

Original Article

Species- and sex-specific adjustments of movement behavior to landscape heterogeneity in butterflies

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Landscapes are often considered as islands of suitable habitat patches located in a hostile and homogeneous matrix. Variation in matrix quality, however, can be differently perceived by individuals, generating variation in movements related to external conditions (i.e., contrasted boundaries, corridors, or barriers) and/or individual phenotypes (i.e., sex or age). Accordingly, movements may differ both among species and among conspecific individuals of various age and/or sex. Here, we quantified sex-dependent and condition-dependent variations in orientation capacity, exploration behavior, and adjustment of flight speed to landscape heterogeneity in 2 related butterfly species (*Boloria aquilonaris* and *B. eunomia*). Using experimental cages, we assessed 1) individual butterfly's ability to discriminate between habitat and matrices of different types and hence to orientate themselves towards their habitat, 2) individual butterfly's ability to adapt their flight behavior to their local environment, and 3) their propensity to cross habitat–matrix boundaries. We showed the existence of species-specific orientation capacity, changes in exploration behavior according to local environment, and differences in behavior at habitat boundaries that are consistent between sexes. The adjustment of flight speed was also species-specific and varied in an opposite way between sexes. We explore how these differences might have arisen from adaptive responses to differences in the spatial configuration of habitats and in life-history traits, and we discuss how they can lead to differences in functional connectivity measurements. *Key words:* *Boloria aquilonaris*, *Boloria eunomia*, cage experiment, dispersal, flight speed, matrix quality. [*Behav Ecol* 22:967–975 (2011)]

INTRODUCTION

In classical metapopulation theory (Hanski and Simberloff 1997), landscapes were considered as either suitable habitat patches or a hostile matrix (Hanski 1999b; Per Sjørgren-Gulve and Hanski 2000). This binary classification was recognized as an oversimplification, and the influence of the landscape heterogeneity was consequently integrated into predictive models (e.g., Ricketts 2001; Thomas et al. 2001). Interpatch connectivity is commonly based on structural estimates of the landscape and usually results in distance-dependant functions of dispersal. However, such estimates may lead to overestimating or distorting the actual landscape connectivity, simply because in heterogeneous landscapes, each particular landscape element can differently facilitate or impede dispersal movements (Taylor et al. 1993). Therefore, we can no longer assume that individuals always use the shortest straight line to move between habitat patches and that dispersal probability is strictly a function of distance. To quantify the effects of matrix heterogeneity on individual movements (i.e., the permeability of different landscape elements), it is of the utmost importance to explicitly consider the interaction between dispersing indi-

viduals and the landscape conditions (e.g., Hein et al. 2003; Revilla et al. 2004; Romero et al. 2009). To tackle this issue, functional connectivity estimates that incorporate behavioral components of matrix use were developed. Among those, most probable path analyses focus on the computation of paths between pairs of patches that maximize the preference over the landscape layer. Resistance values attributed to each landscape cell can be based on 1) arbitrary values (e.g., Wang et al. 2009), 2) expert opinion (e.g., Larue and Nielsen 2008), 3) landscape utilization inferred from resource selection function (e.g., Fall et al. 2007) and radio tracking data (e.g., Driezen et al. 2007), or 4) experimental tests (e.g., Stevens et al. 2006).

Ronce (2007) defined dispersal as “any movement of individuals or propagules with potential consequences for gene flow across space.” Dispersal can be considered either as a by-product of routine movements or as the results of movements specially designed for dispersal (Van Dyck and Baguette 2005; Baguette and Van Dyck 2007). Trajectories associated with these 2 types of movements are topologically different, special dispersal movements often being significantly longer with less directional changes. Variation in dispersal can be examined from 2 different aspects. First, condition-dependent dispersal reflects the influence of environmental conditions on individual behavior and associated evolutionary responses in dispersal (e.g., Schtickzelle et al. 2006, 2007; Clobert et al. 2009). Second, phenotypic-dependent dispersal arises from sex, age, and other individual variations at the intraspecific

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level (Clobert et al. 2009). Several studies demonstrate that variation in dispersal behavior is sex-dependent (Greenwood 1980; Pusey 1987; Tucker et al. 1998; Pardini et al. 2001; Prugnolle and de Meeus 2002). At a higher level, interspecific differences in dispersal are widely accepted and seem rather trivial but still remain poorly investigated (but see Stevens, Pavoine, et al. 2010).

