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ORIGINAL ARTICLE

Spatial population dynamics of a specialist aphid parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae): evidence for philopatry and restricted dispersal

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Within insect communities, the population ecology of organisms representing higher trophic levels, for example, hymenopterous parasitoids, may be influenced by the structure of their insect hosts. Using microsatellite markers and ecological data, we investigated the population structure of the specialist braconid wasp parasitoid, *Lysiphlebus hirticornis* Mackauer attacking *Metopeurum fuscoviride*, a specialist aphid feeding on tansy, *Tanacetum vulgare*. Previous studies revealed that *M. fuscoviride* has a classic metapopulation structure with high subpopulation turnover. In this study, up to 100% of ramets within a host plant genet colonized by aphids were colonized by the parasitoid, yet plants with aphids but no parasitoids were also observed. Genetic differentiation measured by F_{ST}, actual differentiation (D) and relative differentiation (G_{ST}) indicated highly structured parasitoid population demes, with restricted gene flow among and between parasitoid subpopulations at the various sites. Interestingly, both field data and population assignment analysis showed that the parasitoid is highly philopatric. Thus, despite the frequent local extinctions of the aphid host, the parasitoid continuously exploits its aphid host and contributes to the demise of local aphid subpopulations, rather than spreading its genes over many aphid populations. F_{ST} values for the haplodiploid parasitoid were similar to those found in an independent study of the diploid aphid host, *M. fuscoviride*, hence supporting the view that an insect herbivore's population structure directly influences the ecology and genetics of the higher trophic level, in this case the wasp parasitoid.
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Introduction

In recent years, a number of empirical studies have shown that metapopulation structure influences the genetic makeup of subpopulations of various organisms studied (for example, Bay *et al*., 2008; Orsini *et al*., 2008; Purrenhage *et al*., 2009), including aphids (Massonnet *et al*., 2002). General predictions about the genetic structure of metapopulations are difficult to make because of factors such as the population dynamics of local populations, their extinction rate, and the rate and distance of dispersal of the species under consideration (Harrison and Hastings, 1996; Pannell and Charlesworth, 2000). Broadly speaking, a metapopulation with frequent extinctions is expected to show lower overall genetic variability compared with an unstructured population of a similar organism with similar natural history, and subpopulations (colonies or demes) are more or less genetically differentiated depending on the balance between dispersal, colonization and extinction dynamics (Pannell and Charlesworth, 2000). For example, in insect species with metapopulation dynamics, genetic differentiation between populations range from: (1) significant differences between almost all subpopulation pairs investigated, for example, the tansy aphid, *Macrosiphoniella tanacetaria* Kaltenbach (Hemiptera: Aphididae) (Massonnet *et al*., 2002); to (2) some level of genetic differentiation, such as that found in the wasp parasitoid, *Hyposoter horticola* Gravenhorst (Hymenoptera: Ichneumonidae), which attacks the Glanville fritillary butterfly, *Melitaea cinxia* L. (Lepidoptera: Nymphalidae) (Kankare *et al*., 2005); to (3) no genetic differentiation despite the existence of discrete subpopulations, for example, in the dung beetle, *Aphodius fossor* L. (Coleoptera: Scarabaeidae) (Roslin, 2001).

In spatially structured multitrophic systems such as those formed on plants by insect herbivores and their natural enemies, the population ecology of the higher trophic levels, for example, wasp parasitoids, may be influenced by the metapopulation structure of the host insect. However, few studies have to date estimated dispersal and genetic population structure at one or more trophic levels or analysed the influence of species interactions on metapopulation structure of one or more of the organisms involved (Cronin and Reeve, 2005). The few studies performed (for example, Johannesen and

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Seitz, 2003; Kankare *et al*., 2005; Anton *et al*., 2007) show that even though the herbivore host may have a distinct metapopulation structure, which also affects local survival of the parasitoid, the population genetic structure of the parasitoid can be very different.

The host–parasitoid system comprising the specialist wasp parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae) attacking the specialist aphid, *Metopeurum fuscoviride* Stroyan (Hemiptera: Aphididae), which feeds exclusively on tansy, *Tanacetum vulgare* L. (family Asteracae), has a classic metapopulation structure (Weisser, 2000; Zheng *et al*., 2009). In a field study, Weisser (2000) reported frequent extinction events for the aphid, *M. fuscoviride*, and as a direct consequence, of the parasitoid *L. hirticornis*, at the level of tansy plants (=genets) and shoots (=ramets). At the local spatial scale (site), Weisser (2000) found that many tansy plants were not colonized by the aphid and many tansy genets with the aphid were not colonized by the parasitoid. He also reported a mean survival time of 3.1 and 4.8 weeks (mode=1 week) for aphids at the level of ramet and genet, respectively.

In support of the view that *M. fuscoviride* indeed shows a metapopualtion structure, Massonnet (2002), using six microsatellite markers on seven subpopulations (five from the Alsace region of France and two from Germany), reported high population differentiation: F_{ST} values ranged from 0.029 to 0.416, and of the 21 pairwise F_{ST} comparisons performed, 18 were significant ($P<0.05$).

L. hirticornis is a solitary parasitoid (that is, a single wasp develops from a single aphid host) that attacks aggregated aphids and will oviposit eggs in the same aphid colony until virtually all available hosts are parasitized or it becomes egg limited (Völkl, 1997). In addition, although winged, the adult parasitoids have a low dispersal ability (Rauch and Weisser, 2007), but this has not been tested directly using marked individuals—either fluoresecent dyes or molecular DNA markers.

In this study, the population dynamics of *L. hirticornis* on hand-labeled tansy plants colonized by *M. fuscoviride* were surveyed, and parasitoid samples genotyped at 11 polymorphic microsatellite loci to assess the extent of *L. hirticornis* relative isolation and dispersal, the relationship of flight behaviour (or lack of it) to philopatry, and the effect of aphid host population structure (that is, that of *M. fuscoviride*, the second trophic level) on the genetic diversity and differentiation of the parasitoid, *L. hirticornis* (the third trophic level). To investigate these variables, we estimated the level of *L. hirticornis* colonization and dispersal on tansy genets colonized by *M. fuscoviride*, the population genetic differentiation of the parasitoid subpopulations and isolation by distance (IBD), analysed *L. hirticornis* for philopatric behaviour, and compared the population structure of this haplodiploid parasitoid with that of its diploid aphid host. Given the limited knowledge on the population genetic structure of the parasitoid in relation to that of its host, we tested the following hypotheses: (1) there is significant population genetic differentiation between local colonies of *L. hirticornis*; (2) as in the case of the host, there is no IBD in the parasitoid; and (3) the level of genetic differentiation in the parasitoid is as high as in the host aphids.

