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ORIGINAL PAPER

Host associations of *Coenonympha hero* (Lepidoptera: Nymphalidae) in northern Europe: microhabitat rather than plant species

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Abstract Understanding ecological requirements of endangered species is a primary precondition of successful conservation practice. Regrettably, we know surprisingly little about the life history of numerous threatened insects, and about their use of larval host plants in particular. The brown butterflies (Nymphalidae: Satyrinae) have traditionally been considered polyphagous on grasses and indiscriminatory in their oviposition behavior. However, detailed studies on several species have revealed local specialization in host plant use as well as the decisive role of microlimatic conditions as determinants of habitat quality. The present study addresses host plant relationships in the endangered brown butterfly *Coenonympha hero* (L.) at the northern limit of its European distribution. We combine laboratory-based host preference and performance tests with an analysis of microhabitat use by adult butterflies in the field. Both lines of evidence suggest that *C. hero* is polyphagous enough not to be associated with one particular host species. Oviposition choices of *C. hero* are not driven by host plant species but rather by structural characteristics of the substrate. The preferred rigid needle-like structures may serve as cues of 'transparent' vegetation which allows the larvae to benefit from sunlight reaching the lower strata of the tuft. Our results suggest that conservation efforts should prioritize microclimatic parameters, rather than the presence of any particular host plant species, as decisive determinants of habitat quality in *C. hero*.

Keywords Satyrinae · Scarce heath butterfly · Preference performance linkage · Conservation · Growth rate · Habitat management · Grazing · Monophagy · *Coenonympha oedippus* · Habitat use

Introduction

The loss of biodiversity remains a serious concern: it has been estimated that one third of European butterfly species are currently declining (van Swaay et al. 2010). The cornerstone of successful conservation practice is understanding the basic ecological needs of the endangered species: primarily, the set of parameters defining a suitable habitat. Regrettably, we know surprisingly little about the life history of numerous threatened insects (van Swaay and Warren 1999). Deficient knowledge frequently turns conservation work into a guessing game in which there is a "gut feeling" of how the favourable habitat looks, but it may remain largely unknown which elements of it are actually essential for the target species (Dolek et al. 2005; Brückmann et al. 2010).

In the practice of conservation work, a critical mistake would be to assume that species' requirements are wider than they actually are. Indeed, there are a number of cases where butterfly conservation has failed due to such errors

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(Pullin 1996). The risk of this kind of misfortune appears particularly high for the "browns" (Nymphalidae; Satyrinae), which are, in various taxonomic handbooks and field guides, described as generalists on grasses. In addition, grass feeding butterflies are generally believed to be indiscriminate in their choice of oviposition site (e.g. Wiklund 1984; Bergman 2000). However, a different picture has emerged from the data accumulated for some extensively studied satyrine species (e.g. Gotthard 2004). In addition, geographic variation cannot also be neglected in this context: for example, the larvae of the pearly heath *Coenonympha arcania* (Linnaeus, 1761) have been found to use 11 host plants in mainland Europe, whereas only one has been confirmed for Sweden (Nylin and Bergström 2009; Nylin et al. 2014).

Along with specialization to host plants *per se*, butterflies are often highly selective with respect to microhabitats. Microclimate has indeed been frequently shown to be the crucial aspect of habitat suitability, especially at the margins of a species' distribution range (e.g. Roy and Thomas 2003; Eilers et al. 2013; Örvössy et al. 2013; Lawson et al. 2014). This type of selectivity can also limit the set of host plants used for oviposition (Anthes et al. 2008; Gibbs and Van Dyck 2009; Bennie et al. 2013): only some of the potential host species may grow in conditions supporting larval development. For example, in the case of the grizzled skipper *Pyrgus malvae* (Linnaeus, 1758), host plant use was shown to be primarily driven by microhabitat preferences of the candidate plants (Krämer et al. 2012). Among satyrines, microclimatic conditions appear to be a primary criterion for oviposition site selection in the false ringlet *Coenonympha oedippus* (Fabricius, 1787): spring temperatures must be high enough to ensure successful development of the larvae (Čelik et al. 2015). The crucial role of microclimate may imply that even butterflies that are generalists at a larger scale can be functional specialists due to abiotic factors restricting the choice of oviposition sites.

The endangered (Van Swaay and Warren 1999; Van Swaay et al. 2010, 2012) scarce heath butterfly (*Coenonympha hero*, Linnaeus, 1761; Satyrinae, Nymphalidae) is one of the species believed to be a generalist feeding on various grasses (Cassel et al. 2001; Cassel-Lundhagen and Sjögren-Gulve 2007; numerous field guides), with some reports of also using sedges (Bräu and Dolek 2013). However, the idea about a broad ecological niche of this species is not consistent with the patchy distribution pattern of the butterfly, nor with its high sensitivity to landscape change (Soga and Koike 2012). Indeed, *C. hero* is decreasing rapidly in many countries in Central and Western Europe (van Swaay et al. 2012), which calls for increasing the deficient research-based empirical evidence on its host plant preferences and habitat use.

