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Diversity, population structure, and individual behaviour of parasitoids as seen using molecular markers

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Parasitoids have long been models for host–parasite interactions, and are important in biological control. Neutral molecular markers have become increasingly accessible tools, revealing previously unknown parasitoid diversity. Thus, insect communities are now seen as more speciose. They have also been found to be more complex, based on trophic links detected using bits of parasitoid DNA in hosts, and host DNA in adult parasitoids. At the population level molecular markers are used to determine the influence of factors such as host dynamics on parasitoid population structure. Finally, at the individual level, they are used to identify movement of individuals. Overall molecular markers greatly increase the value of parasitoid samples collected, for both basic and applied research, at all levels of study.

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Introduction

The tools of molecular biology have developed rapidly over the past decade, and infiltrated into most aspects of biological research. Neutral genetic markers are short, sometimes repetitive, DNA sequences that can be used to classify individuals as part of a group, or to distinguish among groups, or among individuals based on differences in sequence data. In this brief review I will summarize the current use of neutral genetic markers in studies of parasitoid diversity and community structure, population structure, and individual behaviour and movement (Figure 1). I will then discuss how genetic markers are used at each of these ecological levels in biological control.

Parasitoid diversity and community structure

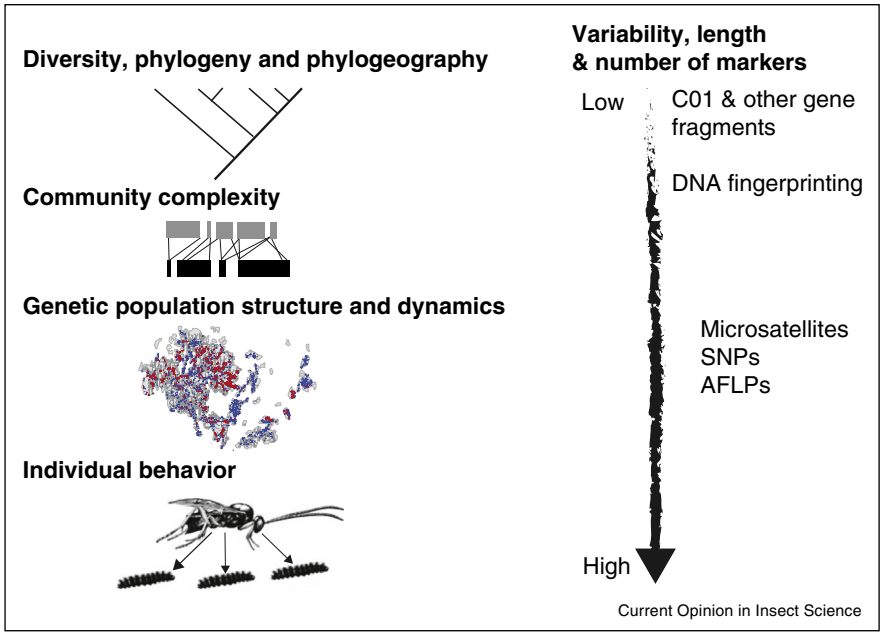
Diversity

Detailed studies of taxonomic groups of parasitoids, using traditional morphology based taxonomy in combination with molecular markers show increased diversity, and the formation of cryptic species groups rooted in long-known single named species [1,2]. In some case these are interpreted in a phylogeographic context in order to understand the ecological or evolutionary context of species geographic and host ranges [3–8]. More recently molecular markers, mostly DNA barcoding, are being used even without morphological study, to elucidate the diversity of parasitoid species, resolve parasitoid phylogeny, and build host–parasitoid food webs. The barcoding approach to quantifying parasitoid diversity started with work based in Costa Rica by Janzen *et al.* [9,10]. That and later studies show very high diversity of parasitoids, with high host specialization in the tropics (e.g. [11,12••,13]). The same techniques have now been used in temperate and even subarctic systems, where less dramatic increases in species diversity have been found [14,15]. The greater increase in diversity in the tropics can be attributed both to there being less previous knowledge of the taxonomy of parasitoids to begin with in the tropics [13], as well as the general trend of high tropical diversity due to resource specialization of species [16].

Host–parasitoid communities

DNA barcoding is also used to determine the structure and complexity of trophically structured communities. Recent reviews by Smith *et al.* [17] and Hrček and Godfray [18•] point out that as the cost of using molecular tools decreases and they become more convenient, host–parasitoid food webs are built with increasingly accurate species diversity, and patterns of trophic linkage. Quantification of species diversity in parasitoid communities has again been exemplified by studies of tropical host–parasitoid communities [9,19], but detailed food webs have also been constructed in temperate [14] and even in high arctic [15] host–parasitoid communities. Though it is still resource and time consuming to sample sufficiently to make a quantitative food web, molecular methods increase the value of these efforts greatly because much more can be learned from each sample if they are associated with molecular markers. For instance, trophic linkages can be identified using molecular methods to detect parasitoids, or the remains of parasitoids in sampled hosts [19]. Using this method Condon *et al.* [12••] found high host specificity in the 18 parasitoid species using 14 *Blepharoneura* fly species on Cucurbit flowers in Peru.

