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Spatial and temporal genetic structure at the fourth trophic level in a fragmented landscape

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A fragmented habitat becomes increasingly fragmented for species at higher trophic levels, such as parasitoids. To persist, these species are expected to possess life-history traits, such as high dispersal, that facilitate their ability to use resources that become scarce in fragmented landscapes. If a specialized parasitoid disperses widely to take advantage of a sparse host, then the parasitoid population should have lower genetic structure than the host. We investigated the temporal and spatial genetic structure of a hyperparasitoid (fourth trophic level) in a fragmented landscape over 50 × 70 km, using microsatellite markers, and compared it with the known structures of its host parasitoid, and the butterfly host which lives as a classic metapopulation. We found that population genetic structure decreases with increasing trophic level. The hyperparasitoid has fewer genetic clusters ($K = 4$), than its host parasitoid ($K = 15$), which in turn is less structured than the host butterfly ($K = 27$). The genetic structure of the hyperparasitoid also shows temporal variation, with genetic differentiation increasing due to reduction of the population size, which reduces the effective population size. Overall, our study confirms the idea that specialized species must be dispersive to use a fragmented host resource, but that this adaptation has limits.

1. Introduction

High trophic-level species, such as predators and parasitoids experience more fragmented habitat than do their prey or host species. This is because with increasing trophic level, the resource pool becomes sparse and locally unstable [1–3]. Parasitoids live at the third and fourth trophic levels and are an important part of virtually all insect communities [4]. Most parasitoid species have a narrow host range, and relatively specific resource requirements [5], thus we expect them to be sensitive to landscape structure, and vulnerable to decline due to habitat fragmentation. However, parasitoids can persist in many landscapes if they have a broad resource niche, or greater dispersal ability than their hosts [6–8].

The consequences of landscape structure for a population should be reflected in its spatial genetic diversity and structure [9]. The degree of genetic differentiation of a host and a parasitoid in a shared landscape has been compared in few cases. Some of these studies have reported the parasitoids having higher genetic differentiation [10–13], whereas other studies have found lower (e.g. [14]), or simply different geographical patterns of genetic differentiation [15]. Host specialization and fluctuating dynamics of the host populations are also known to influence the genetic structure of parasitoids [16–18].

We present an analysis of the large-scale spatial and temporal genetic structure of a hyperparasitoid wasp, which to our knowledge is the first such study of an insect at the fourth trophic level. We compare the genetic structure of this wasp with that of the species it depends on, at trophic levels below it (host parasitoid and host butterfly). We test the hypothesis that in a shared fragmented landscape, specialized species at higher trophic levels have weaker spatial genetic structure

than their hosts, because they must move at a larger scale to find resources that are sparsely distributed. We also studied the temporal changes in hyperparasitoid structure over three years, and its qualitative association with changes in host population size. The species, in this study, live in the Åland islands of Southwest Finland where the host butterfly, *Melitaea cinxia* has a classical metapopulation in a fragmented landscape [19].

(a) Research system

The Glanville fritillary butterfly, *M. cinxia*, (Lepidoptera: Nymphalidae) and the community of parasitoids associated with it in Åland, have been the centre of intensive ecological research for the last 20 years [20–22]. In Åland, there are about 4 000 small habitat patches over an area of 50–70 km. The habitat patches are clustered in the landscape, which we delineate by using the software package SPOMSIM [23], as semi-independent patch networks (later referred to as SINs), which are separated from each other by an unsuitable habitat, and differ in size and connectivity. The butterfly distribution is fragmented. During the period of the study, the average number of local butterfly populations was 510, with a low of 363 and a high of 771. On average, 35% of local populations become extinct and 35% are newly colonized each year [19]. Generally, from 1 to 10 butterfly egg clutches (100–200 eggs in each clutch) can be found in a local population (meadow). The caterpillars live in family groups, feeding gregariously in the summer, and spending winter diapause in a shared silken nest [24].

