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Tanja Koskela

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Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
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ABSTRACT

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Host – parasite interaction is one of the most common ecological interactions. The aim of this thesis was to examine the potential for coevolution in a host plant (*Urtica dioica*) – holoparasitic plant (*Cuscuta europaea*) interaction. An infection experiment suggested that previous parasitism may have selected for quantitative resistance in the host as indicated by the lower parasite performance on hosts from parasitized populations compared to that on hosts from unparasitized populations. In a common garden experiment, previous parasitism was associated with lower allocation to asexual reproduction in the host, indicating that selection by the parasite may have modified this trait. According to a reciprocal cross-infection experiment, parasites had overall higher infectivity on their sympatric hosts than on allopatric hosts, but the level of local adaptation varied among populations. An infection experiment among different host families demonstrated genetic variation in resistance and tolerance as well as costs of resistance and tolerance. Based on allozyme data, gene flow among populations was high. However, local selection seems to be strong since I found evidence for parasite local adaptation, and divergence in host resistance and tolerance among unparasitized and parasitized populations. In summary, coevolution seems possible in this system. I found differences in host and/or parasite traits between "novel" and "familiar" host – parasite combinations (i.e. between unparasitized and parasitized populations, and between allopatric and sympatric combinations). These results indicate effects of past selection by parasitism. Further, I found genetic variation in host's resistance and tolerance, which is a prerequisite for the evolution of these traits.

Key words: *Cuscuta europaea*; genetic variation; host – parasite interaction; local adaptation; resistance; tolerance; *Urtica dioica*.

T. Koskela, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FIN-40351 Jyväskylä, Finland

Author's address

Tanja Koskela
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FIN-40351 Jyväskylä, Finland
e-mail: tamattil@cc.jyu.fi

Supervisors

Docent Veikko Salonen
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FIN-40351 Jyväskylä, Finland

Docent Pia Mutikainen
Department of Biology
University of Oulu
P.O. Box 3000
FIN-90014 Oulu, Finland

Reviewers

Dr. Sharon Y. Strauss
National Science Foundation
Suite 635
4201 Wilson Blvd
Arlington VA 22230
The USA

Dr. Heikki Roininen
University of Joensuu
Department of Biology
P.O. Box 111
FIN-80101 Joensuu
Finland

Opponent

Docent Kari Lehtilä
Södertörns Högskola
Section of Natural Sciences
Division of Population Biology
P.O. Box 4101
SE-14104 Huddinge
Sweden

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. I am the main writer in the papers I-IV and I have performed and planned large part of the work in each paper.

I Koskela, T., Salonen, V. & Mutikainen, P. 2001. Interaction of a host plant and its holoparasite: effects of previous selection by the parasite. Journal of Evolutionary Biology 14:910-917.

II Koskela, T. 2001. Variation in life-history traits among *Urtica dioica* populations with different history in parasitism by the holoparasitic plant *Cuscuta europaea*. Submitted.

III Koskela, T., Salonen, V. & Mutikainen, P. 2000. Local adaptation of a holoparasitic plant, *Cuscuta europaea*: variation among populations. Journal of Evolutionary Biology 13: 749-755.

IV Koskela, T., Puustinen, S., Salonen, V. & Mutikainen, P. 2001. Resistance and tolerance in a host plant – holoparasitic plant interaction: genetic variation and costs. Preliminarily accepted (Evolution).

V Mutikainen, P. & Koskela, T. 2001. Population structure of a parasitic plant and its perennial host. Submitted.

1 INTRODUCTION

1.1 General introduction

Parasites are organisms that obtain all or part of the resources required for their own growth and reproduction from other living organisms, their hosts (e.g. Price 1980). Unlikely free-living organisms, parasites are usually attached to their host during most or all of their life-cycle. The number of species living as parasites is enormous; by some estimates parasites may represent more than half of the organisms (Price 1977, de Meeûs et al. 1998). Consequently, among different types of ecological interactions between organisms, one of the most common and hence most important is that between hosts and parasites (Price 1977, Price et al. 1986).

By obtaining resources from the host, parasites often have substantial negative effects on the fitness of their hosts. There is plenty of evidence showing that parasites affect the evolution of host resistance, as well as behavioural and life-history traits (e.g. Price 1980, Minchella 1985, Minchella & Scott 1991, Michalakis & Hochberg 1994, Jokela & Lively 1995, Rausher 1996, Simms 1996, Mauricio & Rausher 1997, Sorci et al. 1997, Koella et al. 1998). In addition, parasites may affect the structure of host populations and communities (e.g. Price 1980, Dobson & Hudson 1986, Price et al. 1986, Minchella & Scott 1991, Gibson & Watkinson 1992). Further, parasites may play a significant role in the evolution and maintenance of sexual reproduction, and in the maintenance of genetic polymorphism in the host (Haldane 1949, Jaenike 1978, Bremermann 1980, Hamilton 1980, Bell 1982, Hamilton et al. 1990).

About 1 % of all angiosperms, approximately 3000 species in total, are parasitic and obtain part or all of their resources from other plants (Kuijt 1969). Parasitic plants are widespread, and are known to have significant negative effects on both the growth and reproduction of their host plants (e.g. Graves et al. 1992, Musselman & Press 1995, Puustinen & Salonen 1999). However, the evolutionary ecology of host plant – parasitic plant interaction has not been studied extensively in natural populations. In contradiction to most of the previously studied host – parasite systems, parasitic plants are rather similar to

their host species with regard to size, generation time, reproductive rate, and length of life cycle. In addition, many parasitic plants are generalists in their host choice (Musselman & Press 1995). These are characteristics that are likely to affect the evolutionary interaction between the host and the parasite.

1.2 Host – parasite coevolution

For natural selection to operate and lead to evolution in any trait, three conditions must be met: there has to be variation among individuals in the trait, this variation has to lead to fitness differences among individuals, and the trait has to be heritable (Endler 1986). Coevolution is defined as reciprocal natural selection on the phenotypic traits of interacting species, and is regarded as one of the most important processes shaping patterns of adaptation between species (e.g. Janzen 1980, Thompson 1994). In host – parasite interactions, strong selective pressures may act on both interacting species, potentially leading to antagonistic coevolution. Since parasitism decreases the fitness of the host, hosts are believed to evolve defence mechanisms that either prevent infection or limit its extent (resistance), or reduce the detrimental effects of damage after infection (tolerance) under the selective pressure by the parasites (e.g. Dawkins & Krebs 1979, Ebert & Hamilton 1996, Sorci et al. 1997 and references therein, Strauss & Agrawal 1999, Stowe et al. 2000). Similarly, parasites are affected by their hosts; the ability of the host to resist parasitic infection imposes selection on the parasite that may, in turn, evolve strategies that enable them to overcome host resistance and to exploit the host more efficiently (e.g. Burdon 1987, Thompson 1994, Wakelin 1997). Given that resistance traits of the host decrease parasite performance, resistance is expected to impose selection on the parasite. However, there is likely to be no evolutionary pressure for the parasite to overcome tolerance *per se* since tolerance does not directly reduce the fitness of parasites (Rosenthal & Kotanen 1994, Roy & Kirchner 2000).

A prerequisite for the evolution of resistance and tolerance in the host is the existence of genetic variation in these traits for natural selection to act. Indeed, several studies examining plant – herbivore and plant – pathogen interactions have documented the presence of genetic variation in resistance (e.g. Berenbaum et al. 1986, Rausher & Simms 1989, Simms & Rausher 1989, Stowe et al. 1994, Mutikainen et al. 2000a) or tolerance (e.g. Simms & Triplett 1994, Fornoni & Núñez-Farfán 2000) indicating that evolutionary change in these traits is possible. When the risk of parasitism is high, one would expect selection to favour high levels of resistance and/or tolerance. However, selection for increased resistance or tolerance may be constrained by negative genetic correlations (i.e. trade-offs) with other traits that affect host fitness (e.g. growth and reproduction). That is, resistance and tolerance may be costly and these costs may partly maintain genetic variation in resistance and tolerance within populations (Simms & Rausher 1987, Roff 1992, Stearns 1992, Rausher 1996, Sheldon & Verhulst 1996), especially if the risk of parasitism varies in temporal and/or spatial scale.

Parasites, in turn, may be under strong selection to be able to infect common host genotypes. That is, parasites are expected to specialize on the most common host genotypes in the population (e.g. Jaenike 1978, Hamilton 1980, Bell 1982, Hamilton et al. 1990). As a consequence of this specialization process, parasites may become adapted to their local, sympatric host. Locally adapted parasites are expected to have higher infectivity and/or higher fitness on sympatric hosts than on allopatric (novel) hosts of the same species (Lively 1989, Ebert 1994, Ebert & Hamilton 1996, Gandon et al. 1996, Gandon & Van Zandt 1998, Kaltz & Shykoff 1998). In turn, parasite's specialization on the common host genotypes is expected to drive down the frequency of this host genotype and to create an advantage for rare resistant host genotypes (Bell & Maynard Smith 1987, Hamilton et al. 1990, Frank 1996). This type of frequency-dependent selection with a time-lag is believed to be common in host – parasite interactions, and is expected to lead to oscillations in host and parasite genotype frequencies and hence to the maintenance of genetic variation in both host and parasite traits (Clarke 1976, Hamilton 1980, Hutson & Law 1981, Bell & Maynard Smith 1987, Hamilton et al. 1990). Furthermore, parasite-mediated selection for rare genotypes has been suggested to favour sexual reproduction as a means of producing genetically variable progeny (Red Queen hypothesis: Jaenike 1978, Hamilton 1980, Bell 1982).