Figure 1



Schematic drawing of the scales of parasitoid research and types of neutral molecular markers used.

However, analysis of parasitoid tissue in host pupae revealed that many of the parasitoid species actually attacked multiple fly species, but failed to develop in most of them. This study brings to light the role of hosts in determining the apparent specificity of parasitoids. It also demonstrates the risk of overestimating host range of parasitoids by analysing parasitoid samples detected in hosts but not reared from them.

Just as parasitoid remains can be found in hosts, remnants of hosts can also be found in adult parasitoids. Species-specific genetic markers can be used identify parasitoid–host links [19,20•,21••] just as they have been used to identify predator–prey trophic structure [22]. In a recent study of the community associated with high arctic Diptera and Lepidoptera, Wirta *et al.* [21••] detected host remains in parasitoids as well as in the guts of spider and bird predators. With this they showed qualitatively the breadth and overlap of parasitism and predation by higher trophic level species, making the relative role of parasitoids in the community apparent.

Genetic population structure

Genetic markers are used for studying populations of parasitoids as well. Since the data of interest are variation within a species rather than among species, more variable markers than those used for DNA barcoding are needed. These have mostly been DNA-microsatellite markers, and more recently also SNPs [23]. PCR techniques such as AFLPs have also been used [24,25]. Over the past decade the use of these tools for studying population genetics of parasitoids has blossomed, as it has in the study of other taxa. Traditional population genetics measures, such as heterozygosity, population differentiation (F_{st}), effective population size (N_e) and isolation by distance are used to quantify the genetic variability in a population, degree of inbreeding, and its history, such as evidence of founder effects [26]. Researchers go on to quantify how populations are genetically structured, often using multi-locus genotype clustering techniques [27]. Samples taken over a geographic area are used to determine limitations of gene flow due to many factors, such as geography [28], climate [29,30], host species [31–35] and host plant [35–39].

Because parasitoids have a close relationship with their hosts, the host population dynamics and distribution in the landscape impose structure and dynamics on the parasitoid [40]. The genetic structure of some parasitoid species matches that of the host suggesting that the parasitoid population is tracking that of the host. Nyabuga *et al.* [41•] showed that the parasitoid *Lysiphlebus hirticornis* had lower population genetic structure than that of its host aphid. This is expected because aphids are mostly wingless and reproduce clonally for generations on a single host plant, while the parasitoid reproduces sexually and is winged. Nonetheless, Nyabuga *et al.* [41•] argued that the genetic structure of the parasitoid is driven by the small-scale distribution of the host, because both contained structure at the scale of host subpopulation, though the host structure was of higher magnitude.

Mobile parasitoids, even specialized ones, may not track fine scale host dynamics, and instead have more coarse population genetic structure [26,42–45]. Alternatively, some parasitoid species are known to have a finer spatial structure than their hosts suggesting that factors other than host, such as dispersal ability, govern parasitoid spatial genetic structure [26,46]. These differences in relation to host population dynamics are tractable most obviously to dispersal behaviour, but also to host range, and phylogeographic history [47]. While there have been a handful of studies comparing spatial genetic structure of a host and a parasitoid in the same landscape, we are still lacking studies of the spatial genetic structure of parasitoids with a wide host range. We would expect fine structure if individuals do not have to go far to find host species; and coarse structure if individuals must move in the landscape to find hosts.

Studies at the population level have also lead to greater understanding of parasitoid ecology aside from parasitoid relation to host population dynamics or landscape structure. For instance, we know parasitoids use host plant derived cues to locate hosts, and that individuals benefit from learning specific host plant odours [48]. Nonetheless a distinct lack of genetic structure related to host plant species is often found [36,37,44,49], indicating that parasitoids, even those with a narrow host breadth, can be adept at using a range of plant cues to find hosts.

Finally, in some cases genetic structure may be imposed not by the environment or hosts, but by symbionts. For example, many parasitoid species include both sexual and asexual lineages or populations, even sympatrically. In many cases the asexual female lineages are maintained by endosymbionts through male killing, feminization, and induction of thelytokous parthenogenesis [50]. Since members of asexual lineages do not regularly interbreed, there is a loss of genetic variability (e.g. [51]). The lineages become genetically distinct, and can be distinguished using molecular markers (e.g. [52]). Similarly, association of a host with endosymbionts that confer resistance to parasitism has lead to genetically differentiated parasitoid lineages (e.g. [53]).

