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# Population structure of a large blue butterfly and its specialist parasitoid in a fragmented landscape

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**Abstract**

**Habitat fragmentation may interrupt trophic interactions if herbivores and their specific parasitoids respond differently to decreasing connectivity of populations. Theoretical models predict that species at higher trophic levels are more negatively affected by isolation than lower trophic level species. By combining ecological data with genetic information from microsatellite markers we tested this hypothesis on the butterfly *Maculinea nausithous* and its specialist hymenopteran parasitoid *Neotypus melanocephalus*. We assessed the susceptibility of both species to habitat fragmentation by measuring population density, rate of parasitism, overall genetic differentiation ($\theta_{ST}$) and allelic richness in a large metapopulation. We also simulated the dynamics of genetic differentiation among local populations to asses the relative effects of migration rate, population size, and haplodiploid (parasitoid) and diploid (host) inheritance on metapopulation persistence. We show that parasitism by *N. melanocephalus* is less frequent at larger distances to the nearest neighbouring population of *M. nausithous* hosts, but that host density itself is not affected by isolation. Allelic richness was independent of isolation, but the mean genetic differentiation among local parasitoid populations increased with the distance between these populations. Overall, genetic differentiation in the parasitoid wasp was much greater than in the butterfly host and our simulations indicate that this difference is due to a combination of haplodiploidy and small local population sizes. Our results thus support the hypothesis that *Neotypus* parasitoid wasps are more sensitive to habitat fragmentation than their *Maculinea* butterfly hosts.**

*Keywords*: host–parasitoid interaction, isolation, *Maculinea*, metapopulation, population genetics, spatial structure

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## Introduction

The explicit study of landscape complexity and population connectivity is of prime importance for understanding population persistence in fragmented landscapes (Tilman & Kareiva 1997). This is especially true for predator–prey and host–parasitoid systems (Hassell 2000), but there are few studies that have directly estimated dispersal and genetic population structure in spatially structured multitrophic systems (Cronin & Reeve 2005). For insects, we know that habitat fragmentation often affects population viability (e.g. Tscharntke & Brandl 2004), population size and density (Zschokke *et al*. 2000; Matter 2003), migration (Matter *et al*. 2005) and species richness (Fahrig 2003). The dynamics of this process have been modelled for single species, showing that increasing interpopulation distances and decreasing local population sizes reduce the genetic neighbourhood and increase the erosion of genetic diversity (Gilpin 1991). As a consequence, inbreeding may increase so that individual fitness declines (e.g. Joron & Brakefield 2003) and populations become more vulnerable to demographical stochasticity (e.g. Saccheri *et al*. 1998).

Theoretical (Holt *et al*. 1999; Holt 2002) and empirical (Kruess & Tscharntke 1994; Komonen *et al*. 2000) studies indicate that higher trophic-level species, such as predators

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and parasitoids, are more sensitive to habitat loss and population fragmentation than their herbivore prey or host species; but, in particular, parasitoids have been neglected when designing conservation strategies (Hochberg 2000; Shaw & Hochberg 2002). Van Nouhuys (2005) reviewed the effects of habitat fragmentation at different trophic levels but did not find patterns in the comparative data that were consistent with theoretical predictions. Furthermore, the very limited comparisons of genetic structure between trophic levels have so far failed to reveal a consistent pattern (e.g. Johannesen & Seitz 2003; McCoy *et al*. 2005), possibly because the spatial scale of the studies did not match the overall dispersal ability of the focal species (e.g. Cronin *et al*. 2000). Another reason for the poor fit of comparative data with theoretical predictions may be that overall densities are highly variable across empirical studies. Larger consumer populations support larger populations of exploiters that may thus persist longer (Hanski & Singer 2001), but few studies have measured both local densities and genetic variation (Saccheri *et al*. 2004).

Our study focuses on the large blue butterfly *Maculinea nausithous* (Lepidoptera: Lycaenidae) and its specific parasitoid wasp *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae). Female *M. nausithous* deposit their eggs on flowers of the food-plant *Sanguisorba officinalis*. Caterpillars feed on the developing seeds inside the flower-heads for 2–3 weeks and drop to the ground when reaching the fourth instar, to be adopted by workers of the ant *Myrmica rubra*. Once inside the host-ant nest, the caterpillars feed on the ant brood until they pupate the following spring and emerge as adults a few weeks later (Thomas & Elmes 1998). While feeding inside the flower-heads, *M. nausithous* caterpillars are attacked by the specialized parasitoid wasp *N. melanocephalus*, leading to an average of 35% parasitization in infested host populations (Anton *et al*. 2007). The development of parasitoid larvae is synchronized with the development of their *Maculinea* host, as the wasps delay development and emerge relatively late from the pupal cases of *M. nausithous;* just before the next generation of young *Maculinea* caterpillars has become available. A metapopulation in which both *M. nausithous* and *N. melanocephalus* co-occur in discrete habitat patches therefore provides an interesting model system to investigate the effects of isolation on genetic structure and density of a specific bi-trophic interaction, as we may assume that differences in population structure are directly related to the biological properties of the two species involved (Kankare *et al*. 2005).

The objective of the present study was to analyze the genetic structure of the ant-predatory butterfly *M. nausithous* and its specific parasitoid *N. melanocephalus* at a sufficiently small spatial scale to allow the effects of population isolation on host density and on rate of parasitism to be assessed. At the same time, we use the comparative population genetics of diploid *Maculinea* hosts and haplodiploid *Neotypus* parasitoids to obtain insight into the relative importance of ploidy for genetic population structure, as haplodiploidy has been argued to induce both lower genetic variability (Graur 1985) and more pronounced genetic differentiation among local populations in comparison to diploidy (Packer & Owen 2001). We therefore supplemented our empirical study with model simulations to estimate the relative impact of haplodiploidy, population size and migration rate on overall genetic differentiation.

