Population structure of a parasitic plant and its perennial host

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Characterization of host and parasite population genetic structure and estimation of gene flow among populations are essential for the understanding of parasite local adaptation and coevolutionary interactions between hosts and parasites. We examined two aspects of population structure in a parasitic plant, the greater dodder (*Cuscuta europaea*) and its host plant, the stinging nettle (*Urtica dioica*), using allozyme data from 12 host and eight parasite populations. First, we examined whether hosts exposed to parasitism in the past contain higher levels of genetic variation. Second, we examined whether host and parasite populations differ in terms of population structure and if their population structures are correlated. There was no evidence that host populations differed in terms of gene diversity or heterozygosity according to their history of parasitism. Host populations were genetically more differentiated ($F_{ST} = 0.032$) than parasite populations ($F_{ST} = 0.009$). Based on these F_{ST} values, gene flow was high for both host and parasite. Such high levels of gene flow could counteract selection for local adaptation of the parasite. We found no significant correlation between geographic and genetic distance (estimated as pairwise F_{ST}), either for the host or for the parasite. Furthermore, host and parasite genetic distance matrices were uncorrelated, suggesting that sites with genetically similar host populations are unlikely to have genetically similar parasite populations.
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Introduction

Host-parasite coevolution depends on genetic variation, population structure and gene flow of the interacting species (Price, 1980; Thompson, 1994). According to Price (1980), parasite populations will tend to be more strongly structured than their hosts, with low rates of gene flow, leading to local adaptation and host-race formation. Relative migration rates of hosts and parasites are considered important in shaping the outcome of the coevolutionary interaction (Gandon *et al*, 1996; Lively, 1999) since migration affects the spread of resistant and susceptible host genotypes as well as that of virulent and avirulent parasite genotypes. Theoretically, the ability of parasites to adapt to their local hosts depends significantly on the relative migration rates of the host and the parasite. Local adaptation is expected when the parasite has a higher migration rate than the host and when the parasite is highly virulent (Gandon *et al*, 1996; Lively, 1999).

Nonetheless, empirical tests of these predictions are rare (Mulvey *et al*, 1991; Michalakis *et al*, 1994; Nadler, 1995; Dybdahl and Lively, 1996; Davies *et al*, 1999; Delmotte *et al*, 1999) and sometimes in contradiction. For example, populations of freshwater snails can be more strongly structured than those of their parasitic trematodes (Dybdahl and Lively, 1996; Davies *et al*, 1999).

Mulvey *et al* (1991) observed significant population differentiation for a fluke as well as for its host, the white-tailed deer. In contrast, three of the previous cases studied show the opposite pattern. Populations of weedy plants can be less strongly differentiated than those of their seed-eating weevils or fungal pathogens (Michalakis *et al*, 1994; Delmotte *et al*, 1999). Furthermore, a brood parasitic cuckoo showed stronger population structure than its host, the magpie (Martinez *et al*, 1999). In the freshwater snail-trematode interaction the parasites are locally adapted to their hosts (Lively, 1989) and also have higher migration rates than their hosts (Dybdahl and Lively, 1996). In the dioecious plant-fungal pathogen interaction the parasites are not locally adapted to their hosts (Kaltz *et al*, 1999) and show less gene flow than their hosts (Delmotte *et al*, 1999).

We have previously observed local adaptation of the parasitic plant, the greater dodder (*Cuscuta europaea*) to its host, the stinging nettle (*Urtica dioica*) in terms of infectivity, although the degree of local adaptation differed among populations (Koskela *et al*, 2000). Thus, in this case, one might expect that the parasitic plant has a higher migration rate than its host plant. In this study, we tested this prediction. We examined genetic variation and population structure of the parasitic plant (*C. europaea*) and its host (*U. dioica*) using allozyme data from 12 host and eight parasite populations. We addressed two issues. First, by comparing populations that differ in their histories of parasitism we examined whether parasitism is associated with higher level of genetic variation in the host. Host plants from populations free of parasit-

