involve abilities to overcome immune responses or other types of defenses in their hosts, and where correlated defenses in other hosts thereafter can be exploited. One class of potential key innovations in parasitoids may include morphological adaptations in the ovipositor needed for attacking concealed hosts, even though the ancestral state of Apocrita was likely a species attacking wood-living beetle larvae [6]. For instance, parasitoids attacking free-living hosts are typically not able to lay eggs on leafminers [23], and problems may be even larger for hosts living inside of woody tissues. Previous studies suggest that sclerotization of the ovipositor or the presence of teeth on the tip varies depending on the parasitoid life history, where harder ovipositor or more teeth occur on species attacking hosts inside harder tissues [40,41]. On the other hand, the ovipositor length seems to be a much more labile trait that is less predicted by parasitoid phylogeny [42] and is therefore also less likely to constitute a key innovation. Other potential key innovations may be virus domestication in Braconidae and Ichneumonidae [7,14,43–46], which has likely had major consequences on their host use capacity. Recent studies also suggest viral involvement in host

race formation of *Cotesia sesamiae* (Braconidae) [32,47]. Innovations in other offensive traits have similarly received some attention, for instance, that evolution of venom genes often involves co-option of other gene classes and neofunctionalization following gene duplication [48–50], but either of these innovations has yet to be connected to radiation events.

Dynamic host repertoires and diversification

An alternative to the escape-and-radiate hypothesis is the oscillation hypothesis, which posits that diversification of species lineages is rather a consequence of ecological processes acting on standing genetic variation [51]. This hypothesis originates from the observation that host repertoires within a species show high variability both geographically and over time. Even though a species may use only single species locally (realized host repertoire), the fundamental host repertoire may be much broader, which allows for gains or switches of resources when opportunity arises. An expansion of the host repertoire through, for instance, a geographic range shift may trigger host-associated differentiation on the old and novel hosts, where diversification may arise through multiple such events. The mechanisms underlying the oscillation and the adaptive radiation hypotheses are expected to lead to different imprints on the species interaction network, where innovations typically lead to modular networks, whereas resource use variability is expected to lead to more nested networks [52]. Studies of butterfly–plant networks indicate that both processes are involved and typically explain different parts of the historical process [53].

The role of host repertoire variability and range shifts for diversifications in parasitoids is yet unknown, but these mechanisms have recently been proposed for two other highly specialized enemy groups, parasites, and diseases [21,54,55]. The main assumption underlying the oscillation hypothesis is that species tend to have broader fundamental than realized host repertoires. Even though many parasitoid species use only one host locally, it is well known that many species also have the capacity to successfully attack other species, should the opportunity arise, for example, during a range shift. In fact, recommendations underlying the introduction of parasitoid wasps for pest control strongly suggest that alternative host use in the introduced range should be considered before release and in particular for species related to the expected host [56–58]. The literature is also replete with examples of changed resource use following parasitoid introductions (for a recent example, see Ref. [59]). Even though these diet expansions involve human-assisted migrations, natural range shifts in either hosts or parasitoids may similarly cause species to be exposed to novel interactors, with the potential of triggering host-associated diversification. In butterflies