## Materials and methods

### *Study region and sampling sites*

The study region of $30 \times 45$ km is located in the Upper Rhine valley in southwestern Germany (Fig. 1), where the

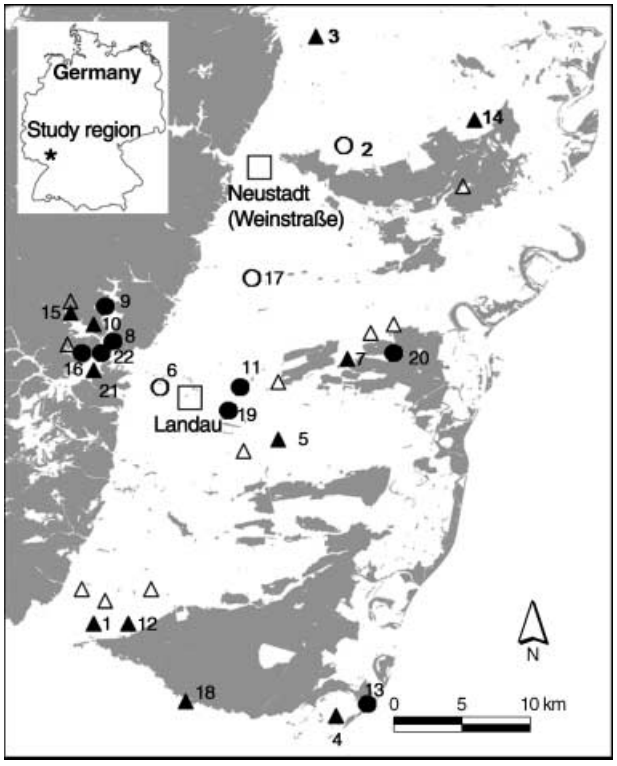


**Fig. 1** Study area in the Upper Rhine valley in southwestern Germany where samples for genetic and demographical analysis of *Maculinea nausithous* and *Neotypus melanocephalus* were collected. Dark grey areas indicate forests, whereas white areas are grasslands or areas used for vineyards and other forms of agriculture. Symbols indicate sites where population density and parasitism were measured. On a subset of sites, parasitoids (filled circles), butterflies (open circles) or both species (filled triangles) were collected for genetic analysis. Open triangles indicate sites where population density and parasitism were measured but no genetic data were available. Large squares indicate the location of Neustadt and Landau villages.

metapopulation dynamics of *Maculinea nausithous* has been studied for more than 10 years (Settele 1997; Settele, unpublished). All local populations in the study area where adult butterflies were observed during the flight period in 2003 were included in the analysis of isolation effects on population density ($N = 34$). Fourteen sites were randomly selected for genetic analyses of population structure in the butterfly host and 19 sites (where parasitism was common enough for sampling at least 15 larvae) for similar analyses of the parasitoid (Kalinowski 2005). After the sampling scheme had been completed we had the opportunity to genotype five additional populations of *Neotypus melanocephalus*, increasing the total to 22.

### *Population densities, population sizes and rates of parasitism*

Butterfly density was estimated for adults, eggs and fourth instar caterpillars. First, adult *M. nausithous* individuals were counted during transect walks between 10.00 am and 17.00 pm. During these walks, which were done three times during the flight period, all individuals spotted within 2.5 m to each side were counted. The egg density of *M. nausithous* was estimated after collecting 20 randomly chosen *Sanguisorba officinalis* shoots spread across each local population. From this sample, we determined the average number of flower-heads per shoot and randomly selected 40 flower-heads to examine the number of eggs per flower-head. Using the obtained knowledge about the number of eggs per flower-head and the number of flower-heads per *S. officinalis* shoot, we determined the density of *S. officinalis* shoots on each site to infer the total number of eggs per site. The density of *S. officinalis* was estimated by counting shoots in 16–50 randomly distributed squares per site ($2 \times 2$ m). The total number of *M. nausithous* eggs was then divided by the size of the available habitat (the area over which the host plant *S. officinalis* grew) to obtain the number off eggs per square meter. Because the egg shells of *M. nausithous* can be found between single flowers until the flower-heads wilt, the estimation of egg density (and consequently population size) is very reliable. Finally, the density of *M. nausithous* preadoption caterpillars was estimated from the above mentioned collection of 20 randomly selected shoots of *S. officinalis*. The caterpillars were collected upon emergence to obtain an estimate of the number of caterpillars per shoot and stored in 96% alcohol for further analyses. Two approaches were used to estimate the adult population size of *M. nausithous*. First, butterfly counts from transect walks were used. The highest number of adults observed during these walks (butterfly abundance) was used as an approximation for *M. nausithous* population size. As a second approach, population size was estimated from the egg counts as described above. The total number of eggs was divided by 80, the mean number of eggs produced per female (Pfeifer *et al*. 2005) to obtain an independent estimate of the population size of *M. nausithous* females.

The nest density of *Myrmica rubra* was previously shown to be correlated with the number of foraging *M. rubra* workers (Glinka & Settele 2005). We therefore estimated ant density with baits (Elmes *et al*. 1998). Depending on the size of the habitat patch, between 16 and 53 baits were laid out in a random stratified manner (Thomas *et al*. 1989). *Myrmica* workers forage up to 2 m from the nest (Elmes *et al*. 1998), so that a minimum distance of 5 m between baits was used to ensure independence of ant-density measures at baits. Baiting began between 8 am and 11 am at each site and baits were checked for ants 1–2 h later. The number of *M. rubra* ants per bait was estimated in categories (1–5 ants, 6–15, 16–35, 36–75, 76–160, > 161). The size of these categories increased two-fold because large numbers of ants are increasingly difficult to estimate. All *Myrmica* workers collected at baits were identified in the laboratory according to Wardlaw *et al*. (1998). If there was another *Myrmica* species besides *M. rubra* in the sample (this was the case in 7% of the samples), we tried to correct the estimate accordingly. Since the percentage of occupied baits correlated positively with the total number of *M. rubra* per bait (Pearson correlation, $r^2 = 0.81$, $N = 34$, $P < 0.0001$), the proportion of occupied baits was used as a measure for *M. rubra* density. By using the presence/absence data of *M. rubra* at the baits, any errors caused by the presence of *Myrmica* species other than *M. rubra* was avoided.