Butterflies are excellent study organisms in spatial ecology: Their ecological requirements are often well documented (e.g., Turlure et al. 2009; Dennis 2010), their habitats occur generally as discrete entities across the landscape (e.g., Thomas et al. 1992; Baguette and Mennechez 2004), and adult butterfly dispersal has been thoroughly analyzed (see Stevens, Turlure, et al. 2010 for a review). Many studies aimed at comparing dispersal abilities through different kinds of matrices (Ricketts 2001; Dennis 2004; Keyghobadi et al. 2005), but surprisingly, only 2 studies on butterflies assessed landscape connectivity using least cost or most probable path analysis so far (see Chardon et al. 2003; Sutcliffe et al. 2003). As suggested by Stevens, Pavoine, et al. (2010), this could be explained by the lack of a mechanistic relationship between individual behaviors and population dispersal rates in butterflies. Functional connectivity estimates require the knowledge of movement rules of the target organism that could subsequently be used to model its displacements (Delattre et al. 2010). Accordingly, here we quantify sex-dependent and condition-dependent (i.e., effect of matrix type) variations in orientation, exploration behavior, and adaptations of flight behavior of 2 related butterfly species (*Boloria aquilonaris* and *B. eunomia*).

To do so, we used the method developed by Stevens et al. (2004, 2006) to assess 1) individual butterfly's ability to discriminate between habitat and matrix and hence to orient toward its habitat, 2) whether individual butterfly's flight behavior changes with respect to the landscape heterogeneity (i.e., in the habitat or in matrices of different types), and 3) the propensity to cross habitat–matrix boundaries, using experimental cages. More precisely, we tested the following hypotheses. 1) Under the hypothesis of the existence of an orientation capacity at the local scale, we expect that individual butterflies released at a habitat–matrix boundary will head first for their habitat. 2) The switch from slow tortuous explorative movements to directed dispersal movements designed for net displacement (Baguette and Van Dyck 2007) may depend on individual location, that is, in the habitat or in the matrix. Therefore, we predict individuals to fly less long, over shorter distances and to explore less systematically the matrix than the habitat, especially in the case of low-quality matrix. 3) The effect of habitat and matrix type on movements (i.e., habitat or matrix resistance) can also be estimated from the adjustment of flight speed to the local environment. We expect the individuals to fly faster in the matrix compared with the habitat and especially in the case of lower quality matrix. 4) Moreover, as boundary permeability is supposed to result from individual habitat preference, we expect to observe less frequent boundary crossings when the quality of the matrix is lower. 5) Finally, the study species and the sexes of conspecific individuals differ both in morphological attributes (see Turlure, Schtickzelle, et al. 2010) and in realized dispersal kernels (see Baguette 2003; Mennechez et al. 2003). Thus, we expect species-specific and sex-dependent adjustments of movement behaviors to landscape elements.

MATERIALS AND METHODS

Study species and sites

The bog fritillary *B. eunomia* and the cranberry fritillary *B. aquilonaris* are specialist species of wet meadows and peat

bogs. We performed the experiments in 2 peat bogs in the “Plateau des Tailles” landscape: the Fange de Pisserotte (lat 50°13'N, long 5°47'E) and the Fange de Crépale (lat 50°16'N, long 5°44'E) nature reserves. These bogs host large populations of *B. eunomia* and *B. aquilonaris*, respectively (Turlure, Chouet, et al. 2010; Turlure C, unpublished data). The vegetation of the 2 bog sites mainly consisted of 1) the habitat (i.e., for *B. eunomia*: patches with *Polygonum bistorta*, the only food plant for both caterpillars and adults; for *B. aquilonaris*: *Vaccinium oxycoccos* [the primary host plant] patches for the caterpillars and nectar patches for the adults); 2) bog and meadow vegetation with a similar vegetation structure as the habitat (20–60 cm height) but without any of the species' food resources; 3) a scrub vegetation with a markedly taller vegetation than the habitat (up to 2 m); and 4) forests mainly composed of spruces, birches, and willows. The last 3 vegetation types can be considered as matrices of contrasted vegetation height and represent the main matrix types occurring between the species' habitat patches in this landscape.

Cage experiment: procedure

In June and July 2009, we constructed an enclosure made of green opaque tissue (5 m wide × 10 long × 1.5 m height; Figure 1a) to test the preference of both species for 1) habitat versus bog vegetation (referred to as HB test) and 2) habitat versus scrub vegetation (=HS test). Each 5 × 5 m² half of the cage (hereafter referred to as a compartment) contained a given vegetation type (i.e., habitat or bog vegetation for the HB test and habitat or scrub vegetation for the HS test, Figure 1b). Within each enclosure, the transition between the habitat and the matrix compartments is hereafter named the boundary.