The intensity and outcome of selection in host – parasite interactions are affected by at least the following factors, and preferably all of these factors should be addressed when examining the potential for coevolution between the host and the parasite. First, the virulence of the parasite (i.e. reduction in host fitness due to infection, e.g. Read 1994, Ebert 1998) to a great extent determines the strength of selection. The higher the virulence, the stronger the selection of parasitic infection on host traits, and of host traits on the parasite. Second, the genetic structure of host and parasite populations has important implications for the interaction. Genetic diversity of the host population can be of great importance as it can give the host a means to prevent the parasite from developing optimal virulence (Jaenike 1978, Bell 1982, Hamilton et al. 1990, Ebert & Hamilton 1996). Further, new resistance and virulence genes can enter the population through gene flow (i.e. migration) among populations. Differences in gene flow between the host and the parasite are likely to affect the evolutionary potential of the counterparts; the one with the lower gene flow is likely to have a lower evolutionary potential since there is less raw material for natural selection to act upon. Third, differences between the host and the parasite in generation times and reproductive rates also affect their evolutionary potential. Conventional wisdom holds that parasites have higher evolutionary potential than their hosts due to their shorter generation times, higher reproductive rates and larger populations sizes (Hamilton et al. 1990, Hafner et al. 1994). Fourth, for the host – parasite interaction to lead to reciprocal evolutionary change (i.e. coevolution), there has to be some degree of reciprocal specialization among the interacting species (e.g. Thompson 1994). Such specialization, and thus potential for coevolution, has been demonstrated even in interactions involving several species (e.g. in generalist parasites) (e.g. Thompson 1994 and references therein, Tripet & Richner 1997). Lastly, selection

pressures that affect both of the interacting species may vary among local populations (the so-called geographic mosaic theory of coevolution; Thompson 1994, 1999). There may differences among populations in the strength and agents of selection by both abiotic and biotic conditions (e.g. in the composition of natural enemies and availability of suitable hosts), and there may be among-population variation in genetic structure (e.g. in gene diversity, gene flow). Consequently, populations may differ in the traits shaped by the interaction (Thompson 1994, 1999).

To sum up, the main assumptions for coevolution are that the parasite has strong negative effects on host fitness so that selective impact is high, that there is genetic variation for host resistance and parasite infectivity so that evolutionary change is possible, and that there are genotype-specific effects on resistance and infectivity for frequency-dependent selection to operate (e.g. Hamilton 1980, Clay & Kover 1996).

1.3 Aims of the study

The general aim of this thesis was to study the potential for coevolution in a host plant (*Urtica dioica*) – holoparasitic plant (*Cuscuta europaea*) interaction by examining the following basic assumptions for coevolution, concentrating mainly on the characteristics of the host plant. In the first two papers, I studied if previous parasitism has selected for higher resistance and/or tolerance against parasitic infection in the host (I), or if it is associated with differences in other life-history traits of the host (II). These questions were examined by comparing host and parasite traits among host plants from previously unparasitized and parasitized natural populations in a greenhouse infection experiment (I) and in a common garden experiment (II). In the third paper, I examined if the parasitic plant is locally adapted to its sympatric host by conducting a reciprocal cross-infection experiment among five populations in the greenhouse (III). In paper four, I studied genetic variation in host resistance and tolerance against parasitic infection, and the potential fitness costs associated with these traits in a greenhouse infection experiment using 20 host plant families originating from one site (IV). In the fifth paper, I examined genetic variation, population structure, and gene flow of both the host and the parasite, and studied if genetic diversity differs among previously unparasitized and parasitized host populations by using neutral genetic markers (allozymes) (V).

2 METHODS

2.1 Study species (I-V)

The stinging nettle, *Urtica dioica* L. (Urticaceae), is a common dioecious (i.e. obligately outcrossing) wind-pollinated perennial plant found in nutrient-rich habitats. It reproduces both by seed (i.e. sexual reproduction) and by spreading vegetatively (i.e. asexual reproduction) by over-wintering underground rhizomes and above-ground stolons. It has stinging trichomes on the stem and on the leaves. The highly specialized structure of the trichomes suggests that they function as defence against mammalian herbivores (Pullin & Gilbert 1989). To my knowledge, the trichomes do not have any function in defence against parasitic plants.

The greater dodder, *Cuscuta europaea* L. (Cuscutaceae), is an annual rootless stem holoparasite that obtains all resources (i.e. carbon, inorganic nutrients, and carbon-containing compounds) from the host plant through haustorial connections that penetrate into the host vascular tissue (Kuijt 1969, Press et al. 1990, Stewart and Press 1990, Parker and Riches 1993). In Finland, *C. europaea* has become less common during the past decades. It occurs mainly in southern parts of Finland, and has a very patchy distribution. After germination, *C. europaea* seedlings can live for some time (1-2 weeks) without a host using the resources reserved in the seed (Kuijt 1969). *C. europaea* attacks the above-ground parts of the host by twining around the host stem and by producing new branches throughout the growing season. *C. europaea* is regarded as a generalist parasite; it is able to parasitize more than just one species (e.g. Parker & Riches 1993, Musselman & Press 1995). However, in the populations studied in this thesis, *U. dioica* is the main host for *C. europaea*. I observed *C. europaea* only rarely on other species than *U. dioica*. Therefore, it seems that *C. europaea* is to a great extent specialized on *U. dioica* in my study populations.

Both *U. dioica* and *C. europaea* reproduce once a year (more precisely, during the growing season which extends on the average from late April to late September). However, based on the fact that *U. dioica* is a perennial plant with

vigorous clonal growth, it is highly likely that *U. dioica* stands are mainly composed of over-wintered plants that represent the same genotypes that were present during the previous growing seasons. On the contrary, *C. europaea* individuals are always established from seed and thus represent new genotypes. Still however, the generation times of the host and the parasite are relatively more equal in this study system compared to, for example, interactions involving bacteria or viruses.

2.2 Obtaining and maintenance of study organisms (I-V)

The experiments were carried out in greenhouse (I, III, IV) and common garden conditions (II) at the Konnevesi Research Station (University of Jyväskylä) (62°37'N:26°21'E). The allozyme analyses (V) were conducted in the facilities of ETH-Zürich and University of Turku. Most of the plant material was collected from Turku area, South-western Finland (62°N:22°E) (I-V), but also from Tampere (61°30'N:23°45'E) (III, V) (Table 1).

TABLE 1 The study populations. The Roman numerals refer to the number of the study. The Arabic numbers refer to the respective population number used in each study. Populations marked with an asterisk (*) are host (*Urtica dioica*) populations that have not been parasitized by the holoparasitic plant *Cuscuta europaea*.

Population	I	II	III	IV	V
Vaarniemi	1	1	1	-	1
Seili	-	-	-	-	2
Seili seashore	-	-	2	-	3
Halistenkoski	2	2	3	1	4
Mynämäki	-	-	4	-	5
Tampere	-	-	5	-	6
Ylioppilaskylä	3	3	-	-	7
Kuoviluoto*	7	7	-	-	8
Nautelankoski*	5	5	-	-	9
Vähäjoki*	8	8	-	-	10
Lieto	4	4	-	-	11
Lieto riverbank*	6	6	-	-	12

The host plant material was collected either as rhizomes (i.e. clones) during spring (I, III) or as seeds (i.e. at least half-siblings) during autumn (II, IV, V). The rhizomes and the seeds of the host were collected from different *U. dioica* patches separated by space where *U. dioica* did not grow (i.e. one rhizome per patch was collected or seeds were collected from one female shoot per patch). This was done to try to make sure that the samples represent separate genetic individuals (genets). The parasitic plants were collected either as seedlings from the ground just after they had started to germinate (I) or as seeds from several parasite and host individuals during autumn (III, IV). In the populations used in this thesis, the host plants start to grow from rhizomes 2-4 weeks before the seeds of the parasitic plant germinate. Therefore, in the infection treatments, the

host plants were first grown without the parasite in order to have experimental conditions that correspond well with the conditions in natural populations. The parasites were planted when the host plants were well established and were, on the average, 13 cm in length (I, III, IV). When infecting the host plants, the small parasite seedlings were carefully placed at the base of the host plant (I, III, IV). The plants were then regularly checked for successful infections.

The host plants collected as rhizomes were individually planted in experimental pots a couple of days after their collection (I, III). In the first experiment (where the rhizomes had already started to grow in nature and the rhizome-derived plants were thus larger), the parasitic plants were planted 3-4 days after planting of the host (I). In the third experiment (where the rhizomes had not yet started to grow in nature), the host plants were grown for four weeks before the planting of the parasite (III).

The seeds of both the host (II, IV, V) and the parasite (III, IV) were stored in paper bags at room temperature until they were used in the experiments. The seeds of *U. dioica* were sown in containers filled with commercially available fertilized soil. Germination occurred within 2-3 weeks. The seedlings were then either kept in the same container were they had been sown (V) or were individually transplanted in the experimental pots (IV) or in the common garden (II). The host seedlings were grown for four weeks before the planting of the parasite (IV). To break the dormancy of *C. europaea* seeds, we used two methods that have previously been used for *Cuscuta* species (see Baskin & Baskin 1998, and references therein). In the first method, the seeds were stored at 0°C in moist peat for four months and were then taken into room temperature where germination occurred within a couple of weeks (III). In the second method, the seeds were soaked in sulphuric acid (98 %) for 20 minutes, rinsed with distilled water, and stored at +4°C between moist filter papers until they started to germinate within 3-5 weeks' time (method modified from Wolswinkel 1974) (IV).