Materials and methods

Study system

Tansy is a perennial composite with bright yellow flowers native to Europe and Asia (Mitich, 1992). It grows mainly along river valleys, but also occurs in well-drained poor soils and on wastelands. It propagates by both sexual and asexual means and a single genet consists of numerous 'genetically identical' ramets produced by vegetative propagation.

The tansy aphid, *M. fuscoviride*, is a monophagous, holocyclic species (that is, with an autumnal/winter sexual phase involving the laying of cold-hardy eggs) that feeds exclusively on tansy (Massonnet, 2002). *M. fuscoviride* colonies are normally attended by ants, especially *Lasius niger* L. (Hymenoptera: Formicidae) (Mackauer and Völkl, 1993). From the aphid, ants obtain an important source of nutrients in the form of honeydew; in return, they act as guards, warding off predators and parasitoids (Völkl, 1997). The parasitoid *L. hirticornis*, is monophagous on *M. fuscoviride* (Starý, 1973) and is thought to mimic the cuticular hydrocarbons of its aphid host to evade ant attacks (Dettner and Liepert, 1994; Liepert, 1996).

Field sites

Parasitoid samples in the form of aphid mummies were collected between early June and late October, 2007 from 11 sites in and around Jena, Germany (50° 54′ N, 11° 35′ E; Figure 1). The sites were composed of ruderal habitats (see Supplementary Appendix 1 for site location and description). The geographical positioning system was used to estimate distances between sampling sites, the furthest points being Göschwitz-Bhf and Dornburg-Bhf, ~15 km apart.

Field surveys and population sampling

Genets at all sites were visited and inspected for *M. fuscoviride* every 10 days, starting June 2007 and ending October 2007. When colonized by aphids, the genet was marked with a label; the numbers of ramets on a genet were then counted along with the number of ramets colonized by aphids. Each aphid colony was estimated numerically (data not shown) and further inspected for aphid mummies. The aphid mummies formed by *L. hirticornis* are dark brownish in colour and readily distinguished from other parasitoid species attacking *M. fuscoviride*, in particular *Aphidius tanacetarius* Mackauer and *Ephedrus* spp. Haliday (Hymenoptera: Braconidae: Aphidiinae).

During subsequent visits, the fate of marked genets with aphid colonies was followed and any new genet colonization was marked and recorded. The dynamics of *L. hirticornis* are based on the occurrence of aphid mummies, which provide a basis for reliable estimates of the presence of the developing offspring of parasitoids. During visits, a maximum of 10 aphid mummies were sampled per genet. These were held in 1.5-ml Eppendorf tubes for transport to the laboratory. Here, they were left to eclose and the sex of emerging adult wasps was determined under a stereo binocular microscope. Males and females from a single genet were stored separately in Eppendorf tubes in 100% ethanol at 4 °C before DNA extraction. The study continued for the

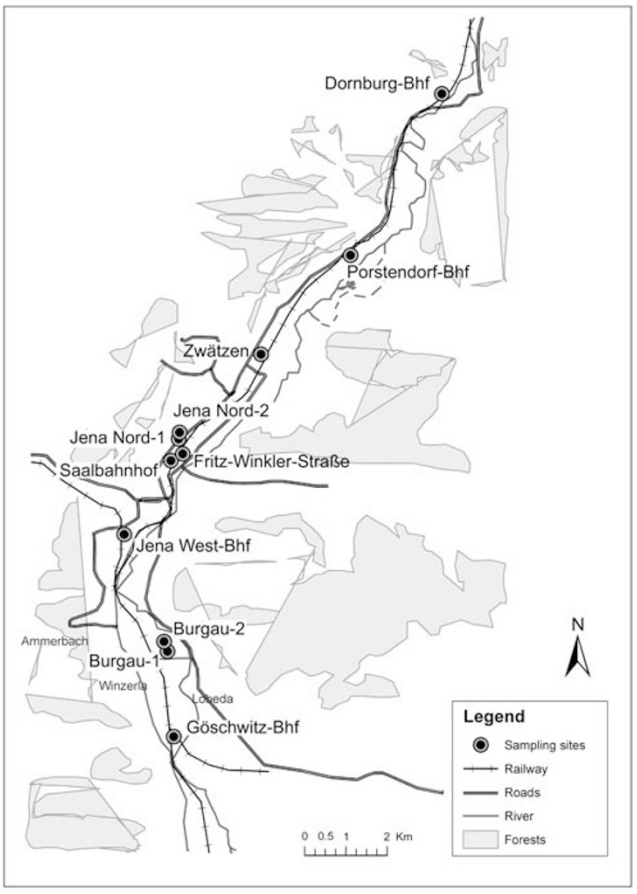

Figure 1 Schematic representation of the sampling sites of *L. hirticornis*. The closest distance was between Jena Nord-1 and Jena Nord-2, 217 m apart, and the furthest, 15 106 m, between Göschwitz-Bhf and Dornburg-Bhf.

entire growing season, until the shoots senesced and the aphid colonies collapsed.

Microsatellite analysis

For genetic analyses, only female parasitoids were used. In total, DNA from 321 female individuals (that is, between 25 and 30 wasps per site, Table 2) was extracted using the 'salting-out' method of Sunnucks and Hales (1996). Eleven polymorphic microsatellite loci were used as follows: nine (*Lhirt 01*, *Lhirt02*, *Lhirt03*, *Lhirt04*, *Lhirt06*, *Lhirt08*, *Lhirt10*, *Lhirt15*, *Lhirt23*) developed from *L. hirticornis*, and two others derived from published results for other *Lysiphlebus* species, that is, *Lysi08* from *L. fabarum* (Marshall) and *Lysi6b12* from *L. testaceipes* (Cresson) (see Nyabuga *et al*., 2009 for details and references therein).