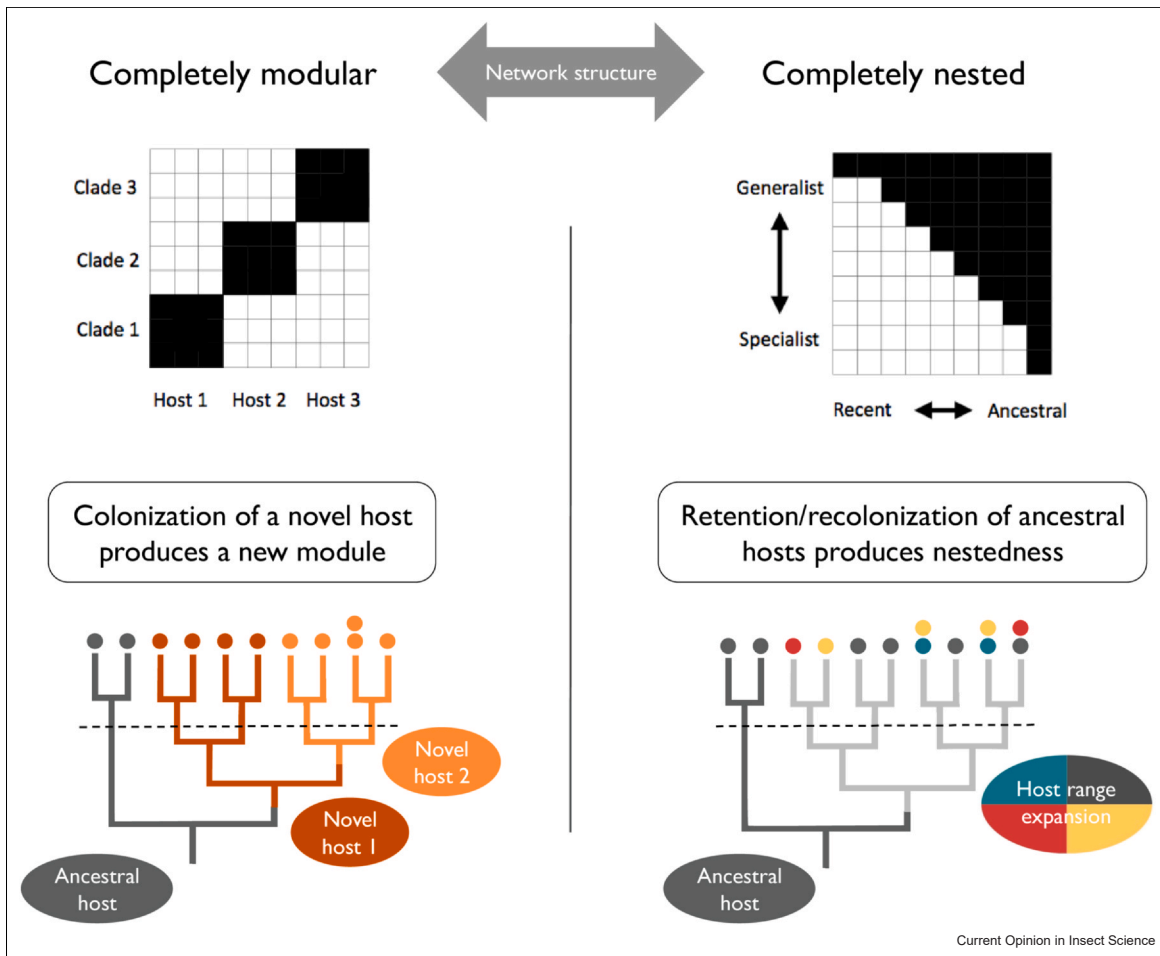
and other herbivores, fundamental niche spaces are typically defined only by chemical or morphological properties of the host plant. In parasitoids, fundamental niches may be determined both by host properties and by properties of the plant host consumed by the herbivore host. For instance, the fundamental niche of a parasitoid attacking leafminers may include species with a similar lifestyle irrespective of taxon identity.

Exploring diversification theories

No studies in host–parasitoid systems have yet attempted to explore the relative role of key innovations and host repertoire variability in host–parasitoid systems, leaving the question unresolved on mechanisms underlying the extreme diversity of parasitoid wasps. Theoretical analyses provide a solution to this question, where different diversification models give different imprints on network properties. Key innovations followed by adaptive radiations will lead to a modular network, which often consists of closely related enemy species but may also involve unrelated enemy species that have diversified in a similar fashion. For instance, the capacity to defuse the mustard bomb in Brassicaceae has caused adaptive radiations in both butterflies and beetles, which then would show up as a joint module in a network involving both groups. Host repertoire variability and range shifts will result in a different network pattern, either fully nested or nested within modules [52]. Thus, a simple way to look for signs of radiations and oscillations is to quantify modularity and nestedness in parasitoid–host networks and then test (i) whether phylogenetic diversity within modules is lower than expected by chance and (ii) whether ancestral hosts are used by many parasitoids widespread in the phylogeny (Figure 1). Before any analyses can be done, however, a robust interaction data set that describes the structure of clade-level parasitoid–host networks need to be compiled, that is, phylogenetic trees for the parasitoid and the host clades and interaction data at the taxonomic level of interest, but such data set are often difficult to compile for many parasitoid group. The last test also requires some knowledge about the coevolutionary history between the studied clades.