The greenhouse experiments were conducted in an unheated greenhouse under natural light conditions (I, III, IV). The pots were organized according to a randomized block design and were rearranged regularly during the experiments to minimize position effects in the greenhouse. The plants were kept well-watered. The plants in the common garden were planted according to a randomized block design, and were watered if necessary (II). At the end of the experiments, the biomass of the host plant was collected (I, II, III, IV). From the infected plants, the biomass of the parasitic plants was also harvested (I, III, IV). In the first experiment, the number of flowers of the parasite was also counted (I). From the host, the above-ground biomass was collected separately for vegetative (i.e. stem and leaves) and for reproductive parts (i.e. flowers, flower stalks, and seeds) (I, II, III, IV). For the host, allocation to reproduction was calculated as the biomass of reproductive parts divided by the total above-ground biomass (vegetative + reproductive biomass) (II, IV). In the first and the second experiment, the root biomass of the host was also collected, either as the total root biomass in the pot (I), or as a root sample taken from the ground with a root drill (II). The roots were then carefully washed free from debris. The plant parts were oven-dried (75°C or 80°C, 24 hours) and weighed.

2.3 Definitions and estimates of resistance and tolerance (I, III, IV)

Karban & Baldwin (1997) define resistance as any plant trait that reduces the preference or performance of herbivores, and tolerance as the degree of herbivore-induced damage in host fitness relative to fitness in the undamaged state. In general, resistance to biological enemies (e.g. herbivores, parasites) is often measured as host traits that completely prevent the infection (i.e. qualitative resistance). Resistance has also been measured in terms of performance of the enemy, for example, as decreased growth or reproduction of the natural enemy on resistant hosts (i.e. quantitative resistance, see e.g. Lane et al. 1993, Karban & Baldwin 1997, Kuiper et al. 1998, Strauss & Agrawal 1999). In parasitic plants, host resistance may include mechanical barriers (e.g. localized necrosis of host tissue around the penetration site of the parasite) as well as different defence chemicals that lead to unsuccessful establishment of a parasitic plant after the initial penetration phase of the parasite (Lane et al. 1993, Parker & Riches 1993, Riopel & Timko 1995 and references therein). Overall, the mechanistic and genetic basis of plant resistance to parasitic plants is, however, not well understood (Graves 1995). In the studies included in this thesis, I recorded the infectivity of the parasite (i.e. qualitative resistance) as well as the performance of the parasite (the total biomass or the number of flowers of the parasite, i.e. quantitative resistance).

Whereas the benefits of qualitative resistance to the host are usually obvious and immediate, the benefits of quantitative resistance may be less so since they are often related to the lowered damage level (i.e. lower parasite performance on resistant hosts leads to lower parasite-induced decrease in host fitness) and expressed at a later stage in the host's life. In this study system, in which the parasite is an annual and the host a perennial, the benefits of this type of resistance may be explained by at least two mechanisms. First, there is a significant positive correlation between parasite biomass and reduction in host fitness due to the parasite (i.e. the damage level is lower for resistant hosts that support lower parasite biomass, see results from the fourth study). Second, because the growth and reproduction of the parasite is lower on resistant hosts, there is likely to be fewer parasites attacking the same host clone and its offspring in subsequent year(s).

Tolerance is the ability of the host to regrow and/or reproduce after damage, and it is defined as the degree to which plant fitness is affected by the natural enemy (for reviews, see Rosenthal & Kotanen 1994, Strauss & Agrawal 1999, Stowe et al. 2000). Mechanisms of tolerance may be related to allocation patterns (e.g. reallocation of below-ground resources to above-ground parts), plant architecture (e.g. increased branching and tillering), and photosynthetic capacity (e.g. McNaughton 1979, Richards 1984, Van der Meijden et al. 1988, Strauss & Agrawal 1999). Tolerance is estimated from a group of related plants (i.e. clones, families) as the fitness of infected individuals relative to that of

uninfected plants of the same genetic group (for reviews, see Strauss & Agrawal 1999, Stowe et al. 2000).

When damage level is fixed (e.g. damage is experimentally imposed at a single level), a statistically significant interaction between damage treatment (i.e. undamaged or damaged) and host genotype (i.e. clone, family) has been used as an indication of genetic variation in tolerance (see e.g. Simms & Triplett 1994). Then, the difference between related damaged and undamaged plants or the proportional fitness of damaged individuals relative to undamaged ones have been used as measures of tolerance (see Strauss & Agrawal 1999 for a review). However, when damage levels are continuous (e.g. proportion of leaf area removed by herbivores) and vary among host individuals, a reaction norm approach is considered more appropriate. In this case, the standard operational definition of tolerance for a group of related individuals is the slope of a regression of fitness on the level of damage sustained by each individual within that genetically related group (Simms & Triplett 1994, Abrahamson & Weis 1997, Mauricio et al. 1997, Tiffin & Rausher 1999). It is important that the undamaged plants are also included in the analyses to represent fitness of each genetic group in the absence of damage to be able to distinguish the effects of damage on fitness from other uncontrolled environmental factors (Stowe 1998, Stowe et al. 2000).

In this thesis, I used two approaches to examine differences in host plant's tolerance to infection by the parasitic plant. First, a significant interaction between infection treatment (uninfected or infected) and host population type (unparasitized or parasitized) was used as an indication of differences in tolerance between these two groups (I). Second, I used the reaction norm approach (I, IV). That is, I measured tolerance as the biomass accumulation of the hosts relative to the parasite burden (measured as parasite biomass). The reaction norm approach was used because infected host plants had varying parasite burdens at the end of the experiment, and thus varying levels of damage. Basically, the reaction norm approach reveals whether there are fitness differences between host genotypes (or host population types) when carrying equal parasite biomasses, indicating differences in their tolerance to parasitic infection.

2.4 Effects of previous parasitism (I, II)

2.4.1 Description of the study populations (I, II)

In these two studies, eight *U. dioica* populations were used. Four of the populations were previously unparasitized and four previously parasitized by *C. europaea*. According to the collections of the Turku University Herbarium, the parasite has been present in the parasitized host populations for at least 20 years, and in some of the parasitized populations even for over 80 years. In general, *C. europaea* has a very patchy distribution in this area, and the occurrences of the parasite are well known by the local botanists (K. Syrjänen,

personal communication). The unparasitized populations were chosen from sites from where there were no previous records of the occurrence of *C. europaea*, and these sites also represent habitat types similar to those of the parasitized populations. Distances among populations varied from 500 meters to 20 kilometers. Distances between unparasitized and parasitized populations were always at least 800 meters.

2.4.2 Infection experiment: resistance and tolerance (I)

The aim of this study was to examine if there are differences in host resistance and/or tolerance against parasitic infection between host plants from previously unparasitized and parasitized populations. To study this, *U. dioica* plants from unparasitized and parasitized populations were grown either uninfected or infected with the parasitic plant *C. europaea* in a greenhouse experiment. Rhizome-derived plants from 20 host clones from each of the populations were used: one plant from each clone was grown uninfected and one plant infected with the parasitic plant. The parasitic plants were collected as seedlings from the sites of the four parasitized *U. dioica* populations just after they had germinated and before they had attached to host plants. In the infection treatments, an equal proportion of parasites from the four parasite populations were used to infect each of the eight host populations. The infectivity, biomass, and number of flowers of the parasite were then compared between parasites grown with hosts from parasitized populations and those grown with hosts from unparasitized populations. Differences in these traits would indicate differences between the two host types in their parasitic resistance. The biomass accumulation of the host plants was compared between uninfected and infected treatments, as well as between the two host types. Differences between uninfected and infected plants would indicate that parasitic infection has an effect on the performance of the host. Differences among the unparasitized and parasitized populations in biomass accumulation would indicate that there are differences between the host types in their tolerance of parasitic infection.

2.4.3 Field survey and common garden experiment: life-history traits (II)

This study is composed of two parts: in a field survey, I examined if there are differences (other than the presence of the parasitic plant, *C. europaea)* between unparasitized and parasitized *U. dioica* populations, and in a common garden experiment, I studied if there are differences in the life-history traits of *U. dioica* between the two population types.

For the field survey, ten circular plots (0.25 m^2 each) from each of the populations were randomly chosen for a vegetation inventory. The number of vascular plant species was monitored, the coverage (%) of each species was estimated, the sex of the *U. dioica* plants, and the presence or absence of *C. europaea* (parasitized populations) were recorded. The Simpson's diversity index (D) was calculated for each of the populations using the formula:

$D = 1/\sum_{i=1}^{S} P_i^2$, where P_i is the proportion for the *i*th species, and *S* is the total number of species in the population. To calculate the diversity index, the population mean coverages of each plant species were used. Soil samples were taken to examine if there are differences in soil nitrogen (N), phosphorus (P), and potassium (K) concentrations (g/kg dry mass) between the population types. The soil samples were analyzed by Novalab Oy (Karkkila, Finland).

For the common garden experiment, the plants were grown from seeds collected from 15 maternal plants per population. The seeds from parasitized populations were collected from individuals not infected by *C. europaea* during that particular season to exclude the possible direct effects of parasitic infection on the quality of seeds. It should be noted, however, that while this procedure may have lessened the importance of maternal effects, it may have selected for more resistant genotypes in the sample. The seeds were weighed prior to the experiment to take into account possible maternal effects in the form of seed weight. Two seedlings from each of the 15 families from each of the populations were planted in the common garden. The plants were grown in the common garden for two growing seasons: years 1999 (when none of the plants flowered yet) and 2000 (when all of the plants flowered). After the first growing season, one plant per family from each population was randomly chosen and their above-ground biomass (i.e. leaves and stems) was collected. During and after the second growing season, the onset of flowering and the sex of each plant was recorded, the number of above-ground stolons (a measure of asexual reproduction) of each plant was counted, and their above-ground biomass was collected separately for vegetative and reproductive parts. Root samples were also taken with a root drill right after harvesting the above-ground biomass to estimate the below-ground biomass (i.e. roots and rhizomes) of the plants.