Genetic diversity in *L. hirticornis* populations

Genetic diversity was quantified from observed heterozygosity (H_O) and the unbiased estimates of expected heterozygosity (H_E) calculated using ARLEQUIN (v.3.1; 2006, Excoffier *et al*., 2005). The number of alleles per locus (*A*) and allelic richness was calculated using FSTAT version 2.9.3.2 (Goudet, 2002). Values for allelic richness were subjected to analysis of variance in SPSS v. 16 (SPSS, 2007) to determine differences in population genetic diversity. Within-sample deviation from Hardy–Weinberg equilibrium (HWE) was tested in GENEPOP (V.4.0; Raymond and Rousset, 1995) under the dual null hypotheses of both heterozygote excess and deficiency for individual loci as well as over all loci. An estimate of private (unique) allele frequency was also performed using GENEPOP as described by Barton and Slatkin (1986).

The hierarchical partitioning of the genetic variance was calculated with ARLEQUIN using an analysis of molecular variance framework with 11 populations (Weir and Cockerham, 1984; Excoffier *et al*., 1992). Pairwise and global F_{ST} according to Weir and Cockerham (1984) were calculated in ARLEQUIN. Absolute differentiation (D_{ST}, the proportion of gene diversity present among populations), relative differentiation (G_{ST}, a measure of genetic differentiation estimated as the gene diversity between populations relative to the combined population) (Nei, 1973) and actual differentiation (*D*) (Jost, 2008) were calculated using SMOGD, version 1.2.5 (Crawford, 2009). In brief, D_{ST} is the difference between total heterozygosity (H_T, heterozygosity of the pooled subpopulations) and the mean within-subpopulation heterozygosity (H_S, the mean heterozygosity of the individual subpopulations): $D_{ST} = H_T - H_S$. Nei (1973) called D_{ST} a measure of absolute differentiation, and he divided this by total diversity (H_T) to obtain what he termed the relative differentiation, G_{ST}: $G_{ST} = D_{ST}/H_T$. Nei's G_{ST} is equivalent to Wright's F_{ST} (Nei, 1973) and has a theoretical minimum of zero indicating no genetic divergence and a theoretical maximum of one indicating fixation for alternative alleles in different subpopulations. The observed maximum, however, is usually much less than one (see Wright, 1978 for further details). Actual differentiation, *D* by Jost (2008) is calculated as: $D = (J_T/J_S - 1)/((1/n) - 1)$ or $((H_T - H_S)/(1 - H_S)/(1 - H_S))(n/(n-1))$, where J_T is the gene identity of the pooled subpopulations, J_S is the gene identity within subpopulation and *n* the number of subpopulations. Values of *D* range between zero (no genetic differentiation) to one (subpopulation fixation). Jost (2008) argued that G_{ST} and its relatives did not rank species correctly in terms of differentiation because they used expected heterozygosity in their calculations and discusses a number of shortcomings. Thus he proposed that actual differentiation (*D*) was a better estimate (see Jost, 2008 for details). In spite of the shortcomings of F_{ST} and its relatives, nevertheless, the index continues to prove useful in many population genetic studies, hence its calculation here.

For each parasitoid population, the proportion of unique genotypes *D** was calculated as the number of genotypes present only in that population divided by the total number of parasitoid wasps analysed (Hunter, 1993).

Bottleneck analysis

A graphical technique was used to test for severe reductions in population size, that is, bottlenecks (Luikart *et al*., 1998). The method 'allele distortion by graphical technique' involves comparing the distribution of allele frequencies observed in a population suspected of having gone through a bottleneck with the distribution expected in a population not having done so. For

selectively neutral loci in a natural population, allele number and frequency distribution result from a dynamic equilibrium between mutation and genetic drift. The graphical method groups alleles from a sample of at least five polymorphic loci into ten allele frequency classes (0.001–0.100, 0.101–0.200, 0.201–0.300, and so on) and then plots a frequency histogram. Populations that have not gone through a bottleneck and are near mutation-drift equilibrium for selectively neutral loci are expected to have a large proportion of alleles at low frequency allele classes (Luikart *et al*., 1998). Low-frequency allele classes are defined as those between 0–0.1; high-frequency allele classes between 0.901–1.00; and the eight intermediate classes between 0.101 and 0.900.

The Garza–Williamson index (G–W index; Garza and Williamson, 2001) was also used to estimate bottlenecks. The index is calculated as: $G-W = k/R+1$, where k is the number of alleles at a given locus in a population sample and R is the allelic range of that particular locus. The statistic has a very low value in a population that has passed through a bottleneck and is close to unity in a stationary population.

IBD

The measure of population differentiation $F_{ST}/(1-F_{ST})$ developed by Rousset (1997) was used to test for IBD. F_{ST} values were used to compute Rousset's (1997) genetic distance, and thereafter all IBD calculations were computed using an application program, Isolation by Distance Web Service (IBDWS Version 3.15; Jensen *et al*., 2005). IBDWS assesses whether there is a statistically significant relationship between the genetic distance (or similarity) matrix and the comparable matrix of geographical distances using Mantel tests (Bohonak, 2002).

Relatedness and assignment of individual genotypes to populations for philopatry analysis

Population assignment was performed using logarithm of likelihood measures calculated in ARLEQUIN. To assign a multilocus genotype (MLG) to a population, an individual's allele frequencies are estimated at each locus. The genotypes' expected frequency at each locus in its sampled population is determined and the value is multiplied across all loci and the product logarithm transformed. The same calculations to estimate the genotypes' frequency in other putative source populations are repeated and the MLG assigned to the population in which it has the highest logarithm likelihood (that is, greatest probability) of occurrence (Paetkau *et al*., 1995; Waser and Strobeck, 1998).

Estimation of relatedness was performed using the program ML-RELATE (Kalinowski *et al*., 2006). Each individual in each population was related to all other individuals and the maximum likelihood values obtained (=102720 pairwise relatedness comparisons) were categorized as follows: 0=unrelated; 0.0001–0.25=weakly related; 0.2501–0.5000=moderately related; 0.5001–0.9999=highly related and 1.0=fully related. Within- and between-population comparisons were then performed using the category frequencies.