The rate of parasitism was estimated as the proportion of parasitized *Maculinea nausithous* caterpillars after dissection of the preadoption caterpillars sampled as described above. Depending on the estimated population size of the host, we dissected between 50 and 150 *M. nausithous* caterpillars. The parasitoid larvae obtained from these dissections were used for genotyping, which allowed us to work with reasonable sample sizes without having to harm the *N. melanocephalus* populations by removing adult wasps.

### *Isolation of local populations*

We used two approaches to measure habitat isolation: connectivity measures according to Moilanen & Nieminen (2002) and nearest-neighbour measures. To analyze the connectivity of populations we used a negative exponential dispersal kernel that takes into account the scaling of immigration into populations:

$$S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j^b$$

where $A_j$ is the patch size of neighbouring habitats and $d_{ij}$ is the distance from the neighbouring habitat ($j$) to the study site ($i$), $\alpha$ describes the effect of distance on migration

($1/\alpha$ is the average migration distance) and $b$ a parameter describing the scaling of emigration and immigration. Because $\alpha$-values are unknown for *M. nausithous* and *N. melanocephalus*, we calculated $S_i$ with $\alpha$'s ranging from 0.002 to 0.3 (*M. nausithous*) and 0.001–3.0 (*N. melanocephalus*) and b using values between 0.1 and 0.5, which are typical for butterflies (Moilanen & Nieminen 2002). These analyses were repeated for *M. nausithous* using the population size of *M. nausithous* (adult butterfly abundance) and for *N. melanocephalus* using the total number of caterpillars instead of the patch size A. However, none of these connectivity measures improved the power of the logistic regression models that analyzed the occurence of host and parasitoid populations. Therefore, we used nearest-neighbour measures for population isolation. In a number of other studies, this simple measure was more powerful (e.g. Krauss *et al*. 2005; Elzinga *et al*. 2007) suggesting that detailed information about the dispersal ability of species is necessary when using complex connectivity measures (van Nouhuys, personal communication). Nearest-neighbour distance was defined as the shortest distance between two sites measured from centre to centre (Kruess & Tscharntke 1994; Krauss *et al*. 2005). To assess the metapopulation dynamics of *M. nausithous* (Settele 1997) and *N. melanocephalus*, we used these measures to obtain two distances with direct biological relevance: For the butterflies, we measured (i) the distance to the nearest other population occupied by *M. nausithous;* and ii) the distance to the nearest potentially suitable habitat. A potentially suitable habitat was defined as a site where the host ants and host plants were present and where *M. nausithous* had been observed within the last five years (Settele, unpublished; not presented in Fig. 1). For the parasitoid we used (i) the distance to the nearest other population with an extant *N. melanocephalus* population; and (ii) the distance to the nearest *M. nausithous* host population irrespective of the presence of parasitoids.

### *Genetic analyses*

DNA from *M. nausithous* was isolated from a small piece of caterpillar tissue using a Chelex protocol incorporating a proteinase K digestion step (Walsh *et al*. 1991). Polymerase chain reaction (PCR) amplification and analysis of *M. nausithous* population structure was carried out using seven polymorphic loci (*Macu3, Macu5, Macu7, Macu9, Macu11, Macu15* and *Macu17*) as described in Zeisset *et al*. (2005). Larvae of the parasitoid *N. melanocephalus* were stored in 100 µL 5% Chelex-100 (10 mM Tris, pH 7.5). Parasitoid DNA was extracted from larvae as described in Anton *et al*. (2006). PCR amplification and analysis of *N. melanocephalus* microsatellites was carried out using seven polymorphic loci (*Neo09, Neo34, Neo35, Neo37, Neo39, Neo40* and *Neo42*) as described in Anton *et al*. (2006).

Microsatellite genotypes of both *M. nausithous* and *N. melanocephalus* were scored relative to internal size markers using GENESCAN 3.1.2 (Applied Biosystems) and GENOTYPER 2.5 (Applied Biosystems). A total of 441 caterpillars of *M. nausithous* and 522 larvae of *N. melanocephalus* were genotyped. When the typical microsatellite pattern of peaks could not be obtained from multiplexed PCRs, single PCRs were carried out to get unambiguous size identifications of alleles. For the parasite with its haplodiploid sex-determining system, all individuals that were genotyped as being homozygous for all loci were considered to be males.

Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using exact probability tests in GENEPOP version 3.4 as implemented for online access (http://wbiomed.curtin.edu.au/genepop; Raymond & Rousset 1995). Only individuals identified as females were used to calculate HWEs and heterozygosities in the parasitoid. Genetic differentiation among populations was assessed with *F*-statistics (Wright 1951) using GENEPOP and the $F_{ST}$ estimator $\theta$ by Weir & Cockerham (1984). Differences between $\theta_{ST}$ values for the host and parasitoid populations were evaluated using *t*-tests.

The effect of geographical distance on the pairwise genetic differentiation of populations was analyzed by regressing $\theta_{ST}/(1-\theta_{ST})$ against the natural logarithm of geographical distance as suggested for two-dimensional study regions (Rousset 1997). The two species were analyzed twice; once using all sampled sites and once with only those sites where both host and parasitoid were present. The significance of the obtained Spearman correlation coefficients was examined with Mantel tests (Mantel 1967) with 10 000 permutations in GENEPOP. The population structures of *M. nausithous* and *N. melanocephalus* were also directly compared by correlating the genetic distances of populations ($\theta_{ST}$) where both host and parasitoid were sampled.

