

Long-term spatio-temporal genetic structure of an accidental parasitoid introduction, and local changes in prevalence of its associated *Wolbachia* symbiont

Anne Duploux¹, Abhilash Nair¹, Toshka Nyman¹, and Saskya van Nouhuys²

¹University of Helsinki

²Cornell University

March 23, 2021

Abstract

Population bottlenecks associated with founder events strongly impact the establishment and genetic makeup of populations. In addition to their genotype, founding individuals also bring along symbionts that can manipulate the phenotype of their host, affecting the host population establishment, dynamics and evolution. Thus, to understand introduction, invasion, and spread, we should identify the roles played by accompanying symbionts. In 1991, the parasitoid wasp, *Hyposoter horticola*, and its associated hyperparasitoid were accidentally introduced from the main Åland islands, Finland, to an isolated island in the archipelago, along with their host, the *Glanville fritillaria* butterfly. Though the receiving island was unoccupied, the butterfly was present on some of the small islands in the vicinity. The three species have persisted as small populations ever since. A strain of the endosymbiotic bacterium *Wolbachia* has an intermediate prevalence in the *H. horticola* across the main Åland population. The infection increases susceptibility of the parasitoid to hyperparasitism. We investigated the establishment and spread of the parasitoid, along with patterns of prevalence of its symbiont using 323 specimens collected between 1992 and 2013, from five localities across Åland, including the source and introduced populations. Using 14 microsatellites and one mitochondrial marker, we suggest that the relatively diverse founding population and occasional migration between islands might have facilitated the persistence of all isolated populations, despite multiple local population crashes. We also show local near-fixation of *Wolbachia*, where the hyperparasitoid is absent, and selection against infected wasp genotypes is relaxed.

Long-term spatio-temporal genetic structure of an accidental parasitoid introduction, and local changes in prevalence of its associated *Wolbachia* symbiont

Authors: Anne Duploux^{1,2*}, Abhilash Nair², Toshka Nyman², Saskya van Nouhuys^{2,3}

¹ Lund University, Department of Biology, Lund, Sweden

² The University of Helsinki, Organismal and Evolutionary Biology Research Program, Helsinki, Finland

³ Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, New York, USA

*Corresponding author:

Anne DUPLOUX: anne.duploux@biol.lu.se/anne.duploux@helsinki.fi

Short title : Population genetic changes after introduction

Conflict of interest: There are no conflicts of interest concerning this article.

Abstract:

Population bottlenecks associated with founder events strongly impact the establishment and genetic makeup of populations. In addition to their genotype, founding individuals also bring along symbionts that can manipulate the phenotype of their host, affecting the host population establishment, dynamics and evolution. Thus, to understand introduction, invasion, and spread, we should identify the roles played by accompanying symbionts. In 1991, the parasitoid wasp, *Hyposoter horticola*, and its associated hyperparasitoid were accidentally introduced from the main Åland islands, Finland, to an isolated island in the archipelago, along with their host, the Glanville fritillary butterfly. Though the receiving island was unoccupied, the butterfly was present on some of the small islands in the vicinity. The three species have persisted as small populations ever since. A strain of the endosymbiotic bacterium *Wolbachia* has an intermediate prevalence in the *H. horticola* across the main Åland population. The infection increases susceptibility of the parasitoid to hyperparasitism. We investigated the establishment and spread of the parasitoid, along with patterns of prevalence of its symbiont using 323 specimens collected between 1992 and 2013, from five localities across Åland, including the source and introduced populations. Using 14 microsatellites and one mitochondrial marker, we suggest that the relatively diverse founding population and occasional migration between islands might have facilitated the persistence of all isolated populations, despite multiple local population crashes. We also show local near-fixation of *Wolbachia*, where the hyperparasitoid is absent, and selection against infected wasp genotypes is relaxed.

Keywords: Genotyping, Gene flow, Endosymbiosis, *Melitaea cinxia* a, *Mesochorus stigmaticus*, Trophic chain **Introduction**

Introduced and invading populations generally show low genetic variability, and a different genetic structure than in their native range, due to small founder populations, and demographic bottlenecks (Hufbauer, Bogdanowicz, & Harrison, 2004). Low genetic variability may in turn influence persistence, population dynamics, and evolutionary potential of introduced populations (Fauvergue, Vercken, Malausa, & Hufbauer, 2012; Szucs, Melbourne, Tuff, & Hufbauer, 2014). The individuals founding new populations may bring along various symbiotic passengers (Hurst & Jiggins, 2005; Lu, Hulcr, & Sun, 2016; Rokas, Atkinson, Brown, West, & Stone, 2001). A common example of such a symbiont is the α -Proteobacterium *Wolbachia pipientis* - a maternally inherited endosymbiotic bacterium that infects over 40% of all arthropod species (Sazama, Bosch, Shouldis, Ouellette, & Wesner, 2017; Weinert, Araujo-Jnr, Ahmed, & Welch, 2015; Zug & Hammerstein, 2012). *Wolbachia* can be intimately involved in the biology of their hosts. In insects, the symbiont is known for manipulating the host reproductive system (O'Neill, Hoffman, & Werren, 1997), susceptibility to predators, parasites or pathogens (Fytro, Schofield, Kraaijeveld, & Hubbard, 2006; Hedges, Brownlie, O'Neill, & Johnson, 2008; Osborne, Iturbe-Ormaetxe, Brownlie, O'Neill, & Johnson, 2012; van Nouhuys, Kohonen, & Duploux, 2016), metabolism (Gruntenko et al., 2017; Gruntenko et al., 2019), or dispersal capacities (Evans et al., 2009). *Wolbachia* -mediated costs and benefits have been shown to affect host population dynamics (Charlat et al., 2009; Duploux, Hurst, O'Neill, & Charlat, 2010; Verne, Johnson, Bouchon, & Grandjean, 2012), select for particular host genotypes (Signor, 2017), or even hamper the evolution of host traits in infected populations (Martinez et al., 2016). Consequently, studying spatio-temporal patterns in the penetrance and prevalence of symbionts in host populations along with the genetic structure of introduced and original host populations, can provide crucial insights into how both intentionally and accidentally introduced species may successfully establish, persist and further spread across habitats (Lu et al., 2016).

The Glanville fritillary butterfly, *Melitaea cinxia* (L.) (Lepidoptera: Nymphalidae) lives as a metapopulation in Åland, Finland (I. Hanski, Pakkala, Kuussaari, & Lei, 1995). The (meta)population ecology and dynamics of the butterfly and associated community of parasitoid species has been extensively studied since the early 90's (van Nouhuys & Hanski, 2005). The butterfly population dynamics dictates the population sizes of its associated parasitoids (van Nouhuys & Hanski, 2002). In August 1991, seventy-two sibling groups of gregarious *M. cinxia* larvae were intentionally introduced on to the previously unoccupied island of Sottunga, on the East side of the Åland archipelago. The larvae originated from Finström, in the main Åland island (Figure 1a), and the introduction was part of an experiment to manipulate the butterfly metapopulation dynamics (Fountain et al., 2018; I. Hanski et al., 2004; I. Hanski et al., 2017). The introduced butterfly larvae were collected from natural populations that were occupied by larval parasitoids. Consequently, the specialist

parasitoid wasp *Hyposoter horticola* (Gravenhorst) (Hymenoptera: Ichneumonidae: Campopleginae), some of which were parasitized by their own specialist hyperparasitoid *Mesochorus cf. stigmaticus* (Hymenoptera: Ichneumonidae: Mesochorinae) (I. Hanski et al., 2004; G. C. Lei, Vikberg, Nieminen, & Kuussaari, 1997; Montovan, Couchoux, Jones, Reeve, & van Nouhuys, 2015; Shaw, Stefanescu, & Van Nouhuys, 2009; van Nouhuys & Ehrnsten, 2004) were accidentally introduced to Sottunga along with the butterfly larvae.

Parasitoids are at the highest trophic levels of insect communities, which makes them extremely sensitive to the spatio-temporal dynamics and structure of their host resources in the landscape (Cronin & Reeve, 2005; Gagic et al., 2012; Gagic et al., 2011; Kaartinen & Roslin, 2011; Nair, Fountain, Ikonen, Ojanen, & van Nouhuys, 2016; van Nouhuys, 2005). Nonetheless, despite occasional strong bottlenecks through local butterfly population crashes in which the population declined to a few gregarious larval butterfly families (Figure 2) (Fountain et al., 2016; I. Hanski et al., 2004; van Bergen et al., 2020), the parasitoids have persisted on Sottunga, which is more than 30km away from the main Åland island (hereafter referred to as the mainland), and more than 12km away from any other small island population. Based on mark recapture studies, pattern of colonization of new sites, and analyses of gene flow based on genetic markers, the islands are outside the usual dispersal distances of the butterfly (van Nouhuys & Hanski 2002), the parasitoid (Couchoux et al 2016), and the hyperparasitoid (Nair et al 2016).