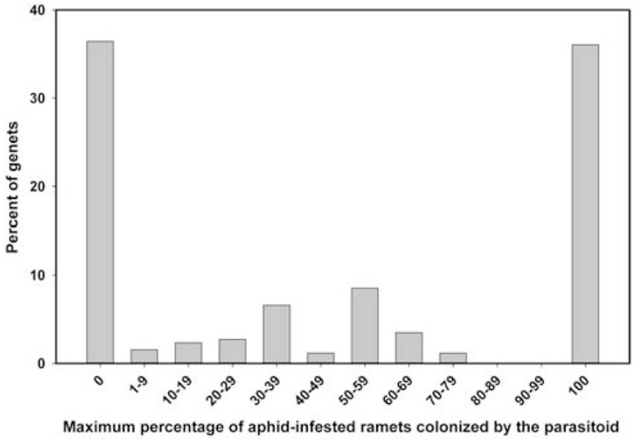

Figure 2 Maximum *L. hirticornis* occupation of aphid colonies within genets. The graph shows the maximum percentage of aphid-infested ramets per genet that also had parasitoids ($N=258$ genets with aphids). For each genet, the maximum derives from the 12 inspections during the season.

Field data and correlations

Field data means and all Pearson's correlations were performed by SPSS version 16 for windows (SPSS, 2007). The significance level was set at $\alpha=0.05$.

Results

L. hirticornis population ecology

Parasitoid dynamics: Mummified aphids were found at all sites, indicating the presence of *L. hirticornis*. After pooling data of genets with aphids from all sites over the entire season; 36% of the genets were not colonized by parasitoids, 36% of the genets had 100% of all aphid colonies colonized by the parasitoid, and on the remaining 28% of genets, between 1 and 79% of their aphid colonized ramets were colonized by the parasitoid (Figure 2). The percentage of genets with aphids colonized by the parasitoid increased from 6% in early June (4–13) to 41.1% in mid-July (4–13), and thereafter began to steadily decline to about 3% by the end of the season (30 October). Within genets colonized by both aphid and parasitoid, the mean percentage of ramets with aphids and colonized by parasitoids increased from 2% in June (4–13) to 40% by the end of the season (30 October), thus showing the infection to have spread locally. Within sites, the percentage of aphid-infested genets with mummified aphids on all aphid-infested ramets ranged from 12% at Porstendorf-Bhf to 69% at Fritz–Winkler–Straße (Supplementary Appendix 1). A total of 1796 *L. hirticornis* parasitoids emerged from the mummies collected; of these, 553 were males, an average sex ratio of 0.31 (sex ratio is calculated as the number of males to total parasitoids). Generally, female biased sex ratios were calculated on all sites and ranged from 0.17 in Porstendorf-Bhf to 0.49 in Saalbahnhof (Table 2).

Microsatellite overall diversity

The observed number of alleles ranged from 2 at locus *Lysi08* and *Lhirt08* to 21 at *Lhirt01* and *Lhirt04*, mean=8.0 (s.d.=6.67). Overall expected heterozygosity ranged from 0.186 at *Lysi6b12* to 0.858 at *Lhirt01* (Table 1). Private alleles were recorded at 5 of the 11 loci tested

(Supplementary Appendix 2). Allelic size distributions varied among loci resulting in differences in their overall variances, for example, the variance of *Lhirt04* was 63 times greater than that of *Lysi08* (Table 1). A few loci showed disjunct allelic size distributions in which size classes were separated by several base pairs. This was particularly so at locus *Lhirt04*, which had an allelic size range of 160–286 bp, but no alleles were found between 160–244 bp; locus *Lhirt15* (allele size range 300–330 bp) had no alleles between 300 and 320; and locus *Lhirt01* (allele size range 182–270 bp) had gaps between 182–192, 196–216 and 238–252 bp.

Within-population genetic diversity

The mean number of alleles per locus for each population ranged from 2.9 (Zwätzen) to 4.4 (Burgau-2) (Table 2). Allelic frequencies ranged from 2% at locus *Lysi08* in Burgau-1 to 100% (fixation) at locus *Lysi6b12* in Fritz–Winkler–Straße (Supplementary Appendix 2). The observed differences in allelic richness among populations sampled were not significant ($F_{10,100} = 51.03$, $P = 0.085$).

Of the MLGs observed, 310 were found for the 321 parasitoids analysed at the 11 loci. Ten MLGs were repeated (multiple repeats): nine were duplicated, and one was found three times. The repeated MLGs were sampled from ten tansy genets from various sites (four from Zwätzen, two from Burgau-1, and one each from Burgau-2, Jena West-Bhf and Fritz–Winkler–Straße). The proportion of unique genotypes (D^*) found per population ranged from 0.87 (Zwätzen) to 1.0 (populations Jena Nord-1, Göschwitz-Bhf, Saalbahnhof, Jena Nord-2 and Porstendorf-Bhf; Table 2). The number of genets from which parasitoids were collected and the proportion of unique genotypes were not correlated ($r = 0.43$, $F_{1,10} = 2.01$, $P = 0.190$). The number of alleles per population and the number of MLGs were marginally correlated ($r = 0.61$, $F_{1,10} = 5.35$, $P = 0.046$); and the number of genets from which the parasitoids were collected was correlated with the number of alleles per population ($r = 0.61$, $F_{1,10} = 5.44$, $P = 0.045$). The correlation between the number of alleles per population and number of parasitoids genotyped was not significant ($r = 0.03$, $F_{1,10} = 0.01$, $P = 0.938$).

Of the 121 Fisher's exact tests performed (11 populations at 11 loci) to measure deviations from HWE, 74 (61.2%) were significant ($P < 0.05$, Table 2); moreover, significant heterozygote deficits were found in 65 of the 74 (87.8%) significant deviations. Only one test showed significant heterozygote excess (Saalbahnhof at locus *Lhirt06*; $P = 0.029$, s.e. $= 0.002$) Over all loci, all the 11 populations had significantly positive F_{IS} values (Table 2). H_O ranged from 0.367 in subpopulation Jena Nord-2 to 0.170 in Burgau-2, mean $= 0.28 \pm 0.18$ (s.d.); H_E from 0.533 in Jena Nord-2 to 0.423 in Burgau-2, mean $= 0.54 \pm 0.23$ (s.d.).