To characterize the genetic diversity of butterfly and parasitoid populations, we used FSTAT 2.9.3 (Goudet 2001) to estimate allelic richness, corrected for differences in sample size by rarefaction (El Mousadik & Petit 1997). We used allelic richness as a measure of genetic diversity in correlation analyses because both theory and experiments indicate that this variable is more sensitive to demographical changes and bottlenecks than heterozygosity and thus represents a better estimate of the evolutionary potential of a population (Leberg 2002). Because FSTAT requires data to be entered as diploid genotypes, male haplotypes were randomly merged to form diploids (Packer *et al*. 2005). This has no effect on the allelic richness estimates because the method uses allelic frequency data and not genotypic data.

### *Simulations*

We used an individual-based model as implemented in the software EASYPOP (Balloux 2001) to generate statistics

describing the genetic differentiation among 16 populations in a two-dimensional stepping-stone model. We used seven loci with an assumed mutation rate of 0.001 for all simulations. At the start of the simulations, genetic diversity was always set at the maximal possible value. Computer populations consisted of 200 males and 200 females (based on the estimated average population size of *M. nausithous*, using egg counts). We analyzed the specific impact of haplodiploidy by setting the initial local population sizes of host and parasitoid at 400 (sex ratio 1 : 1) and assuming a migration rate between habitat patches of 0.01. We then combined smaller population sizes (320, 240 and 160) with higher migration rates (0.02, 0.03, 0.04 and 0.05) to assess the impact of host and parasitoid traits on global $\theta_{ST}$. All simulations we run for 300 generations and replicated 20 times.

### *Data analysis*

Multiple linear regression analysis was used to examine the influence of host ant density, the distance to the nearest *M. nausithous* population, the distance to the nearest potential *M. nausithous* habitat, and interactions between host ant density and both measures of isolation on the density of *M. nausithous* eggs (log($y$ + 1)). The same model was repeated with *M. nausithous* caterpillar density, adult density and population size (based on egg counts and adult counts) as response variable. Parasitism rates were analyzed using a generalized linear model (GLM) with quasi-binomial error structure and a logit link function (ln ($p/1 - p$)) as linear predictor. We tested the potential impact of *M. nausithous* caterpillar density, population size (based on egg counts and adult counts), distance to the nearest *M. nausithous* population, distance to the nearest *N. melanocephalus* population, two-way interactions between *M. nausithous* caterpillar density and both measures of isolation, and two–way interactions between *M. nausithous* population size (both egg and adult counts) and both measures of isolation.

The density of host ants, *M. nausithous* population size (both egg and adult counts), the distance to the nearest *M. nausithous* population and the distance to the nearest potential *M. nausithous* habitat were used as predictor variables in regression models to explain the allelic richness of *M. nausithous* populations. The same technique and variables were used to analyze the mean genetic population differentiation of *M. nausithous* as response variable. The following predictor variables were used to explain the allelic richness of *N. melanocephalus* populations: population size *M. nausithous* (egg counts and adult counts),

**Table 1** Sample size ($N$), allelic richness, observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$) of *Maculinea nausithous* butterflies and *Neotypus melanocephalus* parasitoids across all microsatellite loci. Subscripts f and m indicate females and males, respectively

| | | Maculinea nausithous | | | | Neotypus melanocephalus | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Locality | $N$ | Allelic richness | $H_O$ | $H_E$ | $N_{f/m}$ | Allelic richness | $H_O$ | $H_E$ |
| 1 | Schweighofen | 30 | 5.3 | 0.554 | 0.56 | 27/10 | 5.8 | 0.365 | 0.74 |
| 2 | Haßloch | 38 | 3.9 | 0.55 | 0.58 | | | | |
| 3 | Niederkirchen | 33 | 4.2 | 0.578 | 0.60 | 23/8 | 5.3 | 0.506 | 0.68 |
| 4 | Neuburg-Süd | 27 | 4.9 | 0.64 | 0.57 | 14/10 | 4.2 | 0.415 | 0.63 |
| 5 | Herxheim | 35 | 4.0 | 0.503 | 0.53 | 7/10 | 4.0 | 0.194 | 0.64 |
| 6 | Landau West | 33 | 4.7 | 0.622 | 0.62 | | | | |
| 7 | Zeisskam-Mühle | 34 | 4.8 | 0.571 | 0.52 | 16/9 | 5.4 | 0.629 | 0.77 |
| 8 | Eußertal-Mitte | | | | | 19/7 | 5.2 | 0.370 | 0.66 |
| 9 | Dernbachtal | | | | | 20/6 | 5.0 | 0.424 | 0.71 |
| 10 | Gut Waldeck | 35 | 5.5 | 0.539 | 0.57 | 13/9 | 5.6 | 0.333 | 0.69 |
| 11 | Offenbach-Ost | | | | | 33/8 | 4.1 | 0.449 | 0.65 |
| 12 | Steinfeld | 37 | 5.5 | 0.592 | 0.60 | 14/15 | 5.1 | 0.322 | 0.72 |
| 13 | Neuburg-Ort | | | | | 13/17 | 4.2 | 0.286 | 0.58 |
| 14 | Schifferstadt | 31 | 3.5 | 0.447 | 0.50 | 9/9 | 3.9 | 0.371 | 0.64 |
| 15 | Eußerthal-Ort | 24 | 4.3 | 0.512 | 0.56 | 14/17 | 4.3 | 0.365 | 0.58 |
| 16 | Gräfenhausen | | | | | 10/25 | 4.4 | 0.352 | 0.62 |
| 17 | Venningen West | 32 | 4.7 | 0.575 | 0.55 | | | | |
| 18 | Bienwald | 32 | 3.7 | 0.473 | 0.41 | 16/8 | 3.9 | 0.371 | 0.53 |
| 19 | Offenbach-West | | | | | 28/5 | 3.9 | 0.282 | 0.58 |
| 20 | Ludwigsmühle | | | | | 4/14 | 3.9 | 0.429 | 0.67 |
| 21 | Annweiler | 30 | 4.9 | 0.546 | 0.54 | 12/16 | 3.9 | 0.171 | 0.50 |
| 22 | Alberweiler | | | | | 19/6 | 4.1 | 0.563 | 0.63 |

the distance to the nearest *M. nausithous* population, the distance to the nearest *N. melanocephalus* population, two-way interactions between the population size of *M. nausithous* (adult counts) and the distance to the nearest *M. nausithous* population, and two-way interactions between population size (adult counts) and the distance to the nearest *N. melanocephalus* population. Initially, all variables were implemented in the regression model or the GLM and nonsignificant terms ($P > 0.05$) were subsequently deleted step by step to obtain the minimal adequate model (Crawley 2002). The allelic richness of host and parasitoid populations was compared with a paired *t*-test.