The objective of the current study was to explore host plant use of *C. hero* in Estonia where the species still has a favourable status. We used a combination of approaches with a common goal to evaluate the possibility of *C. hero* being specialised on a particular host species, as opposed to being a generalist feeder on grasses. Laboratory trials were conducted to determine host preference of ovipositing females as well as that of newly hatched larvae. Larval performance on different host plants was measured in rearing experiments. To obtain further information about host plant associations, and to select candidate plants for our laboratory trials, we performed an analysis of habitat use of the butterfly in Estonia. The resulting small-scale model was based on vegetation parameters recorded in the immediate surroundings of resting points of adult butterflies. Finally, we integrate the results of the different sub-studies to discuss the likely causes of host and habitat preferences in *C. hero*.

Materials and methods

Study species

Coenonympha hero is a small (wing span 27–32 mm) slow-flying satyrine butterfly distributed over much of the Palaearctic region, reaching the northern limit of its European range in Estonia. This species typically inhabits seminatural bushy meadows and woodland clearings. *C. hero* is univoltine with the flight period starting from early June and lasting to early July in northern Europe. The grass-feeding larvae overwinter in their third instar, growth resumes in spring, and the larvae pupate having gone through 5 instars (Cassel-Lundhagen and Sjögren-Gulve 2007). As is the case for many satyrines (Tolman and Lewington 1997), the larvae are cryptic and difficult to find in their natural habitats which implies that indirect methods must be used to study the species' use of host plants.

For our laboratory studies on host plant relationships of *C. hero*, we used wild collected females from various sites across Estonia, and their offspring. In most cases, the wild caught females were used in the experiments on the same day. With of longer transportation times, females were kept in a cool transportation box (ca 10 °C) and used in the experiments within 48 h. The main body of laboratory experiments were carried out at the University of Tartu in 2012 and 2013, while field work was conducted in western Estonia in 2013. In 2015, some of the laboratory experiments were repeated to include *Festuca rubra*, a potential host species found to be associated with *C. hero* in the field study.

Oviposition preference

Wild-caught females were subjected to multiple choice tests. In the 2012 experiments, each female was offered five oviposition substrates simultaneously. Four of the substrates used in these experiments were potential host plants: *Festuca ovina, Dactylis glomerata*, *Calamagrostis epigejos* and *C. arundinacea* (all Poaceae). *F. ovina* and *D. glomerata* were selected because these plants had been successfully used to rear *C. hero* caterpillars previously (Cassel et al. 2001; Cassel and Tammaru 2003). The two *Calamagrostis* spp. were added as grasses abundant in several *C. hero* habitats on the Estonian mainland. *F. ovina* differs from the rest of the grass species used in that it has very narrow, needle-like leaves. To test if females may also lay their eggs on substrates completely unsuitable as larval hosts, we used Norway spruce (*Picea abies*) as a control plant (not utilised as a host by any European butterfly). The experiment was repeated in 2015 to include a comparison between *Festuca rubra* and *F. ovina*, with again *Picea abies* as the control.

For the multiple choice tests, adult females were housed singly in transparent boxes (25 × 25 × 15 cm). Similarly sized small (about 12 cm in length) plant bunches were placed, in jars with water, circularly in equal distances between neighbouring jars. The order was randomized for each replicate. Sugar-water solution was offered as food for the female using damp tissue paper located at the middle of the box. Egg laying behaviour was initiated by 18 W fluorescent lamp set above the box which resulted in constant temperature of about 27 °C inside the box. Females were kept in the setting for 48 h, and light was on for 18 h daily. The number of eggs laid on different substrates was recorded thereafter.

The number of eggs laid on each plant in each trial was analysed as dependent on the plant species using a Poisson mixed model accounting for overdispersion, the type III Chi squared test was based on model deviance. Female identity was included in the models as a random factor. To visualize rank order of plant species, the number of eggs on each of the candidate plants was compared against the arbitrarily chosen reference plant (*F. ovina;* also for all other experiments). If not stated otherwise, all statistical analyses were performed in the R environment (R Core Team 2014) using package lme4 (Bates et al. 2014).