ism allocate more of their vegetative biomass to asexual reproduction than host plants from populations with a history of parasitism (Koskela, 2002). This difference may indicate that parasitism has favored resource allocation to sexual reproduction, which promotes high levels of genetic polymorphism (Jaenike, 1978; Hamilton, 1980; Bell, 1982). In this study, we examined whether this difference is reflected in the level of neutral genetic variation measured using allozymes. Using this data, we were also able to characterize the breeding systems of the host and the parasite. Second, we examined whether host and parasite populations differ in terms of population differentiation, and whether their population structures are correlated. A significant correlation between the genetic population structures of host and parasite could indicate that the rates of dispersal are similar or that parasites respond to the local selection imposed by host resistance and hosts in turn respond to selection for resistance. In the latter case, the neutral markers assayed would be linked to the loci under selection. Since we have previously observed local adaptation in the present study system (Koskela *et al*, 2000), local selection seems to occur.

Materials and methods

Study species and populations

The stinging nettle, *U. dioica* L. (Urticaceae), is a wind-pollinated perennial species that is common in nutrient-rich habitats in most of southern Finland. It is dioecious and therefore obligately outcrossing. *Urtica dioica* genets spread by seed and pollen dispersal, and ramets spread by rhizomes. The greater dodder, *C. europaea* L. (Cuscutaceae) is an annual holoparasitic vine that mostly lacks chlorophyll (Machado and Zetsche, 1990; Parker and Riches, 1993), and is thus completely dependent on its host for resources. Holoparasitic plants extract water, nutrients, and carbon predominantly from the phloem of their hosts via haustorial connections (Kuijt, 1969; Press *et al*, 1990; Parker and Riches, 1993). Although *Cuscuta* species are usually generalists in their host choice (eg, Parker and Riches, 1993; Musselman and Press, 1995), the main host of *C. europaea* in the populations studied here was *U. dioica*. *Cuscuta europaea* is pollinated by insects (Kuijt, 1969).

We collected samples for electrophoresis in 12 *U. dioica* populations in southern Finland (Figure 1). Eight of the populations sampled were parasitised by *C. europaea* (populations 1–7, 11; Figure 1), whereas in four of the populations the parasitic plant was absent (populations 8–10, 12; Figure 1). Regardless of their parasitism status, the host populations were similar in terms of habitat type, vegetation and soil nutrient levels (Koskela, 2002). *Cuscuta europaea* was abundant in the parasitised populations, and present in 87.5% of plots (10 plots, each 0.25 m² in area) in which *U. dioica* occurred (Koskela, 2002). Geographic distances among the study populations ranged from 0.5 to 166 km (Figure 1). Distances between nonparasitised and parasitised populations were at least 800 m. The eight parasite populations sampled occurred in the same sites as the eight parasitised host populations (populations 1–7, 11; Figure 1).

Sampling and electrophoresis

In summer 1999, we collected seeds from 20 to 30 host individuals from each of the 12 populations. The seeds were germinated in January 2000 in a greenhouse, and a fresh leaf sample (about 1 cm² in leaf area) from one seedling per family was used for the allozyme analysis. In July 2000, we collected samples from 20 to 40 parasitic plants from each of the eight infected populations. The samples (about 2 cm in stem length) were snap-frozen for electrophoresis and reserved at −81°C.

We performed cellulose acetate electrophoresis (Helena Laboratories, Beaumont, TX, USA) on both host and parasite using the stains and buffer recipes described in Richardson *et al* (1986) and Hebert and Beaton (1989). For both the host and parasite, 10 enzymes were screened. 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, E.C. 1.1.1.44), PGM-1 and PGM-2 (two isozymes of phosphoglycerate mutase, E.C. 2.5.7.3). For the parasitic plant, only PGM (phosphoglycerate mutase, E.C. 2.5.7.3) and DIA (diaphorase, E.C. 1.6.99.-) were reliably resolved and polymorphic.

Data analysis

Allele frequencies and gene diversities within populations were estimated according to Nei (1987) using FSTAT v. 2.8 (Goudet, 1999). Within-population and overall heterozygote deficiency (F_{IS}) were estimated using FSTAT (Goudet, 1999). Standard deviations for within population F_{IS} values were obtained by bootstrapping over loci using MATLAB™ routines written by J Jokela. To test for a significant overall deviation from Hardy-Weinberg proportions (F_{IS}) alleles were randomized over the whole dataset (Goudet, 1999).