2.5 Reciprocal cross-infection experiment: local adaptation of the parasite (III)

To study local adaptation of the holoparasitic *C. europaea* to its host plant, *U. dioica*, a completely reciprocal cross-infection experiment was conducted among five populations. The geographic distance between the populations varied from 9 to 166 km. In the experiment, parasites from all of the five populations were grown with hosts from their local population (sympatric hosts) as well as with hosts from the other four populations (allopatric hosts) in the greenhouse. Twenty female host clones from each of the populations were used. From each host clone, one clonal segment was infected with the sympatric parasite, four clonal segments were infected with parasites from the four allopatric populations, and one clonal segment was grown uninfected. The seeds of *C. europaea* were collected from at least 15 maternal families from parasites growing on the 20 females or on other nettle individuals in the proximity of these females. In the infection treatments, a bulk sample of the field-collected

seeds of the parasite from each of the populations was used (i.e. randomly chosen parasites from several genotypes were used).

To examine local adaptation, the data from the four allopatric host – parasite combinations was pooled (see e.g. Ebert 1994, Kaltz et al. 1999). The infectivity, biomass, and virulence of the parasite were then compared between sympatric and allopatric combinations.

2.6 Infection experiment among host families: genetic variation in resistance and tolerance, and costs of resistance and tolerance (IV)

To study genetic variation in host resistance and tolerance to parasitic infection, a greenhouse infection experiment was conducted with 20 host plant families that originated from a single natural population. In the experiment, *U. dioica* plants from the seeds of the 20 maternal families were grown either uninfected or infected with *C. europaea.* The seeds of *C. europaea* were collected from the same site, and the bulk sample of field-collected seeds was used to infect the host plants. Among-family differences in resistance and tolerance have been used as an indication of genetic variation in these traits (e.g. Simms & Triplett 1994, Mauricio et al. 1997, Mauricio 1998, Tiffin & Rausher 1999). Since differences among families may also represent the effects of maternal environment, the seeds were weighed prior to the experiment to take into account the possible maternal effects in the form of seed weight.

To study costs of resistance and tolerance, correlations between family mean values of resistance or tolerance and family mean values of fitness (vegetative and reproductive biomass) of the uninfected plants were calculated. The logic behind this is that in the absence of natural enemies (i.e. parasites in this case) the benefits of resistance and tolerance are absent and only the costs are manifested (Simms & Rausher 1987, Simms & Triplett 1994). These correlations estimated through the maternal family effect (i.e. dam effect) are likely to reflect underlying genetic correlations.

2.7 Allozyme analysis: population structure (V)

In this study, genetic variation and population structure of both the parasitic plant and the host plant were examined by using allozymes (neutral molecular markers). Further, it was examined whether previous parasitism had affected the level of genetic variation in the host by comparing allozyme variation between unparasitized and parasitized host populations. Altogether, twelve host and eight parasite populations were used in this study. Eight of the host populations were parasitized by *C. europaea* whereas in four of the populations the parasitic plant was not present. The host plant material was collected as

seeds from 20-30 female host individuals from each of the twelve populations. The seeds were germinated, and the seedlings were grown in the greenhouse. For the allozyme analysis, a fresh leaf sample of about 1 cm^2 in area was used. The parasitic plant material was collected from the eight parasitized host populations. Fresh samples of about 2 cm in length were collected from 20-40 parasitic plants from each population. The samples were snap-frozen and kept at -81°C until used for the electrophoresis.

Cellulose acetate electrophoresis (Helena Laboratories, Beaumont, Texas) were conducted using the stains and buffer recipes described in Richardson et al. (1986) and Hebert & Beaton (1989). Ten enzymes were screened for both the host and the parasite. For the host plant, four loci were reliably resolved and turned out to be polymorphic: GPI (glucose-6-phosphate isomerase, E.C. 5.3.1.9), 6-PGD (6-phosphogluconate dehydrogenase, 1.1.1.44), PGM-1 and PGM-2 (two isozymes of phosphoglycerate mutase, 2.5.7.3). For the parasitic plant, PGM (phosphoglycerate mutase, 2.5.7.3) and DIA (diaphorase, 1.6.99.-) were reliably resolved and polymorphic.

Allele frequencies and gene diversities within populations were estimated according to Nei (1987) using FSTAT v. 2.8 (Goudet 1999). Genetic distances among the twelve host populations were calculated as Nei's unbiased measures (Nei 1978) using POPGENE (Yeh et al. 1999). Within-population and overall heterozygote deficiency (F_{IS}) were estimated using FSTAT (Goudet 1999). Differentiation among the eight infected host populations and the eight parasite populations was examined by calculating pairwise and overall F_{ST} values using FSTAT (Goudet 1999). Gene flow among the populations was inferred from the F_{ST} values (Whitlock & McCauley 1999). The associations between genetic distances and geographic distances for both the host and parasite were analyzed by comparing the matrices of pairwise F_{ST} (i.e. genetic distance) and pairwise geographic distances using Mantel test (Mantel 1967). To examine if the genetic distances of the host and the parasite correlate (i.e. if host and parasite populations have correlated structures), the matrix of host pairwise F_{ST} was compared to that of the parasite with Mantel test.

3 RESULTS AND DISCUSSION

3.1 Effects of parasitic infection on host performance (I, III, IV)

The holoparasitic plant, *Cuscuta europaea*, had significant negative effects on the growth and sexual reproduction of its perennial host plant, *Urtica dioica* (I, III, IV). This is consistent with the results from several other studies on parasitic plants (e.g. Wolswinkel 1974, Graves et al. 1992, Parker & Riches 1993, Marvier 1996, Puustinen & Salonen 1999, Mutikainen et al. 2000b). In here, parasitic infection decreased growth and reproduction of the host by 44-48 % and 58-76 %, respectively (III, IV). In the first study, parasitic infection even completely prevented the reproduction of most of the host plants (I). Given its high virulence, *C. europaea* is thus likely to act as a selective agent on host traits that, in turn, are likely to exert selection pressure on parasite traits.

3.2 Effects of previous selection by the parasite (I, II)

3.2.1 Infection experiment: resistance and tolerance (I)

Infectivity of the parasite (measured as the percentage of successful infections) was high and did not significantly differ between hosts from previously unparasitized and parasitized populations (78 and 76 %, respectively). That is, qualitative resistance of the host against parasitic infection did not differ between the two host types. However, parasites grown with hosts from previously parasitized populations had lower biomass and less flowers than parasites grown with hosts from unparasitized populations, indicating host resistance in terms of parasite's performance (i.e. antibiosis, quantitative resistance; Painter 1958, Futuyma 1983, Strauss & Agrawal 1999, Tiffin 2000). In other words, the lower the parasite performance, the higher the resistance of the host. Contrary to the results on resistance, the lower above-ground vegetative

biomass of infected hosts from previously parasitized populations indicate that the tolerance of host plants from previously parasitized populations was lower compared to hosts from previously unparasitized populations. Furthermore, according to the reaction norm approach, with similar parasite burdens, host plants from previously parasitized populations had lower biomass, and thus, lower parasitic tolerance compared to host plants from previously unparasitized populations.

This study is based on the assumption that if the parasite exerts selection on host traits, resistant and/or tolerant genotypes may increase in frequency in parasitized host populations with time. Indeed, our results suggest that parasitism by *C. europaea* may have selected for quantitative resistance in the host but this response is associated with lower tolerance. One possible explanation for the lower tolerance of host plants from previously parasitized populations is a trade-off between resistance and tolerance (see e.g. Van der Meijden 1988, Herms & Mattson 1992, Belsky et al. 1993). In other words, if resistance and tolerance to parasitic plants are negatively genetically correlated, selection would have favoured either resistant or tolerant genotypes in previously parasitized host populations, but selection would have acted against genotypes that are both resistant and tolerant. The results of this study are based on comparisons among host populations with different histories in parasitism (i.e. on phenotypic differences). The obvious next step was to study variation in resistance and tolerance at the within-population level in order to demonstrate genetic variation in these traits and the potential fitness costs associated with these traits (i.e. costs of resistance and tolerance). These assumptions are central for the evolution of these traits and for a resistance-tolerance trade-off, and they were addressed in the fourth study of this thesis.

3.2.2 Common garden experiment: life-history traits (II)

The field survey demonstrated no differences (except for the presence of the parasitic plant) between unparasitized and parasitized *U. dioica* populations. For example, the number of plant species, percentage coverage of *U. dioica*, and nutrient levels in the soil did not differ between the two population types. Therefore, it seems reasonable to assume that differences in life-history traits between unparasitized and parasitized populations would reflect selection by previous parasitism. In general, common garden experiments are one of the most commonly used methods in studying genotypic differentiation in life-history traits among plant populations (e.g. Mazer & LeBuhn 1999). If individuals from different populations (or e.g. habitat types) express variation in life-history traits in a common garden, it is supposed to indicate genetic differentiation among populations, and reflect the effects of among-population differences in selection pressures. However, it should be noted that selection is not the only process that can create differentiation. Differentiation among populations may also be caused by genetic drift, founder effects, mutation, and low gene flow among populations (e.g. Slatkin 1987).