For *B. eunomia*, the cage was placed in a *P. bistorta* patch in the Pisserotte site. In one of the compartments, we left the vegetation untouched (i.e., habitat). In the second

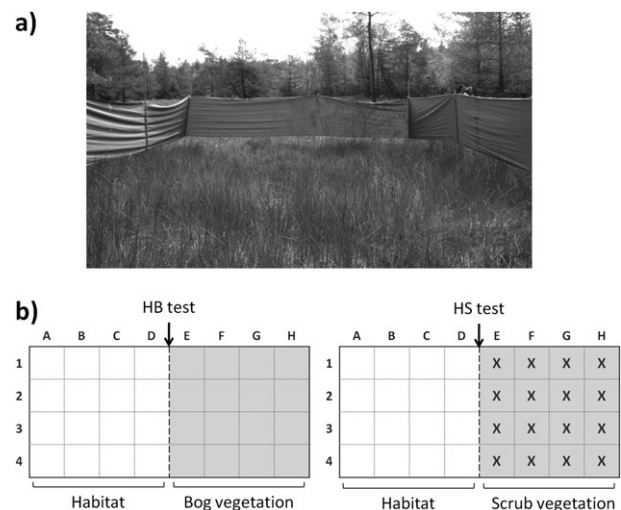


Figure 1

a) Picture of semiopen cage: view of one compartment from the other one. (b) Schematization of the cage used for the experiments. The cage was split into 2 equal compartments of 5 × 5 m² and divided into 32 smaller grid squares of equal size, referenced by letters for the column position and by numbers for line position. The dotted line indicates the habitat–matrix boundary. The black arrow represents the release point. The HB test is represented on the left, with the white area for the habitat and the gray area for the bog vegetation. The HS test is represented on the right, with the white area for the habitat, the gray area for scrub vegetation, and the black crosses for the position of small trees.

compartment (and 5 m around), we first removed all the *P. bistorta* plants manually to mimic a bog vegetation without resources to perform the HB test. Next, to prepare the HS test, 16 small trees of 1.5–2 m height (mainly willows and poplars) were transplanted homogeneously in this compartment (and 5 m around) to mimic the scrub vegetation. For *B. aquilonaris*, the cage was placed in a habitat patch in the Cré-pale site, providing a mixture of both larval and adult resources. Similarly, one of the compartments was left untouched (i.e., habitat), whereas the other was emptied of all host and flowering plants to mimic the bog vegetation without resources for the HB test, and then, 16 small trees were added to mimic the scrub vegetation for the HS test.

The experimental procedure was the same for all tests. A single newly emerged individual captured in the study area just before the experiment was released in the cage at the boundary of the 2 compartments (Figure 1b: black arrow), and its behavior was recorded until it left the cage area. For each individual, we recorded the complete path (time, position, and behavior) within the cage by reporting the individual's position on a virtual grid of 32 squares of 1.25×1.25 m² each (Figure 1b). We released 60 individuals per species (30 males and 30 females) in each cage configuration (HB and HS). All observations were performed under suitable weather conditions for butterfly flight (i.e., no wind, no clouds, air temperature >22 °C).

Data analysis

From the data collected, we first inferred the compartment chosen by each individual at departure (i.e., first individual position = D1 square for the habitat or E1 square for the matrix; Figure 1b). Then, we modeled the effect of species (*B. eunomia* or *B. aquilonaris*), sex, cage configuration (i.e., HB test vs. HS test), and their interactions on this choice using generalized linear models (with a logit-link function and a binomial distribution; Proc Genmod in SAS; SAS Institute Inc., 2003). We also quantified the frequency of boundary crossings and their orientation (i.e., from habitat to matrix or from matrix to habitat) according to species, sex, and compartment. Given the limited number of boundary crossings, we did not perform statistical analysis on this parameter but rather give the general frequency in the results.

Secondly, we separately calculated the total time spent in flight, the total distance covered, and the mean flight speed in each compartment of the cage and then investigated the effects of species, sex, compartment, and their interactions on these 3 parameters using generalized mixed models and appropriate contrasts (Proc Mixed in SAS; SAS Institute Inc., 2003). Individual was added as a random factor in these models. Response variables were log transformed prior to the analysis.

Next, we analyzed the pattern of cage exploration for each combination of species and sex separately. To do this, we counted the number of times each of the 32 grid squares of the cage was flown over by each individual as an index for grid square utilization. Then, we summed these indexes over the 4 squares of each column, and columns were sorted according to their distance from the habitat–matrix boundary: Columns D and E (Figure 1b) were the closest columns to the habitat–matrix boundary, whereas columns A and H were at the outer edge of the cage for the habitat and matrix compartments, respectively. First, we tested for homogeneity of compartment exploration using χ^2 tests (for the 4 compartments separately). Second, we paired the columns of the habitat and the matrix compartments arising out from the same cage configuration according to their distance to the boundary. Then, we tested for differences in the pattern

of exploration between compartments (i.e., habitat vs. matrix) using χ^2 tests and between cage configuration (HM vs. HS) using Cochran–Mantel tests.

RESULTS

Initial preference and boundary crossings

Logistic regression models revealed that individuals generally headed for the habitat first (intercept effect; Table 1; McKillup 2006) and that the preference for the habitat compartment was significantly stronger for *B. aquilonaris* ($81.66 \pm 0.08\%$ of the individuals headed toward the habitat first) than for *B. eunomia* ($68.33 \pm 0.08\%$). This initial choice was similar for the HB and HS tests. No effect of sex or interaction was detected (Table 1).