To examine differentiation among the eight infected host populations and the parasite populations, pairwise and overall F_{ST} values were calculated using FSTAT (Goudet, 1999). For the overall F_{ST}, genotypes were randomized among samples, and the log-likelihood G-statistic was used to test for a significant deviation of F_{ST} from zero (Goudet *et al*, 1996). Gene flow among the populations was inferred from the F_{ST} values (Whitlock and McCauley, 1999). To test for isolation by distance for the host and parasite, we compared the matrices of pairwise F_{ST} (ie, genetic distances) and pairwise geographic distances using Mantel test (Mantel, 1967). For these analyses, the pairwise F_{ST} values were transformed as $F_{ST}/(1 − F_{ST})$ (Rousset and Raymond, 1997) and the geographic distances were log-transformed. To test if the host and parasite had correlated population structures we compared the matrix of host pairwise F_{ST} to the matrix of parasite pairwise F_{ST} using a Mantel test.

Results

General

For all of the 12 host populations, all four loci analysed were polymorphic; the average number of alleles per locus varied between three and six (Table 1). For the parasite, the average number of alleles varied between two and three in the two polymorphic loci analysed (Table 2). Overall, the parasite had a higher level of homozygosity than the host, although both host and
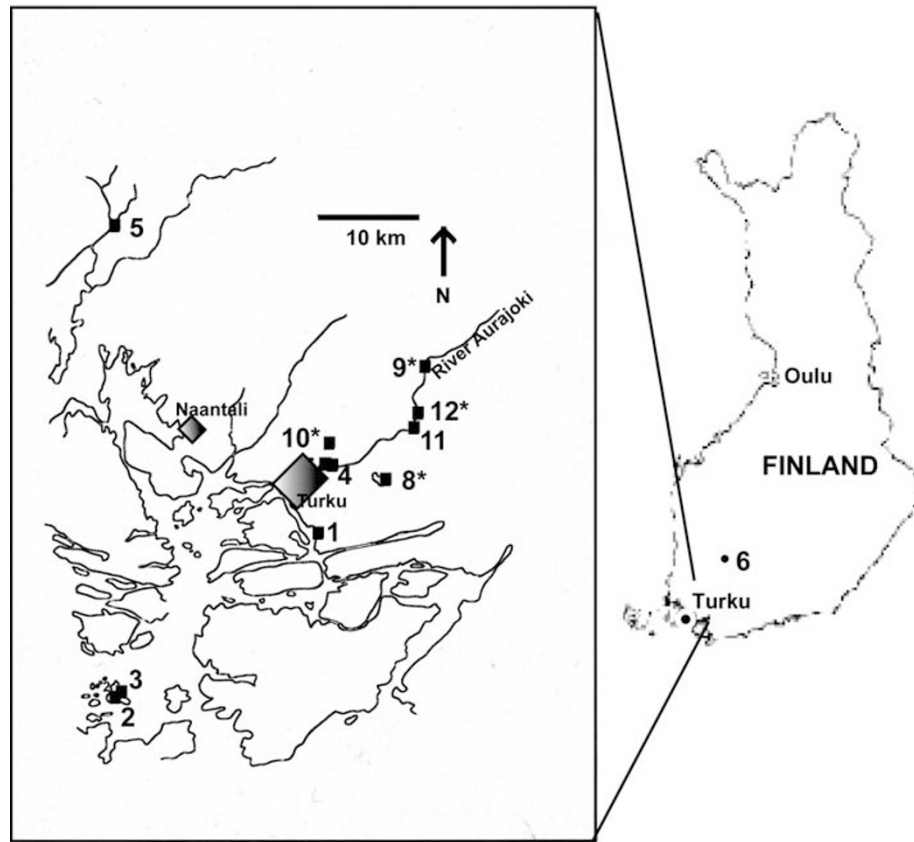
Figure 1 A map showing the locations of the 12 study sites. Sites marked with an asterisk (*) are host (*Urtica dioica*) populations that have not been parasitised by the parasitic plant *Cuscuta europaea*.

parasite had significant heterozygote deficiency (host: F_{IS} = 0.117, parasite: F_{IS}= 0.444) (Table 3). Differences in inbreeding coefficient were larger among host populations than among parasite populations. Three of the host populations actually had an excess of heterozygotes (Table 3).

