Attraction toward alfalfa and wheat aphid-host plant complexes explains the absence of genetic population structure of the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in Chile

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Abstract

The parasitoid *Aphidius ervi* Haliday shows a host range of macrosiphine aphids in Europe, composed mainly of aphids from legumes and infrequently of aphids from cereals, whereas in Chile, it commonly parasitises macrosiphine aphids from both agroecosystems. In order to establish whether different strains of *A. ervi* are present on these agroecosystems in Chile, we first evaluated the parasitoid searching behaviour in choice tests in a wind tunnel between alfalfa (legume) and wheat (cereal), and then the genetic population structure of parasitoids collected in the field on these crops. In general, *A. ervi* from alfalfa showed a tendency towards higher preference, higher percentage of oriented flights and landings, and lower response time towards alfalfa plants. As previously reported in the literature, oviposition experience further enhanced such patterns. Results with *A. ervi* from wheat showed the same trend in most parameters. On the other hand, variation in mitochondrial DNA (mtDNA) segments and RAPD-PCR polymorphism using total DNA, showed high gene flux between *A. ervi* from legumes and cereals, which resulted in the absence of population structure. These results confirm that different host-strains of this parasitoid do not occur in Chile.

Introduction

Chemical signals play an important role in host searching by aphid parasitoids. Chemical signals may be released from the aphid food-plant, from the host aphid, and from the interacting host aphid and aphid food-plant, known as host-plant complex (Powell *et al.*, 1998). Parasitoids may change their response to foraging cues as a result of experience during the foraging process, by responding to previously ignored cues or by enhancing their response (Du *et al.*, 1997; Poppy *et al.*, 1997).

Aphidius ervi Haliday is an oligophagous parasitoid associated in Eurasia with macrosiphine aphids, such as *Acyrthosiphon pisum* Harris on legumes and to a lower degree with *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* (Kaltenbach) on solanaceus food-plants (Takada & Tada, 2000). Although *Sitobion avenae* (Fabricius) on cereals is a suitable host for *A. ervi*, this parasitoid has a minor relevance as an aphid biocontrol agent on the cereal agroecosystem in Europe (Cameron *et al.*, 1984). In contrast, *A. ervi* was successfully introduced in Chile during the 1980s, showing high levels of parasitism on both *A. pisum* on legumes and *S. avenae* on cereals (Starý *et al.*, 1993; Starý, 1993). In order to evaluate whether *A. ervi* in legumes and cereals in Chile represent different strains, we first studied the attraction of alfalfa (legume) and wheat (cereal) plants, as well as the respective aphid-plant complexes, toward experienced and naive females of *A.ervi*, and then the genetic structure of *A. ervi* populations obtained from either alfalfa and wheat agroecosystems.

Materials and methods

A. ervi was obtained from mummies collected on alfalfa or wheat, and was reared in the laboratory on the same hosts they came from. Mated female parasitoids which or without 30-min oviposition experience on the plant-aphid complex from which they came from, were offered in a wind tunnel the choice between alfalfa or wheat plants (undamaged, aphid-damaged or aphid-plant complex), following the general methodology described by Du *et al.* (1997). Parasitoids were released in the wind tunnel and observed for 5 minutes. The behavioural variable determined was preference of the parasitoid during flight for either wheat or alfalfa. This preference may result from either oriented flight, landing on the plant, or both behaviours. Furthermore, time from release of the parasitoid into the wind tunnel until obtaining a response was also evaluated. Fourteen parasitoids were tested per treatment.

RAPD-PCR polymorphism of total DNA and variation at mitochondrial DNA (mtDNA) were studied. DNA was extracted using the "salting out" method described for aphids (Sunnucks & Hales, 1996), adapted for parasitoids by increasing proteinase K concentration and incubation period. RAPD-PCR reactions using two decamer primers (HN9: 5'-AATCGGGCTG-3' and CFa5: 5'-TGCGGCTGAG-3') were performed on 5µl of 1:50 dilution of resuspended DNA. The RAPD phenotypes as well as their variation between host-plants were computed with RAPDistance freeware (Armstrong *et al.*, 1994). Allelic frequencies and structuring were computed using POPGENE freeware (Yeh *et al.*, 1999).

In order to study the variability of mtDNA between *A. ervi* populations, segments of the cytochrome B gene (480 bp) (Cyt B), and of the region overlapping genes for subunits I and II of cytochrome oxidase (600 bp) (COI/II) were PCR-amplified (Sheppard *et al.*, 1994), and digested using restriction enzymes. Useful digestions were only obtained with *Dra*I and *Hinf*I.

Results and discussion

The dual choice wind tunnel experiments with both naive and experienced *A. ervi* from alfalfa showed a significant preference of the parasitoids for the undamaged aphid food plants, host-damaged plants, and host-plant complexes involving the plant on which they had been reared (Table 1). For *A. ervi* from wheat, both naïve and experienced parasitoids preferred the host-plant complexes and the aphid-damaged plants, but only experienced parasitoids preferred the undamaged plants on which they had been reared (Table 1). These results agree with reports that suggest attraction by induced volatiles of host-plant complexes and of aphid-damaged plants in the *Vicia faba-A. pisum-A. ervi* system (Du *et al.*, 1997; 1998). The preference and lack of preference of *A. ervi* from alfalfa and wheat, respectively, for undamaged host plants (Table 1), suggest an innate preference for alfalfa, as expected since the pea aphid-legume interaction is known as the main host-plant system at the genetic diversity centre for *A. ervi* in Eurasia (Starý *et al.*, 1993).