The wasp *Hyposoter horticola* (Hymenoptera: Ichneumonidae) is a solitary egg-larval endoparasitoid. It is a specialist, using only the host *M. cinxia* [25]. The adult is extremely mobile and parasitizes a third of the host caterpillars throughout the landscape every year, resulting in a population size that is a fixed fraction of the host population size [21]. *Mesochorus* cf. *stigmaticus* (Hymenoptera: Ichneumonidae) [26] is a solitary hyperparasitoid wasp that lays eggs into larval parasitoids within *M. cinxia* caterpillars. It is specific to parasitoids of only *M. cinxia*, and uses almost exclusively *H. horticola* as a host in Åland [24], where it is found in most local host populations [27].

2. Material and methods

(a) Sampling

Three caterpillars were collected from each *M. cinxia* nest found during the systematic annual survey [22]. Thus, the sampling effort is uniform over the entire landscape each year (electronic supplementary material, figure S1). After overwintering as larvae in the laboratory and reaching adulthood, the wasps were stored in 96% ethanol at −20°C until further use. We used female hyperparasitoids reared from caterpillars collected over four years (2008–2011). The number ranged from 22 to 175 individuals per year, depending on host butterfly population size, the rate of parasitism, and rearing conditions. For comparison, we calculated the population structure of the parasitoid *H. horticola* using 407 samples (for details, see [28]) and the host butterfly *M. cinxia* using samples ($n = 421$) collected across Åland in 2010.

(b) Genotyping

For the hyperparasitoid, DNA was extracted from abdominal tissue of females using a DNeasy isolation kit (Qiagen), and genotyped using species-specific microsatellite loci [29]. The fluorescent dyes FAM, HEX, and TAMRA (DNA Technology A/S) were used to label the forward primers. The tested loci were arranged in multiplex panels based on non-overlapping size ranges for each dye. The PCR reactions were performed in a total reaction volume of 10 µl which consisted of 1× Qiagen Multiplex PCR solution, Q solution, 0.2 µM of each primer, distilled H_2O (dH_2O), and 10–20 ng of template DNA. The PCR conditions used for amplification were as follows: 95°C for 15 min, followed by 30 cycles of 95°C for 30 s, 55–60°C for 1.5 min, and 72°C for 1 min with a final step at 60°C for 10 min. The diluted PCR products were electrophoresed on an ABI 3730 automated sequencer (Applied Biosystems) and the sizes were determined using Genescan-500 ROX size standard (Applied Biosystems). The genotypes were scored manually using Gene Mapper version 5 (Applied Biosystems).

For the host butterfly, larval tissue was homogenized prior to extraction using TissueLyser (Qiagen) at 30 s^{-1} for 1.5 min with Tungsten Carbide Beads, 3 mm (Qiagen). DNA was extracted using the NucleoSpin 96 Tissue Core Kit (Macherey-Nagel). Where DNA yield was low, extracted DNA underwent two rounds of whole genome amplification (WGA) (LGC Genomics). Genotyping was performed using a panel of 40 SNP markers on the Kompetitive Allele Specific PCR (KASP) system (LGC Genomics). Single nucleotide polymorphisms (SNPs) markers were selected from putatively neutral regions of the genome [30]. Full marker information and details of SNP calling and validation are given in [31] and in the electronic supplementary material, table S1.

(c) Data analyses

For the hyperparasitoid, the locus-specific inbreeding coefficient (F_{IS}, [32]) and deviations from the Hardy–Weinberg equilibrium (HWE) for the microsatellite loci were tested using the software GENEPOP, (v. 4.2; [33]) for all the samples at the level of SINs. The indices of genetic diversity (H_E) and allelic richness (A_r, rarefaction method) per locus and per SIN were estimated using program FSTAT v. 2.9.3 [34]. The same program was used to estimate the neutral genetic differentiation (F_{ST}) among and between populations/SINs, respectively. The standard error of the F_{ST} was calculated by jackknifing over loci.