Comparison of non-parasitised and parasitised host populations

The non-parasitised and parasitised populations did not differ from each other in terms of gene diversity (mean (SD) non-parasitised: H_E = 0.480 (0.018), parasitised: H_E = 0.520 (0.021); t = 1.230, d.f. = 10, P = 0.247) or in terms of inbreeding coefficient (non-parasitised: F_{IS} = 0.017 (0.052), parasitised: F_{IS} = 0.087 (0.053); t = 0.836, d.f. = 10, P = 0.423).

Population differentiation

Since we wanted to compare host and parasite population differentiation, we included only the eight infected host populations in the following analyses. Host populations were more genetically differentiated (F_{ST} = 0.032) than parasite populations (F_{ST} = 0.009). Both F_{ST} values differed significantly from zero (log-likelihood G, P < 0.05). We found no significant correlation between geographic distance and pairwise F_{ST} either for the host (Mantel test: r = −0.114, P > 0.351; Figure 2a) or for the parasite (Mantel test: r = 0.199, P > 0.810; Figure 2b). Lastly, host and parasite pairwise F_{ST} matrices were not significantly correlated (Mantel test: r = −0.316, P > 0.114; Figure 2c).

Discussion

Our results are consistent with the results of a few previous studies on host and parasite population genetic structures in that we found stronger differentiation for the host plant than for the parasite. The low F_{ST} value observed for the parasite could indicate either higher gene flow or lower genetic drift in the parasite populations than in the host populations. Since not all host individuals are infected, the parasite populations are typically smaller, and are also likely to be younger than the host populations. Thus, one would expect more genetic drift and stronger population differentiation in the parasite (Whitlock and McCauley, 1990; Giles and Goudet, 1997). High gene flow is thus likely to explain the low differentiation observed for the parasite.

Interestingly, the results of two previous cases in which both local adaptation and population structure were studied in the same host-parasite system support the theoretical models that emphasize the importance of the relative migration of host and parasite (Gandon *et al*, 1996; Lively, 1999). Here, inferred gene flow in the parasite was higher than that in the host, theoretically consistent with local adaptation. Nonetheless, parasite gene flow as high as observed here may reintroduce parasite genetic diversity and prevent local adaptation and formation of local host races. Furthermore, differences in the level of local adaptation become more likely, and local adaptation more difficult to detect, as the parasite migration rate increases (Lively, 1999). Thus, the local adaptation previously observed in this study system (Koskela *et al*, 2000)

Table 1 Gene diversity (H_e) and allele frequencies for four allozyme loci in 12 populations of the host plant (*Urtica dioica*). Four of the populations were not parasitised (marked with an asterisk *)