In general, oviposition experience resulted in an increase in oriented flights (Table 1). This increase was not always statistically significant, possibly on account of the strong preferences of naive parasitoids. Similarly, increased landing responses and reductions in the mean response times were also shown by experienced parasitoids. The effect was particularly strong towards the host-plant complexes (Table 1), which constitute the most attractive among the stimuli tested. These results agree with previous work by Du *et al.* (1997) and Powell *et al.* (1998).

Table 1: Effect of oviposition experience (naive or experienced) on behavioural patterns of *Aphidius ervi* females from different origins (alfalfa or wheat), confronted with alfalfa and wheat plants in a wind tunnel[a,b].

Origin - Stimulus	Preference[c,d]		Oriented flight[d]	Landing[d]	Response time[e]
	naive	exp.	naive *vs.* exp.	naive *vs.* exp.	naive vs. exp.
Parasitoids from alfalfa					
Undamaged plants	**	***	E ns	E ns	E ns
Damaged plants	*	**	E ns	E ns	N ns
Host-plant complex	***	***	EN ns	E ***	E ***
Parasitoids from wheat					
Undamaged plants	ns	***	E *	E ns	E ns
Damaged plants	**	***	E ns	E ***	E ***
Host-plant complex	**	***	E ns	E ***	E ***

[a]Levels of significance: ns= >0.05; *= <0.05; **= <0.01; ***= <0.005.

[b]Behaviours: E= attraction or lower response time by experienced female towards plant of origin; N= attraction or lower response time by naïve female towards plant of origin.

[c] Parasitoids always preferred the plant of origin.

[d] χ^2 test.

[e] U test.

A slightly higher genetic polymorphism was found in parasitoids from wheat with respect to those from alfalfa, but no significant differences in genetic composition were found between populations. The Nei genetic distances showed a high degree of relatedness between populations (>99%). Differences in the allelic structure were not detected, and no deviations from the Hardy-Weinberg equilibrium were found, either considering both populations separately, or the complete sample. No allelic structure was noted at any locus (G_{ST}<1% for all loci studied). An estimation of gene flow between populations showed a high degree of gene flow for several loci. An Ewens-Watterson test for neutrality for both separated and joint populations did not reveal differences at any locus using 1000 simulated samples, indicating free mating between parasitoids from both populations. When the analysis was performed on aphid parasitoids with a known type of flight response in the wind tunnel, no allelic or genotypic differences were found between individuals with preference for wheat or for alfalfa.

After digestion with the restriction enzymes, six restriction patterns were recognisable (Table 2). No significant differences were observed at the restriction pattern frequencies between parasitoid populations collected from wheat and alfalfa. Thus, no restriction pattern could be associated with a given population, suggesting no structuring or specialised haplotype from the markers studied.

The molecular results showed the absence of host-based population structure, both at the individual and multilocus levels. Additionally, no preferential distribution of any allele was observed among individuals with a given allele or genotype. In spite of the fact that both populations appeared as genetically homogeneous, the wind tunnel results show they are different with respect to host preferences of non-experienced parasitoids, and with respect to changes in host preferences upon oviposition experience. This situation could be explained by conditioning of adults parasitoids during emergence from the aphid mummies, which is subsequently modified by parasitoid associative learning based on oviposition experience (van Emden *et al.*, 1996; Poppy *et al.*, 1997; Storeck *et al.*, 2000).

Table 2: Frequencies for the restriction patterns found on parasitoid populations living on aphids from wheat and alfalfa.

Fragment/Restriction enzyme	Pattern	Wheat (26)[a]		Alfalfa (49)[a]	
		n	Frequency	N	Frequency
CytB / *DraI*	A	5	0.19	5	0.10
	B	21	0.81	44	0.90
COI/II / *DraI*	C	3	0.12	6	0.12
	D	23	0.88	43	0.88
COI/II / *HinfI*	E	4	0.15	4	0.08
	F	22	0.85	45	0.92

[a] Number of individuals whose mtDNA PCR-amplified satisfactorily under the conditions employed. Total number analysed: 35 from wheat and 71 from alfalfa, including 7 from wheat and 9 from alfalfa, whose responses in a wind tunnel had been previously determined.

The high gene flow shown by the data is further evidence of the presence of a single genetic pool for the markers utilised. This result supports the hypothesis put forward by Starý *et al.* (1993) and Starý (1993) to account for the presence of *A. ervi* in both legumes and cereals in Chile. Perennial legumes would serve as year-round reservoirs for parasitoids, particularly during the period when wheat is not available (end of summer, autumn and part of winter). When the vegetative period of wheat starts (late winter and spring), migration of *A. ervi* would occur from legumes to cereals and also from cereals to legumes, maintaining populations of parasitoids on both crops.

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