In this common garden experiment, none of the plants flowered during the first growing season (1999). During the second growing season (2000) plants

from previously parasitized populations started to flower somewhat later compared to plants from unparasitized populations. However, it should be noted that the difference in the onset of flowering between the two population types was very small (22.7 versus 24.9 days). Thus, this difference is likely to be of a minor biological significance. This is further supported by the fact that the difference in the onset of flowering between parasitized and unparasitized populations was not associated with consequent differences in reproductive biomass or biomass allocation to reproduction in terms of sexual reproduction. Several theoretical and empirical studies predict that when the risk of parasitism is high, selection should favour early reproduction in the host. This would, especially, be expected if the parasite has strong detrimental effects on host fitness (e.g. results in host castration or death), if the prevalence of infection increases with age, if the host can compensate for later losses due to the infection by reproducing early, and if early reproduction is a mechanism of escape from parasitism (e.g. Law 1979, Minchella 1985, Hochberg et al. 1992, Lafferty 1993, Jokela & Lively 1995, Shykoff et al. 1996, Koella et al. 1998, Koella & Agnew 1999). That biologically significant differences between unparasitized and parasitized populations in their timing of reproduction seem not to occur in this study system could be explained by the perenniality of the host plant and by the general characteristics of the parasitic plant infection. *U. dioica* is an iteroparous long-lived perennial species that can reproduce several times during its lifetime. If conditions are unfavourable for reproduction during a particular growing season, instead of reproducing sexually, it can allocate resources to vegetative growth, and postpone sexual reproduction to following growing seasons. Further, *C. europaea* seems rarely to kill the host plant in nature and seems to parasitize host plants throughout the growing season. Given further that the infection rates are rather high, early reproduction is not likely to provide a mechanism of escape from *C. europaea* infection for *U. dioica*. In general, the conditions under which parasitism is expected to lead to selection for early reproduction may not be met in this study system.

All of the plants reproduced by sexual means (i.e. produced pollen and/or seeds), and most of them (71 %) produced also stolons (i.e. reproduced asexually). No differences between the population types in their reproductive biomass or biomass allocation to reproduction were found in terms of sexual reproduction. As such, this result seems to contradict two predictions regarding sexual reproduction. First, it has been predicted that sexual reproduction should be favoured over asexual reproduction when the risk of infection is high, since sexually produced genetically variable progeny should be better able to evade coevolving parasites (Levin 1975, Jaenike 1978, Hamilton 1980, Bell 1982, Hamilton et al. 1990, Howard & Lively 1994). According to the second prediction, parasitism may select for lower reproductive effort in each reproductive event if the negative effects of parasitic infection increase with increasing investment in current reproduction (see e.g. Roff 1992, Stearns 1992, Thomas et al. 2000). However, I found differences between the population types in asexual reproduction. On the average, plants from previously parasitized populations had a lower number of stolons compared to plants from unparasitized populations. Since there were no differences between the

population types in their above-ground vegetative biomass, flower or seed biomass, or in biomass allocation to sexual reproduction, these results in fact indicate that there are differences in allocation patterns to sexual and asexual reproduction between the population types. More precisely, plants allocating more of their vegetative biomass to the production of stolons (i.e. plants from unparasitized populations) actually had a relatively lower biomass of other plant parts (i.e. stem, flowers, seeds) compared to plants that allocate less to asexual reproduction (i.e. plants from previously parasitized populations). Thus, in the presence of parasitism, it may be more profitable to allocate most available resources to sexual reproduction and vegetative growth than to asexual reproduction.

In summary, the results of this study indicate that previous parasitism may have indirectly selected for increased resource allocation to sexual reproduction. Whether this difference has a genetic basis, and whether it is associated with resistance and/or tolerance of the parent plant and/or its offspring requires further studies.

3.3 Local adaptation of the parasite (III)

Overall, parasites were more infectious to sympatric (local) hosts than to allopatric (novel) hosts. From the total of five populations, parasites from three of the populations were significantly more infectious to sympatric hosts. However, parasites from one of the populations were more infectious to allopatric hosts. The infectivity of one of the parasite populations did not significantly differ between sympatric and allopatric hosts. Parasites from one of the populations showed local adaptation in terms of performance, that is, had a significantly higher biomass on sympatric hosts compared to allopatric hosts. On the contrary, parasites from two of the populations tended to have a higher biomass on allopatric hosts. In other words, variation among parasite populations in their local adaptation was found both in terms of infectivity and performance of the parasite. Among-population variation in local adaptation has also been observed by, for example, Kaltz et al. (1999) in the *Microbotryum violaceum* - *Silene latifolia* system (though, overall, they found higher infectivity of the fungal pathogen on allopatric hosts).

In general, evidence for parasite local adaptation has been variable. According to a recent review by Kaltz & Shykoff (1998), local adaptation was not detected in half of the studies conducted by that time. On one hand, local adaptation has been observed in several study systems: in studies including plants and pathogens (Parker 1985, 1991), plants and herbivores (Mopper et al. 1995), snails and their trematode parasites (Lively 1989, Manning et al. 1995, Lively & Jokela 1996), fish and their trematodes (Ballabeni & Ward 1993), and *Daphnia* and their microsporadian parasites (Ebert 1994). On the contrary, other studies representing also several host – parasite systems have not found evidence for local adaptation (e.g. Parker 1989, Ennos & McConnell 1995, Davelos et al. 1996, Dufva 1996, Strauss 1997, Imhoof & Schmid-Hempel 1998,

Mutikainen et al. 2000b). Furthermore, Kaltz et al. (1999) and Oppliger et al. (1999) demonstrate higher overall infectivity of the parasite (a fungal pathogen and a haemogregarian blood parasite, respectively) on allopatric hosts (a plant and a lizard, respectively). In at least some of these cases, the lack of evidence for local adaptation may be explained by low virulence of the parasite or by migration rates. According to recent theoretical studies, local adaptation of the parasite is more likely when the virulence of the parasite is high and when the migration rate (gene flow among populations) of the parasite is higher than that of the host (Gandon et al. 1996, Lively 1999). For example, the hemiparasitic plant, *Rhinanthus serotinus*, had a rather low virulence in terms of relative decrease in host's biomass (44 %), and was not locally adapted to its host (*Agrostis capillaris*) (Mutikainen et al. 2000b). In turn, the fungal pathogen, *Microbotryum violaceum*, was not adapted to sympatric hosts (*Silene latifolia*) (Kaltz et al. 1999), and had a lower gene flow among populations compared to the host (Delmotte et al. 1999). In the *Cuscuta – Urtica* interaction, the virulence of the parasite is high, especially in terms of host reproduction (I, III, IV). Therefore, the virulence assumption seems to be fulfilled in this study system. Gene flow among parasite and host populations was addressed in the fifth study of this thesis.

There are at least five possible explanations for the among-population variation in the level of local adaptation. First, the variation in parasite adaptation may be completely random. Second, Ebert (1994) suggests that as the distance between the origins of the host and the parasite increases, the level of local adaptation decreases. However, in the present study system, the observed variation among populations in the level of local adaptation was not affected by geographical distances between host and parasite populations. Third, the emergence of new resistant host genotypes and infectious parasite genotypes may lead to time-lagged coevolutionary cycles in host and parasite genotype frequencies following the dynamics of negative frequency-dependent selection (e.g. Hamilton 1980, Bell & Maynard Smith 1987, Hamilton et al. 1990, Frank 1996). Thus, the degree of local adaptation may fluctuate in time within populations (Gandon et al. 1996, Morand et al. 1996, Lively 1999). Since different populations may be in different phases of their coevolutionary cycles, the pattern of local adaptation may vary among populations (e.g. Lively 1999). Fourth, if there are fitness costs associated with resistance, selection should favour resistant genotypes when parasite pressure is high and, in turn, when parasite pressure is low, one would expect selection to favour non-resistant genotypes. Changes in the frequencies of resistant and non-resistant host genotypes may then affect parasite's adaptation to host genotypes. Fifth, there may be variation among populations in the strength and agents of selection (i.e. different arrays of natural enemies or differences in abiotic conditions), and in their genetic population structure (e.g. gene diversity, gene flow) acting on both of the interacting species (the so-called geographic mosaic theory of coevolution; Thompson 1994, 1999). Consequently, the strength of selection affecting the level of local adaptation may vary spatially. Due to all of these processes, the magnitude of local adaptation may fluctuate both in temporal

and spatial scale and lead to variation in local adaptation both within and among populations.

To unequivocally demonstrate local adaptation of the parasite, one should follow up the coevolutionary changes in genotype frequencies of both the host and the parasite for an adequately long time. In other words, one should demonstrate that parasites are actually tracking the common host genotypes and that this leads to selection favouring the rare resistant host genotypes in the population. Evidence for both of these processes have been found by Dybdahl & Lively (1995, 1998) and Lively & Dybdahl (2000) in a snail – trematode interaction where local adaptation of the parasite has also been demonstrated (Lively 1989). Further, coevolutionary cycles in host – parasite interactions are usually thought to occur in systems where infectivity and susceptibility are determined according to a matching alleles model; infection is only successful when there is an exact match of host and parasite genotypes (for a review, see Clay & Kover 1996). On the other hand, recent evidence shows that oscillations in genotype frequencies are also possible for polygenic, quantitative resistance traits (Dieckmann et al. 1995, Clay & Kover 1996, Gavrilets 1997, Berenbaum & Zangerl 1998).

Basic assumptions for the processes leading to changes in the frequencies of resistant and non-resistant host genotypes are the existence of genetic variation in host's resistance and genetic correlations between resistance and other fitness-related traits of the host. These assumptions were tested in the fourth paper of this thesis. Furthermore, host and parasite population structures that are central for the understanding of local adaptation were examined in the fifth study.