Population	1	2	3	4	5	6	7	8*	9*	10*	11	12*	All
GPI													
n	24	21	19	26	21	24	22	24	22	25	17	18	
1	–	–	0.026	0.019	0.024	–	–	–	–	0.020	–	–	0.008
2	0.063	0.048	0.026	0.115	0.119	0.042	0.250	0.188	0.182	0.080	0.059	0.083	0.106
3	0.479	0.333	0.316	0.346	0.452	0.479	0.477	0.417	0.227	0.220	0.294	0.444	0.375
4	0.354	0.524	0.500	0.481	0.310	0.333	0.205	0.354	0.273	0.440	0.500	0.417	0.388
5	0.104	0.095	0.132	0.038	0.095	0.104	0.068	0.042	0.250	0.220	0.059	0.056	0.106
6	–	–	–	–	–	0.042	–	–	0.068	0.020	0.088	–	0.017
H_e	0.638	0.614	0.645	0.643	0.692	0.661	0.677	0.676	0.789	0.714	0.671	0.629	0.671
												H_e SD=	0.014
PGD													
n	26	21	20	27	21	23	22	22	22	25	17	19	
1	0.096	0.071	0.075	0.093	0.095	0.196	0.114	0.045	0.091	0.040	0.059	0.105	0.091
2	0.788	0.667	0.775	0.741	0.643	0.696	0.864	0.932	0.864	0.840	0.912	0.895	0.798
3	0.115	0.262	0.150	0.167	0.262	0.109	0.023	0.023	0.045	0.120	0.029	–	0.111
H_e	0.365	0.501	0.384	0.427	0.529	0.477	0.246	0.132	0.249	0.287	0.169	0.193	0.330
												H_e SD=	0.040
PGM-1													
n	24	17	20	25	18	20	22	23	15	24	17	17	
1	–	–	–	0.020	–	–	0.023	0.065	–	–	–	0.059	0.014
2	0.625	0.265	0.425	0.340	0.333	0.500	0.318	0.304	0.367	0.229	0.324	0.382	0.370
3	0.125	0.441	0.350	0.500	0.472	0.425	0.500	0.413	0.467	0.563	0.441	0.382	0.421
4	0.250	0.206	0.225	0.140	0.139	0.075	0.136	0.152	0.167	0.167	0.147	0.147	0.163
5	–	0.088	–	–	0.056	–	0.023	0.065	–	0.042	0.088	0.029	0.031
H_e	0.545	0.706	0.663	0.623	0.663	0.588	0.642	0.722	0.640	0.616	0.689	0.706	0.650
												H_e SD=	0.015
PGM-2													
n	23	21	19	21	20	24	22	24	19	23	17	16	
1	–	–	–	–	–	–	0.023	–	0.079	–	–	0.031	0.010
2	–	0.381	0.158	0.095	0.300	0.042	0.227	0.021	0.079	0.109	0.206	0.094	0.139
3	0.848	0.405	0.684	0.786	0.550	0.750	0.750	0.792	0.763	0.696	0.735	0.844	0.717
4	0.152	0.214	0.158	0.119	0.150	0.208	–	0.188	0.079	0.196	0.059	0.031	0.135
H_e	0.267	0.669	0.497	0.371	0.600	0.406	0.392	0.351	0.412	0.477	0.426	0.285	0.429
												H_e SD=	0.034

Table 2 Gene diversity (H_e) and allele frequencies for two allozyme loci in eight populations of the parasitic plant *Cuscuta europaea*

Population	1	2	3	4	5	6	7	11	All
PGM									
n	11	19	24	23	15	14	19	7	
1	0.045	–	–	–	–	–	–	0.071	0.008
2	0.364	0.500	0.313	0.478	0.300	0.464	0.395	0.643	0.417
3	0.591	0.500	0.688	0.522	0.700	0.536	0.605	0.286	0.576
H_e	0.545	0.509	0.438	0.512	0.438	0.516	0.491	0.524	0.497
								H_e SD=	0.014
DIA									
n	20	23	33	32	23	20	23	12	
1	0.200	0.022	0.061	0.203	–	–	0.022	–	0.073
2	0.550	0.609	0.606	0.531	0.543	0.800	0.609	0.500	0.594
3	0.250	0.370	0.333	0.266	0.457	0.200	0.370	0.500	0.333
H_e	0.621	0.513	0.534	0.623	0.518	0.337	0.511	0.545	0.525
								H_e SD=	0.031

is likely to be a consequence of strong local selection on parasite infectivity and virulence.

Contrary to previous studies (Dybdahl and Lively, 1996; Delmotte *et al*, 1999), we observed no significant correlation between genetic (estimated as pairwise F_{ST}) and geographic distances among the populations either for the host or the parasite. Furthermore, host and para-site genetic structures were uncorrelated, suggesting that sites with genetically similar host populations are unlikely to have genetically similar parasite populations. Similarly, Mulvey *et al* (1991), who studied white-tailed deer and its fluke, and Michalakis *et al* (1994), who studied a thistle and a seed-eating weevil, found that the pat-terns of genetic distances in the hosts were not concord-

Table 3 Gene diversity (H_e), F_{IS} and its standard deviation (SD) for 12 populations of the host plant (*Urtica dioica*) and for eight populations of the parasitic plant (*Cuscuta europaea*). Eight of the host populations were from the same sites as the parasite populations; four of the host populations were not parasitised (marked with an asterisk *). Mean values of H_e and the overall F_{IS} values for the host are based on the eight parasitised population. Both overall F_{IS} values (marked with two asterisks **) differ significantly from zero