In single substrate oviposition trials, conducted in 2013, wild caught females were placed singly in 500 ml transparent boxes, accompanied by a bunch (or twigs) of one out of three plants: *F. ovina*, *D. glomerata* or *P. abies*. The selection of the substrates offered was motivated by the results of the multiple choice tests. After 72 h, the experiment was terminated, and the eggs were counted. The influence of plant species on the number of eggs laid was tested using an ANCOVA with host plant as the categorical factor, and remaining life span of the female as a covariate (an index of female age: females living longer in the laboratory were likely younger when captured). Further, to obtain the ranking order for host plants, the number of eggs on each plant was compared to the reference plant (*F. ovina).*

Larval preference

In 2012, the host plant preference of neonate larvae was tested using a set of plant species identical to that in the female multiple plant choice test: *F. ovina*, *D. glomerata*, *C. epigejos* and *C. arundinacea.* The neonates were offspring of the butterflies used in the oviposition preference tests. The larvae were allowed to choose between sections of two plant species which were offered in all six possible combinations. A Petri dish was prepared with damp filter paper at the bottom and equally sized (ca 3 cm) leaf sections from each plant were placed on the opposite sides of the dish (Lindman et al. 2013), with a newly hatched caterpillar in the middle. After 24 h, larval preference was recorded on the basis of caterpillar location and eating marks. In the typical case, the larva was found resting on the plant it had eaten, which made recording the preference straightforward. The cases where larva had died during the trial were excluded. Laboratory temperature was 23 °C during the experiment.

To infer the overall preference rank order of the four plant species from (all possible) pairwise comparisons, we used Bradley–Terry model (a type of generalized linear model, Bockenholt 2001), with a random "judge" factor to incorporate the effect of brood. This analysis was performed using an original SAS (SAS Institute Inc. 2008) script, available from the authors upon request.

In 2015, the experiment was repeated so that *Festuca rubra* and *F. ovina* were compared in pairwise settings. The results were analysed with a binomial mixed effect model including female identity as a random factor.

Larval performance

Larval performance on different hosts was tested using a partly different set of candidate plants, adjusted considering the data obtained in the course of oviposition experiments (above), and the field study (below). In 2013, *F. ovina* and *D. glomerata* were included as plants preferred in the oviposition choice experiment. In addition, *Sesleria caerulea* and *Helictotrichon pratense* were included as these grasses appeared to be of high abundance in the sites in which the field study was performed. The identically designed experiments of 2015 compared the performance on three grass species: *F. ovina* (preferred for oviposition), *F. rubra* (positively associated with butterfly presence in the field, see below) and *D. glomerata* (a grass the butterfly is unlikely to be specialized to, due to habitat differences).

Newly hatched larvae of *C. hero* were placed singly in 60 ml jars, with a bunch of about 5 cm long plant sections that were renewed on a daily basis. Larval survival was checked daily, and surviving larvae were weighed at the age of one week. Mortality rate was analysed as dependent on host species using a Cox proportional hazards model for clustered data (to accommodate the effect of brood), with survival probability being modelled using *F. ovina* as the reference plant.

Differences in larval weight between the plants were tested by mixed ANOVA with Kenward–Roger ddf correction. The model included brood as a random factor. In order to obtain ranking order of the plants offered, the reference plant (*F. ovina*) was compared to other plants.

Host plant associations in the field

The search for plants potentially associated with the presence of *C. hero* relied on comparison of the points in which a butterfly had been observed (presence points, hereafter), with control points selected within the same habitat patch (=site, hereafter; area 4–18 ha; Sang et al. 2010; Tiitsaar et al. 2013). In the resulting microhabitat use model, predictor variables included abundances of particular plant species: both those considered as potential hosts, as well as those indicative of abiotic parameters of the site, the latter primarily functioning as covariates in the analyses of potential host plant associations.

The study was performed at six sites (>5 km apart) on the islands of Saaremaa and Muhu in western Estonia. In that area, *C. hero* is a relatively common species in suitable habitats, semi-natural calcareous grasslands with a deep soil layer. None of the sites occupied by *C. hero* were currently being managed although our preselection sample (i.e. patches of 'butterfly habitat' being surveyed by the authors: Sang et al. 2010; Tiitsaar et al. 2013; unpublished data) included both grazed (16 sites, with *C. hero* being absent from all of those) and unmanaged grasslands (45 sites). For the present study, we selected all these six sites in which *C. hero* was known to occur in 2007 or 2008, and was found again in both 2012 and 2013. All these sites represented abandoned grassland in various stages of overgrowth, surrounded by forest or agricultural land which made delimiting the habitat patches straightforward.

Within the sites, we systematically searched for resting *C. hero* adults. The exact resting point (i.e. the presence point) was marked and the individual was captured to determine its sex. Control points were selected within the same site 10 m apart from the occupied point. Care was taken to ensure that selection of the control points occurred in a random manner though it was obviously reasonable (and, mostly, also technically inevitable) to avoid habitats unsuitable for any grassland butterflies (forests, *Juniperus* thickets, water beds). Naturally, the control points cannot be treated as true absence points as some of them might have been occupied by *C. hero* butterflies at a different time point. Nevertheless, a comparison of points occupied and not occupied at a particular moment must contain information relevant to microsite preferences of the butterfly. We aimed at selecting equal number of female, male and control points.