Mean genetic differentiation of populations was obtained by averaging all population-level pairwise $\theta_{ST}$ values. In case of non-normal distribution of the residual error terms, we tried to achieve normal error distributions with a log-transformation. When this was not successful, we used a GLM with a linear predictor. The global genetic differentiation of *M. nausithous* and *N. melanocephalus* populations (after 300 generations) was compared using *t*-tests after testing for normal distributions. All models and tests were performed with the software R version 2.2.0 (http://cran.r-project.org/).

## Results

### Maculinea nausithous *density, population size and rate of parasitism*

The density of *Maculinea nausithous* eggs and caterpillars was not significantly influenced by host-ant density or interactions between host-ant density and population isolation (see also Fig. 2A). The density of adult *M. nausithous* correlated with the density of the host ant (slope = 0.05 ± 0.0001, $r^2 = 0.27$, $P = 0.002$). There was a marginal significant correlation between the population size of *M. nausithous* (adult counts) and the density of host ants (slope = 0.01 ± 0.005, $r^2 = 0.12$, $P = 0.05$), but no correlations were found when egg counts were used to estimate population size.

The proportion of parasitism by *Neotypus melanocephalus* systematically decreased with distance to the nearest neighbouring population of the butterfly host (Fig. 2B), but measures of *M. nausithous* population size, density and the interactions between spatial isolation and *M. nausithous* population size or density did not have any impact on the proportion of caterpillars that were parasitized.

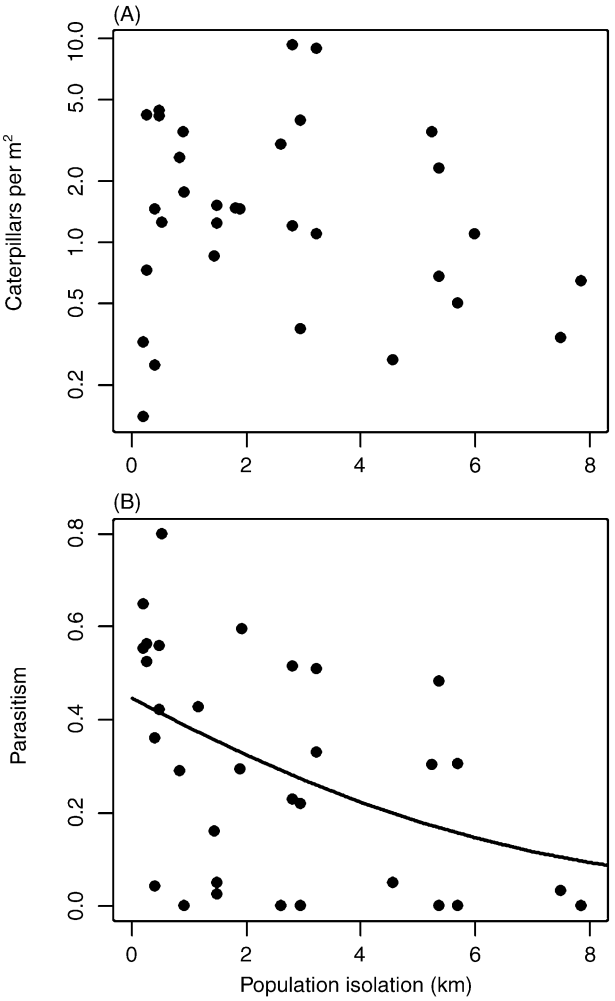


**Fig. 2** (A) Population density of *Maculinea nausithous* caterpillars in relation to the nearest potentially suitable habitat (linear regression: slope = 0.04 ± 0.0004, $r^2 = 0.03$, $P = 0.69$, $N = 34$, $P = 0.69$) and (B) proportion of *M. nausithous* caterpillars parasitized by *Neotypus melanocephalus* in relation to the distance to the nearest *M. nausithous* population (GLM: $F_{2,32} = 8.2$, $P = 0.01$). Note: two sites where we did not find caterpillars are not shown.

### *Genetic diversity of* M. nausithous

The seven *M. nausithous* microsatellite loci were polymorphic in all populations. A total of 62 alleles were detected across all populations, ranging from three (*Macu11*) to 20 (*Macu5*) per locus. Allelic richness per population varied from 3.5 (locality 14, Fig. 1) to 5.5 (localities 10 & 12, Fig. 1). One *M. nausithous* population (locality 21) was not in HWE, mainly because of a heterozygote deficit at the loci *Macu17* and *Macu15*, but these deficiencies did not reveal a consistent pattern across loci and populations. Out of the 98 locus × population combinations, four failed to meet HWE conditions. Two population-combination randomizations gave a larger $F_{IS}$ than the observed values and for two combinations the randomized values were smaller than the expected ones. Given that these deviations occurred in < 5% of the comparisons, we inferred that they were due to chance. Allelic richness of *M. nausithous* populations was also not correlated with isolation (both distance to nearest *M.*

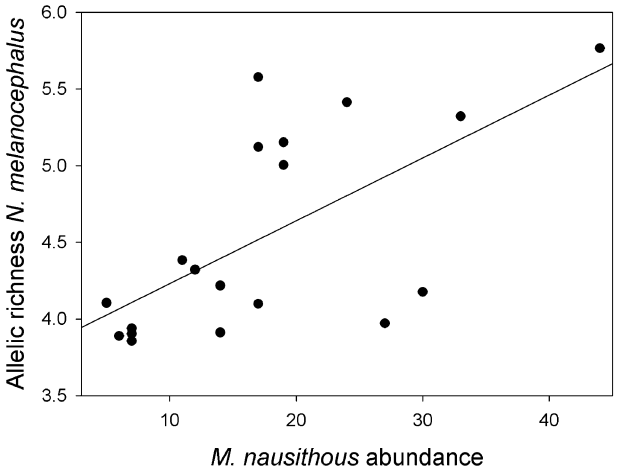


**Fig. 3** Allelic richness of the *Neotypus melanocephalus* parasitoid as a function of adult *Maculinea nausithous* abundance (maximum numberof adult butterflies; linear regression: slope = 0.04 ± 0.01, $r^2 = 0.42$, $P = 0.003$, $N = 19$).