In the Åland system *Wolbachia* only infects the parasitoid wasp *H. horticola* (Duploux, Couchoux, Hanski, & van Nouhuys, 2015) and the infection occurs at an intermediate and stable rate of [?]50% of the wasp population (Duploux et al., 2015). The local prevalence of the bacterium however differs between the mainland and neighbouring isolated islands (Duploux et al., 2015), and the infection is more often associated with one of two mitochondrial host haplotypes (Duploux et al., 2015). Finally, the infection is not known for manipulating its host reproductive system either through cytoplasmic incompatibility or any other sex-ratio distorting phenotypes (Duploux et al 2015), and it has no direct impact on several other fitness traits of the wasps, including metabolic rate, longevity and egg production (Duploux et al 2015). The infection is nonetheless costly to its host, as it increases the susceptibility of infected individuals to *M. cf. Stigmaticushyperparasitoid* nearly two-fold (from 40 to 74% parasitism) (van Nouhuys et al., 2016); perhaps by decreasing the mobility of the larval wasp in the host, or by decreasing the host immune response to the hyperparasitoid (van Nouhuys et al 2016). The prevalence and rate of hyperparasitism varies across local populations in Åland (Montovan et al., 2015; Nair et al., 2016).

We analysed spatio-temporal variations in both the genetic structure of the parasitoid host, *H. horticola*, and the infection rate of the parasitoid by the endosymbiont *Wolbachia* on the island of Sottunga, and four other regions in the Åland islands (Figure 1a-b). We used 14 nuclear microsatellite markers and one mitochondrial marker to genotype 323 wasps over a 22year period (1992-2013), and screened the wasps for infection with *Wolbachia* over the same period, to infer history and outcome of the accidentally introduced small population for the host and the symbiont. We investigated (1) whether migration occurred after the accidental introduction of the parasitoid species on the island of Sottunga, potentially supporting persistence of the neighbouring island populations despite occasional population crashes (Fountain et al., 2016; I. Hanski et al., 2004; van Bergen et al., 2020), and (2) whether variations in the local levels of hyperparasitism selected for *Wolbachia* -infected or uninfected host genotypes in isolated local populations.

Material and methods:

Insect material

The entire metapopulation of the Glanville fritillary butterfly (*Melitaea cinxia*, L.) in the Åland archipelago, Southwest Finland (60deg13'N 19deg55'E, Figure 1), has been surveyed for more than two decades as part of a long-term ecological study of its metapopulation dynamics (Ojanen, Nieminen, Meyke, Poyry, & Hanski, 2013). Caterpillars and the larval parasitoids in them were collected occasionally from each of the regions in the 1990's, and early 2000's, and were systematically collected as part of the annual survey starting in 2008 (Fountain et al., 2016; I. A. Hanski, 2011). About a third of the field collected caterpillars are naturally parasitized by the solitary endoparasitoid wasp *H. horticola*, some of which in turn are hyperparasitized

by *M. stigmaticus* (Montovan et al., 2015; Shaw et al., 2009; van Nouhuys & Hanski, 2005). About half the *H. horticola* in Åland are infected by *Wolbachia* (Duploux et al., 2015; van Nouhuys et al., 2016).

Before 1991 (Figure 1c), the Glanville fritillary butterfly, the parasitoid and the hyperparasitoid did not inhabit Sottunga, but the butterfly at least was known to inhabit nearby islands of Foglo, Seglinge and Kumlinge (I. Hanski et al., 2004; Murphy, Wahlberg, Hanski, & Ehrlich, 2004) (G. Lei & Hanski, 1998). Since 1991, the butterfly (Fountain et al., 2016) the parasitoid (Couchoux, Seppa, & van Nouhuys, 2016), and the hyperparasitoid populations (Nair et al., 2016) have persisted despite going through occasional strong local population bottlenecks (Figure 2).

We selected 323 *H. horticola* parasitoid individuals, including both males and females, reared from different butterfly host nests sampled from five localities in the Åland archipelago between 1992 and 2013 (Ojanen et al., 2013). The butterfly and its parasitoids are not classified as endangered or protected and hence no permits are required for their collection in the Åland Islands. In total, we used 40 wasps from the island of Sottunga (60deg07'N 20deg40'E), 43 from the northern islands of Foglo (60deg03'N 20deg32'E, in areas called Jyddo, Noto, and Overo), 44 from the closely adjacent islands of Seglinge-Kumlinge (60deg14'N 20deg46'E, 14 samples from Kumlinge, others from Seglinge), 95 from northern Finstrom (60deg32'N 19deg95'E) and 101 from Saltvik (60deg16'N 20deg03'E) on the main Åland island (Figure 1a). See Table 1 for sample size for each year for different populations. The chance of collecting full-siblings in this sample is low, as a previous study designed to detect siblings using samples from the same collections found very low incidence of siblings outside of those within a gregarious host group (Couchoux, Seppa, & van Nouhuys, 2015a). The coast-to-coast distances between Sottunga and Seglinge, and between Sottunga and Foglo, are of 8.5km and 6.5km, respectively, while the closest distance between the two suitable habitat patches on different islands is 12km and 13km, respectively. The distance from a mainland area to a suitable patch on Sottunga is about 30km.

Sample preparation

All *H. horticola* wasps emerging from the field-collected caterpillars were individually preserved in ethanol in the freezer (-20degC) until use. The DNA was extracted from the abdomen of each wasp for the purpose of another study (Duploux et al 2015), using a Qiagen DNeasy blood and tissues extraction kit, following the manufacturer's protocol (Cat. #69506, Qiagen, USA). We amplified the mitochondrial *COI* gene by PCR using the primer pair LCO/HCO and PCR conditions developed by Folmer et al. (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and including two positive samples from Duploux et al (2013) and a negative control, to check for the quality of the DNA extracts.

Mitotypes and *Wolbachia*-infection status

Cytoplasmic entities such as the mitochondria and *Wolbachia*symbionts can have profound impacts on their hosts but are passed on only from mothers. These maternally inherited entities can thus change frequency in a population at different rates than genotypes determined using nuclear microsatellite markers. To evaluate maternal inheritance in the Åland *H. horticola* population, we look at differences in the prevalence of the mitochondrion and *Wolbachia* -infection in most of the male and female samples genotyped in this study. For this, we used sequences of the 5'end of the mitochondrial gene *COI* and screenings of *Wolbachia* that were previously done by Duploux et al. (2015). The same authors (Duploux et al., 2015) characterized two common mitotypes (GenBank #KF722993 & 94, for mitotype-C and -T, respectively) from 222 of the 323 *H. horticola* wasps used in the present study, including 37 wasps from Finstrom, 41 from Foglo, 43 from Seglinge-Kumlinge, 63 from Saltvik and 38 from Sottunga. The 5'end of the *COI* gene was Sanger sequenced with an ABI 3730 DNA Sequencer (Applied BiosystemsTM, USA). Additionally, Duploux et al. (2015) showed earlier that about 50% of the *H. horticola* population on the Åland islands is infected by the *Wolbachia* strain *w* Hho (ST435 from the *Wolbachia* -PubMLST database, (Baldo et al., 2006)). They screened for *w* Hho in 296 of the 323 wasps used in this study, including 90 from Finstrom, 41 from Foglo, 40 from Seglinge-Kumlinge, 86 from Saltvik and 39 from Sottunga, using the primer pair 81F/691R to amplify the conserved *Wolbachia* surface protein (*wsp*) using PCR conditions described by (Zhou, Rousset, & O'Neil,

1998), with two positive controls from (Duploux et al., 2013) and a negative control.

Microsatellite genotyping

To document temporal and spatial nuclear genetic variation in *H. horticola* wasps, we genotyped 323 wasps (including 195 females) using the 14 microsatellite-loci and genotyping conditions developed by Couchoux et al. (Couchoux, Seppa, & van Nouhuys, 2015b). Couchoux et al. (2015b) demonstrated that there is no linkage disequilibrium between each pair of these microsatellite loci. The forward primers were labelled with either FAM, HEX, or TAMRA fluorescent dye (DNA Technology A/S), and used in multiplex non-overlapping PCR reactions using Qiagen Multiplex PCR kit (Cat. #206143, Qiagen, USA) as described by Couchoux et al. (2015b). Diluted PCR products were genotyped on an automated ABI 3730 DNA Sequencer (Applied BiosystemsTM, USA). The sizes were called using Genescan-500 ROX size standard. We manually curated the genotypes for each sample using the GeneMapper(r) Software 5 (Applied BiosystemsTM, USA).

