Chapter II

Does history of parasitism by *Urophora cardui* influence the genotypic and genetic diversity of *Cirsium arvense*?

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Abstract: Several studies in controlled conditions have demonstrated that the parasite (*U. cardui*) has profound negative effects on life history traits of its host (*C. arvense*). Such negative effects may affect the neutral genotypic and genetic diversity of natural populations of the host plant. To test this hypothesis, we investigated eight populations of *C. arvense* with different history of infestation by *U. cardui*. Half of the populations were in the last 5 years infested by *U. cardui*, whereas the other half was not. We investigated genotypic and genetic diversity with AFLP markers. Contrary to what we expected, average genotypic diversity, clonal evenness and molecular variance did not differ between infested and not-infested populations (0.73 ± 0.21 *versus* 0.78 ± 0.26 (U = 6; $P > 0.56$) for clonal diversity; 0.58 ± 0.27 *versus* 0.71 ± 0.34 (U = 5.5; $P > 0.46$) for clonal evenness). Molecular variance due to infestation state of populations was not significant (1.81 ± 1.05 versus 2.22 ± 0.82; $P > 0.11$) and explained less than five percent of the total variance. Hence, our results suggest that selection imposed by *U. cardui* on *C. arvense* was weaker on a population and/or metapopulation scale than it was supposed in experimental studies. This can be explained by the complex spatio-temporal population dynamics of the *C. arvense*-*U. cardui* system. Within each population, we did not find any significant correlation between the genetic dissimilarity matrix of *C. arvense* shoots and the corresponding "infestation state" matrix. In our study, *C. arvense* shoots appear to be randomly infested by *U. cardui.*

Key words: host plant-parasitic interaction, neutral genotypic and genetic diversity, AFLP, *Urophora cardui*, *Cirsium arvense*, population infestation.

Introduction

The tephritid fly *Urophora cardui* is a gall-forming insect which attacks the common weed *Cirsium arvense*. In this plant-herbivore system, *U. cardui* is able to occur only within a narrow range of environmental conditions (Peschken *et al.*, 1997) as the clonal *C. arvense* as well as *C. setosum* are the only host plants of this species (Frenzel et al., 2000). Adults of *U. cardui* emerge in early summer (June to July) and females lay eggs into suitable shoots of the host. With the development of the larvae the plant is forced to produce conspicuous multilocular stem galls (Peschken and Harris, 1975, Peschken *et al.*, 1982; Peschken and Derby, 1992).

C. arvense is a perennial clonal weed of considerable economic importance. (Moore, 1975; Donald, 1990). Furthermore, it is an aggressive invader throughout North American continent since the 17th century. Hence, the potential of *U. cardui* as a biocontrol agent for *C. arvense* was intensively investigated during the last decades (Peschken *et al.*, 1997; Peschken *et al*., 1982). Experimental studies demonstrated that *U. cardui* can influence the fecundity and survival of infested plants. For example *U. cardui* may reduce the production of mature seed-heads (Laing, 1977) as well as below and above ground biomass (65% and 47% respectively). In extreme *U. cardui* may cause the death of infested ramet (Peschken and Harris, 1975). Furthermore, Gange and Nice (1997) showed that the larvae manipulate the nitrogen metabolism of infested ramets in order to maintain an optimal nutrient level within galls. Hence, galls act as sinks (Shorthouse and Watson, 1976). Thus, ramets with galls are at a disadvantage, especially in nitrogen-limited habitats.

Although the impact of phytophagous insects on host fitness is well-known, their effect on other life history traits is less obvious. (see Thomas *et al.*, 2000 for review). For example, phytophages may influence the dispersal of their host (Heeb *et al.*, 1999). In the *C. arvense*-*U. cardui* system, the growth of galls leads to a reduction of plant height (Peschken and Harris, 1975; Peschken *et al*., 1982). In turn this may reduce dispersal distances of seeds (Sheldon and Burrows, 1973) which may affect genotypic diversity and the genetic structure of the host populations. Furthermore, reduced fecundity and survival of infested plants may reduce effective population size and thus may also influence genetic diversity. On the other hand, in *C. arvense*, a reduced growth of infested ramets can lead to an increased growth of axillary shoots (Peschken and Harris, 1975). This suggests a reallocation of resources between infested and non-infested shoots within clones. This evidence of changes in life history of

host plants after infestation calls for an analysis of the repercussions of the attack by *U. cardui* in the population genetic of *C. arvense*.