Table 1 Allelic richness and allelic size variance for the 11 microsatellite loci used in population analysis

Microsatellite loci	*Number of alleles*	*Allelic size variance (bp)*	*Expected heterozygosity*
Lhirt01	21	88	0.858
Lhirt02	8	14	0.447
Lhirt03	10	40	0.815
Lhirt04	21	126	0.854
Lhirt06	9	24	0.612
Lhirt08	2	3	0.336
Lhirt10	3	5	0.591
Lhirt15	4	30	0.417
Lhirt23	5	28	0.640
Lysi08	2	2	0.202
Lysi6b12	3	8	0.186

Population differentiation and IBD

In all, 18 out of the total 88 alleles were private (Supplementary Appendix 2). There were six such alleles at locus *Lhirt01* distributed among six populations and eight at locus *Lhirt04*, distributed as follows: three in Burgau-1, two in Saalbahnhof, two in Jena Nord-2 and one in Jena West-Bhf. Two private alleles at locus *Lhirt06* were found in populations Jena Nord-1 and Saalbahnhof. A single private allele each at loci *Lhirt15* and *Lysi6b12* was recorded in populations at Fritz–Winkler–Straße and Saalbahnhof, respectively. A mean frequency of 0.097 private alleles was calculated. Analysis of molecular variance in ARLEQUIN revealed that 15% of the

Table 2 Details of population genetic diversity in *L. hirticornis* samples at the 11 microsatellite loci

	N_{genets}	*Sex ratio*	$N_{parasitoids}$	$N_{genotypes}$	D*	*MNA (s.d.)*	H_E *(s.d.)*	H_O *(s.d.)*	F_{IS}	*HW*	*HD*	*HE*	G_{ST}	D_{ST}	D
Burgau-1	3	0.39	30	28	0.93	3.64 (2.46)	0.42 (0.26)	0.17 (0.14)	0.603***	8	8	0	0.5	0.416	0.784
Burgau-2	17	0.35	30	29	0.97	4.36 (2.46)	0.46 (0.20)	0.25 (0.13)	0.473***	7	7	0	0.459	0.386	0.779
Dornburg-Bhf	2	0.39	30	29	0.97	3.91 (2.07)	0.44 (0.24)	0.25 (0.12)	0.442***	7	5	0	0.494	0.419	0.807
Fritz–Winkler–Straße	5	0.42	25	28	0.93	3.91 (2.43)	0.51 (0.27)	0.27 (0.26)	0.473***	9	8	0	0.417	0.361	0.802
Göschwitz-Bhf	7	0.23	26	26	1	3.64 (1.37)	0.52 (0.20)	0.35 (0.22)	0.325***	8	6	0	0.393	0.33	0.740
Jena Nord-1	11	0.26	30	30	1	4.00 (2.22)	0.48 (0.22)	0.29 (0.18)	0.414***	6	5	0	0.447	0.383	0.802
Jena Nord-2	19	0.29	30	30	1	4.27 (2.86)	0.53 (0.20)	0.37 (0.16)	0.316***	5	4	0	0.381	0.323	0.748
Jena West-Bhf	11	0.27	30	29	0.97	4.09 (2.31)	0.44 (0.26)	0.25 (0.14)	0.426***	6	6	0	0.494	0.418	0.803
Porstendorf-Bhf	7	0.17	30	30	1	3.55 (1.67)	0.44 (0.23)	0.27 (0.20)	0.385***	6	5	0	0.487	0.406	0.782
Saalbahnhof	10	0.49	30	25	1	3.64 (1.92)	0.44 (0.20)	0.31 (0.16)	0.285***	4	5	1	0.495	0.418	0.801
Zwätzen	6	0.28	30	26	0.87	2.91 (1.08)	0.43 (0.19)	0.27 (0.12)	0.390***	8	6	0	0.493	0.416	0.799

Abbreviations: D^*, proportion of unique genotypes found per parasitoid population; D, actual differentiation (Jost, 2008); D_{ST}, absolute differentiation (Nei, 1973); F_{IS}, individual inbreeding coefficient in relation to subpopulation; G_{ST}, relative differentiation (Nei, 1973); HD, number of heterozygote deficits; H_E, expected heterozygosity; HE, number of heterozygote excesses; H_O, observed heterozygosity; HW, number of loci showing significant deviations from HWE; MNA, mean number of alleles per locus.
For each population, number of genets (N_{genets}) from which the sample ($N_{parasitoids}$) were collected; sex ratio (males: total parasitoids); number of different multilocus genotypes deduced ($N_{genotypes}$).
***$P < 0.001$.

variance was due to 'among-populations' differences, 35% due to 'among individuals within populations' and 50% due to 'within individuals' differences.

Absolute differentiation, D_{ST}, ranged from 0.419 in Dornburg-Bhf to 0.323 in Jena Nord-2 (Table 2). Pairwise F_{ST} values ranged from 0.065 (between Jena Nord-2 and Dornburg-Bhf) to 0.233 (between Zwätzen and Saalbahnhof; Table 3) and overall global F_{ST} was 0.148. Relative differentiation, G_{ST}, showed Burgau-1 to be the most genetically differentiated parasitoid subpopulation (0.5) and Jena Nord-2 the least (0.381) with mean G_{ST} value of 0.46. Actual differentiation, D, ranged from 0.74 in Göschwitz-Bhf to 0.807 in Dornburg-Bhf with a mean of 0.786, indicating high levels of subpopulation differentiation (Table 2).

A Mantel test performed for matrix correlation between genetic distance ($F_{ST}/(1-F_{ST})$) and logarithm of geographical distance showed no IBD relationship ($Z=34.88$, $r=-0.21$, intercept $=0.84\pm0.09$ s.e.; slope $=-0.18\pm0.03$ s.e.; $N=55$: one-sided $P=0.86$ from 1000 randomizations).

Bottlenecks

Graphical distortions of allele frequencies showed that the majority of alleles in all populations studied to be in the low-frequency class (0.001–0.1) and therefore had an ordinary distribution of alleles, an indication that bottlenecks were not important in determining the population genetic makeup observed. In contrast, at the level of individual loci, the G–W index revealed some low values (<0.1) at three loci to a mean 0.636 at *Lysi08* (Table 4). Over all loci, means ranged from 0.203 in population Burgau-1 to 0.264 in Burgau-2. At loci *Lhirt08* and *Lhirt10*, all populations had a mean of 0.5, and at locus *Lysi08*, ten populations had a mean of 0.667. The low values of the G–W index sometimes encountered (3 of 11 values; Table 4) suggests that these populations may have gone through bottlenecks at the various loci tested.