For the purpose of the genetic analyses described below, we separated the samples into the five localities they were collected from (North Finstrom, North Foglo, Seglinge-Kumlinge, Saltvik or Sottunga, Figure 1b), or into 12 spatio-temporal groups according to their geographic origin and their collection time interval (Finstrom 1992-97, 2003-08, 2009-11, Foglo 2000-09, 2010-13, Seglinge-Kumlinge 2000-09, 2010-11, Saltvik 1999, 2005-09, 2010-13, and Sottunga 2002-04, 2005-09; Figure 3, Table 2). Each of the 12 spatio-temporal groups was designed to include specimens from roughly either the 90's, the 2000's, and the 2010's, from each locality, but due to the smaller sample size, the samples from Sottunga were divided between two temporal groups: the early and the late 2000's.

Data analyses

Hymenoptera males are haploid, and females are diploid. We analysed the genetic structure of female wasps only (N=195), to avoid overestimating the impact of the haploid males, from the five localities and across the 22 years of collection using three analyses implemented in the BAPS software (Corander, Marttinen, Siren, & Tang, 2008; Corander, Waldmann, & Sillanpaa, 2003). First, we ran a Bayesian clustering of individuals analysis, using the whole Aland as a unique population ($N_{pop}=1$), to identify the maximum number of genetic clusters across all our samples. Then, we ran two independent spatial Bayesian clustering analyses with the 'clustering of groups of individuals' ($N_{pop}=5$ or $N_{pop}=12$), and with respective admixture analyses settings (Figure 3) to evaluate the degree of admixture at each locality, and in each locality through time. Multiple 'k' parameters were provided for each run and the parameter was not set as a strict limit; and the Bayesian model in BAPS estimated the posterior probabilities for the optimal number of clusters (Appendix 1-3) (Corander et al., 2003).

We used the software GenoDive 3.04 (Meirmans & van Tienderen, 2004) to calculate population genetics measures using only the samples that were successfully genotyped for at least seven microsatellite markers using either only diploid females (N=195), or combined diploid females and haploid males dataset (N=313). We tested *Hardy-Weinberge* equilibrium at each locus in each locality, and for each of the 12 spatio-temporal groups, using the Least-Square Amova Method with 999 permutations (estimator *Fis* given in Appendix 1C and 3C). We calculated the F_{ST} values between the five localities and between the 12 spatio-temporal groups. Finally, we calculated the observed (H_O) and expected heterozygosity (H_E) at each locus, as well as the inbreeding coefficient (Gis) between each locality (N=5), and each spatio-temporal group (N=12) (Appendix 1B and 3B).

To test whether samples from the Seglinge-Kumlinge islands were more often *Wolbachia* -infected, or carry the T-mitotype over the C-mitotype more than the rest of the Aland population, we used Fisher exact tests for count data (two-sided) in R 3.6.3 (R Core Team, 2020).

Results

Genotyping Hyposoter horticola across Aland

After two rounds of genotyping, we successfully genotyped 7-14 microsatellite markers from 313 samples

(Table S1), with majority of the samples in each spatio-temporal group being genotyped with at least 11 markers. The spatial Bayesian clustering analysis of 12 spatio-temporal groups ($N_{\text{pop}}=12$, $k=4$, posterior probability=0.999, Appendix 1A), and the spatial Bayesian clustering analysis of five localities ($N_{\text{Pop}}=5$, $k=4$, posterior probability=0.999; Appendix 3A), detected only four genetically differentiated population clusters; while the Bayesian clustering analysis of female individuals ($N_{\text{pop}}=1$) resolved to 16 genetic clusters ($k=16$, posterior probability=0.982, Appendix 2). Additionally, spatial Bayesian analyses including all genotyped female individuals based on either the five localities and the 12 spatio-temporal groups, show little signature of shared history (e.g. restricted gene flow) between the four clusters, and across generations, with little admixture between the different groups.

Both the five localities and the 12 spatio-temporal groups analyses suggest that Sottunga and Seglinge-Kumlinge are genetically similar to one another (Figure 3). In the Bayesian clustering of localities ($N_{\text{Pop}}=5$) analysis, Sottunga and Seglinge-Kumlinge populations made up one cluster ($F_{\text{ST}}=0.014$, $p=0.045$, Table 3), and Foglo, Finstrom and Saltvik each made up the three other clusters (Figure 3, $F_{\text{ST}}>0.05$, $p=0.001$, Table 3). Although the clustering analysis of the 12 spatio-temporal groups generally supports the formation of these same four clusters, it also reveals more detailed spatio-temporal changes, especially in the early samples from Foglo and Finstrom. All samples from Saltvik, group in the same cluster (Figure 3). The two island populations of Sottunga and Seglinge-Kumlinge are always found to be genetically different from Foglo ($F_{\text{ST}}=0.113$, $p=0.001$, Table 3). The shared ancestry of the Sottunga and Finstrom female samples is visible, the samples collected in Sottunga belong to six genotypes, most of which are also found in samples collected from Finstrom in 1992 and 1993 (Table S1), in Seglinge-Kumlinge, and/or in other localities. For example, the genotype ‘8’ is found in two samples from Sottunga, five from Finstrom, eight from Saltvik, and eight from Foglo, while the genotype ‘15’ is found in 14 samples from Sottunga, 11 from Seglinge-Kumlinge and two from Foglo (Table S1). In general, the admixture analyses revealed little interbreeding between Foglo and Sottunga or between Foglo and Saltvik, which would have been possible through dispersal through several generations, or steppingstone events, as well as dispersal over water. In contrast the analysis showed ongoing geneflow between the two mainland populations of Saltvik and Finstrom (Figure 2).

Consistent with Couchoux et al. (2016), we show that inbreeding occurs in Åland at similarly low levels in each of the five localities, despite the mainland populations being more connected, and of larger sizes. The inbreeding coefficient (G_{is}), generally about 0.215 across Åland, ranges from 0.165 in Saltvik, to 0.270 in Foglo (Table 1; Appendix 3B); while reaching 0.206 in Sottunga (based on the diploid females data only). The values of heterozygosity are the lowest in Foglo and Seglinge-Kumlinge ($H_o = 0.277$ & 0.284 , $H_s = 0.379$ & 0.383 , respectively) and the highest in Finstrom and Saltvik ($H_o = 0.405$ & 0.408 , $H_s = 0.523$ & 0.488 , respectively), with Sottunga showing intermediate heterozygosity values ($H_o = 0.345$, $H_s = 0.410$) (Table 1). At the level of the 12 spatio-temporal groups, the three temporal groups from Finstrom and the three temporal groups from Saltvik also show the highest H_o and H_s values (i.e. generally $H_o > 0.31$ and $H_s > 0.47$; Table 2), but in that case, the multi-locus dataset for each spatio-temporal group show that they are not all at Hardy-Weinberg equilibrium (Appendix 1C).

The parasitoid *H. horticola* in Åland has experienced both global and local population crashes through the years (Figure 2), some of which resulted in detectable changes in local genetic structure. For example, the genotype characterizing Foglo after the population crash of 2010 (Figure 2) is from a different genetic cluster than any of the three other clusters characterized from the other four populations. In contrast, other local crashes, for example in 1999 and 2006 in Finstrom, and in 1999 in Saltvik, did not seem to affect the genotypic clusters of these populations, maybe due to their expected larger population sizes. Unfortunately, we lack data to directly address the genetic consequences of the population bottlenecks that occurred in both Seglinge-Kumlinge and Sottunga.

Mitochondrial haplotypes

Finally, in agreement with a previous study by Duploup et al. (2015), the majority of the *H. horticola* parasitoids carry the C-mitotype ($N=145$, 67%), while the remaining specimens carry the T-mitotype (Table 1, Figure 3). Noticeably, the C-mitotype is prevalent in all localities (57% in Saltvik, 71% in Sottunga, 84%

in Finstrom, 95% in Foglo) except Seglinge-Kumlinge (28%) (Table 1, Figure 3). These general patterns hold across the different spatio-temporal groups (Table 2, Figure 3). The T-mitotype is significantly more prevalent in Seglinge-Kumlinge than in the rest of the Aland islands (Fisher exact-test $P = 0.0001$), and this holds if we only take into account the wasps carrying one of the five genotypes characterized from Seglinge-Kumlinge: three wasps carry the C-mitotype and 12 wasps carry the T-mitotype in Seglinge-Kumlinge, compared to 18 wasps carrying the C-mitotype and eight wasps carrying the T-mitotype in the rest of Aland (Fisher exact-test $P = 0.0036$).