3.4 Genetic variation in resistance and tolerance, and costs of resistance and tolerance (IV)

Significant variation among host plant families in parasite performance (measured as parasite biomass) were found indicating among-family differences in quantitative resistance of the host. Further, significant differences among host plant families in parasitic tolerance were found in terms of reproductive biomass of the host (but not when tolerance was measured in terms of vegetative biomass). The observed among-family differences indicate genetic variation in these traits (e.g. Simms & Triplett 1994, Mauricio et al. 1997, Mauricio 1998, Tiffin & Rausher 1999). Natural enemies, such as pathogens, herbivores, and parasites are believed to affect the evolution of host traits conferring resistance and tolerance given that there is heritable variation in these traits. On the other hand, if resistance and/or tolerance are beneficial in terms of plant fitness, one would expect plant populations to evolve towards the highest level of resistance and tolerance and to lose genetic variation in these traits (Fisher 1930). However, numerous studies have observed genetic variation in resistance (e.g. Berenbaum et al. 1986, Maddox & Root 1987,

Rausher & Simms 1989, Simms & Rausher 1989, Stowe et al. 1994, Mutikainen et al. 2000a), tolerance (e.g. Simms & Triplett 1994, Fornoni & Núñez-Farfán 2000) or both (e.g. Fineblum & Rausher 1995, Mauricio et al. 1997, Agrawal et al. 1999, Tiffin & Rausher 1999) within plant populations. Thus, in many plant species these traits are found at an intermediate level.

The correlations between mean family values of resistance, tolerance, and fitness of the uninfected hosts indicated costs of resistance in terms of host growth and reproduction, and costs of tolerance in terms of host reproduction. Thus, these costs may constrain the selection for increased resistance and/or tolerance, and may contribute to the maintenance of genetic variation in these traits (see e.g. Simms & Rausher 1989, Roff 1992, Stearns 1992, Rausher 1996, Sheldon & Verhulst 1996, Agrawal et al. 1999, Tiffin & Rausher 1999). In general, previous studies with plant – herbivore and plant – pathogen interactions have yielded variable evidence for costs of resistance and tolerance (for a review, see Bergelson & Purrington 1996). In some studies, cost of resistance (e.g. Coley 1986, Mauricio & Rausher 1997, Mauricio 1998, Elle et al. 1999) or tolerance (e.g. Simms & Triplett 1994, Tiffin & Rausher 1999) have been detected. On the contrary, Simms & Rausher (1987, 1989), Parker (1990), Ågren & Schemske (1993), Mauricio et al. (1997), Agrawal et al. (1999), and Fornoni & Núñez-Farfán (2000) found no evidence for such costs. It is notable, however, that in most of these cases, fitness costs of resistance or tolerance have been determined only in terms of host reproduction. In the present study, the fact that no indication of costs of tolerance was found in terms of vegetative biomass of the host, even though tolerance was costly in terms of reproductive biomass, is in accordance with the finding that genetic variation in tolerance was observed only in terms of reproduction. Thus, the lack of a correlation between resistance and/or tolerance and fitness may simply be explained by the lack of genetic variation in these traits.

To sum up, the differences among host plant families in their resistance and tolerance observed here indicate that there is genetic variation in these traits, and that these traits can evolve as a response to, for example, parasite-mediated selection. Further, the results indicate that there are costs of resistance and tolerance (at least in terms of reproduction of the host). Vegetative and reproductive biomass yielded differential results in terms of genetic variation in tolerance and costs of tolerance. These differences could, for example, be related to the mechanisms of resistance and tolerance: tolerance seems to be related to genetic variation in allocation to reproduction (as indicated by the significant negative correlation between allocation to reproduction and tolerance in terms of reproduction) whereas no such association was found between resistance and reproductive allocation. Thus, measuring more than just one trait when studying these issues seems especially important in perennial plants in which allocation to one function (e.g. vegetative growth, reproduction, defence) is likely to affect allocation to other functions not only during that particular growing season but during consecutive growing seasons as well.

3.5 Population structure (V)

In this study, two specific questions were addressed. First, by comparing populations that differ in their histories of parasitism (i.e. previously unparasitized or parasitized populations), it was examined if parasitism is associated with higher levels of genetic variation in the host. Parasites are believed to play a significant role in the maintenance of general genetic polymorphism in the host (Haldane 1949, Jaenike 1978, Bremermann 1980, Hamilton 1980, Bell 1982, Hamilton et al. 1990). Second, it was examined if host and parasite populations differ in population genetic structure that has important implications for parasite local adaptation and the coevolutionary interactions between hosts and parasites. These questions were examined by using allozymes (selectively neutral genetic markers).

Unparasitized and parasitized host populations did not differ in terms of gene diversity (H_E = 0.480 versus 0.520, respectively) or heterozygosity (F_{IS} = 0.017 versus 0.087, respectively). These results suggest that the observed differences in parasite resistance and tolerance (I), and in other life-history traits (II) between hosts with different histories of parasitism are not associated with differences in the allozyme variation. In other words, even if these differences among the two host population types were caused by selection by the parasite, this selection is not reflected in the level of neutral genetic variation.

Both host and parasite populations were slightly but significantly genetically differentiated (measured as F_{ST}). However, host populations were more strongly differentiated than parasite populations (0.032 versus 0.009). Studies in which the population structures of both the host and the respective natural enemy have been compared are, however, scarce. One of these studies demonstrates significant population differentiation for both the host and the parasite (Mulvey et al. 1991). Higher population differentiation of the host compared to that of the parasite has been found by Michalakis et al. (1994), Dybdahl & Lively (1996), and Davies et al. (1999). On the contrary, Delmotte et al. (1999) and Martinez et al. (1999) found higher population structures for a fungal pathogen and a brood parasite, respectively, compared to their respective hosts.

The low F_{ST} values probably indicate that gene flow is high among both host and parasite populations (Whitlock & McCauley 1999). Further, the lower F_{ST} value for the parasite suggests that parasite's gene flow is higher than that of the host. Relative migration rates of the interacting species are of central importance in shaping the outcome of the coevolutionary interaction and for the understanding of parasite local adaptation (Gandon et al. 1996, Lively 1999). On one hand, high gene flow is believed to counteract local adaptation (Slatkin 1987). Especially, when both organisms have either large or similar migration rates, no local adaptation is expected according to the model by Gandon et al. (1996). On the other hand, gene flow among populations introduces genetic variability on which selection can act (e.g. Frank 1991, Ladle et al. 1993, Thompson 1994), which may be especially important in antagonistically coevolving species. According to the model by Gandon et al. (1996), local

adaptation of the parasite is expected when parasite's migration rate is higher than that of the host. This is based on the idea that if parasite's gene flow is higher, the appearance of new infectious genotypes is higher than that of the new resistant host genotypes, and this will promote local adaptation of the parasite. Thus, according to this prediction, parasites in this study system would be expected to show local adaptation. However, a recent model by Lively (1999) that combines virulence and migration rate of the parasite (but not that of the host) with local adaptation, predicts that variation in the level of local adaptation becomes more likely (and local adaptation more difficult to detect) when the parasite's migration rate increases. In any case, our previous study demonstrated that the parasite overall had higher infectivity on sympatric hosts (i.e. showed local adaptation in terms of infectivity) (III). This result indicates that despite high gene flow among populations, as suggested by the results from the present study, local selection seems to occur.

Both the host and the parasite indicated significant heterozygote deficiency (measured as F_{IS}). However, the parasite had a lower level of heterozygosity than the host (0.444 versus 0.117) suggesting that it has a higher level of inbreeding. These differences are most likely related to differences in mating systems. The host is dioecious, that is, obligately outcrossing and wind-pollinated. So, in the host plant, all inbreeding must occur between relative plants (i.e. biparental inbreeding). The parasite is insect-pollinated (Kuijt 1969), but otherwise its mating system (e.g. in terms of ability and commonness of self-pollination) is not well known. Seed dispersal of both the host and the parasite is likely to occur within short distances. Given that wind-pollinated species usually have higher rates of gene flow through pollen migration compared to animal-pollinated species (Hamrick 1989, Hamrick & Godt 1990), one would have actually expected the parasitic plant to have a higher level of population differentiation than the host plant.

There was no association between genetic distances and geographic distances neither for the host nor the parasite as indicated by the non-significant correlations between the two measures of isolation. This result is contradictory to those observed by Dybdahl & Lively (1996) and Delmotte et al. (1999). The lack of correlation between genetic and geographic distances, and the fact that populations were genetically differentiated suggests that the level of differentiation does not depend only on the geographic isolation among populations. In addition, there was no significant correlation between host and parasite pairwise genetic distances. This result indicates that local selection has not led to concordance in host and parasite population structures.

In here, population structure was measured using neutral markers, allozymes (i.e. alleles that are unlinked to selected loci). It should be noted that measuring the differentiation in terms of adaptive phenotypic traits that are under selection pressure in host – parasite relations (i.e. parasite's infectivity and virulence, and host's resistance and tolerance) might yield differential results. Indeed, when population differentiation is measured in terms of both adaptive phenotypic traits and neutral markers, stronger differentiation is often found for adaptive phenotypic traits (e.g. Lande & Barrowclough 1987, Goodnight 1988, Podolsky & Holtsford 1995).