Philopatry

Population assignment using ARLEQUIN revealed that in 4 of the 11 populations studied (that is, Burgau-2, Jena Nord-1, Saalbahnhof and Zwätzen), all analysed individuals originated from their natal populations (Supplementary Appendix 3, Figures a–d). In the remaining subpopulations, most individuals were most likely to have originated from their natal patches, and only a few individuals (1–6) are predicted to have come from other subpopulations, for example, in Burgau-1, one individual may have originated from either Göschwitz-Bhf or Dornburg-Bhf, and in Fritz–Winkler–Straße, six individuals showed a high likelihood of having originated from other subpopulations (Supplementary Appendix 3, Figures e–k).

Analysis of within- and between-population relatedness using ML-RELATE showed that all populations had high proportions of full (1.0000), high (0.5001–0.9999) or moderate (0.2501–0.5000) degrees of relatedness within the population (Figure 3). In contrast, relatedness among populations was very low, as seen from the high proportions of unrelatedness (that is, zero) and low relatedness (0.0001–0.2500) values among pairs of individuals of different populations.

Table 3 Pairwise F_{ST} values (under the diagonal) of each population comparison according to Weir and Cockerham (1984) and their level of significance (above the diagonal)

	Burgau-1	*Burgau-2*	*Dornburg-Bhf*	*Fritz–Winkler–Straße*	*Göschwitz-Bhf*	*Jena Nord-1*	*Jena Nord-2*	*Jena West-Bhf*	*Porstendorf-Bhf*	*Saalbahnhof*	*Zwätzen*
Burgau-1		***	***	***	***	***	***	***	***	***	***
Burgau-2	0.111		***	***	***	***	***	***	***	***	***
Dornburg-Bhf	0.105	0.121		***	***	***	***	***	***	***	***
Fritz–Winkler–Straße	0.161	0.141	0.154		***	***	***	***	***	***	***
Göschwitz-Bhf	0.145	0.159	0.084	0.119		***	***	***	***	***	***
Jena Nord-1	0.196	0.161	0.226	0.187	0.226		***	***	***	***	***
Jena Nord-2	0.101	0.070	0.065	0.124	0.094	0.169		***	***	***	***
Jena West-Bhf	0.174	0.089	0.165	0.137	0.176	0.198	0.103		***	***	***
Porstendorf-Bhf	0.091	0.096	0.078	0.130	0.140	0.204	0.069	0.093		***	***
Saalbahnhof	0.219	0.202	0.221	0.207	0.236	0.220	0.198	0.214	0.223		***
Zwätzen	0.206	0.126	0.122	0.159	0.170	0.233	0.099	0.158	0.160	0.244	

*** $=P<0.001$.

Table 4 Garza–Williamson indices per locus for each parasitoid population sample and means per locus for each population

	Lhirt01	Lhirt02	Lhirt03	Lhirt04	Lhirt06	Lhirt08	Lhirt10	Lhirt15	Lhirt23	Lysi08	Lysi6b12	*Mean G–W index/population*
Burgau-1	0.056	0.133	0.171	0.071	0.120	0.500	0.500	0.065	0.172	0.333	0.111	0.203
Burgau-2	0.079	0.200	0.195	0.063	0.280	0.500	0.500	0.065	0.138	0.667	0.222	0.264
Dornburg-Bhf	0.090	0.267	0.146	0.055	0.120	0.500	0.500	0.065	0.138	0.667	0.222	0.252
Fritz–Winkler–Straße	0.112	0.267	0.146	0.047	0.120	0.500	0.500	0.097	0.103	0.667	0.111	0.243
Göschwitz-Bhf	0.067	0.267	0.122	0.039	0.120	0.500	0.500	0.097	0.172	0.667	0.222	0.252
Jena Nord-1	0.090	0.200	0.146	0.063	0.160	0.500	0.500	0.065	0.138	0.667	0.222	0.250
Jena Nord-2	0.112	0.267	0.171	0.071	0.080	0.500	0.500	0.065	0.138	0.667	0.222	0.254
Jena West-Bhf	0.101	0.267	0.122	0.063	0.160	0.500	0.500	0.065	0.138	0.667	0.222	0.255
Porstendorf-Bhf	0.079	0.200	0.146	0.039	0.080	0.500	0.500	0.097	0.138	0.667	0.222	0.243
Saalbahnhof	0.045	0.200	0.122	0.071	0.120	0.500	0.500	0.097	0.138	0.667	0.222	0.244
Zwätzen	0.045	0.133	0.122	0.031	0.080	0.500	0.500	0.065	0.138	0.667	0.222	0.228
Mean G-W index/locus	0.080	0.218	0.146	0.056	0.131	0.500	0.500	0.076	0.141	0.636	0.202	0.244

Abbreviation: G–W index, Garza–Williamson index.
Values close to zero = population or locus in bottleneck, whereas values close to one = non-bottlenecked locus or population.

Discussion

This study highlights variations in levels of genetic diversity and differentiation among different *L. hirticornis* subpopulations (demes) comprising the metapopulation. Another main finding is the restricted level of aerial dispersal of the winged adult parasitoids and the lack of significant IBD as calculated from the molecular genetic data over the geographical scale studied. Population assignment measures indicate small amounts of genetic 'leakage' (gene flow) between populations, but with highly related individuals within sites compared with between sites, a characteristic of philopatric behaviour. Given the similar F_{ST} and heterozygosity results for *L. hirticornis* in this study to those of the earlier independent study of *M. fuscoviride* by Massonnet (2002), we suggest that *L. hirticornis* population genetic/ecological structuring and dynamics are directly influenced by the metapopulation genetic structure of its aphid host.

Parasitoid metapopulation ecology in the field

In the field study, winged adult *L. hirticornis* were observed to fly as well as walk between ramets within individual tansy genets harbouring aphid colonies. It is therefore highly probable that *L. hirticornis* flies, at least occasionally, to nearby genets and ramets. Our field results show that the number of ramets occupied by the parasitoid increased over time. Although cases of up to 100% aphid mummification on a ramet or genet were recorded, aphid colonies on some ramets and genets had no mummies throughout the season (Figure 2). These results thus confirm those of Weisser (2000) who also found that many genets colonized by aphids within a tansy stand were not attacked by the parasitoid, even though instances in which 100% of aphids in a ramet and/or nearby genet were parasitized. Rauch and Weisser (2007) similarly reported up to 100% rates of parasitism and noted that *L. hirticornis* was rarely found on plants on which it had not been released.