Wolbachia infection status

Wolbachia was detected in samples from all five localities. The mean infection rate across our samples is 50%, which is consistent with the work of Duploux et al. (2015), who had previously investigated the prevalence of the *w* Hho strain across the entire Aland archipelago. Among the localities, Seglinge-Kumlinge shows the highest infection rate (95%), greatly contrasting with the lower infection rates of Finstrom (42%), Foglo (32%), Saltvik (59%), and Sottunga (23%) (Table 1, Figure 3). *Wolbachia* is significantly more prevalent in Seglinge-Kumlinge than in the rest of the Aland islands (Fisher exact-test $P = 0.0001$), and this holds when we only consider the wasps carrying one of the five genotypes characterized from Seglinge-Kumlinge: one uninfected wasp and 13 *Wolbachia* -infected wasps characterized in Seglinge-Kumlinge, compared to 28 uninfected and 12 infected wasps in the rest of the Aland population (Fisher-exact test $P = 0.0001$).

Among all *Wolbachia* -infected wasps sampled, 57% carry the T-mitotype, which is equal to or slightly lower than the infection rate characterized by Duploux et al. (2015)go. However, the proportion of infected wasps carrying the T-mitotype differed among localities. In Saltvik, Finstrom and Foglo, 60%, 33% and 0% of *Wolbachia* -infected wasps carry the T-mitotype, respectively. In contrast, Sottunga and Seglinge-Kumlinge are more similar to each other than to any other population, with the great majority of *Wolbachia*-infected wasps carrying the T-mitotype (75% and 79%, respectively) rather than the C-mitotype. This is despite the two island populations showing contrasting proportions of specimens carrying the T-mitotype (see above).

Discussion

The introduction and long-term persistence of the parasitoid wasp *H. horticola* in the small island of Sottunga, on the East side of the Aland archipelago, offered a unique opportunity to investigate the spatio-temporal changes in the genetic structure, spread, and dynamics of a parasitoid species introduced into a new habitat along with its host butterfly. Because the parasitoid carried along its *Wolbachia* symbiont, we could also investigate how symbionts might affect the spread of different host genotypes after introduction. Our results suggest that the *H. horticola* population that established in Sottunga persisted despite strong bottlenecks during population fluctuations across its 22 years of persistence, and potentially in the founding generation. Additionally, the introduction might have affected the genetic pool of the population inhabiting the nearby islands through dispersal of individuals carrying the mainland genotypes, and by the establishment of populations carrying a costly *Wolbachia* strain.

Spatio-temporal genetic changes – the role of a local introduction

In a previous study, Couchoux et al. (2016) suggested some ongoing genetic mixing between the *H. horticola* parasitoid population in Sottunga and the neighbouring northern population inhabiting the Seglinge-Kumlinge islands. Many genotypes found in the original mainland population of Finstrom were evident in wasps from both Sottunga and Seglinge-Kumlinge islands. The movement of genotypes over long distances such as the one separating the islands from the mainland might occur over several generations of ongoing gene flow through interbreeding (Couchoux et al., 2016; DiLeo, Husby, & Saastamoinen, 2018; Slarkin, 1985), but it is simply more likely that, instead, the genotypes introduced on Sottunga in 1991 have persisted over time on the island, and that dispersal between Sottunga and the islands of Seglinge-Kumlinge in the North has occurred. In contrast, the mainland genotypes are not found in *H. horticola* from the southern islands of Foglo, which is about the same distance from Sottunga as Seglinge is, but further away from the mainland populations.

The coast-to-coast distances between Sottunga and the neighbouring islands of Seglinge or Foglo are at least 6.5km, with about 12km as the shortest distances between known suitable habitat patches on the islands (Ojanen et al., 2013). The parasitoid *H. horticola* is more dispersive than its butterfly host (van Nouhuys & Hanski, 2002), commonly flying over 1km distance, and potentially travelling up to 7.5km over land within a breeding season (Couchoux et al., 2016). The flight capacity of the parasitoid allows it to disperse across unsuitable habitats on the mainland, but is most likely not sufficient to cross stretches of open water separating two islands, or the 30km separating the mainland from the shores of Sottunga. Insects can however move across large unsuitable habitats under prevailing winds (Compton, 2002; Pasek, 1988). In the Baltic Sea, a dominant wind blowing from the South (Bierstedt, Hunicke, & Zorita, 2015) could transport wasps from Sottunga to Seglinge-Kumlinge, as well as inhibit southward movements towards Foglo. Insects can also be moved by humans intentionally or incidentally (Kritani & Yamamura, 2003). One of the host plants of the Glanville fritillary butterfly, *Veronica spicata* (Kuussaari, van Nouhuys, Hellmann, & Singer, 2004) produces indigo blue flower spikes that may be of interest to gardeners on the different islands. The human-assisted migration of *H. horticola* within their host caterpillars feeding on plants (Carlsson, Haeggstrom, & Sundberg, 2014), although possible, has not been suggested from any population genetic studies conducted on the Aland butterfly populations (Fountain et al., 2018; Fountain et al., 2016), and is thus thought unlikely.

While the butterfly was known to occupy Seglinge-Kumlinge prior to year 2000, there is no local historical record of the parasitoid *H. horticola*, and we did not find any unique genotypes nor mitotypes in those islands. Rather, the current parasitoid population on Seglinge-Kumlinge resembles to the introduced parasitoids from Sottunga, suggesting that *H. horticola* from Sottunga may have colonized Seglinge-Kumlinge. If this is true, prior to the *H. horticola* introduction, the Seglinge-Kumlinge butterfly population may have been free of the parasitoid. This is significant for the butterfly because where present, *H. horticola* parasitizes about 30% of the host larvae (Montovan et al., 2015). To date, it remains unclear whether the introduction of the parasitoid to Seglinge-Kumlinge, where the butterfly may have persisted without a specialist parasitoid, has had any influence on the eco-evolutionary dynamics of this local butterfly population.

There are many examples of the effect of isolation on the genetics of introduced Island populations (Hufbauer et al., 2004; Mattila et al., 2012; Miller, Eldridge, Morris, Zenger, & Herbert, 2011; Szucs et al., 2014; Urquia et al., 2019), including a study of human population on the island of Sottunga (O'Brien, Jorde, Ronnlof, & Eriksson, 1988). Generally, these small and isolated populations show low allelic diversity, low heterozygosity and high inbreeding values (Fauvergue et al., 2012; Mattila et al., 2012; Nei, Marutama, & Chakraborty, 1975). The Sottunga population of the parasitoid wasp *H. horticola*, as well as the isolated populations of Foglo and of Seglinge-Kumlinge, show slightly lower observed heterozygosity (H_o [?]0.3) than the large mainland Aland populations (H_o [?]0.4). This is probably due to loss of genetic diversity in the islands following local population crashes. However, heterozygosity remains relatively high compared to studies from other similarly isolated animal populations (Fountain et al., 2016; O'Brien et al., 1988; Sarhan & Kokko, 2007), which may be due to the relatively large founding populations of 71 larval hosts nests. Most of these nests would have contained *H. horticola* from different families (Couchoux et al., 2015a). The processes of species invasion as well as intentional introduction for biological control are often hindered by the genetic consequences of small founding populations (Fauvergue et al., 2012; Hufbauer et al., 2004; Hufbauer, Rutschmann, Serrate, Vermeil de Conchard, & Facon, 2013). Furthermore, while there is some evidence of occasional strong inbreeding (Fis values appendix 1c), all five populations show similar degrees of overall inbreeding without strong differences between mainland and island populations (Gis =0.18 and 0.23 in the mainland populations, while Gis values vary between 0.21 and 0.24 in the three island populations). Rapid population growth after bottleneck and high dispersive ability are known to counteract the effect of small population size and isolation on both the loss of heterozygosity and inbreeding (Nei et al., 1975), our study might represent such example of a clear ancestry being still visible despite local bottlenecks and long-term isolation of some populations.

Spatio-temporal genetic changes – the effect of Wolbachia infection

Endosymbionts such as *Wolbachia*, are selfish entities that have evolved to promote their own prevalence in their host populations through increasing the fitness of the infected individuals over their uninfected counterparts (O'Neill et al., 1997). The success of symbioses is however often context-dependent (Ferrari & Vavre, 2011; Hajek, Morris, & Hendry, 2019). While the *Wolbachia* strain *w* Hho occurs at an intermediate prevalence across the Aland Islands (Duploux et al., 2015), on the islands of Seglinge-Kumlinge, it has been almost at fixation since the year 2000, and potentially earlier. As shown by Duploux et al. (2015), this *Wolbachia* strain appears not to affect dispersal capacity of *H. horticola*, thus the high *Wolbachia* prevalence in Seglinge-Kumlinge is unlikely to be due to differential dispersal of infected wasps to these islands. However, *Wolbachia* increases the susceptibility of the wasp *H. horticola* to hyperparasitism by the wasp *Mesochorus* cf. *stigmaticus* (van Nouhuys et al., 2016). The hyperparasitoid is common across the Aland mainland (Nair et al., 2016), and is also present in Sottunga and Foglo, but is absent from Seglinge-Kumlinge islands (van Nouhuys & Hanski, 2005). The hyperparasitoid wasp restrains the spread of *w* Hho in *H. horticola* by keeping the infection at lower prevalence when highly abundant (van Nouhuys et al., 2016), while the absence of *M. cf. stigmaticus* in Seglinge-Kumlinge (Nair et al., 2016; van Nouhuys & Hanski, 2005; van Nouhuys et al., 2016) releases the selection pressure on *w* Hho-infected *H. horticola* wasps, and allows the spread of the symbiont in this isolated wasp population (van Nouhuys et al., 2016). Although primarily vertically transmitted, *Wolbachia* has been suggested to also occasionally transfer horizontal between hosts, with parasitoids being one of the suggested ecological routes supporting such transfer (Duploux, Pranter, Warren-Gash, Tropek, & Wahlberg, 2020; Vavre, Fleury, Lepetit, Fouillet, & Bouletreau, 1999). *Mesochorus* cf. *stigmaticus* wasps are however not known to carry *Wolbachia*, nor to vector the symbiont between hosts in the Aland population of their food web insect community (Duploux. Pers. Obs.).