Genetic structuring between populations was visualized for the hyperparasitoid using a Bayesian clustering analysis [35,36], using the spatial clustering of individuals model implemented in the program BAPS [37]. We also used analysis of molecular variance (AMOVA) to partition the genetic variation between and among SINs using the program Arlequin [38]. Similar spatial clustering of individuals from different SINs distributed across Åland was also undertaken for (i) the host parasitoid, using 14 microsatellites markers [39] and (ii) the host butterfly, using 40 neutral SNP markers developed for the butterfly [31].

The effective population size (N_e) of the hyperparasitoid was estimated for each year using the sibship method implemented in COLONY2 [40]. The program was run with no prior information on candidate parents and sibship sizes, under the full-likelihood model for a haplodiploid system assuming a monogamous mating of females and a polygamous mating of males. This program was also used to identify the full siblings among the individuals. We calculated the geographical distances between siblings in different patches, in order to assess the dispersal range of the mother during egg laying, using the geographical coordinates of each habitat patch [22]. Isolation by distance (IBD) was tested by correlating the degree of pairwise differentiation between SINs (parametrized by F_{ST}) and geographical distances between them using the program ISOLDE as implemented in GENEPOP (v. 4.2; [33]). IBD analysis was calculated for each year separately, as well as for data from all the years combined.

3. Results

(a) Genetic diversity of the hyperparasitoid

All 25 microsatellite loci genotyped for the analyses were polymorphic and did not deviate from HWE across different SINs.

Table 1. Genetic diversity of the hyperparasitoid each year. H_0 is observed heterozygosity, H_e is expected heterozygosity, A_r is allelic richness, F_{ST} is fixation index, and F_{IS} is the inbreeding coefficient.

year	SIN ID	H_o	H_e	A_r	F_{ST}	F_{IS}
2008–2009	SIN13	0.412	0.47	3.059		
	SIN17	0.45	0.453	3.102		
	SIN21	0.386	0.482	3.296	0.015 ± 0.007	0.167 ± 0.046
	SIN51	0.396	0.471	2.92		
	SIN67	0.393	0.507	3.078		
2009–2010	SIN2	0.4	0.422	2.27		
	SIN13	0.446	0.484	2.47		
	SIN16	0.405	0.429	2.32		
	SIN17	0.4	0.418	2.33		
	SIN18	0.375	0.434	2.35	0.074 ± 0.007	0.104 ± 0.048
	SIN21	0.449	0.471	2.5		
	SIN37	0.362	0.418	2.25		
	SIN44	0.407	0.451	2.38		
	SIN51	0.292	0.364	2.17		
	SIN61	0.381	0.367	2.05		
	SIN67	0.368	0.453	2.45		
2010–2011	SIN37	0.283	0.397	2.04	0.121 ± 0.043	0.231 ± 0.071
	SIN67	0.395	0.505	2.27		
2011–2012	SIN21	0.429	0.499	2.68	0.057 ± 0.013	0.162 ± 0.051
	SIN51	0.446	0.436	2.53		
	SIN67	0.345	0.451	2.53		

The overall expected heterozygosity (H_e) and A_r were low across different SINs/populations and ranged from 0.364 to 0.507 and 2.04 to 3.29, respectively, across different years (table 1). A_r and H_e did not differ between SINs within each year (electronic supplementary material, figure S2 and table S2) or between years (A_r: ANOVA: $F_{3,96} = 0.253$, $p = 0.859$; H_e: ANOVA: $F_{3,96} = 0.083$, $p = 0.969$; electronic supplementary material, figure S3). Thus, the overall genetic diversity was consistent over the landscape, and there was no observed difference in genetic diversity between years.

(b) Genetic structure of the hyperparasitoid and comparison with the host and butterfly