Genetic variability and differentiation between populations

Genetic diversity for *L. hirticornis* in our study measured as observed heterozygosity was between 0.170–0.367, and hence is in the range of other parasitoid populations studied using microsatellites, for example, $H_O = 0.171–0.629$ in *Neotypus melanocephalus* Gmelin (Hymenoptera: Ichneumonidae) (Anton *et al*., 2007); 0.486–0.594 in *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae) (Hufbauer *et al*., 2004); and 0.250–0.538 in *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae: Aphidiinae) (Baker *et al*., 2003). Global population differentiation in *L. hirticornis* ($F_{ST} = 0.148$) is slightly higher than in *N. melanocephalus* ($F_{ST} = 0.116$) (Anton *et al*., 2007). *Cotesia melitaearum* Wilkinson (Hymenoptera: Braconidae) and *H. horticola* had F_{ST} values of 0.378 and 0.063, respectively (Kankare *et al*., 2005). It seems, however, that *L. hirticornis* has dispersal behaviour similar to *N. melanocephalus* and *C. melitaearum*. Population assignment measures indicate small amounts of genetic leakage between the subpopulations and relatedness analysis shows that individuals within a site were highly related compared with between sites, findings that further support the view that *L. hirticornis* displays philopatric behaviour.

Compared with G_{ST} and F_{ST} values, actual differentiation (*D*) values were higher and closer to unity, consistent with Jost (2008). The three measures indicated high levels of differentiation within populations and unlike Jost (2008), our results show that despite the presence of private alleles at a number of loci, *L. hirticornis* metapopulations shared many alleles.

With regard to population bottlenecks in our study, distorting allele frequencies and using graphical method (Luikart *et al*., 1998) to test for severe reduction in population size failed to show such restrictions. In contrast, the G–W index gave low values (means < 0.3), and as values close to unity are expected in populations that have not been through bottlenecks (Garza and Williamson, 2001), it is probable that bottlenecks are at least partially responsible for the population genetic structuring seen in *L. hirticornis*.

Departures from HWE, as found in all populations, may be due to either null alleles or violations of other assumptions inherent in fulfilling HW expectations. These include non-random mating, migration, selection, genetic drift, mutation and severe bottlenecks (discussed in preceding paragraph), and, in the case of the essentially neutral molecular markers used here, could be due to close linkage to areas under selection (hitchhiking). Failed amplifications were experienced in some individual parasitoids at some loci, even when the

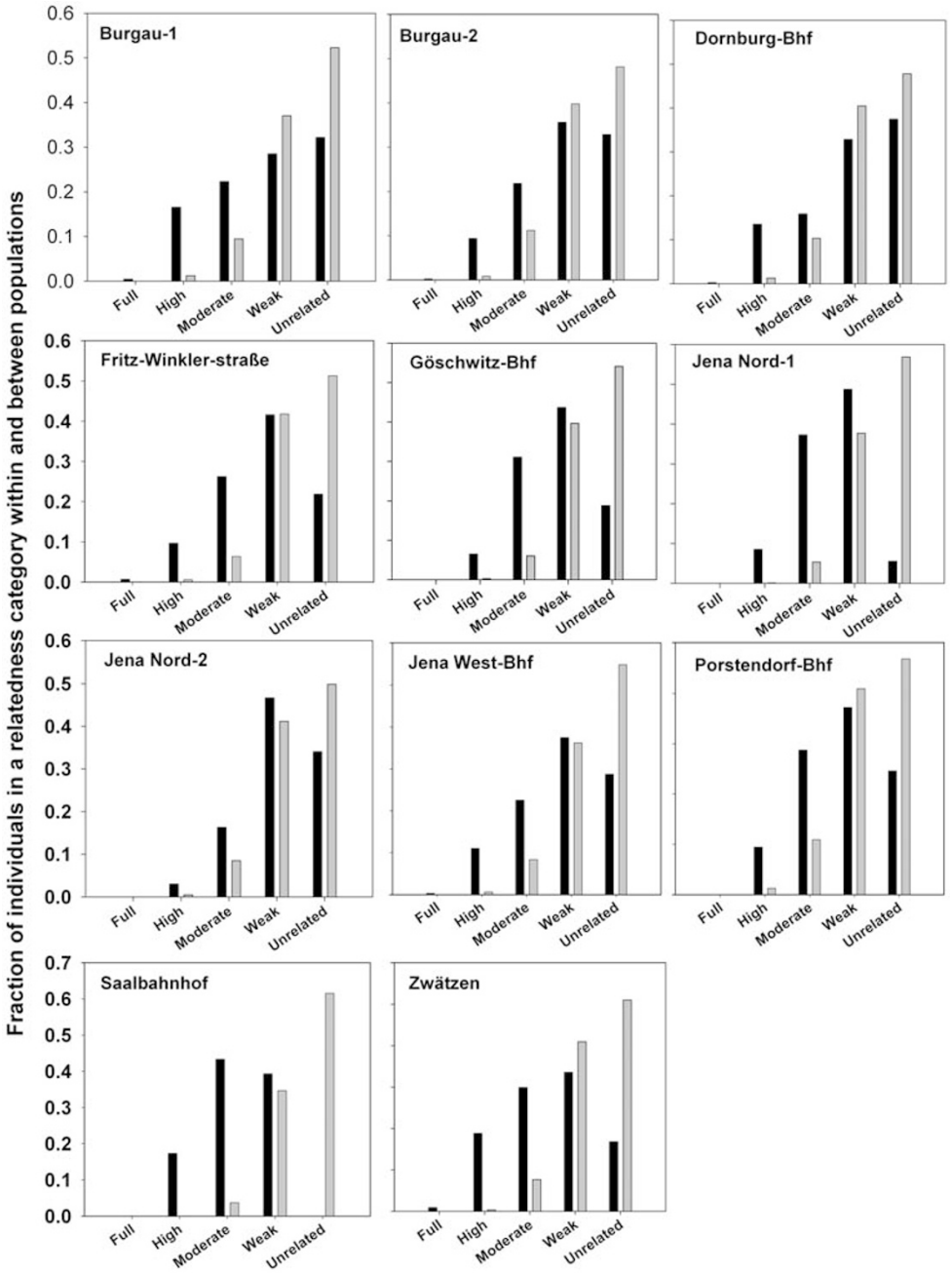

Figure 3 Within- and among-population relatedness of *L. hirticornis* individuals. For each of the 11 populations, pairwise levels of relatedness were calculated between individuals of the same population (within-population, full black bars) and between individuals of a population to individuals of other populations (among-population, light grey bars). Relatedness values were grouped as follows: 0 = unrelated; 0.0001–0.25 = weakly related; 0.2501–0.5000 = moderately related; 0.5001–0.9999 = highly related and 1.0 = fully related.