Some of the captured females were retained for laboratory experiments (see above), males and excess females were released. The released individuals were marked to avoid multiple recording. All the field data were collected during the active flight time of butterflies (9:30–19:00, temperature above 16 °C, and minimum of 60 % of sunshine). Vegetation parameters were recorded within a circle of ($r = 1$ m) around each of the selected points. In particular, cover of all the vascular plant species present, shrub cover and vegetation cover were estimated visually, whereas vegetation height was measured as average height of dominant herbs in the circle. All estimations and measurements were done by the same person, with the expert botanist being unaware of the type of the point (presence vs. control).

Generalized linear mixed models for binary data were constructed to discriminate the presence points from control points, with 'site' being included as a random variable. Predictor variables included the cover of the seven most common (recorded at least 15 of 58 sampling points) *Poaceae* species (as potential hosts). The rationale was that if any particular host plant species was, indeed, an essential determinant of the *C. hero* presence, it cannot be too rare at the surveyed sites: all the sites had held *C. hero* for at least 3 years prior to the study. The rest of the predictors were environmental parameters which were either measured directly (shrub cover, vegetation height and overall vegetation cover) or were estimated on the basis of the floristic composition of the sampling point. In particular, Ellenberg cover-weighted fertility, light and moisture indices were calculated for each point on the basis of estimated cover of each plant species, and corresponding species-specific Ellenberg values (Schaffers and Sykora 2000; Diekmann 2003) using the freeware program MAVIS (2000). To avoid collinearity among predictor variables, we excluded those *Poaceae* species that were already included separately (see above). As a result, the values of thirteen different parameters were calculated for each sampling point.

To compare models with different sets of predictors, we used the variable ranking procedure based on the Akaike information criterion (as described by Anderson et al. 2000; Burnham and Anderson 2004; Johnson and Omland

2004). Models with all possible combinations of predictors were run, and the models were ranked using the AIC_C value. Subsequently, model averaging was used to estimate the importance of each parameter. Initially, we analysed the data for male and female butterflies separately. However, as the results were highly consistent for the two sexes, we present the analyses with sexes pooled, i.e. for all the presence points compared to the control points.

Results

Oviposition preference

In 2012 a total of 27 females were used in multiple choice tests in the laboratory. Of these, 21 females laid eggs, 522 eggs altogether (range 1–69; mean 19.3 ± 3.0 SE), of which 329 were attached to any of the plant species, and could thus be considered in further analyses (the rest were laid on cage walls, floor etc.). Although at least a few eggs were recorded on all plants species (Table 1), the number of eggs clearly differed between the plants offered (GLZ assuming Poisson distribution, $\chi^2 = 34.1$, $df = 4$, $p < 0.001$). *F. ovina* was strongly preferred over *C. arundinacea*, *C. epigejos* and *D. glomerata*. Surprisingly, in 2012, the number of eggs on the control plant, Norway spruce (*P. abies*), was equal to the number of eggs on *F. ovina*. In 2015, the 8 females which were allowed to choose between three candidate plants clearly preferred *F. ovina* over *F. rubra* and *Picea abies* (GLZ assuming Poisson distribution, $\chi^2 = 11.7$, $df = 2$, $p = 0.003$; Table 1).

Table 1 Results of multiple choice tests

	N	Range	Estimate	SE	z value	*p*
2012						
C. arundinacea	11	0–6	−3.38	0.95	−3.56	**<0.001**
C. epigeios	2	0–1	−4.43	1.13	−3.93	**<0.001**
D. glomerata	7	0–3	−3.33	0.93	−3.58	**<0.001**
P. abies	154	0–45	0.12	0.78	0.16	>0.05
F. ovina	155	0–31	–	–	–	–
2015						
F. rubra	39	0–35	−3.19	1.19	−2.67	**0.007**
P. abies	11	0–9	−3.96	1.27	−3.13	**0.002**
F. ovina	145	0–35	–	–	–	–

Significances <0.05 are marked in bold

Ovipositing *C. hero* females were provided plants to choose from, and the number of eggs laid on a plant was modelled as dependent on host plant species using Poisson mixed effects model with overdispersion accounted for. Egg numbers laid on a given plant species were tested against the reference plant, *F. ovina*. N—number of eggs laid on a plant, summed over the females in the experiment (27 in 2012, 8 in 2015). Range—the range of the number of eggs laid on particular plant species in a replicate

Single substrate oviposition trials were conducted with 34 wild caught females, equally divided between 3 plant species: *F. ovina*, *D. glomerata* or *P. abies*. During the 72 h experiment, the females laid a total of 972 eggs (2–73 per female, 28.6 ± 2.8 SE on average). Although eggs were laid on all substrates offered, the number of eggs depended on plant species (ANOVA: $F_{2,31} = 4.62$, $p = 0.018$). In particular, females laid significantly more eggs on *F. ovina*, as compared to *D. glomerata* (or the control plant *P. abies*, Table 2). The effect of the covariate—female age—was not significant ($F_{1,31} = 0.27$, $p = 0.61$).