In all, we observed 74 boundary crossings, mostly arising from *B. eunomia* individuals (65%). Contrary to what was expected, the number of boundary crossings did not differ between matrix type (*B. aquilonaris*: 13 boundary crossings in the HB test vs. 13 in the HS test; *B. eunomia*: 22 in the HB test vs. 26 in the HS test). Three types of boundary crossings occurred: 1) 18% occurred when individuals headed first to the matrix and came back to the habitat; 2) 15% were crossings from habitat to matrix, followed by the direct exit from the cage; and 3) most (67%) were the result of loopy movement in the matrix (Figure 2). Loops observed in the HS test tend to penetrate less into the matrix compartment than those in the HB test (only 23% of the loops went over the first column in the HS test vs. 60% in the HB test).

Behavior in the cage

The total time spent in flight (Table 2a; Figure 3a) was significantly greater for *B. eunomia* (8.70 ± 2.92 s) than for *B. aquilonaris* (2.32 ± 0.35 s). Furthermore, both species were in flight for significantly longer periods of time in the habitat than in the matrix (pooled bog and scrub; see details of contrasts in Table 3a). Effect of sex and interactions on time spent in flight were not significant.

The total distance covered in the cage (Table 2b; Figure 3b) was significantly greater for *B. eunomia* (6.42 ± 1.59 m) than for *B. aquilonaris* (3.41 ± 0.47 m), and greater distances were traversed in the habitat compartments for both species when compared with the matrix compartments (Habitat_{HB}: 7.69 ± 2.01 m; Habitat_{HS}: 7.88 ± 2.36 m; Bog: 2.29 ± 0.73 m; Scrub: $1.79 \pm$

Table 1

The cage compartment chosen at departure (i.e., habitat or matrix) was analyzed using a generalized linear model with a binomial distribution and a logit-link function

Parameter	χ^2	P
Intercept	4.36	0.0367
Test	0.07	0.7868
Species	4.51	0.0337
Sex	0.12	0.7282
Species \times sex	0.02	0.9018
Species \times test	1.79	0.1808
Test \times sex	0	0.9543

Species, sex, test, and their interactions were used as explanatory variables in the model. Individuals more often headed for the habitat first (significant intercept effect), and the preference for the habitat compartment was significantly stronger for *B. aquilonaris* compared with *B. eunomia* (significant species effect) independently of the habitat preference tests performed. Effects of the other variables were not significant.

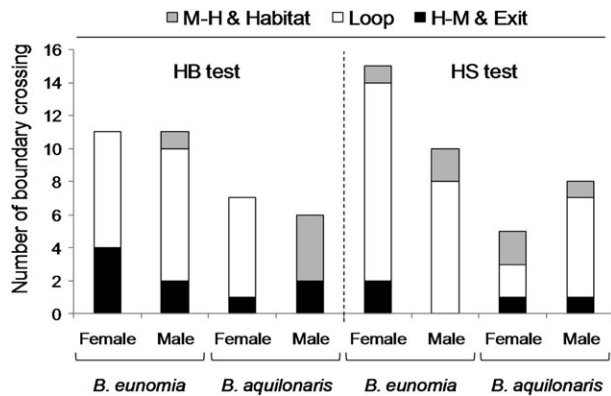


Figure 2

Number of boundary crossings according to cage configuration (HM test = habitat vs. bog vegetation; HS test = habitat vs. scrub vegetation), species, and sex. Three types of boundary crossings occurred: 1) M-H and Habitat = individuals headed first to the matrix and came back to the habitat, 2) Loop = loopy movement to explore the matrix compartment, and 3) H-M and Exit = crossing from habitat to matrix followed by a direct exit from the cage.

0.52 m; see details of contrasts in Table 3b). The species \times compartment effect was also significant. For *B. eunomia*, the total distance covered was longer in the habitat than in the matrix for both tests and similar in both habitat compartments and in both matrix compartments. For *B. aquilonaris*, the total distance covered was longer in the habitat than in the matrix for both tests, similar in both habitat compartments but longer in the matrix compartment of the HB test than in the matrix compartment of the HS test (Figure 3b).

The mean flight speed (Table 2c; Figure 3c) of *B. aquilonaris* ($1.82 \pm 0.12 \text{ ms}^{-1}$) was significantly greater compared with *B. eunomia* ($1.19 \pm 0.07 \text{ ms}^{-1}$). The species \times compartment was also statistically significant. *Boloria eunomia* were faster in the matrix than in the habitat for both tests, whereas *B. aquilonaris* had a similar speed in both compartments within each test but were generally faster in the HS test than in the HB test. The significant species \times sex effect indicated that females *B. aquilonaris* flew faster than males, whereas in *B. eunomia*, the opposite situation was observed.