Population		Host			Parasite		
		H_e	F_{IS}	SD	H_e	F_{IS}	SD
1	Vaarniemi	0.444	0.068	0.189	0.556	0.461	0.186
2	Seili	0.604	0.208	0.173	0.496	0.270	0.411
3	Seili seashore	0.533	0.046	0.098	0.474	0.538	0.379
4	Halistenkoski	0.506	0.014	0.173	0.553	0.514	0.233
5	Mynämäki	0.603	0.134	0.111	0.458	0.621	0.245
6	Tampere	0.517	0.360	0.123	0.409	0.504	0.340
7	Ylioppilaskylä	0.480	−0.138	0.037	0.485	0.359	0.219
8*	Kuoviluoto	0.459	0.133	0.178			
9*	Nautelankoski	0.509	−0.031	0.074			
10*	Vähäjoki	0.512	0.069	0.104			
11	Lieto	0.474	0.006	0.054	0.500	0.411	0.477
12*	Lieto riverbank	0.442	−0.103	0.137			
Mean		0.520	0.117**		0.491	0.444**	
SD		0.058			0.048		

ant with those of their parasites. Michalakis *et al* (1994) suggest that this lack of concordance is caused by the ephemerality of the thistle populations that selects for increased migration between the weevil populations. This explanation is unlikely to work in our study system since the host plant is a relatively long-lived perennial and the populations are rather stable. In contrast to the other studies, Dybdahl and Lively (1996) found concordant patterns of genetic distances between a freshwater snail and its trematode parasite. In that case, the concordant population differentiation between the host and the parasite was more likely to be explained by the similar dispersal patterns of the two interacting species than by responses of the host and parasite to the local selection by each other (Dybdahl and Lively, 1996).

Our results may be affected by two additional factors. First, we mostly examined population differentiation over a small geographic scale; the distance among the sites varied from 0.5 to 166 km. Relative population differentiation of the host and parasite might have been different if studied over a larger geographic scale. However, site 6 was more than 130 km away from all of the other populations and did not significantly differ from the other populations. Second, the use of indirect estimates of gene flow, such as the F_{ST} values, is not the best possible way to estimate gene flow among populations and must be interpreted with caution (Whitlock and McCauley, 1999). However, even if the assumptions for inferring gene flow from the F_{ST} values are not likely to be fulfilled in the present study system, they are likely to be violated to the same degree in the host and the parasite.

The difference in the level of heterozygosity between the host and the parasite (ie, $F_{IS} = 0.117$ *vs* 0.444) probably reflects the differences in the mating systems of these two species. The host is dioecious and thus an obligate outcrosser whereas the mating system of the parasite is poorly known. According to our results, the parasite is highly inbreeding. In addition, parasite populations are usually clearly smaller than host populations since only a part of the host individuals is typically infected. These two species also differ in their pollination biology: the

host is wind-pollinated whereas the parasite is insect-pollinated. In general, animal-pollinated species tend to have less gene flow than wind-pollinated species (Hamrick, 1989; Hamrick and Godt, 1990). In addition to pollen, both species disperse by relatively large seeds (compared with other parasitic plants) that probably do not disperse long distances. Given these facts, and opposite to what we observed, one could actually expect higher population differentiation for the parasite. The levels of migration and population differentiation observed here are similar to, or even higher, than those usually observed for outcrossing, wind-pollinated species (Hamrick, 1989; Hamrick and Godt, 1990).

According to our results, populations of the stinging nettle did not differ in their level of genetic variation according to their history of infection by the parasitic plant. We have previously observed differences between the two types of host populations (previously nonparasitised and previously parasitised) in parasite resistance and tolerance (Koskela *et al*, 2001) as well as in allocation to asexual reproduction (Koskela, 2002). The results of the present study suggest that if these differences in host traits are adaptations caused by parasite-mediated selection, this is not reflected in the levels of neutral genetic variation estimated using allozymes.