The aim of our work was twofold. Firstly, using AFLP markers we characterised the genotypes of infested and non-infested *C. arvense* shoots in local populations. Although the biology of the *U. cardui*-*C. arvense* system is well-known, it still remains elusive how *U. cardui* chooses suitable shoots for oviposition. We wanted to investigate whether *U. cardui* selects particular genotypes for oviposition. Secondly, we explored at a landscape scale the effects of the phytophage on the genetic diversity of its host. For that purpose, we compared the genotypic and genetic diversity of populations with different history of parasitism.

Material and methods

Sampling

Our study area in north-eastern Bavaria (11°50'E 49°35'N) was a 15 km² rural area composed of diverse array of habitats (meadows, agricultural fields, wastelands, old fallows, roadsides). Within this area, the biology and local dynamics of both *C. arvense* and *U. cardui* are well-known (Eber and Brandl, 1994; Eber and Brandl, 1996; Eber and Brandl, 1997; Eber and Brandl, 2003). For the present study, we focused on eight populations of *C. arvense*. All populations were in a similar successional phase, judged from other co-occurring plant species. Half of these eight populations were infested by *U. cardui* for at least five years, whereas there were no previous records of infestation for the other half. In September 1999 we recorded the number of *C. arvense* shoots. Fresh thistle leaves were sampled at all sites. In thistle populations with *U. cardui* galls we collected leaves from infested and non-infested thistle shoots separately. Details of the samples are presented in Table 1.

Table 1: Demographic, ecological, genotypic and genetic characteristics of eight populations of the clonal weed *C. arvense*. In brackets we give the number of shoots with galls of the tephritid fly *U.cardui*, which were sampled additionally in infested populations.

Population labels	Infestation group	Population size (number of shoots)	Number of plants analysed (N)	Number of genotypes detected (G)	Genotypic diversity (i)	Evenness index ($E_{1/D}$)	Molecular variance
8	infested	500	46 (7)	42	0.79	0.58	2.48
61	infested	3000	44 (10)	52	0.96	0.93	2.92
119	infested	400	18 (10)	13	0.46	0.29	1.07
155	infested	8000	10 (8)	13	0.72	0.5	0.76
60	non-infested	30	20	20	1.00	1	3.01
120	non-infested	40	12	6	0.50	0.33	1.06
148	non-infested	800	11	11	1.00	1	2.42
156	non-infested	100	8	5	0.63	0.5	2.39

Genotypic and genetic variability among infested and non-infested populations

AFLP were processed according to Solé et al., 2004. Genotypic diversity (i) was estimated according to Ellstrand and Roose (1987; i = G/N where G is the number of genotypes and N the number of sampled shoots). Clonal evenness, which estimates the relative abundance of each genotype within a population, was calculated according to Williams (1964; $E_{1/D} = \frac{1/D}{G}$, where D is Simpson's index ($D = \sum_{i=1}^{G} p_i^2$; p_i = the relative abundance of the i^{th} genotype). We compared the average genotypic diversity (i) and the clonal evenness ($E_{1/D}$) of infested and non-infested populations with a Mann-Whitney U-test. To study the partitioning of genetic variance among populations we performed an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using the program Arlequin (Schneider *et al.*, 2000). As we were interested in comparing genetic variability of infested and non-infested populations, we constructed a hierarchical model where genotypes were nested within populations and populations were nested within either an infested or non-infested group. The program calculated the associated variance components (σ_a^2, σ_b^2, σ_c^2; see Table 3 for explanations), the significance of estimated parameters was then tested by a permutation procedure (Excoffier *et al*., 1992). We used the number of pairwise differences as genetic distance. For each population we also calculated the molecular variance (sum of number of pairwise differences within a population divided by 2N (N-1), where N equals the number of samples). The molecular variance is a measure of genetic diversity within populations. We tested for a difference in the average molecular variance of infested and non-infested populations using a Mann-Whitney U-test.