The spread of a maternally inherited symbiont, such as *Wolbachia*, leads to a simultaneous increase in prevalence of the host mitochondrial haplotypes associated with the symbiont (Charlat et al., 2009; Duploux et al., 2010; Schuler et al., 2016). In *H. horticola* in Aland, C- and T-mitotypes associate with the *w* Hho infection. The C-mitotypes are however less associated with the infection, potentially because the trans-generation transmission of this *Wolbachia* strain is less efficient in females carrying the C- over the T-mitotype (Duploux et al., 2015). Consequently, we expected that the T-mitotype would be found at low frequency in Seglinge-Kumlinge, as it was in the original population of Finstrom (16%), as well as in Sottunga (29%). Instead, we found that the T-mitotype is prevalent in Seglinge-Kumlinge (72%). The spread of the wasps carrying the T-mitotype in Seglinge-Kumlinge could result from: (I) the selective sweep of the T-matriline during the spread of *Wolbachia* in Seglinge-Kumlinge in the absence of pressures from the hyperparasitoid, (II) strong bottlenecks randomly selecting for individuals from the T-matriline over the C-matriline in Seglinge-Kumlinge during migration events from Sottunga; and (III) uncharacterized fitness benefits associated with the T- mitotype. According to our data, Seglinge-Kumlinge is colonized by several genotypes also found in the rest of Aland, which suggests that several migration events have occurred between the two populations over the 22 years period of our study. This rate of migration considerably reduces the probability of a high frequency of the T- mitotype in Seglinge-Kumlinge due to bottlenecks, especially because the C-mitotype is significantly more prevalent in the potential source population of Sottunga. It is thus more likely that selection acts on the wasps after migration in Seglinge-Kumlinge. Additionally, there is currently no evidence that the T-mitotype provides any benefit to its host that the C-mitotype would not, and it remains generally rare across Aland (Duploux et al., 2015). Consequently, the high prevalence of the T-mitotype in Seglinge-Kumlinge is most likely due to the spread of *w* Hho under relaxed pressures of the hyperparasitoid.

Conclusion

The study of invasion and of accidental or intentional introductions of species are central to much of contemporary population and community ecology (Davis, 2009; Lockwood, Hoopes, & Marchetti, 2013). Parasitoids are used as insect biological control agents (Wang, Liu, Shi, Huang, & Chen, 2019). Consequently, they are intentional introduced into both agricultural and natural environments (Grangirard, Hoddle, Petit, Roderick, & Davies, 2009) These introductions, however, do not persist in the long term (Goldson et al., 2014), and if they do, their genetic structure both bares the signature of the origin and differs from it (Hufbauer

et al., 2004). Associated symbionts brought along during the introduction events, can impact the success story of their host populations, by affecting the phenotypes and genotypes of their hosts (Charlat et al., 2009; Hornett et al., 2006). We use genetic evidence to document the trajectory of a successfully introduced parasitoid population over 22 years. We show persistence of the wasp on the previously uninhabited island, and spread of the transplanted genotypes to surrounding islands, along with the associated symbiotic bacterium *Wolbachia*. The shuffling of the nuclear and matrilineally inherited markers among islands suggests differential selection for the infection-associated genotypes in the local host genetic pools under relaxed predation pressures on an island where the hyperparasitoid species is absent (van Nouhuys et al., 2016). The invasion by newly introduced parasitoid genotypes might contribute to either the persistence of the isolated island wasp populations, or the replacement of the local wasp populations, or both. It may have also changed environment for the pre-existing nearby isolated butterfly host population, which previously did not have the parasitoid. Like many other butterfly and insect species, the Glanville fritillary butterfly is vulnerable to loss of genetic variability and extinction in the Baltic Sea region due to habitat fragmentation, and has gone extinct from the Turku archipelago in southwest Finland (Fountain et al., 2016). The spread of an introduced parasitoid population can represent another pressure for such host population, as well as any competing local parasitoid populations (Benson, Van Driesche, Pasquale, & Elkinton, 2003; Benvenuto, Cheyppé-Buchmann, Bermond, Ris, & Fauvergue, 2012). This is especially true if newly introduced parasitoid genotypes show greater fitness than native genotypes (Dupas, Dubuffet, Carton, & Poirie, 2009; Kraaijeveld, Van Alphen, & Godfray, 1998; van Nouhuys, Niemikapee, & Hanski, 2012).

Acknowledgements:

We thank C. Couchoux for advice on the genotyping work, S. Ikonen for rearing the insects, and S. Ojanen and many students from the University of Helsinki, who conducted the annual survey and collected the samples over the years. We are grateful to I. Hanski and P. Seppa for their interest in and constructive discussions on the study. This work was supported by the Academy of Finland (grant #266021 to AD, and #255553 to SvN). **Diversity, Equality and Inclusion statement:** The authors highly value equity, diversity and inclusion in science. We acknowledge the international character of our team, which significantly contributed to the completion and quality of the study. It includes researchers from different countries, backgrounds and career stages. Authors are from France, India, Hungary and USA. Three co-authors are female and one is male. We cite a large body of studies from a potentially male-biased author-list. We wish to address these DEI shortcomings in future work.

References

- Baldo, L., Hotopp, J. C., Jolley, K. A., Bordenstein, S. R., Biber, S. A., Choudhury, R. R., . . . Werren, J. H. (2006). Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl. Environ. Microbiol.*, 72, 7098-7110.
- Benson, J., Van Driesche, R. G., Pasquale, A., & Elkinton, J. (2003). Introduced braconid parasitoids and range reduction of a native butterfly in New England. *Biological control*, 28 (2), 197-213. doi:10.1016/S1049-9644(03)00058-6
- Benvenuto, C., Cheyppé-Buchmann, S., Bermond, G., Ris, N., & Fauvergue, X. (2012). Intraspecific hybridization, life history strategies and potential invasion success in a parasitoid wasp. *Evolutionary Ecology*, 26, 1311-1329. doi:10.1007/s10682-011-9553-z
- Bierstedt, S. E., Hunicke, B., & Zorita, E. (2015). Variability of wind direction statistics of mean and extreme wind events over the Baltic Sea region. *Tellus A: Dynamics Meteorology and Oceanography*, 67 (1), 29073. doi:10.3402/tellusa.v67.29073
- Carlsson, R., Haeggstrom, C.-A., & Sundberg, K. (2014). Ruderal vascular plants on a waste ground in the island of Dano, Aland Islands, SW Finland. *Memoranda Societatis pro Fauna et Flora Fennica*, 90, 55-66.
- Charlat, S., Duploux, A., Hornett, E. A., Dyson, E. A., Davies, N., Roderick, G. K., . . . Hurst, G. D. (2009). The joint evolutionary histories of *Wolbachia* and mitochondria in *Hypolimnas bolina*. *BMC Evol*

Biol, 9 , 64. doi:10.1186/1471-2148-9-64

Compton, S. G. (2002). Sailing with the wind: dispersal by small flying insects. In J. M. Bullock, R. E. Kenward, & R. S. Hails (Eds.), *Dispersal ecology: 42nd symposium of the British ecological society* (Vol. 113-133). Oxford: Blackwell.

Corander, J., Marttinen, P., Siren, J., & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics*, 9 , 539.

Corander, J., Waldmann, P., & Sillanpää, M. L. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics*, 163 , 367-374.