Cage and compartments exploration

Results of the χ^2 tests (Table 4) indicated that compartment exploration, from the boundary to the outer edge of the cage,

was homogeneous in the following cases: in the habitat compartment for both sexes and both tests in *B. eunomia* and in the habitat compartment of the HB test for *B. aquilonaris* females. In all other cases, the compartment exploration was not homogeneous; individuals flew more often in columns close to the boundary and explored less often the outer edge of the cage (Figure 4).

As expected, compartment exploration differed significantly between habitat and matrix for all cage configurations, species, and sex, except for *B. eunomia* females in the HB test (Table 5). Differences in butterfly frequentation from the boundary to the outer edge of the cage were more pronounced in the matrix compartment. Given the limited amount of data collected in the matrix compartment for *B. aquilonaris* males for the HS test, the low number of expected values for the last 2 columns did not allow for a formal statistical test in that case. Finally, compartment exploration between habitat and matrix was different for both species and sex (Table 5): The outer edge of the cage was less explored in the scrub matrix as compared with the bog matrix for both species and less explored in the habitat of the HS test as compared with the habitat in the HB test for *B. aquilonaris* (Figure 4).

DISCUSSION

We showed the existence of species-specific orientation capacity, adjustment of exploration behavior to landscape heterogeneity, and differences in behavior at habitat boundaries that are consistent between sexes. The adjustment of flight speed was also species-specific and varied in an opposite way between sexes, resulting in species-specific and sex-specific resistances to habitat and matrices of different types. After discussing these results, we explore how these differences might have arisen from adaptive responses to differences in the spatial configuration of habitats and life-history traits. Finally, we conclude by assessing the consequences of these differences for the estimation of functional connectivity.

The capacity to navigate and explore their environment is species specific in butterflies

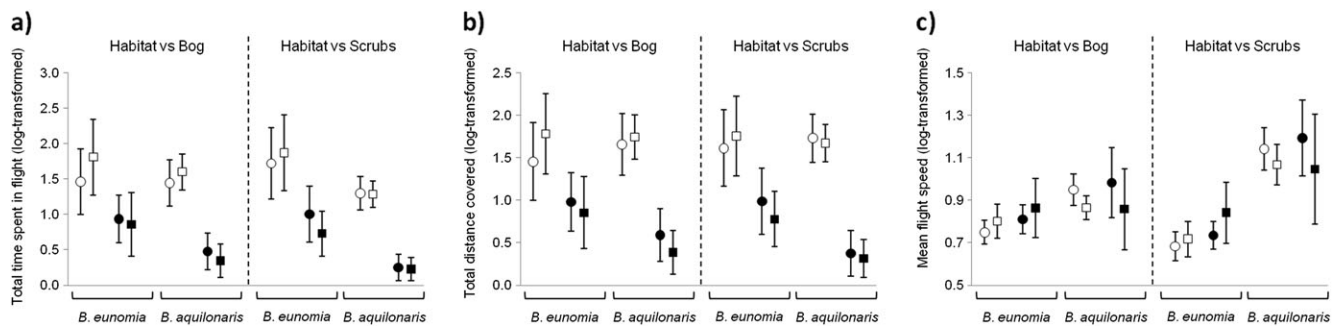
Species-specific orientation capacities have been demonstrated in other taxa (Derosa and Taylor 1980; Andrews 1984; Franz and Wcislo 2003; Gillies and Saint Clair 2010). Our study demonstrates the existence of an orientation capacity in the 2 different, but related, butterfly species. Indeed, as expected, most of the individuals released in the cage headed toward the habitat first (Table 1). Moreover, among the individuals that headed first to the matrix, 32% of *B. aquilonaris* and 10% of *B. eunomia* returned

Table 2

(a) Total time spent in flight, (b) total distance covered, and (c) mean flight speed in both compartments of the cage were analyzed using generalized mixed models

Parameter	(a) Total time spent in flight			(b) Total distance covered			(c) Mean flight speed		
	df	F	P	df	F	P	df	F	P
Species	232	25.22	<0.0001	232	6.43	0.0119	35	83.88	<0.0001
Sex	232	0.04	0.8512	232	0.03	0.8722	35	0.78	0.3841
Compartment	232	41.44	<0.0001	232	47.62	<0.0001	35	4.59	0.0082
Species \times sex	232	0.05	0.8162	232	0.29	0.5906	35	13.93	0.0007
Species \times compartment	232	1.66	0.1757	232	3.35	0.0199	35	10.78	<0.0001
Sex \times compartment	232	1.13	0.3394	232	1.03	0.3803	35	0.15	0.9313
Species \times sex \times compartment	232	0.34	0.794	232	0.27	0.8483	35	1.11	0.3576

Effect of species, sex, compartment, and their interactions were estimated. Individual was added as a random factor. Response variables were log transformed prior to the analysis. df = degrees of freedom.