The degree of genetic differentiation of the hyperparasitoid was generally low, but differed between years. The pairwise F_{ST} between SINs showed a different degree of differentiation but the overall population differentiation between SINs was low for each year ($F_{ST} = 0.015$–0.121, table 1). There were signs of inbreeding, with an F_{IS} of 0.10–0.23 between years (table 1). The AMOVA showed that the genetic variation was partitioned mostly within SINs (87.21–97.95%), but there was also significant variation between SINs (2.04–12.78%; table 2). The degree of genetic differentiation between SINs differed between years; with 2008–2009 ($F_{ST} = 0.01$) showing less differentiation than 2009–2010 ($F_{ST} = 0.074$), 2010–2011 ($F_{ST} = 0.12$), and 2011–2012 ($F_{ST} = 0.057$). Additionally, the pairwise F_{ST} between samples for the years 2008–2009 and 2011–2012 was significantly higher than that between samples for the years 2008–2009 and 2009–2010 (table 3). The effective population size ($N_e = 133$–718) of the hyperparasitoid also differed considerably among years (figure 1*b*, electronic supplementary material, table S3). Finally, the number of genetic clusters, based on the Bayesian spatial mixture clustering of the individuals, also varied among years (electronic supplementary material, figure S4). It was lowest in 2008–2009 ($K = 2$, posterior probability = 0.99) and then increased subsequently in years 2009–2010 ($K = 4$, posterior probability = 0.99) and then 2011–2012 ($K = 12$, posterior probability = 0.73). In similar analyses of just one year, the host butterfly had the most genetic clusters (2010–2011, $K = 27$, figure 2*a*), the host parasitoid had an intermediate number (2009–2010, $K = 15$, figure 2*b*), and the hyperparasitoid had just 4 (2009–2010, $K = 4$, figure 2*c*).

We did not find IBD for the hyperparasitoid in the years 2008–2009 and 2009–2010 (2008–2009: $r^2 = 0.015$, $p = 0.73$; 2009–2010: $r^2 = 0.06$, $p = 0.08$). The analysis for IBD for the other two years could not be performed because there were too few samples from each SIN. An analysis of the combined data for all the years also shows no effect of IBD ($r^2 = 0.048$, $p = 0.07$; electronic supplementary material, figure S5). There were 55 sibling groups in the dataset, with the remaining 244 individuals having no siblings in the sample. Most of the siblings were located within the same host butterfly habitat patch. Among those found in different patches, the longest distance between siblings was 2 000 m (figure 3).

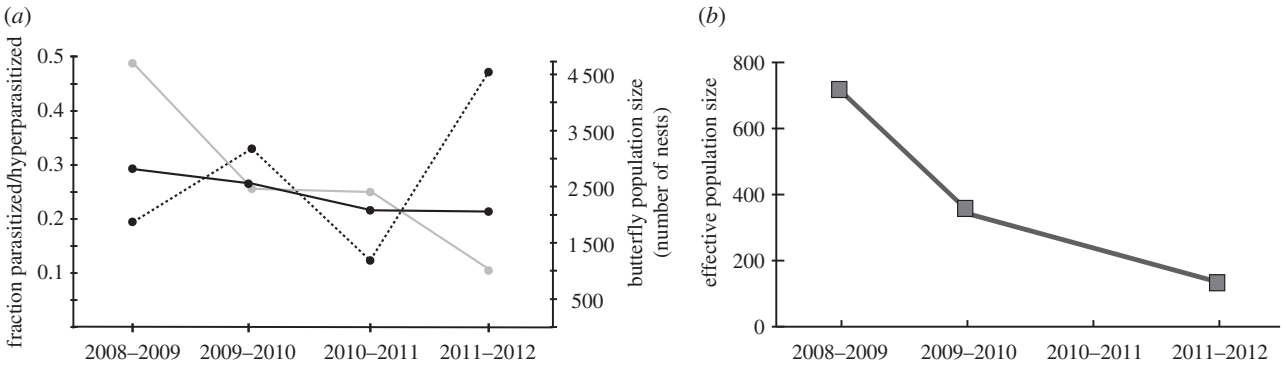

Figure 1. (*a*) The population size of the host butterfly, *Melitaea cinxia* (dashed line, right-hand axis) measured as number of larval nests in the autumn, the fraction of hosts parasitized by *Hyposoter horticola* (black line, left axis) adjusted for differential laboratory mortality, and the fraction of *H. horticola* hyperparasitized by *Mesochorus* cf. *stigmaticus* (grey line, left axis) 2008–2011 (*b*) The effective population size (N_e) of the hyperparasitoid for different years (also the see electronic supplementary material, table S3).