Pattern of infestation within populations

For the four populations with *U. cardui* galls, we calculated a matrix of genetic dissimilarities based on the AFLP data for all possible pairwise comparisons of sampled shoots (1-Jaccard coefficient, Jaccard, 1908), and a matrix which codes for the infestation state. In the second matrix, a shared state (compared shoots either infested or not infested) was coded by 0 and individuals having a contrasting state were coded by 1. Finally, we used matrix correlation to test whether the genetic similarity of shoots with contrasting infestation states was lower than compared to pairs of shoots with identical infestations states (negative matrix correlation) using the NTSYS-pc-p package (Rohlf, 1993).

Results

Our AFLP-fingerprints allowed to analyse 93 polymorphic loci. With these 93 loci we distinguished 162 haplotypes among the 194 sampled *C. arvense* shoots (i.e. 83% distinguishable genotypes). Genotypes shared by several individuals (32 out of 194) always occurred in the same population. Within the 169 shoots not infested by *U cardui* we found 135 individual genotypes (85%), whereas within the 35 shoots infested by *U cardui* we found 27 individual genotypes (77%).

Average genotypic diversity, clonal evenness and molecular variance did not differ between infested and not-infested populations (Table 2).

Table 2: Average genotypic diversity, clonal evenness and molecular variance (± standard deviation) in total eight populations of *C. arvense* according to their infestation by the tephritid fly *U. cardui* (see Table 1 for raw data).

	Genotypic Diversity (i)	Clonal Evenness (E1/D)	Molecular Variance
Infested populations	0.73 (± 0.21)	0.58 (± 0.27)	1.81 (± 1.05)
Non-infested populations	0.78 (± 0.26)	0.71 (± 0.34)	2.22 (± 0.82)
Mann-Whitney U-test	U = 6; P > 0.56	U = 5.5; P > 0.46	U = 7; P > 0.77

Most of the genetic diversity was found among populations within groups (58 %; Table 3). Nevertheless, a large amount of diversity was still present within populations (37%). The variance due to grouping into infested and non-infested populations was not significant ($P > 0.11$) and explained less than five percent of the total variance.

Table 3: Results of a hierarchical analysis of molecular variance between infested versus non-infested populations, among populations within each infestation group and within populations. σ_a^2 was tested by random permutation of genotypes of whole populations across infested and non-infested populations. σ_b^2 was tested by random permutation of individuals across populations but within the same group. σ_c^2 was tested by permuting AFLP phenotypes randomly among populations and between groups. The significance tests are based on 1000 permutations

Variance component		Variance	% total	Significance
Among groups ("*infestation effect*")	σ_a^2	0.56	4.66	P > 0.11
Among populations within groups	σ_b^2	7.02	58.38	P < 0.001
Within populations	σ_c^2	4.44	36.97	P < 0.001

Within each population, we did not find any significant correlation between the genetic dissimilarity matrix of *C. arvense* shoots and the corresponding matrix coding for the similarity in the infestation state (Table 4). In the studied populations, shoots of *C. arvense* appear to be infested randomly by *U. cardui*.

Table 4: Results of matrix correlation for the four infested populations. For each population we correlated the matrix of genetic dissimilarity (1-Jaccard coefficient) with matrix coding for the similarity in the infestation state of individual shoots. Error probabilities are based on 1000 randomisations.

Populations	Matrix correlation	Probability
8	r = -0.02	P > 0.39
61	r = -0.003	P > 0.48
119	r = 0.06	P > 0.19
155	r = -0.02	P > 0.38

Discussion

U. cardui may affect population genetics of *C. arvense* along two pathways: (1) If infestation imposes selection pressure on morphological, phenological or physiological traits with a genetic background, some non-neutral genetic differentiation between infested and non-infested host plants should emerge within populations. However, the chance to detect such differences depends on the number of loci affected and on whether selection influences reproductive isolation. (2) If infestation is random with respect to the traits, neutral genetic effects are expected if infestation changes the demography of the population. An increased mortality or decreased fecundity decreases effective population size and hence influences the neutral genetic diversity.