**Figure 3**

Means and 95% confidence interval of (a) total time spent in flight, (b) total distance covered, and (c) mean flight speed in the each compartment for both cage configuration and for each combination of species and sex. White symbols: habitat compartment. Black symbols: matrix compartment. Circles: females. Squares: males.

rapidly to the habitat after what we could call a “misorientation.” The initial preference for the habitat compartment and the re-orientation were more pronounced in *B. aquilonaris*, meaning that they had a more efficient capacity to orient than *B. eunomia*. Individuals of both species flew over greater distances and for longer duration in the habitat than in the matrix. These differences observed between the cage compartments support the hypothesis that butterflies can discriminate between habitat and matrix and hence that they can orientate themselves in heterogeneous landscapes. We also found that *B. aquilonaris* can distinguish between matrices of different types as they were observed to fly for a shorter duration and over shorter distance in the scrub matrix than in the bog matrix (Figure 3).

The perceptual range (i.e., the distance to which the habitat can be perceived; Olden et al. 2004) of *B. eunomia* ranged from 15 to 30 m (Schtickzelle et al. 2007). As the longest distance in the cage was 11.2 m, we can assume that *B. eunomia* individuals can perceive the whole cage environment independently of their location in the cage. Perceptual range has never been assessed for *B. aquilonaris* so far, but because it is phylogenetically closely related to *B. eunomia*, we expect it to be similar or at least larger than 10 m as for several other butterfly species. Our results showed that *B. eunomia* thoroughly explored the habitat compartments (Figure 4). This was not the case in *B. aquilonaris* in which individuals, and especially males, avoided the outer edge of the habitat compartment. The exploration of the matrix compartment was generally less complete than that of the habitat compartment: Only *B. eunomia* females relatively well explored this compartment. Moreover, the difference of exploration between both matrices was stronger for *B. aquilonaris* than for *B. eunomia*. Altogether, this indicated that matrix in general and scrub matrix were less likely to be explored than habitat and bog matrix, respectively. This may indicate that matrix types differed in quality, scrub matrix being of lower quality compared

with bog matrix. Those differences are especially pronounced for *B. aquilonaris*. Overall, *B. aquilonaris* individuals showed a reduced explorative behavior; they used the matrix compartments less often and also performed fewer loops to explore the matrix from the habitat compartment (Figures 2 and 4).

Adjustment of flight speed to the landscape heterogeneity is species- and sex-specific

Overall, *B. aquilonaris* flew faster than *B. eunomia* (Figure 3c). However, the differences observed between cage compartments suggest that individuals of both species are able to adjust their flight speed to local conditions prevailing in habitat and matrices of different types and hence qualities. In the case of *B. eunomia*, individuals were observed to fly faster in the matrix than in the habitat independently of the matrix type. In the case of *B. aquilonaris*, faster flights were recorded in low-quality matrix only. This may indicate that condition-dependent adjustment of flight speed is stronger in *B. aquilonaris* and that the adjustment arises at finer spatial scale for *B. eunomia*. Notice that similar flight speeds as well as the differences between species and sex were recorded on unhandled individuals, which rules out the possibility that the results reported here could be affected by manipulation (Turlure C, unpublished data). Our results also demonstrate the complex relationship between a behavioral trait (flight) and the phenotype (sex). The adjustment of flight speed to local environment was indeed different between sexes with an inverse relationship between species.

Biological origin of these differences

Looking at the historical repartition of both species provides clues on the observed interspecific differences in orientation

Table 3

Results of the contrasts tested on the (a) total time spent in flight, (b) total distance covered, and (c) mean flight speed in both compartment of the cage using generalized mixed models

Contrast	(a) Total time spent in flight			(b) Total distance covered			(c) Mean flight speed		
	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
Habitat _{HB} vs. Habitat _{HS}	232	0.3	0.7649	232	-0.27	0.7912	35	-2.61	0.0131
Bog vs. scrubs	232	0.83	0.4049	232	0.73	0.467	35	-1.75	0.0884
Habitat _{HB} vs. bog	232	7.59	<0.0001	232	7.94	<0.0001	35	-1.78	0.0832
Habitat _{HS} vs. scrubs	232	8.13	<0.0001	232	8.93	<0.0001	35	-1.67	0.1046
Pooled habitat vs. pooled matrix	232	11.11	<0.0001	232	11.93	<0.0001	35	-2.44	0.02

Individual was added as a random factor. Response variables were log transformed prior to the analysis. df = degrees of freedom.