Larval preference

In 2012, a total of 757 neonatae larvae (offspring of 13 females) were used in larval preference tests; 605 of them survived until the end of the experiment. Larval host plant preference could unambiguously be recorded for 289 larvae; the remaining ones were found in the experimental arena in situations other than resting on any of the plant fragments. In concordance with female host choice experiments, larvae preferred *F. ovina* over other plants (Table 3), the least preferred plant species being *D. glomerata*. In 2015, the test was repeated to assess the preference between *F. ovina* and *F. rubra*. From 45 larvae (9 broods), a clear majority (77 %) selected *F. rubra* (binomial mixed-effects model: $z = 3.49$, $p < 0.001$).

Despite the selectivity, in both years and in all combinations, all the offered plant species were accepted and eaten by some of the larvae (see Table 3). In the 2012 experiment, the probability to make a recordable choice did not depend on plant combination offered ($\chi^2 = 7.2$, $df = 5$, $p = 0.21$).

Larval performance

In 2013, 277 (43.6 %) of neonate larvae survived to the age of 7 days, this value being higher in 2015 (62 out of 88: 70.5 %). In 2013, survival during the first week of larval development did not differ between the grasses offered (Cox proportional hazard model for clustered data: Robust's score = 5.46, $p = 0.14$). In 2015, survival on *D. glomerata* and *F. rubra* was significantly higher compared to *F. ovina* (Robust's score = 7.23, $p = 0.027$; the contrast between *F. ovina* and *D. glomerata*: $z = -2.50$, $p = 0.012$, *F. ovina* vs. *F. rubra*: $z = -5.22$, $p < 0.001$).

Among-plant differences in growth performance, measured as larval weight at the age of 7 days, were not dramatic (Table 4) but attained statistical significance (2013: $F_{3,274} = 8.37$, $p < 0.001$, 2015: $F_{2,43} = 15.45$, $p < 0.001$).

Table 2 Results of single substrate oviposition trials with *C. hero* females

	Aver.	Estimate	SE	t value	*p*
D. glomerata	21.6	−23.94	7.20	−3.34	**0.003**
P. abies	27.1	−16.25	6.59	−2.47	**0.012**
F. ovina	43.0	–	–	–	–
Life span after experiment		−0.41	0.62	−0.64	>0.05

Significances <0.05 are marked in bold

The number of eggs laid on *F. ovina* was compared to the number of eggs on other plants. *Aver.* average number of eggs laid during the 72 h experiment

Table 3 The results of the larval preference test

	Choice %	Estimate	SE	*DF*	t	*p*
C. arundinacea	46.8	−0.57	0.25	48.22	−2.29	**0.03**
C. epigeios	47.3	−0.57	0.25	47.79	−2.28	**0.03**
D. glomerata	39.8	−0.61	0.25	50.03	−2.42	**0.02**
F. ovina	64.3	–	–	–	–	–

Significances <0.05 are marked in bold

The probability of choosing a particular species is compared against that of the reference plant, *F. ovina* (Bradley–Terry GLZ model with random "judge" effect). Choice % shows how often the species was chosen when it was one of the two plants offered

In 2013, the larvae reared on *F. ovina*, *D. glomerata* and *H. pratense* were similar in weight whereas larvae reared on *S. caerulea* remained smaller compared to those on *F. ovina* (Table 4). In 2015, larval weights were significantly lower on *F. ovina* compared to either *F. rubra* or *D. glomerata* (Table 4).

Host plant associations in the field

The microhabitat use model was based on a total of 58 points which were described from six studied *C. hero* sites on Saaremaa and Muhu islands: 19 female and 19 male presence points compared to 20 control points. The surveyed grasslands were highly species rich: a total of 148 vascular plant species were recorded during the survey, 21 on average in each circle. Various species characteristic of calcareous grasslands were prevalent: *Carex flacca* (documented in 42 circles), *Sesleria caerulea* (39), *Galium boreale* (36), *Briza media* (35), *Galium verum* (35), *Inula salicina* (30), *Helictotrichon pratense* (29), *Filipendula vulgaris* (29), *Festuca rubra* (28), *Centaurea jacea* (25), and *Poa angustifolia* (27). Of the potential host plants, the estimated cover values of 7 most common grasses—*H. pubescens, H. pratense, F. rubra, F, ovina, S. caerulea, B. media* and *P. angustifolia*—were included as separate predictor variables in the analyses (see "Materials and methods" section for variable selection; Table 5).