*nausithous* population and distance to nearest potential habitat), *M. nausithous* population size (both egg and adult counts) or density of the host ant.

### *Genetic diversity of* N. melanocephalus

The seven *N. melanocephalus* microsatellite loci were polymorphic in all populations. A total of 91 alleles were detected across all populations, ranging from five (*Neo37*) to 22 (*Neo42*) per locus, allelic richness varied from 3.9 (locality 14, Fig. 1) to 5.8 (locality 1, Fig. 1). None of the parasitoid populations except one (locality 20) were in HWE overall (females only), and this pattern was recurrent across many of the loci. Out of the 133 locus × population combinations, 80 combinations had genotype distributions that were significantly different from expectations of HWE, and in 100% of these cases this was due to heterozygote deficiency. Some of these deviations from HWE may have been due to null alleles, as 2.9% of the PCRs (520 individuals × seven loci combinations) did not amplify after a single PCR. However, this cannot explain more than a small fraction of the overall heterozygote deficiency, as a fraction of 0.029 homozygote individuals for null alleles ($p$) would (under HWE) imply a fraction of 0.056 ($2p(1-p)$) heterozygotes that would have erroneously been scored as homozygotes. Allelic richness of *N. melanocephalus* populations was not affected by spatial isolation, population size (based on egg counts and adult counts) or the interactions between isolation and population size, but correlated positively with the abundance of adult butterflies (Fig. 3).

Population specific allelic richness of *M. nausithous* was significantly correlated with allelic richness of *N.*

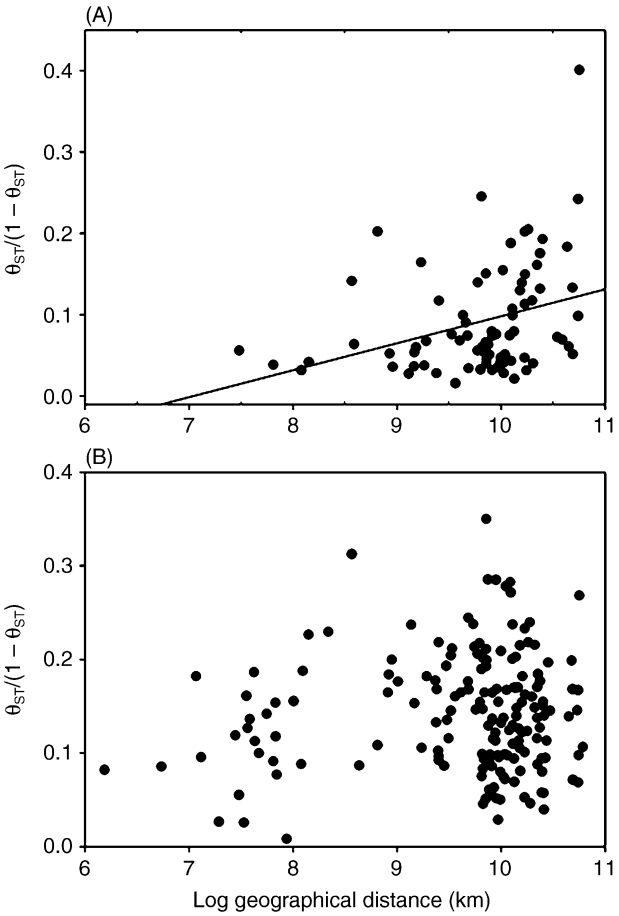


**Fig. 4** Pairwise geographical and genetic distances among (A) 14 populations of *Maculinea nausithous* (Mantel test: $r^2 = 0.26$, $P = 0.005$) and (B) 19 populations of *Neotypus melanocephalus* (Mantel test: $r^2 = 0.001$, $P = 0.62$).

*melanocephalus* in the same population (Pearson correlation: $r^2 = 0.67$, $P = 0.024$, $N = 11$), but allelic richness in local populations did not differ significantly between the two species (paired $t$-test: $t = 0.34$, $P = 0.74$, $N = 11$).

### *Genetic population structure*

The global population differentiation in *N. melanocephalus* ($\theta_{ST} = 0.116$) was significantly higher than in *M. nausithous* ($\theta_{ST} = 0.068$, $t$-test: $t = 4.3$, $P = 0.0003$). The mean pairwise $\theta_{ST}$ value of *N. melanocephalus* populations decreases with increasing butterfly abundance (slope = 0.44 ± 0.01, $P = 0.0001$), and with smaller distances to the nearest *M. nausithous* population (slope = 0.00002 ± 0.04, $P = 0.001$). In contrast, the mean pairwise $\theta_{ST}$ estimates for the butterfly host were not affected by host-ant density, population size (egg and adult counts) and distance to the nearest *M. nausithous* population or potential habitat. Isolation by distance effects were found both for all *M. nausithous* populations (Fig. 4A) and for the subset of populations

where *N. melanocephalus* was present (Mantel test: $r^2 = 0.07$, $P = 0.043$, $N = 11$; figure not shown). In contrast, no effects of isolation by distance were detected in analyses comprising all *N. melanocephalus* populations (Fig. 4B) or the subset of populations (Mantel test: $r^2 = 0.007$, $P = 0.47$, $N = 11$). Finally, the pairwise genetic distances between butterfly populations were not correlated with the genetic distances of corresponding pairs of parasitoid populations (Mantel test: $r^2 = 0.01$, $P = 0.17$, $N = 11$). The mean $\theta_{ST}$ values of host and parasitoid populations did not correlate either (Pearson correlation: $r^2 = 0.19$, $P = 0.18$, $N = 11$).