Table 2. Results of AMOVA for SIN grouping of the hyperparasitoid each year.

year	source of variation	sum of squares	variance components	percentage variation
2008–2009 (average over 24 loci)	among populations	39.06	0.126	2.05
	within populations	913.49	6.021	97.95
	total	952.55	6.147	
2009–2010 (average over 25 loci)	among populations	170.91	0.469	7.82
	within populations	1601.35	5.534	92.18
	total	1772.26	6.003	
2010–2011 (average over 21 loci)	among populations	8.73	0.696	12.79
	within populations	43.02	4.749	87.21
	total	51.75	5.445	
2011–2012 (average over 24 loci)	among populations	20.76	0.407	7.95
	within populations	199.96	4.719	92.05
	total	220.72	5.126	

Table 3. Pairwise F_{ST} for the hyperparasitoid samples between different years. The number above the diagonal indicates the *p*-value. (The '0' in different rows form the diagonal.)

	2008–2009	2009–2010	2010–2011	2011–2012
2008–2009	0	0.0008	0.295	0.0008
2009–2010	0.0026	0	0.289	0.0005
2010–2011	0.0026	−0.0013	0	0.0295
2011–2012	0.0073	0.0079	0.0082	0

4. Discussion

(a) Genetic differentiation of the hyperparasitoid population and effective population size over time

Overall, the pattern of genetic structure of the hyperparasitoid indicates that it disperses widely in the landscape and the population is genetically well mixed. This corresponds to its known distribution, as it is present throughout the island, even in small and relatively isolated localities, in spite of strong local extinction–colonization dynamics of the host butterfly [20]. The degree of structure of the hyperparasitoid is similar to what has been found in some previous studies of primary parasitoid wasps on a regional scale using several different types of molecular markers [41], but is clearly less structured than other systems, such as *Eurytoma robusta*, a parasitoid of the gall fly *Urophora cardui* [10] and the aphid parasitoid *Lysiphlebus hirticornis* which is differentiated even at the level of host aphid colony on a single plant [42]. We expect that the pattern we found should be common for hyperparasitoids that have a narrow host range. Those that are facultative or have a wide host range may have greater spatial structure, because individuals would not necessarily have to move on a large scale to find hosts.

The extent of genetic differentiation and effective population size can vary due to processes, such as migration or