In general, no model or single predictor received overwhelming support in models discriminating between the presence and control points (Tables 5, 6). Both model averaging and examination of top ranked models revealed that butterfly presence was positively related to parameters of physical environment: shrub cover, Ellenberg light and moisture value. Of particular host plants, only the cover of *F. rubra* appeared among the high ranked variables. By contrast, butterflies appeared to avoid locations with high cover of *H. pubescens*. Other variables had substantially lower predictive power (Tables 5, 6). Finally, we used a permutation test to assess the possibility that the high AIC rankings were a result of a chance only. Null hypothesis of no useful information in the model was rejected with $p = 0.0011$, as based on z values of five highest ranked predictor variables.

Table 4 Larval weight at 1 week of age as dependent on host species

	Aver. weight (mg)	Estimate	SE	*t* value	*p*
2013					
H. pratense	0.77	−0.11	0.059	−1.93	>0.05
D. glomerata	1.00	0.11	0.062	1.78	>0.05
S. caerulea	0.71	−0.17	0.058	−2.98	**0.003**
F. ovina	0.89	–	–	–	–
2015					
F. rubra	1.11	0.68	0.096	7.13	**<0.001**
D. glomerata	1.14	0.68	0.12	5.86	**<0.001**
F. ovina	0.42	–	–	–	–

Significances <0.05 are marked in bold

Performance on *F. ovina* is compared to that on other candidate plants using *t* tests

Table 5 Vegetation parameters and their relative importance measured in model averaging

Parameter	Relative importance	Medians (quartiles) of		The no. of points where present	Unit
		Presence point	Control point		
Ellenberg moisture	0.93	4.9 (4.5–5.6)	4.6 (4.4–4.9)	–	Score 1–10
Shrub cover	0.77	7.5 (0–20.0)	1.0 (0–5.0)	–	Coverage (%)
Ellenberg light	0.65	7.0 (6.9–7.3)	7.0 (6.9–7.1)	–	Score 1–10
Helictotrichon pubescens	0.60	0 (0–1.0)	0 (0–1.0)	20	Coverage (%)
Festuca rubra	0.44	0.5 (0–5.0)	0 (0–1.0)	28	Coverage (%)
Vegetation height	0.35	25.0 (20.0–30.0)	25.0 (20.0–30.0)	–	cm
Vegetation cover	0.34	84.0 (80.0–95.0)	90.0 (75.0–95.0)	–	Coverage (%)
Briza media	0.27	2.2 (0–0.6)	0.3 (0–0.8)	35	Coverage (%)
Sesleria caerulea	0.26	0.8 (0–1.0)	1.5 (0–1.3)	39	Coverage (%)
Festuca ovina	0.24	0.3 (0–1.0)	0 (0–0.3)	19	Coverage (%)
Ellenberg fertility	0.22	3.4 (2.8–3.9)	3.5 (3.2–4.0)	–	Score 1–10
Poa angustifolia	0.22	1.6 (0–0.3)	0.5 (0–0.3)	27	Coverage (%)
Helictotrichon pratense	0.22	4.6 (0–5.0)	0.2 (0–10.0)	29	Coverage (%)

The models were built to discriminate between butterfly presence and control points (see "Materials and methods" section for further details)

Discussion

Laboratory experiments showed that *Coenonympha hero* can indeed be considered a generalist feeder on grasses: no potential host species offered was refused by the larvae. Larval performance on different grasses did not show substantial differences even if the growth tended to be somewhat better on "fleshy" grasses like *D. glomerata* and *F. rubra,* as opposed to *F. ovina* and *S. caerulea*. In addition, females readily laid their eggs on substrates other than plants, which is a pattern characteristic of polyphagous Lepidoptera (Tammaru et al. 1995; Janz and Nylin 1997; Nylin et al. 2000). Field observations on habitat use were consistent with the lab-based results: environmental factors other than the presence of any particular host plant species ranked highest in the models of microhabitat use.

In some conflict with the suggested larval polyphagy, oviposition behaviour of *C. hero* females was far from indiscriminatory: the butterflies strongly preferred *Festuca ovina* and, surprisingly, in one of the experiments, also the control plant Norway spruce (*P. abies*). The high rank of *F. ovina* as well as laying eggs on non-host plants was confirmed in single substrate oviposition trials. Even if not directly testing for host preference, single-substrate tests provide information complementary to that delivered by multiple choice tests (Tammaru et al. 1995). In particular, in enclosures with multiple plant species, eggs may be laid on non-host plants due to the confounding effect of the proximity of higher ranked hosts while one substrate designs are free of this problem. In such experiments, the number of eggs laid during a certain (short) period of time is a measure of host acceptability (Javoiš and Tammaru 2004, 2006; Gamberale-Stille et al. 2014; Friberg et al. 2015).