### *Simulations of host and parasitoid population genetics*

Haplodiploid inheritance appears to result in significantly stronger differentiation of parasitoid populations compared to their diploid hosts (Fig. 5A). Based on the same total population size of 400 individuals and an identical migration rate (0.01), overall $\theta_{ST}$ was 26% higher in parasitoid populations ($0.115 \pm 0.001$ vs. $0.087 \pm 0.002$, $t = -19.6$, $P < 0.0001$, Fig. 5A). Reduced population sizes lead to significant increases in genetic differentiation among populations in both host and parasite (Fig. 5B, C) but maintained the overall differences between diploid hosts and haplodiploid parasitoids.

## Discussion

Our data show that the overall genetic differentiation across local populations is moderate in the butterfly *Maculinea nausithous* and high in the specialist parasitoid *Neotypus melanocephalus*. The simulations presented indicate that this difference is due to a difference in the breeding system (diploid host vs. haplodiploid parasitoid) and to smaller population sizes in the parasitoid (Fig. 5). Genetic differentiation ($\theta_{ST}$) across parasitoid populations increased with distance to the nearest *M. nausithous* host population and decreased with host population size. However, genetic differentiation did not result in reduced allelic richness of isolated populations in either species. Population-specific allelic richness in the parasitoid increased with the adult population size of the host (Fig. 3). A pattern of isolation by distance was found in the butterfly host but was absent in the parasitoid (Fig. 4). In addition, the rate at which *N. melanocephalus* parasitized the caterpillars of their *M. nausithous* host decreased with isolation (Fig. 2B), whereas the density of caterpillars (Fig. 2A) and the density of eggs and adults were not affected by spatial isolation.

### *Population genetic differentiation and isolation by distance*

The overall genetic differentiation found in this study and the previous extinction-colonization studies on the same

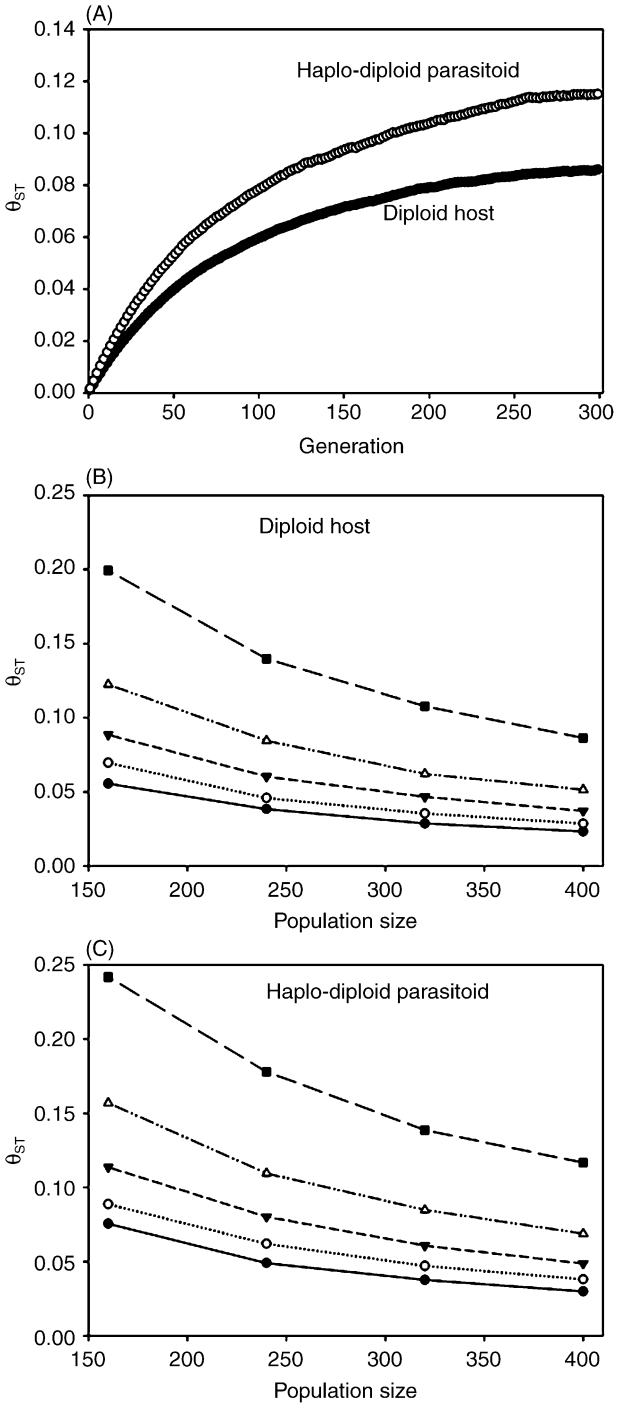


**Fig. 5** (A) The build-up of global genetic differentiation ($\theta_{ST}$) in a diploid host and a haplodiploid parasitoid with the same metapopulation size of 6400 individuals distributed over 16 local populations that are linked by a migration rate of 0.01. (B, C) The effect of increasing migration rates and decreasing population sizes on the $\theta_{ST}$ values obtained for a metapopulation of, respectively, diploid hosts and haplodiploid parasitoids. Migration rates increase from top to bottom in increments of 1% from 0.01 to 0.05 as indicated with different symbols. Each point represents the mean global differentiation across 16 populations in 20 simulations. See Materials and methods section for details.