same individuals amplified satisfactorily at other loci. Nyabuga *et al*. (2009) report the possibility of null alleles at 6 of the 11 loci used in this study. It is unlikely, however, that null alleles were a main source of HW deviation because 88% of the male haploid parasitoids genotyped consistently gave the expected hemizygous genotype pattern at each of the 11 loci tested.

The F_{IS} values were all positive (homozygote excess) in all subpopulations, indicating that non-random mating occurred. Private or unique alleles were also recorded for the different subpopulations at different loci and these results support the view that the parasitoid tends to undergo inbreeding at a very local level. The 10 repeated MLGs derived from parasitoid populations on aphids infesting a total of 10 tansy plants at 5 of the 11 sites examined. One site (Zwätzen) had four repeated MLGs deriving from four genets. This implies, importantly, that the unit of random mating is within a genet or a group of closely located genets, rather than at the level of the whole site.

Previous behavioural studies (see review by Völkl, 1997) established that a single *L. hirticornis* female oviposited many eggs (up to 30) within aggregated aphid colonies. Recent laboratory studies showed that

L. hirticornis stayed on the natal patch for up to 4 h after emergence, and 52 out of 58 individuals (~90%) observed had matings on the natal patch (FN Nyabuga *et al*., unpublished), emphasizing the philopatric nature of *L. hirticornis* involving inbreeding.

Comparison of *L. hirticornis* genetic differentiation with that of its aphid host

Previous studies indicate that the haplodiploid nature of parasitoids (haploid males are produced from unfertilized eggs, diploid females from fertilized eggs) results in higher population genetic differentiation than in diploid organisms (Johannesen and Seitz, 2003; Anton *et al*., 2007). For example, Anton *et al*. (2007) reported higher F_{ST} values in the parasitoid *N. melanocephalus* (0.115) compared with its host butterfly, *M. nausithous* (0.087). Using a simulation study, they found that only part of the higher genetic differentiation of the parasitoid can be attributed to haplodiploidy. Massonnet (2002), working on *M. fuscoviride*, reported an overall F_{ST} of 0.222, with values ranging from 0.066 to 0.230 in Germany (Bayreuth and Münster) and an even lower F_{ST} (0.148) for populations from Alsace in France. Populations of the aphid host *M. fuscoviride* (Massonnet, 2002) and *L. hirticornis* (this study; $F_{ST} = 0.148$) are thus both highly genetically differentiated, with no difference in F_{ST} values between the two species, the parasitoid at small spatial scale and the aphid on a larger geographical scale. Perhaps the failure to find higher F_{ST} values for the parasitoid compared with the host is due to the different sampling period or to the different sampling parameters, that is, localities and spatial levels. Massonnet (2002) reported H_E values ranging from 0.27 to 0.52 for *M. fuscoviride*, whereas our results ranged from 0.423 to 0.533 for *L. hirticornis*, essentially rather similar.

Philopatry and survival in a metapopulation context

The ecological and genetic data presented here, in addition to unpublished behavioural data (Nyabuga *et al*., in preparation), reveal a high level of *L. hirticornis* philopatry within *M. fuscoviride* colonies with little aerial dispersal between them. Thus, even though local extinctions of the aphid host are frequent, the parasitoid continues to exploit its host and contributes greatly to the demise of local aphid colonies, rather than spreading its genes over many aphid subpopulations, thereby (potentially at least) increasing genetic heterogeneity. In fact, this study system represents one of the few field examples involving metapopulations in which host colony extinction is directly due to natural enemy attack (Weisser, 2000). The low probability of the host population's survival raises the question as to why this particular behaviour involving philopatry has evolved, rather than a behaviour that would spread the risk, that is, an oviposition strategy that distributes eggs over several aphid colonies, as observed in many other parasitoids (Mackauer and Völkl, 1993). The parasitoid is apparently able to evade ant attacks by chemical mimicry, as found in *Lysiphlebus cardui* (Marshall) attacking the black bean aphid, *Aphis fabae cirsiiacanthoidis* Schrank on creeping thistle (Liepert and Dettner, 1996). We argue that a key trait of the parasitoid—the ability to use chemical mimicry to evade attack by aphid-tending ants—explains this counter-intuitive behaviour. As in many *Lysiphlebus* species, chemical signals on the epicuticular surface of *L. hirticornis* mimic those of its aphid host, hence making the parasitoid chemically undetectable to ants (Dettner and Liepert, 1994; Liepert, 1996). As a consequence, the parasitoid is able to exploit ant-tended aphid colonies and the offspring obtain protection by ants from attack by even higher trophic levels, that is, hymenopterous hyperparasitoids (Völkl, 1992). Ant-tended colonies also persist longer than untended ones. By concentrating ovipositions into already encountered ant-tended colonies, parasitoids apparently realize higher reproductive success than would otherwise be possible by costly dispersal and oviposition into several aphid colonies, many of which do not survive for very long (a mean survival time of 4.8 weeks has been reported for aphids at the genet level; Weisser, 2000).

In conclusion, this study, the first on an aphid–parasitoid metapopulation system, shows that *L. hirticornis* has limited dispersal between resource patches or interpopulation movement coupled with philopatry and inbreeding. Genetic differentiation measurements clearly identified similarities between genetic structuring in *L. hirticornis* and its aphid host, *M. fuscoviride*.

Conflict of interest

The authors declare no conflict of interest.

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