4 CONCLUSIONS

Host – parasite interactions are believed to be determined by antagonistic reciprocal coevolution: changes in host resistance, tolerance and other life-history traits cause reciprocal evolutionary changes in parasite infectivity and virulence and *vice versa*. The general aim of this thesis was to examine prerequisites for coevolution in a host plant (*Urtica dioica*) – holoparasitic plant (*Cuscuta europaea*) interaction. In agreement with several other studies with different host – parasite systems (e.g. Price 1977, Hochberg et al. 1992), I found that the parasite had significant negative effects on both the growth and reproduction of its host (I, III, IV). Thus, parasitic infection is likely to have exerted strong selection on host traits that confer resistance and/or tolerance to parasitic infection. Consequently, one would expect that differences in host traits, especially in terms of defensive traits, impose selection on the parasite.

Based on comparisons between unparasitized and parasitized *U. dioica* populations, previous parasitism may have selected for quantitative resistance (see e.g. Painter 1958, Futuyma 1983, Tiffin 2000) in the host as indicated by the lower parasite performance on hosts from previously parasitized populations (I). However, the lower tolerance of host plants from parasitized populations compared to those from unparasitized populations (I) contradicts with the prediction that high levels of tolerance would be selected for in the presence of the parasite. In the common garden, I found that host plants from previously parasitized populations had lower asexual reproduction (measured as the production of stolons) compared to hosts from unparasitized populations (II). This finding, and the fact that plants from unparasitized and parasitized populations did not, however, differ in their vegetative and reproductive biomass (in terms of sexual reproduction), indirectly suggests that selection by parasites may have favoured resource allocation to sexual reproduction. In the presence of parasitism, selection is generally believed to favour sexual reproduction over asexual reproduction because genetically variable offspring may have a better chance of escaping from infection by local coevolving parasites (Levin 1975, Jaenike 1978, Hamilton 1980, Bell 1982).

Differences in selection pressures (e.g. due to parasitism) is not the only process that may lead to divergence among populations. The level of

divergence among populations can also be affected by genetic drift, founder effects, gene flow, and mutation (e.g. Slatkin 1987). To explicitly demonstrate that differences among populations are caused by differences in selection pressures, the relative importance of all of these processes in shaping the traits of individuals should be known. However, the observed phenotypic differences between the host plant populations with different histories in parasitism (I, II) are likely to reflect genetic differences and the effect of selection exerted by the parasite for the following reasons. First, these studies were conducted in greenhouse (I) and common garden (II) conditions (i.e. growing conditions were controlled for). Second, I found differences among host plant families in their resistance and tolerance indicating genetic variation in these traits, and that these traits can thus respond to selection (IV). Of course, strong inferences about the role of parasites as a selective factor on host traits can only be made when presence and absence of the parasite and changes in levels of defensive traits of the host can reliably be documented (e.g. Rausher 1996).

I found no evidence that previous parasitism had affected the level of neutral genetic variation in the host (V). Thus, the observed differences in host resistance and tolerance (I), and in host life-history traits (II) among unparasitized and parasitized populations (i.e. potentially indicating the effects of selection by the parasite) were not reflected in the level of neutral genetic variation. However, studying the population structure of loci coding for parasite's infectivity and virulence, and for host's resistance and tolerance (i.e. potentially adaptive traits that are under selection) might have given differential results.

I found evidence for costs of resistance in terms of both growth and reproduction, and costs of tolerance in terms of reproduction of the host (IV). These costs may constrain the evolution of increased levels of host resistance and tolerance (e.g. Simms & Rausher 1989, Roff 1992, Stearns 1992). Since both resistance and tolerance were negatively correlated with host reproduction (IV), it would seem that the most plausible explanation for the higher resistance but lower tolerance of the hosts from parasitized populations (I) is a trade-off between resistance and tolerance. However, this requires further studies.

In the antagonistic coevolutionary interaction between hosts and parasites, parasites may become locally adapted to their sympatric hosts and have a higher infection success and/or fitness on their sympatric (local) than on allopatric (novel) hosts (e.g. Lively 1989, Ebert 1994). In here, the parasitic plant was overall more infectious to sympatric hosts (III), in spite of the fact that the low F_{ST} values suggest high gene flow among populations (V). In general, high levels of gene flow are believed to counteract selection for local adaptation (e.g. Slatkin 1987, Gandon et al. 1996, Lively 1999). Thus, my results suggest that local selection imposed by the host on the parasite and *vice versa* is strong enough to counteract the effects of high gene flow. The importance of local processes is further supported by the fact that I found slight but still significant differentiation among both host and parasite populations in allozyme variation (V).

The level of local adaptation varied among parasite populations both in terms of infectivity and performance of the parasite (III). In other words,

parasites from some of the populations succeeded better in infecting hosts from their sympatric population and/or had higher biomass on their local hosts than on allopatric hosts, while the opposite was true for some of the parasite populations (III). This variation was not affected by geographic distances between populations (III). Correspondingly, there was no correlation between geographic and genetic distances neither for the host nor for the parasite (V). In the fourth study, I found evidence for genetic variation in resistance as well as costs of resistance (IV). Thus, the observed variation among populations in local adaptation could be due to changes in the frequencies of resistant and non-resistant host genotypes caused either by time-lagged coevolutionary cycles in host and parasite genotype frequencies within populations (e.g. Gandon et al. 1996, Morand et al. 1996, Lively 1999) or by costs of resistance. Changes in the frequencies of resistant and non-resistant host genotypes caused by either of these processes may then lead to temporal fluctuations in the level of local adaptation within populations. However, it remains unanswered whether there are genotype-specific effects in this host – parasite system, that are one of the prerequisites for frequency-dependent selection and host – parasite coevolution (e.g. Hamilton 1980, Thompson 1994 and 1999, Clay & Kover 1996). In other words, further studies are required to determine if the host expresses resistance and/or tolerance specific to particular parasite genotypes, and if the parasite shows infectivity specific to particular host genotypes.

To sum up, coevolution seems possible in this host – parasite system according to the basic assumptions tested for in this thesis. I found differences in host and/or parasite traits between "novel" and "familiar" host – parasite combinations (i.e. between unparasitized and parasitized host populations, and between allopatric and sympatric combinations) (I, II, III). These results suggest for the past selection by parasitism, and that local selection is strong despite high gene flow among populations (V). In addition, I found evidence for genetic variation in host plant's resistance and tolerance against parasitic infection which is a prerequisite for the ongoing evolution of these traits (IV).

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YHTEENVETO

Isäntäkasvin ja täysloiskasvin välinen vuorovaikutus: edellytyksiä koevoluutiolle?

Isäntä – loinen –suhde on yksi yleisimmistä ja tärkeimmistä ekologisista vuorovaikutussuhteista (interaktioista). Yli 50 % eliöistä on arvioitu olevan loisia, eli eliöitä, jotka ottavat resursseja omaan kasvuunsa ja lisääntymiseensä toisilta eliöiltä (isänniltä). Loiset eivät yleensä menesty ilman isäntää, ja ne elävätkin usein isäntänsä sisällä tai pinnalla. Ottamalla resursseja isännältään, loiset vaikuttavat suoraan isäntiensä kasvuun ja lisääntymiseen. Lisäksi loiset vaikuttavat epäsuorasti isäntiensä runsauteen sekä niiden populaatio- ja yhteisörakenteeseen ja muovaavat isännän ominaisuuksia evolutiivisessa mielessä.

Jos loinen vaikuttaa merkittävissä määrin isäntänsä kasvuun ja lisääntymiseen, voidaan olettaa, että luonnonvalinta suosii loisresistenssin (vastustuskyky loisinfektiota vastaan) ja/tai loistoleranssin (loisinnan sietokyky) kehittymistä isännässä. Loisinnan arvellaan myös vaikuttavan isäntänsä elinkiertopiirteiden evoluutioon, kuten esimerkiksi lisääntymisajankohtaan, –panostukseen ja –tapaan. Isännän piirteet (esim. loisresistenssi) puolestaan aiheuttavat valintapainetta loisen elinkiertopiirteissä. Luonnonvalinnan oletetaan suosivan loisessa ominaisuuksia, jotka lisäävät sen kykyä infektoida isäntiä tehokkaammin (esim. kyky purkaa isännän resistenssi). Loisten on muun muassa esitetty sopeutuvan evolutiivisesti omaan paikalliseen isäntäpopulaatioonsa; loisen oletetaan kykenevän infektoimaan tehokkaammin oman isäntäpopulaation isäntiä ja/tai menestyvän paremmin oman isäntäpopulaation isännillä kuin saman lajin muista populaatioista olevilla isännillä. Yleisesti loisten ja isäntien välillä katsotaankin olevan käynnissä jatkuva koevolutiivinen kilpavarustelu: isännät kehittävät kykyään minimoida loisinnan haittoja, kun taas loiset kehittyvät entistä optimaalisimmiksi isäntiensä hyväksikäyttäjiksi.

Arviolta noin 1 %, eli yhteensä noin 3000 lajia, koppisiemenisistä kasvilajeista on loisia. Loiskasvit ottavat osan (puoliloiskasvit) tai kaikki (täysloiskavit) kasvuun ja lisääntymiseen tarvitsemansa resurssit isäntäkasvilta isännän johtojänteisiin kasvattamiensa imujuurten, haustorioiden, välityksellä. Useat tutkimukset ovat osoittaneet, että loiskasveilla on merkittäviä haitallisia vaikutuksia isäntäkasviensa kasvuun ja lisääntymiseen. Loiskasvit ovat isäntäkasviensa kaltaisia fysiologiansa, kokonsa, elinkiertonsa pituuden ja lisääntymisnopeutensa suhteen. Lisäksi monet loiskasvit ovat generalisteja eli loisivat useilla eri isäntäkasvilajeilla. Kaikki edellä mainitut tekijät voivat vaikuttaa siihen, että loiskasvin ja isäntäkasvin välinen interaktio saattaa poiketa merkittävästi muista jo aiemmin tutkituista isäntä – loinen –ryhmistä, missä loinen on yleensä ollut isäntäänsä huomattavasti pienempi, sen elinkierto lyhyempi ja populaation kasvunopeus (lisääntymisnopeus) suurempi. Isäntä- ja loiskasvien välisen suhteen ekologiaa ja evoluutiobiologiaa on tutkittu huomattavan vähän luonnonpopulaatioissa.