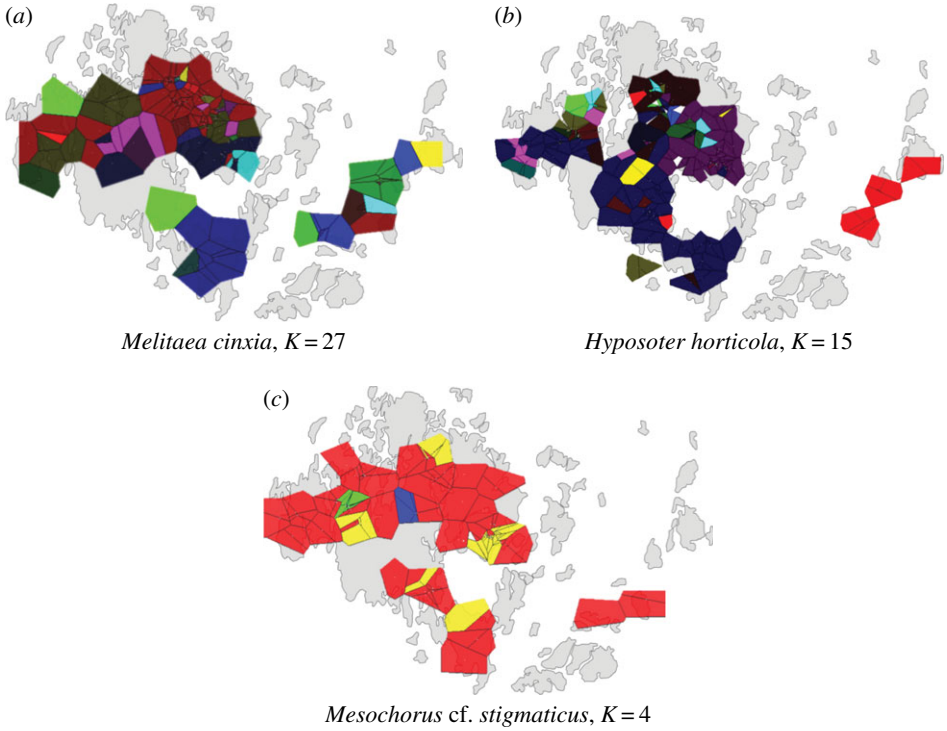

Figure 2. Comparative genetic structures of the three trophic levels in Åland. (*a*) The host butterfly *Melitaea cinxia*, (*b*) the parasitoid *Hyposoter horticola* year 2009–2010 (adapted from [28]), and (*c*) the hyperparasitoid *Mesochorus* cf. *stigmaticus* year 2009–2010 (this study).

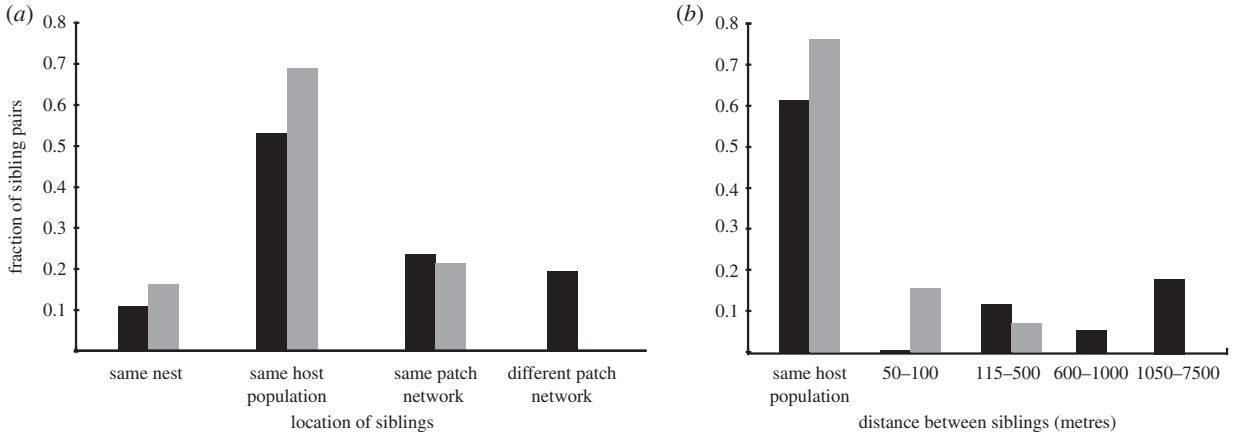

Figure 3. Distribution in the landscape of the offspring of the parasitoid *Hyposoter horticola* (black) (data from [28]) and the hyperparasitoid *Mesochorus* cf. *stigmaticus* (grey) indicated by the proportion of sibling pairs presented as (*a*) a hierarchy of localities and (*b*) distance apart in metres (distance between siblings from the same habitat patch was not measured).

large changes in population size [43,44]. A decline of population size and local founder events can create spatio-temporal genetic differentiation in a population [45]. For example, Nyabuga *et al.* [18] found temporal differences in genetic differentiation of the aphid parasitoid *L. hirticornis* due to local extinction and colonization events and high inbreeding. We found an increase in the overall genetic differentiation over years. This goes along with the observed decrease in overall population size of the hyperparasitoid. The decrease in the population size is both in conjunction with decreasing host population size (year 2010–2011), and independent of host population size (years 2009–2010 and 2011–2012). Specifically, the fraction of parasitoids that was hyperparasitized decreased significantly from year 2008–2009 to 2009–2010, and from 2010–2011 to 2011–2012, despite the increase in the density of host butterfly between these years (figure 1*a*), indicating a significant reduction in population size of the hyperparasitoid. This further explains the drastic reduction of effective population size over time (figure 1*b*; electronic supplementary material, table S3). The spatial analysis shows that the spatial genetic structure of the hyperparasitoid was low in year 2008–2009 ($K = 2$) and increased in year 2009–2010 ($K = 4$, figure 2*c*). Again, after the significant population crash in year 2010–2011 the observed number of spatial genetic clusters increased considerably in the subsequent year 2011–2012 ($K = 12$). However, the majority of the individuals in the year 2011–2012 belong to a single genetic cluster (52%), which is widespread over the island (electronic supplementary material, figure S5*c*). The other genetic clusters comprised