Table 4
Homogeneity of compartment utilization was tested using χ^2 for each combination of species and sex separately

Species	Sex	Compartment	χ^2	P
<i>B. eunomia</i>	Female	Habitat _{HB}	5.32	0.07
		Habitat _{HS}	4.38	0.1119
		Bog	16.53	0.0003
	Male	Scrubs	18.7	<0.0001
		Habitat _{HB}	3.13	0.2093
		Habitat _{HS}	0.56	0.7558
<i>B. aquilonaris</i>	Female	Bog	18.77	<0.0001
		Scrubs	88.13	<0.0001
		Habitat _{HB}	2.09	0.351
	Male	Habitat _{HS}	8.12	0.0172
		Bog	17.67	0.0001
		Scrubs	14.84	0.0006
	Female	Habitat _{HB}	16.44	0.0003
		Habitat _{HS}	19.09	<0.0001
		Bog	22.45	<0.0001
Male	Scrubs	16.4	0.0003	

capacities and habitat selection. *Boloria aquilonaris* is a glacial relict species that inhabits, in Belgium, peat bogs only on the top of the Ardenne shields. Those small ecosystems were strongly fragmented by spruce plantations since the middle of the 20th century in the study area (Petit and Lambin 2002).

We can assume that those butterflies that lived in progressively isolated habitats were forced to develop efficient searching strategy and hence a better ability to orientate themselves in the landscape during dispersal (but see the case of the butterfly *Pararge aegeria* in Merckx et al. 2003). At the opposite, *B. eunomia* habitats were spread all along the rivers in the valleys, resulting in relatively connected population networks. Individuals had then a relatively higher probability to reach a new patch after leaving their natal one, just by flying along valleys. These differences in the spatial configuration of their respective habitats might thus ultimately lead to the evolution of contrasted abilities to orientate themselves. It is worth mentioning that these differences match the divergent dispersal kernels described in both species: *B. aquilonaris* individuals were observed to move longer distances (up to 13 km) compared with *B. eunomia* (up to 4.6 km) in the same landscape (Baguette and Nève 1994; Nève et al. 1996; Baguette 2003; Mennechez et al. 2003).

The difference in flight speed between sexes is more difficult to interpret. We propose that it could have emerged from differences in life history (Hansen and Urban 1992), such as resource use and egg-laying strategies. First of all, *B. aquilonaris* females are single-egg layers, whereas *B. eunomia* females lay batches of eggs. This means that *B. aquilonaris* females have to cover longer distances to lay all their eggs than *B. eunomia*. Moreover, females of *B. aquilonaris* are harassed by males throughout the flight season, whereas female harassment is time limited in *B. eunomia* because the species is protandrous (i.e., adult males emerge before females; Schtickzelle et al.