Individual behaviour and movement

Using many polymorphic DNA-microsatellite markers we can study population structure and dynamics at a fine scale [54], and even identify sibling groups [55,56], which allows use to determine foraging (oviposition) behaviour of individuals. The locations of hosts containing parasitoid sibling indicate the minimum area over which a mother distributes her offspring. Thus, we know for instance, that most *Hyposoter horticola* developing in a single gregarious host caterpillar nest are the progeny of a single mother, and that the same mother can move on a scale of kilometres to parasitize another host nest [57,58]. In contrast female *Diaeretiella rapae* parasitizing the cabbage aphid, *Brevicoryne brassicae*, in an agricultural field were found to parasitize only hosts found on a few neighbouring plants [54]. A recent study by Zepeda-Paulo *et al.* [59•] used the distributions of full sib and paternal half sibs to show that female and male *Aphidius ervi* parasitoids disperse differently, with males, which are known to mate multiple times, moving more than females.

Several researchers have gone on to use the distribution of parasitoid offspring under natural conditions to study basic theories in population ecology and life history. Tentelier *et al.* [60] did this by testing the classical theory of ideal free distribution (IFD) of progeny using *Lysiphlebus testaceipes*, a parasitoids of the aphid *Aphis nerii*. As expected, they found that the number of foundresses using a patch increased with host density, resulting in aggregation in better habitat patches. However, counter to the expectations of IFD, family size increased with number of foundresses, indicating that competition was not limiting fitness at those high quality patches.

As the individual foraging behaviours of more parasitoids are characterized using molecular markers we will no doubt learn more about the roles of host distribution and dynamics in parasitoid foraging behaviour, as well as intraspecific competition, and the relative fitness of different individuals in populations. Genetic markers are also being used for more esoteric aspects of parasitoid population ecology and life history, such as those related to their mode of sex determination and sex ratio at the population level [61]. De Boer *et al.* [23] recently used molecular markers to show that the parasitoid *Cotesia vestalis* avoids sibling mating under natural conditions. This is not surprising because, for this and many other hymenoptera, mating between close relatives can result in sterile diploid sons, due to complementary sex determination [62].

Applications biological control

Molecular markers are used at the community, population and individual levels (Figure 1) in all stages of biological control. For collection of potential biological control agents molecular markers can be used to establish or confirm species identity (e.g. [63]), which is necessary for the importation of parasitoids, especially when there is little traditional taxonomic knowledge of the taxa. To determine the host range of a parasitoid in its native or introduced range, molecular markers are used to identify parasitoid individuals reared from different hosts [33], and more recently to identify the host species from collected adult parasitoids [64]. Once a parasitoid has been selected for mass rearing one has to choose the number of individuals to collect and how many different sites to collect from so that the stock is genetically diverse in order avoid inbreeding and to increase the probability of establishment. DNA-microsatellite markers can be

helpful for determining the amount of genetic variability present in a population, in a collection, and in the individuals to be released after mass rearing [54]. In cases where a parasitoid of an invasive pest is already established, genetic variability can be compared between native and introduced regions [28,65], and molecular markers can be used to detect where it originated from, or if it originated from an intentional introduction or established accidentally (e.g. [66]).

For conservation biological control, conditions are sought to support existing parasitoids and predators in a diversified landscape. Maintenance of a complex parasitoid community, and movement of individuals between crop and non-crop habitat are essential. Towards this end, molecular markers have been used to measure gene flow among parasitoids using hosts in the crop and non-crop habitat [38] as an measure of movement in the landscape. For instance, Lavandero *et al.* [44] determined that there was no genetic differentiation among individuals of the parasitoid *Aphelinus mali* using aphid pests in apple orchards and on the surrounding *Pyracantha coccinea* hedges in central Chile, indicating that the hedges are a suitable refuge for the parasitoid.

Summary and future directions

Neutral genetic markers have been integrated into all aspects of study of parasitoids, in both a basic and applied contexts. Their use should increase as markers become more convenient, along with the mathematical and statistical tools for interpreting their patterns of variation. Then we will get a better idea of the diversity of parasitoids, what causes diversity, and the roles of parasitoids in communities. We will also learn more about what determines host–parasitoid population dynamics, and how we might manipulate it to increase the effectiveness of biological control. An exciting next step will be to use molecular markers that are not neutral in order to determine what traits are under natural selection [67], and how that selection is related to ecological factors we are interested in, such as host species, population dynamics, community complexity and landscape structure.

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