only a small fraction of individuals (3–9%), many of them made up of siblings ($K = 6$). These sibling groups, presumably caused by few females reproducing in isolated host nests, result in a higher number of genetic clusters. In sum, reduction in population density and effective population size have probably led to the increased genetic differentiation and genetic structure between populations/SINs in the years following 2008–2009.

It is not known why the hyperparasitoid population size, and more drastically the effective population size, declined despite the average abundance of the host butterfly and the parasitoid host. One explanation is that when the host population was small, the wasp inbred, producing diploid males (A Nair 2015, personal observation). Diploid males are the result of mating between relatives in haplodiploid species with complimentary sex determination [46]. They are known to impose a genetic load because they are either unviable or sterile [47], which can lead to a severe population crash [48]. An alternative explanation is infectious disease, such as microsporidia [49]. The microsporidian *Nosema* has been detected in the parasitoids and in the host butterfly in Åland (A Duplouy 2015, personal observation), though the prevalence and fitness costs, if any, have not been measured.

(b) Spatial population structure of the hyperparasitoid in comparison to the trophic levels below it

The host butterfly, host parasitoid, and hyperparasitoid interact strongly in a shared landscape in Åland. As predicted, if species at higher trophic levels must move increasingly more to find unstable resources [1], their degree of population structure decreases with increasing trophic level. The host butterfly population is strongly structured at the level of the habitat patches and at the level of SINs owing to habitat fragmentation and dispersal limitations [19,27], resulting in high genetic differentiation ($F_{ST} = 0.1$; [50]), a strong pattern of spatial genetic structuring, and high geographically segregated Bayesian genetic clustering ($K = 27$, figure 2*a*). The structure we found for the butterfly is qualitatively the same as found for samples collected in 2002, using four microsatellite markers and 10 neutral SNPs [51] suggesting that the butterfly has had a relatively stable degree of population structure over time. The primary parasitoid, occupying the next trophic level, also shows a spatial genetic structure at the level of SINs, but the genetic differentiation is weak ($F_{ST} = 0.07$, [28]), with fewer spatially defined genetic clusters ($K = 15$) than the host butterfly. Using data from the same year as were used for the parasitoid (2009–2010), the hyperparasitoid had just $K = 4$ genetic clusters and little spatial pattern in the observed genetic structure.

In order for the spatial genetic structure to decrease with increasing trophic level, the higher trophic-level species must be mobile. This is not always the case, which is illustrated by another parasitoid in the research system, *Cotesia melitaearum* (Hymenoptera: Braconidae), at the same trophic level as *H. horticola*. This wasp is localized and dispersal limited, undergoing local extinctions at a higher frequency than the host butterfly populations [27,52]. Consequently, a previous study showed that it has high spatial genetic differentiation ($F_{ST} = 0.378$, [11]) in Åland. Thus, in this community, the host butterfly *M. cinxia* and primary parasitoid *C. melitaearum* show a metapopulation structure [19,53], wherein the lack of gene flow between spatially isolated populations primarily shapes their genetic structure. However, the metapopulation model does not fit the primary parasitoid *H. horticola* (third trophic level) [28] and the hyperparasitoid *M.* cf. *stigmaticus* (fourth trophic level), as both these species are highly dispersive moving at a scale larger than local host butterfly populations. This movement results in a weak spatial genetic structure. The contrast between species is further supported by lack of IBD in the hyperparasitoid (electronic supplementary material, figure S5), strong IBD in the parasitoid *C. melitaearum* [11], and comparatively weaker IBD in the parasitoid *H. horticola* [28].