If selection occurs, resistance or tolerance in host plants will evolve in order to reduce the costs of phytophages or pathogens (Crawley, 1983; Marquis, 1992). The system *C. arvense*-*U. cardui* should be no exception. Resistance or tolerance of certain genotypes are expected to create non-random infestation patterns in natural populations as well as genetic variation in host plant resistance. Significant genetic differentiation and variation associated with resistance have been demonstrated for several plant species (Mopper *et al.*, 1991; Vargas *et al.*, 2002; Strong *et al.*, 1993; Krabel and Petercord, 2000). However, in most of these cases it is not clear whether genetic differentiation of the host is a consequence of selection induced by infestation or whether differential infestation is a consequence of genetic differentiation due to other causes.

In our analyses, populations infested by *U. cardui* were not dominated by particular genotypes. In an UPGMA cluster analysis (results not shown), genotypes clustered according to the population, and we found no clusters of either infested or non-infested genotypes within populations. This supports the fact that we found no correlation between the similarity of the infestation state and the genetic similarity of shoots. Hence, in our study area, infestation of *C. arvense* by *U. cardui* appears to be random among and within populations referring to the genetic markers used.

Like mentioned above, if infestation is random, effects on genetic diversity of the host plant can only be expected if infestation changes the demography of the population. In species with mixed reproduction system (like in clonal plants), the clonal propagation of genotypes may decrease the genetic evenness of the population and thus promote the evolution of parasite specialization to particular host genotypes (Barrett, 1981). Hence, sexual reproduction

appears to be an efficient strategy to escape from parasitism. In the system *Urtica dioica-Cuscuta europaea* experiments which compared resource allocation into sexual and asexual reproduction showed that plants from infested populations had been selected for sexual reproduction (Koskela, 2002). Consequently, if one expects that parasitism selects for sexual reproduction, this should lead to an increase of genetic diversity within the infested populations. Although a decrease in the fitness of *C. arvense* by the attack of *U. cardui* has been demonstrated (Peschken and Harris, 1975), we found no effect of the phytophagous insect on the genotypic and genetic diversity of its host.

The failure to detect neutral genetic patterns in relation to infestation may be explained by at least two arguments. Firstly, the capacity of *C. arvense* to reallocate resources after infestation provides an "ecological buffer" for the host which reduces the direct effects of the phytophage on its host plant. Secondly, it may be dangerous to generalize the effects of *U. cardui* on *C. arvense* found in small-scale laboratory experiments (e.g. Peschken and Harris, 1975; Peschken *et al*., 1982) to the complex spatial and temporal dynamic of the *C. arvense-U.cardui* system on the landscape scale. The importance of spatial and temporal dynamics in host-parasite has already been intensively investigated for non-neutral genetic variability (Thrall and Burdon, 1997; Frank, 1997; Frank, 1996) Our previous studies of the temporal and spatial dynamics of local thistle populations suggested that *U. cardui* forms a very dynamic metapopulation system (Eber and Brandl, 1994; Eber and Brandl, 1996). Thereby, the spatial and temporal dynamics of *C. arvense* population is crucial for the spatial and temporal abundance of *U. cardui* (Eber and Brandl, 2003). Consequently, the dynamics of the system may preclude any consistent selection within populations. These results also comply with the fact that the introduction of *U. cardui* as a biocontrol agent for *C. arvense* failed in Canada (Peschken and Derby, 1992; Peschken *et al.*, 1997). *U. cardui* was not able to reduce the density of thistles at a landscape scale. Hence, selection pressure imposed by *U. cardui* on *C. arvense* appeared to be weaker on a population and/or metapopulation scale than it was supposed to be in experimental studies.

Moreover, other factors like competition may also be important for the impact of a phytophageous insect on the fitness of its host. In *C. arvense*, Peschken *et al*. (1982) showed that infested and non-infested thistle shoots cultivated without competitors showed no significant difference in vigour. Plants grown with competitors, however, showed significant differences. Hence, simple experiments with isolated plants may lead to a biased view about the impact of the phytophageous insect at the landscape level.

In conclusion, we suggest that in the *C. arvense-U. cardui* system the spatial scale of the host plant-phytophage interaction may be crucial to estimate the effects of the phytophage on host plants at a landscape scale. In contrast to experiments with isolated plants, infestation in natural populations of *C. arvense* occurs at the ramet level, whereas natural selection occurs at a genet level. Thus, the metapopulation dynamics may preclude any consistent selection within local populations. In part this may explain the persistence of the *C. arvense-U. cardui* system.

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