The most thorough way to quantify the contributions of radiations and oscillations for a given clade would be to reconstruct network evolution using a model such as that of Braga et al. [60]. A caveat, however, is that the current model does not account for host evolution (i.e. the host phylogeny is fixed across the diversification of the symbiont clade), which implies that the model works less well when the host clade diversifies at a rate similar to the enemy clade, which could be the case for host–parasitoid systems. The model may nevertheless provide relevant results when applied to data at the level of higher host taxonomic groups (e.g. families or genera), provided that

Figure 1



Network structure reflects the evolution of parasitoid–host interactions. Modularity and nestedness are not mutually exclusive but represent the ends of a gradient. A network, for example, between parasitoid genera and host genera, that is completely modular and closely related parasitoids interact with the same host suggests that the modules were formed by host shifts to the novel hosts followed by radiations. On the other hand, a completely nested network where specialists interact with the ancestral host suggests that oscillations in host range have happened, with at least one event of host range expansion (i.e. colonization of new hosts while also maintaining ancestral hosts) followed by specialization closer to the tips of the tree. In this example, the tips of the phylogenetic trees represent parasitoid species, and the dashed lines represent an arbitrary clustering into parasitoid genera. Different colors represent different host genera, and each circle at the tips of the tree represent interaction with a host species.

host use is sufficiently conserved. Assumptions underlying the oscillation hypothesis can also be tested directly for parasitoids, such as the presence of a ‘genetic memory’ (expressed in the fundamental host repertoire), which could be a mechanism underlying phylogenetic conservatism in host use, as shown in butterflies [61,62].

A complicating factor for these analyses is that host use in parasitoids may cluster not by the host insect directly but rather by the host plant of their insect host, by the host feeding mode or even by habitat [22]. For instance, it seems that unrelated leaf miners with a similar lifestyle share parasitoid enemies, suggesting an additional dimension for host repertoire and speciation dynamics. Network analysis can be useful in identifying the factors

that determine parasitoid clustering (taxonomy or traits of hosts or host plants). These networks may be tritrophic (plant–herbivore–parasitoid) but may be subdivided into bipartite networks for analyses. For each network, module composition can be investigated to determine whether closely related parasitoids tend to attack similar herbivores or herbivores that use similar host plants. If modules are not composed of closely related parasitoids, that finding indicates that phylogenetic conservatism in host use is low. The number of tritrophic networks involving plants, herbivores, and parasitoids is indeed increasing [22,63–65], providing useful templates for further analysis. Depending on how well herbivore–plant and parasitoid–herbivore interactions are described, an alternative approach could be to build networks between parasitoids

and herbivore–plant pairs. This approach would allow direct comparisons between the importance of herbivores and plants for parasitoid host choice but would only make sense if sampling effort is high enough to avoid false negatives (herbivore–plant pairs that have not yet been identified as hosts by the parasitoids).

Conclusions

Processes explaining the mega-diversity of parasitoid Hymenoptera are yet poorly understood, and a more comprehensive analysis of diversification processes is obviously hampered by limitations in host use data and phylogenetic information. Possibly, this information can be more rapidly gathered using metabarcoding tools [63], even though these tools do not distinguish between successful and unsuccessful host attacks. In addition, there is a need to understand whether key innovations are equally important for diversification in parasitoid wasps as for herbivore groups, and what those key innovations may be. The escape-and-radiate hypothesis assumes that key innovations open a wide resource base providing novel opportunities for diversification. Such untapped opportunities could be due to previous adaptations in defense traits, for example, egg laying inside plant tissues, that may have caused host diversifications. The potential importance of such a chain of events in host–parasitoid systems is yet unknown and therefore also the role of key innovations in parasitoid diversification. Similarly, the oscillation hypothesis for diversification in parasitoid Hymenoptera deserves further exploration. In either case, perhaps Askew [9] was right all along, that the common pattern of sib-mating coupled with the haplodiploid sex determination system, leading to rapid local adaptation, is the major explanation underlying the extreme diversity of parasitoid wasps. However, testing such a hypothesis will be a major challenge.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

Acknowledgements

This work has benefited from previous discussions with colleagues and the experimental work of several PhD students (L. Fors, X. Yang, T. Verschut).

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