Couchoux, C., Seppa, P., & van Nouhuys, S. (2016). Strong dispersal in a parasitoid wasp overwhelms habitat fragmentation and host population dynamics. *Mol Ecol*, 25 (14), 3344-3355. doi:10.1111/mec.13696

Couchoux, C., Seppa, P., & van Nouhuys, S. (2015a). Behavioural and genetic approaches to evaluate the effectiveness of deterrent marking by a parasitoid wasp. *Behaviour*, 152 (9), 1257-1276. doi:10.1163/1568539X-00003277

Couchoux, C., Seppa, P., & van Nouhuys, S. (2015b). Microsatellites for the parasitoid wasp *Hyposoter horticola*. *Conservation Genetics Resources*, 7 , 595-597.

Cronin, J. T., & Reeve, J. D. (2005). Host parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proceedings of the Royal Society B: Biological Sciences*, 272 (1578), 2225-2235. doi:10.1098/rspb.2005.3286

Davis, M. A. (2009). *Invasion biology* : Oxford University Press.

DiLeo, M. F., Husby, A., & Saastamoinen, M. (2018). Landscape permeability and individual variation in a dispersal-linked gene jointly determine genetic structure in the Glanville fritillary butterfly. *Evolution Letters*, 2 (6), 544-556. doi:10.1002/evl3.90

Dupas, S., Dubuffet, A., Carton, Y., & Poirie, M. (2009). Local, geographic and phylogenetic scales of coevolution in Drosophila-parasitoid interactions. *Adv Parasitol*, 70 , 281-295. doi:10.1016/S0065-308X(09)70011-9

Duploup, A., Couchoux, C., Hanski, I., & van Nouhuys, S. (2015). *Wolbachia* Infection in a Natural Parasitoid Wasp Population. *PLoS One*, 10 (8), e0134843. doi:10.1371/journal.pone.0134843

Duploup, A., Hurst, G. D., O'Neill, S. L., & Charlat, S. (2010). Rapid spread of male-killing *Wolbachia* in the butterfly *Hypolimnas bolina*. *J Evol Biol*, 23 (1), 231-235. doi:10.1111/j.1420-9101.2009.01891.x

Duploup, A., Iturbe-Ormaetxe, I., Beatson, S. A., Szubert, J. M., Brownlie, J. C., McMeniman, C. J., . . . Woolfit, M. (2013). Draft genome sequence of the male-killing *Wolbachia* strain *w* Bol1 reveals recent horizontal gene transfers from diverse sources. *BMC Genomics*, 14 , 20. doi:10.1186/1471-2164-14-20

Duploup, A., Pranter, R., Warren-Gash, H., Tropek, R., & Wahlberg, N. (2020). Towards unravelling *Wolbachia* global exchange: a contribution from the *Bicyclus* and *Mylothris* butterflies in the Afrotropics. *BMC Microbiol*, 20 (1), 319. doi:10.1186/s12866-020-02011-2

Evans, O., Caragata, E. P., McMeniman, C. J., Woolfit, M., Green, D. C., Williams, C. R., . . . McGraw, E. A. (2009). Increased locomotor activity and metabolism of *Aedes aegypti* infected with a life-shortening strain of *Wolbachia pipientis*. *J Exp Biol*, 212 (Pt 10), 1436-1441. doi:10.1242/jeb.028951

Fauvergue, X., Vercken, E., Malausa, T., & Hufbauer, R. A. (2012). The biology of small, introduced populations, with special reference to biological control. *Evol Appl*, 5 (5), 424-443. doi:10.1111/j.1752-4571.2012.00272.x

Ferrari, J., & Vavre, F. (2011). Bacterial symbionts in insects or the story of communities affecting communities. *Philos Trans R Soc Lond B Biol Sci*, 366 (1569), 1389-1400. doi:10.1098/rstb.2010.0226

- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*, 3 (5), 294-299.
- Fountain, T., Husby, A., Nonaka, E., DiLeo, M. F., Korhonen, J. H., Rastas, P., . . . Hanski, I. (2018). Inferring dispersal across a fragmented landscape using reconstructed families in the Glanville fritillary butterfly. *Evolutionary Applications*, 11 (3), 287-297. doi:10.1111/eva.12552
- Fountain, T., Nieminen, M., Siren, J., Wong, S. C., Lehtonen, R., & Hanski, I. (2016). Predictable allele frequency changes due to habitat fragmentation in the Glanville fritillary butterfly. *Proc Natl Acad Sci U S A*, 113 (10), 2678-2683. doi:10.1073/pnas.1600951113
- Fytrou, A., Schofield, P. G., Kraaijeveld, A. R., & Hubbard, S. F. (2006). *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proc Biol Sci*, 273 (1588), 791-796. doi:10.1098/rspb.2005.3383
- Gagic, V., Hanke, S., Thies, C., Scherber, C., Tomanovic, Z., & Tscharnkte, T. (2012). Agricultural intensification and cereal aphid-parasitoid-hyperparasitoid food webs: network complexity, temporal variability and parasitism rates. *Oecologia*, 170 (4), 1099-1109. doi:10.1007/s00442-012-2366-0
- Gagic, V., Tscharnkte, T., Dormann, C. F., Gruber, B., Wilstermann, A., & Thies, C. (2011). Food web structure and biocontrol in a four-trophic level system across a landscape complexity gradient. *Proc Biol Sci*, 278 (1720), 2946-2953. doi:10.1098/rspb.2010.2645
- Goldson, S. L., Wratten, S. D., Ferguson, C. M., Gerard, P. J., Barratt, B. I. P., Hardwick, S., . . . Tomasetto, F. (2014). If and when successful classical biological control fails. *Biological control*, 72 , 76-79. doi:10.1016/j.biocontrol.2014.02.012
- Grangirard, J., Hoddle, M., Petit, J., Roderick, G., & Davies, N. (2009). Classical biological control of the glassy-winged sharpshooter, *Homalodisca vitripennis*, by the egg parasitoid *Gonatocerus ashmeadi* in the Society, Marquesas and Austral archipelagos of French Polynesia. *Biological control*, 48 (2), 155-163. doi:10.1016/j.biocontrol.2008.10.005
- Gruntenko, N. E., Ilinsky, Y. Y., Adonyeva, N. V., Burdina, E. V., Bykov, R. A., Menshanov, P. N., & Rauschenbach, I. Y. (2017). Various *Wolbachia* genotypes differently influence host *Drosophila* dopamine metabolism and survival under heat stress conditions. *BMC Evolutionary Biology*, 17 (252). doi:10.1186/s12862-017-1104-y
- Gruntenko, N. E., Karpova, E. K., Adonyeva, N. V., Andreenkova, O. V., Burdina, E. V., Ilinsky, Y. Y., . . . Rauschenbach, I. Y. (2019). *Drosophila* female fertility and juvenile hormone metabolism depends on the type of *Wolbachia* infection. *J Exp Biol*, 222 (Pt 4). doi:10.1242/jeb.195347
- Hajek, A. E., Morris, E. E., & Hendry, T. A. (2019). Context-dependent interactions of insects and defensive symbionts: insights from a novel system in siricid woodwasps. *Curr Opin Insect Sci*, 33 , 77-83. doi:10.1016/j.cois.2019.03.006
- Hanski, I., Ehrlich, P. R., Nieminen, M., Murphy, D. D., Hellmann, J. J., Boggs, C. L., & McLaughlin, J. F. (2004). Checkerspots and conservation biology. In P. R. Ehrlich & I. Hanski (Eds.), *On the wings of checkerspots: A model system for population biology* : Oxford University Press.
- Hanski, I., Pakkala, T., Kuussaari, M., & Lei, G. (1995). Metapopulation persistence of an endangered butterfly in a fragmented landscape. *OIKOS*, 72 (1), 21-28. doi:10.2307/3546033
- Hanski, I., Schulz, T., Wong, S. C., Ahola, V., Ruokolainen, A., & Ojanen, S. P. (2017). Ecological and genetic basis of metapopulation persistence of the Glanville fritillary butterfly in fragmented landscapes. *nature communications*, 8 , 14504. doi:10.1038/ncomms14504