sites by Settele (1997) indicate that both host and parasitoid have an intact metapopulation structure in the Upper Rhine valley. The overall genetic differentiation of *M. nausithous* is comparable to what has been found in other butterfly studies on similar spatial scales (e.g. Goulson 1993; Saccheri *et al*. 1998; Figurny-Puchalska *et al*. 2000; Krauss *et al*. 2004), but the differentiation among the local parasitoid populations was remarkably high. As demonstrated in our simulations of the overall levels of genetic differentiation of host and parasitoid metapopulations with similar characteristics, haplodiploidy results in a global genetic differentiation that is 26% higher than the global genetic differentiation of an otherwise similar diploid host (Fig. 5). These results confirm the general pattern of a more pronounced genetic structure of haplodiploid hymenopteran parasitoids as compared to their diploid lepidopteran hosts (Packer & Owen 2001). Given the same census size, haplodiploidy results in smaller effective population sizes (75%). The empirical data from our field study indicate a difference of 39% between the overall genetic differentiation of *M. nausithous* and *N. melanocephalus* populations, indicating that smaller population sizes and lower migration rates may also play a role as suggested by the results of the simulations.

Our simulations showed that a rate of parasitism of 40% (our empirical data give 35% on average) requires a fairly high migration rate of 3% for the parasitoids to establish the degree of global genetic differentiation similar to that found ($\theta_{ST} = 0.11$), whereas the diploid host reached a global genetic differentiation of 0.08 with only 1% migrating individuals. The simulations of genetic population structure and the empirical data therefore indicate that *N. melanocephalus* in our study populations can be expected to have either larger proportions of migrating individuals or longer dispersal distances than their butterfly host in spite of having genetically more differentiated populations.

Allelic richness of *N. melanocephalus* populations was higher in large *M. nausithous* populations, similar to what has been reported for a specialized parasitoid of *Melitaea* butterflies (Lei & Hanski 1997). Our finding that the mean genetic differentiation among local parasitoid populations is lower in large rather than in small host populations suggests that small host populations result in small parasitoid populations with limited gene flow to neighbouring populations. Thus, large *Maculinea* host populations may be important to maintain long-term viable populations of *Neotypus* parasitoids. High $\theta_{ST}$ values among *N. melanocephalus* populations were also associated with large distances to the nearest host populations, suggesting that isolation aggravates the drift effects induced by small population size, consistent with standard theory (Whitlock 2004). This inference assumes that the distance to the nearest host population is a good approximation for the distance to the nearest other parasitoid population, which seems reasonable because our limited dissection sampling has probably failed to detect small parasitoid populations in a number of cases. The fact that genetic differentiation among *M. nausithous* host populations does not increase with isolation (at least at the scale of our metapopulation) suggests that effective gene flow is less influenced by isolation at this lower trophic level where populations are generally larger. According to Cronin & Reeve (2005), spatially discrete host–parasitoid systems may in fact be quite variable in their genetic coherence, ranging from classic metapopulations with high connectedness (Lei & Hanski 1997; Johannesen & Seitz 2003) to subdivided populations where gene flow is constrained (Dempster *et al*. 1995). Despite this variability, parasites have often been found to have more strongly structured populations (Althoff & Thompson 1999; McCoy *et al*. 2005), which appears to be compatible with our findings.

Genetic differentiation with increasing geographical distance arises from the joint effects of gene flow, genetic drift and adaptation to local conditions (Wright 1943; Hutchison & Templeton 1999). While gene flow reduces the degree of genetic differentiation at short distances, the effect will become proportionally less at longer distances when population fluctuations become more random (Hutchison & Templeton 1999). Unlike the butterfly host populations, no pattern of isolation by distance was found in the parasitoid populations, and a large scatter of $\theta_{ST}$ values irrespective of geographical distance indicates that drift is more important than gene flow in this species. Genetic drift may also have caused the deviations from HWE in *N. melanocephalus*. A deficit of heterozygotes across numerous loci was also found in other parasitoid species (Kitthawee *et al*. 1999; Hufbauer *et al*. 2001) and could also be due to strong inbreeding effects or selection against heterozygotes.

### *Population density, population size and parasitism*

Figure 2B shows that the rate of parasitism by *N. melanocephalus* decreases with increasing distance to the nearest host population. This result and the effects described above of population isolation on the genetic structure and genetic diversity of *N. melanocephalus* suggest that the parasitoid wasp is more dependent on a functional metapopulation structure for maintaining local population sizes than the butterfly host, as the density and population size of *M. nausithous* was not affected by isolation. This is in agreement with the theoretical expectation that negative effects of habitat fragmentation amplify at higher trophic levels, and that habitat fragmentation should affect specialized organisms more than generalists (Holt *et al*. 1999). In a comparative study of several parasitoid species attacking the specialist herbivore *Hadena bicruris*, it was shown that a solitary parasitoid species was

more strongly affected by isolation (Elzinga *et al*. 2007). However, solitary and specialist parasitoids such as *N. melanocephalus* might be relatively good dispersers owing to their large body size, which may compensate for dispersal inhibiting factors such as small local population size relative to the host.

### *Implications for conservation*

The demographical data presented in this work suggest that a specialized parasitoid like *N. melanocephalus* may reach a threshold of habitat fragmentation that inevitably induces extinction sooner than a host like *M. nausithous* (see also Kankare *et al*. 2005). This appears to be largely due to the haplodiploid breeding system of the parasitoid, which erodes genetic variation more effectively even when the parasitoid has a more pronounced dispersal capacity than the host. This inference predicts that any hypothetical diploid parasitoid of the same *Maculinea* butterfly might be able to maintain stable metapopulations with either a lower density or a more restricted dispersal behaviour. As *N. melanocephalus* has not been reared from any other host species in Central Europe (C. Anton, unpublished), it may be even more endangered than its butterfly host. Conversely, the presence of the parasitoid may be a useful indicator of an intact and functional metapopulation of the butterfly host. Our results thus indicate that it is crucial to include natural parasites when designing conservation strategies of target flagship species such as *Maculinea* butterflies.

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