When interpreting our results, one has to keep in mind that we have examined population differentiation in this host-parasite interaction using neutral markers. In general, neutral alleles that are unlinked to selected loci are more likely to become established in the populations, leading to lower allozymic differentiation compared to differentiation measured in terms of adaptive phenotypic traits (eg, Lande and Barrowclough, 1987; Goodnight, 1988; Podolsky and Holtsford, 1995). However, the outcome of a coevolutionary interaction depends on the population structure of loci coding for infectivity and virulence of the parasite, and for resistance and tolerance of the host. Since we have previously observed divergence in host resistance and tolerance that is at least partly genetically determined (Koskela *et al*, 2001), it seems that local selection for parasite infectivity, viru-
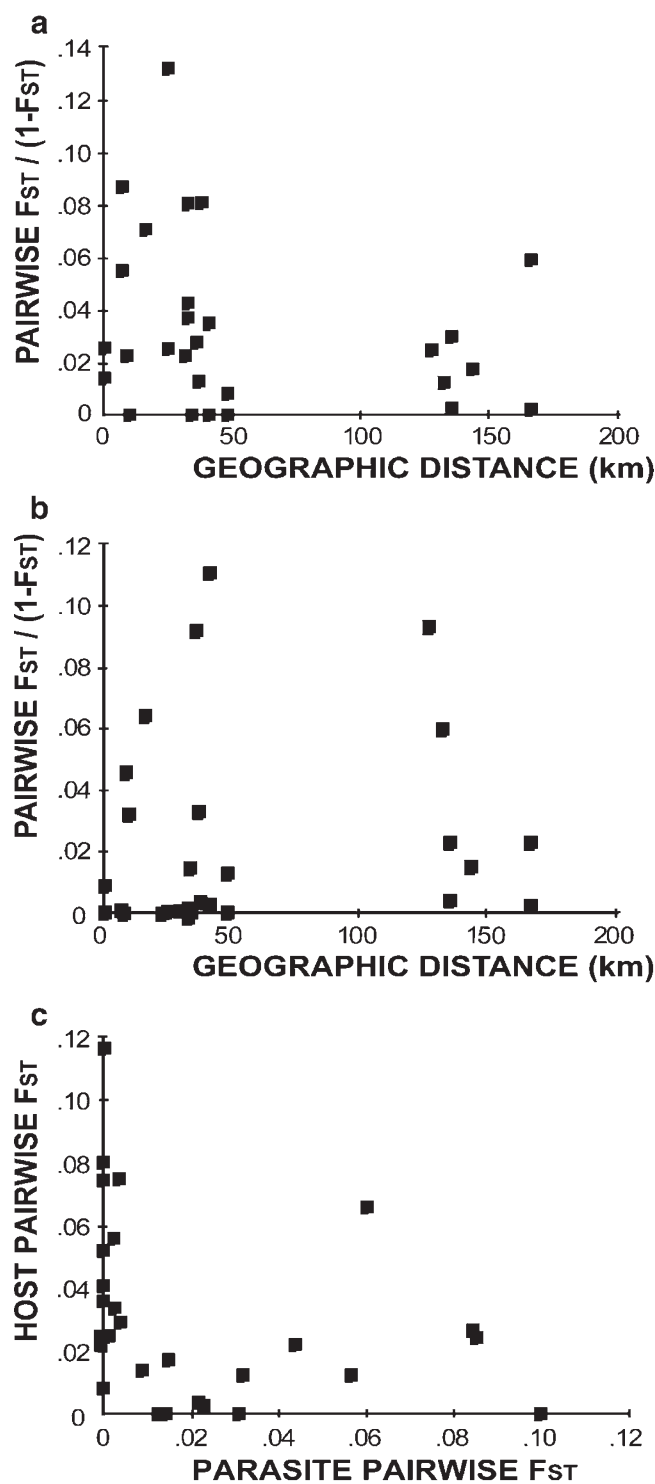
Figure 2 Pairwise F_{ST} between (**a**) host and (**b**) parasite populations in relation to geographic distances between the populations. Pairwise F_{ST} values were transformed as $F_{ST}/(1 - F_{ST})$. (**c**) Pairwise F_{ST} between host populations plotted with the pairwise F_{ST} between the parasite populations.

lence, and local adaptation, as well as for host resistance occurs in this study system. This suggests that the natural selection imposed by the host on the parasite and *vice versa* is strong enough to counteract the effects of high gene flow.

Acknowledgements

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