- Hanski, I. A. (2011). Eco-evolutionary spatial dynamics in the Glanville fritillary butterfly. *Proc Natl Acad Sci U S A*, 108 (35), 14397-14404. doi:10.1073/pnas.1110020108
- Hedges, L. M., Brownlie, J. C., O'Neill, S. L., & Johnson, K. N. (2008). *Wolbachia* and virus protection in insects. *Science*, 322 (5902), 702. doi:10.1126/science.1162418
- Hornett, E. A., Charlat, S., Duploux, A. M., Davies, N., Roderick, G. K., Wedell, N., & Hurst, G. D. (2006). Evolution of male-killer suppression in a natural population. *PLoS Biol*, 4 (9), e283. doi:10.1371/journal.pbio.0040283
- Hufbauer, R. A., Bogdanowicz, S. M., & Harrison, R. G. (2004). The population genetics of a biological control introduction: mitochondrial DNA and microsatellite variation in native and introduced populations of *Aphidus ervi*, a parasitoid wasp. *Molecular Ecology*, 13 (2), 337-348.
- Hufbauer, R. A., Rutschmann, A., Serrate, B., Vermeil de Conchard, H., & Facon, B. (2013). Role of propagule pressure in colonization success: disentangling the relative importance of demographic, genetic and habitat effects. *J Evol Biol*, 26 (8), 1691-1699. doi:10.1111/jeb.12167
- Hurst, G. D., & Jiggins, F. M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proc Biol Sci*, 272 (1572), 1525-1534. doi:10.1098/rspb.2005.3056
- Kaartinen, R., & Roslin, T. (2011). Shrinking by numbers: landscape context affects the species composition but not the quantitative structure of local food webs. *J Anim Ecol*, 80 (3), 622-631. doi:10.1111/j.1365-2656.2011.01811.x
- Kraaijeveld, A. R., Van Alphen, J. J., & Godfray, H. C. (1998). The coevolution of host resistance and parasitoid virulence. *Parasitology*, 116 Suppl, S29-45. doi:10.1017/s0031182000084924
- Kritani, K., & Yamamura, K. (2003). Exotic insects and their pathways for invasion. In J. Carlton (Ed.), *Invasive species: vectors and management strategies* (pp. 44-67). Washington D.C.: Island Press.
- Kuussaari, M., van Nouhuys, S., Hellmann, J. J., & Singer, M. C. (2004). Larval biology of checkerspots. In P. R. Ehrlich & I. Hanski (Eds.), *On the wings of checkerspots: A model system for population biology* (pp. 138-160). Oxford: Oxford University Press.
- Lei, G., & Hanski, I. (1998). Spatial dynamics of two competing specialist parasitoids in a host metapopulation. *Journal of Animal Ecology*, 67 (3), 422-433. doi:10.1046/j.1365-2656.1998.00204.x
- Lei, G. C., Vikberg, V., Nieminen, M., & Kuussaari, M. (1997). The parasitoid complex attacking Finnish populations of the Glanville fritillary *Melitaea cinxia* (Lep: Nymphalidae), an endangered butterfly. *Journal of Natural History*, 31 (4), 635-648. doi:10.1080/00222939700770301
- Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). *Invasion Ecology*. John Wiley & Sons.
- Lu, M., Hulcr, J., & Sun, J. (2016). The role of symbiotic microbes in insect invasions. *Annual Review of Ecology, Evolution, and Systematics*, 47, 487-505. doi:10.1146/annurev-ecolsys-121415-032050
- Martinez, J., Cogni, R., Cao, C., Smith, S., Illingworth, C. J., & Jiggins, F. M. (2016). Addicted? Reduced host resistance in populations with defensive symbionts. *Proc Biol Sci*, 283 (1833). doi:10.1098/rspb.2016.0778
- Mattila, A. L. K., Duploux, A., Kirjokangas, M., Lehtonen, R., Rastas, P., & Hanski, I. (2012). High genetic load in an old isolated butterfly population. *Proceedings of the National Academy of Sciences of the United States of America*, 109 (37), E2496-E2505. doi:10.1073/pnas.1205789109
- Meirmans, P. G., & van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4, 792-794.

- Miller, E. J., Eldridge, M. D. B., Morris, K. D., Zenger, K. R., & Herbert, C. A. (2011). Genetic consequences of isolation: island tammar wallaby (*Macropus eugenii*) populations and the conservation of threatened species. *Conservation Genetics*, 12 (6), 1619-1631. doi:10.1007/s10592-011-0265-2
- Montovan, K. J., Couchoux, C., Jones, L. E., Reeve, H. K., & van Nouhuys, S. (2015). The puzzle of partial resource use by a parasitoid wasp. *Am Nat*, 185 (4), 538-550. doi:10.1086/680036
- Murphy, D. D., Wahlberg, N., Hanski, I., & Ehrlich, P. R. (2004). Introducing Checkerspots: taxonomy and ecology. In P. R. Ehrlich & I. Hanski (Eds.), *On the wings of Checkerspots: a model system for population biology* (pp. 17-33). New York: Oxford University Press.
- Nair, A., Fountain, T., Ikonen, S., Ojanen, S. P., & van Nouhuys, S. (2016). Spatial and temporal genetic structure at the fourth trophic level in a fragmented landscape. *Proc Biol Sci*, 283 (1831). doi:10.1098/rspb.2016.0668
- Nei, M., Marutama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, 29 , 1-10.
- O'Neill, S., Hoffman, A., & Werren, J. (1997). *Influential passengers, inherited microorganisms and arthropod reproduction*. NY: Oxford University Press Inc.
- Ojanen, S. P., Nieminen, M., Meyke, E., Poyry, J., & Hanski, I. (2013). Long-term metapopulation study of the Glanville fritillary butterfly (*Melitaea cinxia*): survey methods, data management, and long-term population trends. *Ecol Evol*, 3 (11), 3713-3737. doi:10.1002/ece3.733
- Osborne, S. E., Iturbe-Ormaetxe, I., Brownlie, J. C., O'Neill, S. L., & Johnson, K. N. (2012). Antiviral protection and the importance of *Wolbachia* density and tissue tropism in *Drosophila simulans*. *Appl Environ Microbiol*, 78 (19), 6922-6929. doi:10.1128/AEM.01727-12
- O'Brien, E., Jorde, L. B., Ronnlof, B. F., J. O., & Eriksson, A. W. (1988). Inbreeding and genetic disease in Sottunga, Finland. *American Journal of Physical Anthropology*, 75 , 477-486.
- Pasek, J. E. (1988). Influence of wind and windbreaks on local dispersal of insects. *Agric Ecosyst Environ*, 22 (23), 539-554.
- Rokas, A., Atkinson, R. J., Brown, G. S., West, S. A., & Stone, G. N. (2001). Understanding patterns of genetic diversity in the oak gallwasp *Biorhiza pallida*: demographic history or a *Wolbachia* selective sweep? *Heredity*, 87 (Pt 3), 294-304. doi:10.1046/j.1365-2540.2001.00872.x
- Sarhan, A., & Kokko, H. (2007). Multiple mating in the Glanville fritillary butterfly: a case of within-generation bet hedging? *Evolution*, 61 (3), 606-616. doi:10.1111/j.1558-5646.2007.00053.x
- Sazama, E. J., Bosch, M. J., Shouldis, C. S., Ouellette, S. P., & Wesner, J. S. (2017). Incidence of *Wolbachia* in aquatic insects. *Ecol Evol*, 7 (4), 1165-1169. doi:10.1002/ece3.2742
- Schuler, H., Koppler, K., Daxbock-Horvath, S., Rasool, B., Krumbock, S., Schwarz, D., . . . Riegler, M. (2016). The hitchhiker's guide to Europe: the infection dynamics of an ongoing *Wolbachia* invasion and mitochondrial selective sweep in *Rhagoletis cerasi*. *Mol Ecol*, 25 (7), 1595-1609. doi:10.1111/mec.13571
- Shaw, M. R., Stefanescu, C., & Van Nouhuys, S. (2009). Parasitoids of European butterflies In J. Settele, T. G. Shreeve, M. Konvicka, & H. Van Dyck (Eds.), *Ecology of butterflies in Europe*. Cambridge: Cambridge University Press.
- Signor, S. (2017). Population genomics of *Wolbachia* and mtDNA in *Drosophila simulans* from California. *Sci Rep*, 7 (1), 13369. doi:10.1038/s41598-017-13901-3
- Slarkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics*, 16 (1), 393-430.