Tässä väitöskirjatyössäni tutkin nokkosen (*Urtica dioica*) ja humalanvieraan (*Cuscuta europaea*) välistä interaktiota ja erityisesti sitä, toteutuvatko koevoluution edellytykset tässä isäntä – loinen -suhteessa. Nokkonen on monivuotinen kasvi, joka lisääntyy sekä suvullisesti (siemenistä) että suvuttomasti maavarsien avulla. Humalanvieras on yksivuotinen täysloiskasvi, joka lisääntyy siementen avulla. Väitöskirjatyössäni käyttämissäni populaatioissa nokkonen on humalanvieraan pääisäntä.

Humalanvierasloisinta vähensi merkittävästi nokkosen kasvua ja lisääntymistä loisimattomiin nokkosyksilöihin verrattuna. Kahdessa ensimmäisessä osatutkimuksessa vertailin, onko aiemmin loisimattomista ja loisituista luonnonpopulaatioista peräisin olevien isäntäkasvien välillä eroa loisresistenssissä ja/tai -toleranssissa tai muissa elinkiertopiirteissä. Erot loisittujen ja loisimattomien populaatioiden välillä antaisivat viitteitä siitä, että loisinta on aiheuttanut valintapainetta isännässä. Ensimmäisessä osatutkimuksessa kasvatin loisimattomista ja loisituista populaatioista olevia nokkosia ilman loista ja loisen kanssa. Tämän infektointikokeen tulosten perusteella loisituista populaatioista olevien isäntäkasvien loisresistenssi (mitattu loisen kasvuna ja lisääntymisenä: kvantitatiivinen resistenssi) oli korkeampi, mutta loistoleranssi (mitattu loisittujen isäntien kasvuna) alhaisempi verrattuna loisimattomista populaatioista oleviin isäntiin. Nämä tulokset viittaavat siihen, että loisinnan aiheuttama valintapaine on suosinut loisresistenssin kehittymistä isännässä. Kenttäkokeen (ns. common garden -koe) perusteella loisituista populaatioista olevat isännät tuottivat vähemmän suvuttoman lisääntymisen rakenteita ("rönsyjä") verrattuna loisimattomista populaatioista oleviin isäntiin. Koska loisittujen ja loisimattomien populaatioiden välillä ei kuitenkaan ollut eroja versobiomassassa tai lisääntymisrakenteiden (kukat ja siemenet, suvullisen lisääntymisen rakenteet) biomassassa, tämä tulos antaisi epäsuorasti viitteitä siitä, että loisen aiheuttama valintapaine on suosinut resurssien allokoimista suvulliseen lisääntymiseen. Yleinen oletus onkin, että loisintapaineen ollessa suurta, valinta suosii isännän suvullista lisääntymistä. Tämä oletus perustuu siihen, että suvullinen lisääntyminen mahdollistaa geneettisesti vaihtelevien jälkeläisten tuottamisen, joilla oletetaan olevan parempi kyky välttää loisinta ja/tai loisinnan aiheuttamat haitat verrattuna suvuttomasti tuotettuihin geneettisesti ei-vaihteleviin jälkeläisiin.

Kolmannessa osatutkimuksessa tutkin ristiininfektointikokeella viiden populaation välillä, kykeneekö humalanvieras infektoimaan paremmin oman (sympatrisen) isäntäpopulaation kasveja ja/tai menestyykö se paremmin oman isäntäpopulaation kasveilla kuin muista populaatioista olevilla (allopatrisilla) isännillä. Tulosten mukaan loiskasvi kykeni infektoimaan keskimäärin paremmin sympatrisia isäntiään kuin allopatrisia isäntiä. Tämä tulos antaa tukea hypoteesille, jonka mukaan loinen sopeutuu evolutiivisesti omaan sympatriseen isäntäpopulaatioonsa. Loispopulaatioden välillä oli kuitenkin eroa; osa loispopulaatioista kykeni paremmin infektoimaan sympatrisia isäntiä ja/tai kasvoi paremmin sympatrisilla isännillä, kun taas osassa populaatioista tulos oli päinvastainen tai loisten infektointikyvyssä ja kasvussa ei ollut eroa sympatristen ja allopatristen isäntien välillä. Nämä erot saattavat johtua esimerkiksi siitä, että populaatioiden välillä voi olla eroa valintapaineessa (sekä valintaa aiheuttavissa tekijöissä että valintapaineen voimakkuudessa) sekä siitä, että isäntä – loinen

-suhde on dynaaminen vuorovaikutussuhde, jossa resistenttien isäntägenotyyppien ja infektointiin kykenevien loisgenotyyppien yleisyydet (frekvenssit) voivat vaihdella ajallisesti yksittäisten populaatioiden sisällä interaktion toisen osapuolen aiheuttaman valintapaineen vuoksi.

Neljännessä osatutkimuksessa tein infektointikokeita käyttäen useita isäntäkasviperheitä. Kasvatin yhdestä populaatiosta peräisin olevien yhteensä kahdenkymmenen emokasvin siemenistä kasvatettuja nokkosia ilman loista ja loisen kanssa, eli kokeessa oli kaksikymmentä isäntäkasviperhettä. Havaitsin, että perheiden välillä on eroa niiden loisresistenssissä ja toleranssissa. Nämä erot isäntäkasviperheiden välillä antavat viitteitä siitä, että isäntäkasvin resistenssissä ja toleranssissa on populaatioiden sisällä geneettisiä eroja yksilöiden välillä, ja siten luonnonvalinnan seurauksena evoluutio näissä piirteissä on mahdollista.

Viidennessä osatutkimuksessa tutkin sekä isäntä- että loiskasvin geneettistä vaihtelua, populaatiorakennetta ja populaatioiden välistä geenivirtaa käyttäen allotsyymientsyymielektroforeesimenetelmää. Lisäksi tutkin, onko aiemmin loisimattomien ja loisittujen isäntäkasvipopulaatioiden välillä eroa niiden geneettisessä vaihtelussa. Tulosten perusteella sekä isäntä- että loiskasvipopulaatiot olivat geneettisesti eriytyneitä toisistaan huolimatta siitä, että geenivirta eri populaatioiden välillä näyttäisi olevan suurta etenkin loisen osalta. Nämä tulokset antavat viitteitä siitä, että paikalliset populaatioiden sisällä tapahtuvat prosessit vaikuttavat merkittävästi sekä isäntään että loiseen. Loisittujen ja loisimattomien isäntäpopulaatioiden välillä ei ollut eroa geneettisessä vaihtelussa, eli vaikka ensimmäisen ja toisen osatutkimuksen perusteella loisimattomien ja loisittujen populaatioiden välillä oli eroa loisresistenssissä ja –toleranssissa sekä suvuttomassa lisääntymisessä, nämä mahdollisesti loisinnan aiheuttamat erot eivät kuitenkaan näy allotsyymimuuntelussa.

Väitöskirjatyöni osoittaa, että isännän ja loisen välinen koevoluutio on mahdollista tässä tutkimassani isäntäkasvin (nokkonen) ja loiskasvin (humalanvieras) välisessä interaktiossa. Tutkimusteni mukaan isäntäkasviyksilöiden välillä on geneettisiä eroja loisresistenssissä ja -toleranssissa, ja siten evoluutio näissä piirteissä on ensinnäkin mahdollista. Toiseksi, havaitsin eroja isännän ja loisen piirteissä aiemmin loisimattomien ja loisittujen isäntäpopulaatioiden välillä sekä sympatristen ja allopatristen lois–isäntä –kombinaatioiden välillä. Nämä tulokset antavat viitteitä siitä, että loisinta aiheuttaa valintapainetta isännän piirteissä ja isännän piirteet, esimerkiksi loisresistenssi, taas voivat aiheuttaa valintapainetta loisen piirteissä. Tulokset viittaavat myös siihen, että paikallinen populaatioiden sisällä tapahtuva loisen aiheuttama valintapaine isännässä (ja päinvastoin) on voimakasta siitäkin huolimatta, että geenivirta populaatioiden välillä näyttäisi olevan suurta.

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Original papers

I

Interaction of a host plant and its holoparasite: effects of previous selection by the parasite

by

Tanja Koskela, Veikko Salonen and Pia Mutikainen, 2001

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II

Variation in life-history traits among *Urtica dioica* populations with different history in parasitism by the holoparasitic plant *Cuscuta europaea*

by

Tanja Koskela

Manuscript (submitted)

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III

Local adaptation of a holoparasitic plant, *Cuscuta europaea*: variation among populations

by

Tanja Koskela, Veikko Salonen and Pia Mutikainen, 2000

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IV

Resistance and tolerance in a host plant – holoparasitic plant interaction: genetic variation and costs

by

Tanja Koskela, Susanna Puustinen,
Veikko Salonen and Pia Mutikainen

Evolution 2002:56: 899-908

https://doi.org/10.1111/j.0014-3820.2002.tb01403.x

V

Population structure of a parasitic plant and its perennial host

by

Pia Mutikainen and Tanja Koskela

Heredity 2002: 89: 318-324

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