The species in this study occupy an island archipelago, so they are isolated from immigration. Additionally, there is strong yearly (generation) fluctuation and spatial variation of population sizes owing to the environmental variation and the host metapopulation dynamics [19,54]. This scenario can lead to loss of genetic variability, especially when the population size is small [55,56]. Indeed, the effects of population isolation and inbreeding are well documented in the host butterfly [57,58]. The parasitoid *H. horticola* [28] and hyperparasitoid also show signs of a general lack of variation and inbreeding.

(c) Individual movement and landscape-scale genetic structure

Although the spatial genetic structure of the hyperparasitoid indicates that it is highly mobile, sibling analysis showed that individual females tend to forage locally for hosts, laying eggs within a single habitat patch and not moving between habitat patches (figure 3). Therefore, the females must be dispersing before reproducing. This is possible because they are adults for several weeks before hosts are available for oviposition [20], and given the high rate of turnover of local host populations, many must move in order to find any hosts. The parasitoid *H. horticola* females behave very differently, distributing their progeny widely in the landscape (figure 3) [28]. Differences in competition may explain the strong difference between the foraging strategies of the parasitoid and the hyperparasitoid. The adult female parasitoid experiences both interspecific competition [59] and very strong intraspecific competition for hosts [21,60], which leads to the behaviour of monitoring of potential host patches by multiple individuals over weeks [61]. This may drive them to disperse widely during egg laying. Study of the foraging strategies of parasitoids has shown that in the presence of high competition, females can leave a habitat patch to explore and forage in other patches in the presence of conspecifics [62,63]. By contrast, the hyperparasitoid has no direct interspecific competitors and apparently no or low intraspecific competition [64].

(d) Conclusion

We found that the genetic population structure of a specialist hyperparasitoid is low, suggesting that instead of tracking the local population dynamics of the host, which might lead to a fine-scale population genetic structure (e.g. [14]) it avoids local host dynamics by being mobile. This makes sense if the host is locally unstable, so that a parasitoid cannot stay in one place for multiple generations. By comparing the genetic structure of the hyperparasitoid with that of its host parasitoid and the host butterfly upon which it depends, we found a pattern of decreasing spatial structure that corresponds to an increasing need to move to find resources in a fragmented landscape.

The host butterfly population sizes fluctuate locally because the butterfly lives as a metapopulation. Hence, the local availability of the host parasitoid also fluctuates. The hyperparasitoid is generally able to accommodate for that by dispersing. However, there are also global (the whole Åland islands) fluctuations in the butterfly population size primarily owing to weather. Large fluctuations are occurring increasingly frequently [54] and affect the overall resource availability to the wasp. Over the four years of the study, the hyperparasitoid population size decreased, both independent of the host population (years 2009–2010 and 2011–2012) and in association with the host population size (year 2010–2011; figure 1*a*). The population size of the host butterfly in the year 2010–2011 was the lowest recorded over the last 20 years, and appears to have had a lasting effect on the population size of the wasp. The decrease in hyperparasitoid population size is reflected in its increase in genetic differentiation and decreasing effective population size between years. While the wasp has persisted due to its dispersal abilities, the observed decline suggests that it is at some risk of extinction both stochastically, due to small population size and as a result of the loss of genetic diversity. This highlights the vulnerability of high trophic-level species in anthropogenically disturbed habitats [65].

Data accessibility. The genotyping data for the hyperparasitoid and host butterfly are available from Dryad (http://dx.doi.org/10.5061/dryad.51j99/1). The genotyping data for the parasitoid *H. horticola* is available from Dryad (doi:10.5061/dryad.s5k6k).

Authors' contributions. S.v.N. and A.N. conceived and designed the study. S.P.O., S.I., and S.v.N. collected, reared, and preserved the samples. A.N. performed the molecular laboratory work. A.N., T.F., and S.v.N. performed the data analysis. A.N. and S.v.N. drafted the manuscript. All authors gave the final approval for publication.

Competing interests. We have no competing interests.

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