- Szucs, M., Melbourne, B. A., Tuff, T., & Hufbauer, R. A. (2014). The roles of demography and genetics in the early stages of colonization. *Proceedings of the Royal Society B-Biological Sciences*, 281 (1792). doi:10.1098/rspb.2014.1073
- Team, R. C. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria Retrieved from <https://www.R-project.org/>
- Urquia, D., Gutierrez, B., Pozo, G., Pozo, M. J., Espin, A., & Torres, M. L. (2019). Psidium guajava in the Galapagos Islands: Population genetics and history of an invasive species. *PLoS One*, 14 (3), e0203737. doi:10.1371/journal.pone.0203737
- van Bergen, E., Dallas, T., DiLeo, M. F., Kahilainen, A., Mattila, A. L. K., Luoto, M., & Saastamoinen, M. (2020). The effect of summer drought on the predictability of local extinctions in a butterfly metapopulation. *Conserv Biol* . doi:10.1111/cobi.13515
- van Nouhuys, S. (2005). Effects of habitat fragmentation at different trophic levels in insect communities. *Annales Zoologici Fennici*, 42 (4), 433-447.
- van Nouhuys, S., & Ehrnsten, J. (2004). Wasp behavior leads to uniform parasitism of a host available only a few hours per year. *Behavioral Ecology*, 15 (4), 661-665. doi:10.1093/beheco/arh059
- van Nouhuys, S., & Hanski, I. (2002). Colonization rates and distances of a host butterfly and two specific parasitoids in a fragmented landscape. *Journal of Animal Ecology*, 71 , 639-650.
- van Nouhuys, S., & Hanski, I. (2005). Metacommunities of butterflies, their host plants and their parasitoids. In M. Holyoak, M. A. Leibold, & R. D. Holt (Eds.), *Metacommunities: Spatial dynamics and ecological communities* (pp. 99-121). Chicago, USA: University of Chicago Press.
- van Nouhuys, S., Kohonen, M., & Duploux, A. (2016). *Wolbachia* increases the susceptibility of a parasitoid wasp to hyperparasitism. *J Exp Biol*, 219 (Pt 19), 2984-2990. doi:10.1242/jeb.140699
- van Nouhuys, S., Niemikapee, S., & Hanski, I. (2012). Variation in a host-parasitoid interaction across independent populations. *Insects*, 3 (4), 1236-1256. doi:10.3390/insects3041236
- Vavre, F., Fleury, F., Lepetit, D., Fouillet, P., & Bouletreau, M. (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Mol Biol Evol*, 16 (12), 1711-1723. doi:10.1093/oxfordjournals.molbev.a026084
- Verne, S., Johnson, M., Bouchon, D., & Grandjean, F. (2012). Effects of parasitic sex-ratio distorters on host genetic structure in the *Armadillidium vulgare* - *Wolbachia* association. *J Evol Biol*, 25 (2), 264-276. doi:10.1111/j.1420-9101.2011.02413.x
- Wang, Z., Liu, Y., Shi, M., Huang, J., & Chen, X. (2019). Parasitoid wasps as effective biological control agents. *Journal of Integrative Agriculture*, 18 (4), 705-715. doi:10.1016/S2095-3119(18)62078-7
- Weinert, L. A., Araujo-Jnr, E. V., Ahmed, M. Z., & Welch, J. J. (2015). The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc Biol Sci*, 282 (1807), 20150249. doi:10.1098/rspb.2015.0249
- Zhou, W., Rousset, F., & O'Neil, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc Biol Sci*, 265 (1395), 509-515. doi:10.1098/rspb.1998.0324
- Zug, R., & Hammerstein, P. (2012). Still a host of hosts for *Wolbachia* : analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS One*, 7 (6), e38544. doi:10.1371/journal.pone.0038544

Data accessibility

Raw data and analyses are available in the supplementary material.

Author contributions

AD, AN & SvN designed the research. AD and TN produced the data. AD and AN analysed the data. AD took the lead in writing the paper, which all authors contributed to.

Tables

Table 1: Sample size, observed (H_O) and expected heterozygosities (H_E), and inbreeding coefficient (Gis) for *Hyposoter horticola* from each locality, and penetrance of mitotype C and *Wolbachia* strain $wHho$ in each locality. Values of H_O , H_E and Gis are calculated based on diploid female data only (N=195), with diploid female plus haploid male data (N=313) in brackets. Penetrance of the mitotype-C and the *Wolbachia* infection ($wHho$) are based on both male and female data.

Locality	Sample size	Statistics Females / (Female + Male)	Statistics Females / (Female + Male)	Statistics Females / (Female + Male)	Penetrance and Sample size	Penetrance and Sample size	Penetrance and Sample size	Penetrance and Sample size
	N_{female} / N_{total}	H_O	H_E	Gis	Mitotype C %	Mitotype C N=	$wHho$ %	$wHho$ N=
Finström	50 /95	0.405 / (0.254)	0.523 / (0.522)	0.226 / (0.514)	84	31/37	42	38/90
Föglö	21 /43	0.277 / (0.146)	0.379 / (0.413)	0.270 / (0.646)	95	39/41	32	13/41
Seglinge- Kumlinge	19 /44	0.284 / (0.135)	0.383 / (0.357)	0.258 / (0.622)	28	12/43	95	38/40
Saltvik	85/101	0.408 / (0.357)	0.488/ (0.489)	0.165/ (0.269)	57	36/63	59	51/86
Sottunga	20 /40	0.345 / (0.185)	0.410 / (0.383)	0.158 / (0.517)	71	27/38	23	9/39
Total	195 /323	0.342 / (0.215)	0.437 / (0.433)	0.215 / (0.514)	65	145 /222	50	149 /222

Table 2: Genetic clusters and total sample size of the 12 spatio-temporal groups. Observed (H_O) and expected heterozygosities (H_E), and inbreeding coefficient (Gis) were calculated using only the data from diploid females, with male and female data combined in brackets.

Locality	Period	Clusters (Fig. 3)	N= (N_{female})	H_O	H_E	Gis	Penetrance Mitotype C (N=)	Penetrance $wHho$ (N=)
Finström	1992-98	red	28 (12)	0,460 (0.269)	0,491 (0.471)	0.063 (0.403)	88,9% (8/9)	7,1% (2/28)
Finström	2001-08	green	12 (8)	0.429 (0.286)	0.535 (0.512)	0.197 (0.442)	87.5% (7/8)	35.7% (5/14)
Finström	2009-11	green	51 (30)	0.381 (0.240)	0.517 (0.501)	0.264 (0.521)	80% (16/20)	62% (31/50)
Föglö	2000-09	red	18 (7)	0.325 (0.142)	0.439 (0.486)	0.259 (0.707)	88.2% (15/17)	17.6% (3/17)
Föglö	2010-13	blue	24 (14)	0.255 (0.148)	0.313 (0.315)	0.184 (0.528)	100% (24/24)	41.7% (10/24)

Locality	Period	Clusters (Fig. 3)	N= (N _{female})	H _O	H _E	G _{is}	Penetrance	Penetrance
Seglinge-Kumlinge	2000-09	yellow	29 (15)	0.306 (0.167)	0.345 (0.356)	0.112 (0.530)	27.6% (8/29)	96.2% (25/26)
Seglinge-Kumlinge	2010-11	red	13 (4)	0.185 (0.061)	0.449 (0.325)	0.589 (0.813)	28.6% (4/14)	92.9% (13/14)
Saltvik	1999	red	2 (2)	0.318 (0.318)	0.477 (0.477)	0.333 (0.333)	0% (0/2)	100% (2/2)
Saltvik	2005-09	red	73 (64)	0.442 (0.391)	0.472 (0.474)	0.065 (0.174)	48.7% (19/39)	59.3% (35/59)
Saltvik	2010-13	red	23 (19)	0.274 (0.228)	0.538 (0.536)	0.490 (0.575)	69.6% (16/23)	64% (16/25)
Sottunga	2002-04	yellow	22 (9)	0.304 (0.126)	0.367 (0.372)	0.172 (0.660)	81% (17/21)	31.8% (7/22)
Sottunga	2005-09	yellow	18 (11)	0.377 (0.251)	0.407 (0.307)	0.073 (0.321)	58.8% (10/17)	11.8% (2/17)

Table 3: F_{ST} values and p -values for all pairs of localities after pairwise differentiation analysis based on 313 samples from five localities, under and above the diagonal, respectively, with male and female data combined (1000 permutations). Bold values highlight the results of the comparison between Sottunga and Seglinge-Kumlinge. (*) Significance for $\alpha < 0.05$, and () $\alpha < 0.01$.**

F_{ST}/p -values	Finström	Föglö	Seglinge-Kumlinge	Saltvik	Sottunga
Finström	-	$p=0.001^{**}$	$p=0.001^{**}$	$p=0.001^{**}$	$p=0.001^{**}$
Föglö	0.068	-	$p=0.001^{**}$	$p=0.001^{**}$	$p=0.001^{**}$
Seglinge-Kumlinge	0.124	0.135	-	$p=0.001^{**}$	$p=0.045^{*}$
Saltvik	0.051	0.076	0.126	-	$p=0.001^{**}$
Sottunga	0.109	0.113	0.014[*]	0.115	-

Hosted file

Revised-Figure1-March2021.pdf available at <https://authorea.com/users/352051/articles/515033-long-term-spatio-temporal-genetic-structure-of-an-accidental-parasitoid-introduction-and-local-changes-in-prevalence-of-its-associated-wolbachia-symbiont>

Hosted file

Revised-Figure2.pdf available at <https://authorea.com/users/352051/articles/515033-long-term-spatio-temporal-genetic-structure-of-an-accidental-parasitoid-introduction-and-local-changes-in-prevalence-of-its-associated-wolbachia-symbiont>

Hosted file

Revised-Figure3.pdf available at <https://authorea.com/users/352051/articles/515033-long-term-spatio-temporal-genetic-structure-of-an-accidental-parasitoid-introduction-and-local-changes-in-prevalence-of-its-associated-wolbachia-symbiont>