Despite the well expressed oviposition substrate preference, there was no evidence of preference-performance linkage: the preferred *F. ovina* could not be shown to be a host supporting larval development better than its alternatives. Notably, in the experiments of 2015, *F. ovina* clearly bypassed *F. rubra* in terms of oviposition preference whereas the situation was the opposite for larval preference and performance. Moreover, even if *F. ovina* was present at five out of six of our fieldwork sites, the overlap with the occupied points was marginal and the relative importance of this covariate was low (Table 5). Furthermore, in one of the experiments, Norway spruce, a plant definitely not suitable for larval development, proved to be a highly ranked oviposition substrate. Spruce twigs were readily accepted also in the single substrate oviposition trials, showing that the stimuli from a suitable host plant are not essential for eliciting oviposition behaviour in *C. hero* (cf. Tammaru and Javoiš 2000*)*.

Combining several lines of evidence, the following scenario appears likely. *C. hero* females have selective oviposition preferring grasses (and perhaps other plants: to be confirmed in the field) with narrow and rigid leaves as substrates. We suggest that such a preference is not adaptive in terms of providing the offspring with a host plant of the 'right' species but is rather 'designed' to provide the larvae with suitable microclimatic conditions (see Krämer et al. 2012; Čelik et al. 2015; for similar results). The common feature of the preferred *F. ovina* and *P. abies* is

Table 6 Parameter estimates for the ten highest ranking models applied to discriminate between butterfly presence and control points

Ellenberg moisture	Shrub cover	Ellenberg light	*Helictotrichon pubescens*	*Festuca rubra*	Vegetation height	Vegetation cover	*Briza media*	*Sesleria caerulea*	*Festuca ovina*	Ellenberg fertility	*Poa angustifolia*	*Helictotrichon pratense*	*df*	logLik	AICc	ΔAICc
1.78	1.10	0.84	−0.61	–	–	0.08	–	–	–	–	–	–	7	−27.6	71.4	**0.00**
1.45	1.07	0.77	–	–	–	0.07	–	–	–	–	–	–	6	−29.05	71.7	**0.36**
1.01	0.96	–	−0.77	0.80	–	–	–	–	–	–	–	–	6	−29.08	71.8	**0.43**
1.29	0.82	0.63	−1.02	0.74	0.67	–	–	–	–	–	–	–	8	−26.51	72.0	**0.59**
1.54	1.18	0.72	−0.81	0.57	–	0.07	–	–	–	–	–	–	8	−26.64	72.2	**0.85**
1.04	1.01	–	−1.07	0.89	0.56	–	–	–	–	–	–	–	7	−28.01	72.3	**0.88**
1.2	0.87	0.51	−0.71	0.68	–	–	–	–	–	–	–	–	7	−28.01	72.3	**0.89**
1.2	0.65	0.60	–	–	–	–	–	–	–	–	–	–	5	−30.60	72.4	**0.98**
1.40	–	0.87	−0.70	–	0.75	–	–	–	–	–	–	–	6	−29.36	72.4	0.99
1.55	0.58	0.77	−0.73	–	0.62	–	–	–	–	–	–	–	7	−28.11	72.5	**1.09**

Significances <0.05 are marked in bold

Habitat parameters are ranked from left to right according to their relative importance

the presence of narrow and rigid needle-like structures which can therefore be hypothesized to serve as oviposition stimuli for *C. hero*. Consistently, such structural stimuli have been proposed to be decisive in oviposition site selection also in the related *C. oedippus* (Bräu et al. 2010; Sielezniew et al. 2010). The females can afford to lay their eggs without considering the species composition of surrounding vegetation (see Cassel and Tammaru 2003; Bräu and Dolek 2013 for some field observations) as the larvae appear polyphagous enough. Moreover, as shown by this study, the larvae are also capable of active host selection (see Bonelli et al. 2010; Lindman et al. 2013, for other satyrines). Indeed, oviposition indiscriminate with respect to host quality is expected to evolve in those species whose hosts are abundant enough, with higher oviposition rate (Tammaru et al. 1995; Janz and Nylin 1997; Nylin et al. 2000) and perhaps higher selectivity with respect to abiotic conditions being the associated benefits.

In agreement with observations on some other butterflies (Möllenbeck et al. 2009; Beyer and Schultz 2010), it has been recently shown for the related *C. oedippus* that direct solar radiation is critical for successful development of the larvae in spring. Being exposed to sunshine is therefore suggested to be a factor largely determining microhabitat suitability for that species (Bräu et al. 2010; Čelik et al. 2015). We thus hypothesize that the adaptive significance of preferring narrow-leaved grasses is in the 'transparency' of the plant cover: vegetation consisting of plants like certain *Festuca* spp. allows sunshine to reach the ground. The result is also in line with a positive effect of Ellenberg light index on the presence of *C. hero* in the field study.

Indeed, other environmental variables rather than the presence of any particular plant species ranked highest in our analysis of *C. hero* microhabitat use (Table 5). Even if fully consistent with the authors' experience and some published observations (i.e. Bergman et al. 2004), we cannot exclude the possibility that the positive effect of bush cover (and perhaps also Ellenberg light index) may have been inflated by a methodological artefact. As the butterflies were frequently observed next to juniper bushes exposed to the sun—which they may actually prefer as resting sites—we could often select the control points only by moving in the direction of decreasing bush cover (avoiding thickets: see "Materials and methods" section). Nevertheless, we cannot see a similar potentially confounding effect which could explain the high rank of moisture—in the models simultaneously including bush cover in particular.

The results are again congruent with those for *C. oedippus*: Šašic (2010) similarly demonstrated that the butterfly was more frequently present in patches with a higher Ellenberg moisture index. We have currently no data available to address the question of why moisture and the presence of shrubs positively affects habitat quality for the butterfly. The reason might be as simple as sensitivity of larvae and eggs to desiccation. Nevertheless, it appears likely that a combination of high soil moisture and light exposure is favoured by *C. hero* as this ensures that the host grasses do not dry out in the second half of summer, i.e. during the pre-hibernation larval development, but provides warm microclimate during spring development. The sensitivity to host quality in terms of wilting has been shown to be high both for *C. hero* (Cassel et al. 2001) as well as for some other satyrine butterflies (Bräu et al. 2010; Lindman et al. 2013). Moreover, the mortality of *C. hero* larvae in the laboratory was quite high (relative to various other lepidopteran species reared under analogous conditions, pers. obs. of the authors), even on the best host plants and variable between years. This is in line with the idea that *C. hero* is highly sensitive to environmental conditions during larval development: an aspect of undeniable importance also in the conservation-ecological context.

Festuca rubra was the only potential host plant species whose cover appeared among the reasonably strong predictors of the presence of *C. hero* butterflies (Table 5). To evaluate the possibility that the Estonian populations of *C. hero* may, despite the potential polyphagy, still be specialised on *F. rubra*, we repeated most laboratory experiments with this grass now included in the sample. *F. rubra* was shown not to be preferred as an oviposition substrate by *C. hero* females, neither did it support larval growth notably better than the alternative generalist grass *Dactylis glomerata.* We are thus inclined to conclude that the microhabitat-scale association of *C. hero* and *F. rubra* is a result of similar habitat requirements of these two species, rather than reflecting a direct biological link between them.

As a part of the current study, we evaluated the usefulness of recording butterfly resting points as a cue for the species' ecological preferences. With our primary focus on host plant associations, we found such a small-scale habitat analysis preferable to approaches such as transect counts, or map-based analyses of landscape use. Only in our small study plots were we able to describe plant cover in sufficient detail. Immediate surroundings of a field-recorded individual must be informative with respect of habitat suitability to a greater or lesser extent. Even if the resting spots recorded do not necessarily coincide with oviposition sites, the slow- and low flying *Coenonympha* butterflies appear to be intimately linked to their habitat (Cassel-Lundhagen and Sjögren-Gulve 2007, personal observations of the authors) being thus promising objects for this type of research. The clearly non-random—and, at least partly, expected—picture emerging from the analysis of respective data for *C. hero* appears encouraging. We also do not see any methodological bias in the analyses which aim at evaluating the effect of particular plant species, especially by means of multi-way models including abiotic parameters as covariates. This is despite the potential ambiguities which may be related to assessing the effect of the abiotic parameters themselves (see above).

In the context of species conservation, the present study points at the priority of microclimatic conditions (exposure to sun, soil moisture) over the presence of particular host plant species as determinants of habitat suitability. As moderate shrub cover appears to be preferred by *C. hero*, changing the extent of shrubs as well as causing major changes in the structure of vegetation cover (such as grazing) should thus be applied with extreme care. Indeed, *C. hero* appears to avoid grazed sites similarly to *C. oedippus* (Čelik et al. 2015)—none of the Estonian populations of *C. hero* known to the authors appears to inhabit grazed sites (unpublished data; Sang et al. 2010). The preference of (semi)open but unmanaged habitats implies that rotational grazing or temporary abandonment is necessary where species conservation is concerned.

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