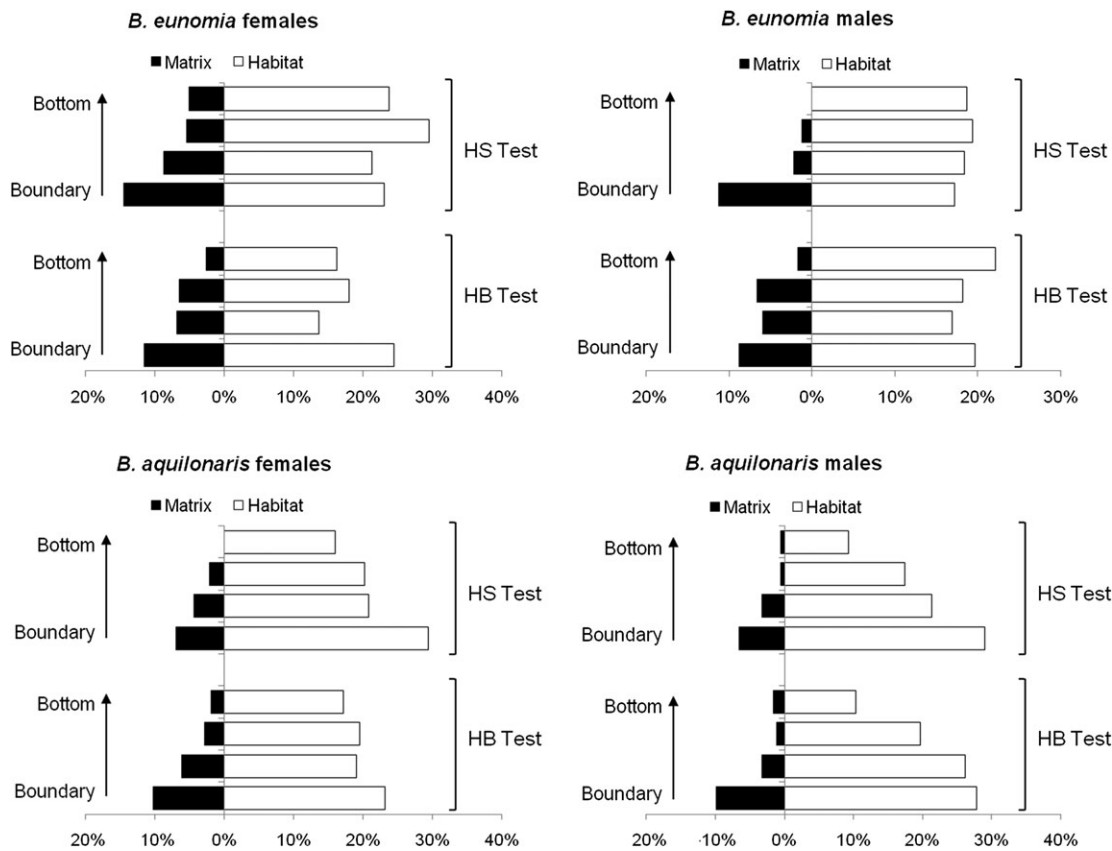


Figure 4
Pattern of cage exploration for each combination of species and sex separately. Bars represent the total number of times each column of the cage was flown over by individual for each compartment (habitat and matrix) and test (HB and HS). Columns were sorted and paired according to their proximity to the habitat-matrix boundary for each test. White bars = columns of the habitat compartment. Black bars = columns of the matrix compartment.

Table 5
Differences in the pattern of exploration were tested between compartments (i.e. habitat vs. matrix) within each cage configuration using χ^2 tests and between cage configuration using Cochran–Mantel tests for each combination of species and sex separately

Species	Sex	Test	χ^2	P
<i>B. eunomia</i>	Female	Habitat _{HB} vs. bog	7.17	0.0665
		Habitat _{HS} vs. scrubs	17.52	0.0006
		Between cage configuration	20.33	0.0001
	Male	Habitat _{HB} vs. bog	19.02	0.0003
		Habitat _{HS} vs. scrubs	68.42	<0.0001
		Between cage configuration	64.55	<0.0001
<i>B. aquilonaris</i>	Female	Habitat _{HB} vs. bog	9.51	0.0232
		Habitat _{HS} vs. scrubs	7.65	0.0539
		Between cage configuration	16.39	0.0009
	Male	Habitat _{HB} vs. bog	9.61	0.0222
		Habitat _{HS} vs. scrubs	too small sample size	
		Between cage configuration	14.19	0.0027

2002). Finally, *B. eunomia* females use the same plant as nectar and egg-laying resource, whereas for *B. aquilonaris*, the different nectar-feeding resources are often spatially separated from oviposition plants (Turlure, Schtickzelle, et al. 2010). This spatial segregation implies that *B. aquilonaris* females may have to fly faster than *B. eunomia* females to optimize their time budget. Besides, females flying among spatially discontinuous resources are more conspicuous to patrolling males, which is another additive reason to adopt a rapid flight in the matrix.

Implications regarding dispersal and connectivity estimates

Understanding the factors that affect movement and dispersal of animals became a primary concern in conservation biology in light of the dramatic worldwide decline of biodiversity due to habitat fragmentation (Poethke et al. 2003; Bowne and Bowers 2004). Most studies dealing with animal dispersal have focused so far on the effects of patch area and isolation (Hanski 1994, 1999a; Hill et al. 1996; Moilanen and Hanski 2001). Structural connectivity estimates may be used in case of an invariant and ecologically neutral matrix (Taylor et al. 2006), which is really unlikely to occur in the real world. Several authors stated that connectivity may differ according to the species considered and to the landscape through which the species must move (Belisle 2005; Baguette and Van Dyck 2007; Fahrig 2007; Clobert et al. 2009). Hence, species-specific behavior in the matrix and the resulting mechanistic rules predicting individual movements should be carefully estimated. Given that these parameters vary between groups of individuals, functional connectivity estimates should be at least species centered or, better, population centered (Hansen and Urban 1992).

In this vein, our results support the key messages regarding landscape connectivity delivered by Taylor et al. (2006). First of all, landscape connectivity must be assessed on a species-specific basis, at least. Results of this study demonstrated this for 2 closely related species: The differential responses to habitat-matrix boundaries make the landscape differently permeable for both species. These differences may arise from the species plasticity of response to changes and may be related to species life-history traits too. Second, the matrix is not of homogeneous quality, and this variation can be differently perceived by individuals (see Bakker and Van Vuren 2005 for an example), which

generates condition- and/or phenotypic-dependent responses. Variation in mobility and morphology related to resource grain (i.e., the spatial heterogeneity of resources) were observed among populations of *B. eunomia* and *B. aquilonaris* (Turlure, Schtickzelle, et al. 2010). In a damselfly, morphological variations between populations were also related to landscape composition (Taylor and Merriam 1995). Such differences among populations ultimately reflect the existence of interindividual phenotypic differences within populations, being plastic or genetic responses to the landscape heterogeneity. Our study demonstrated how orientation, exploration behavior, and the adjustment of flight behavior can differ between categories of individuals (here sexes). This variability highlights the potential for a local adaptation of those traits in natural populations.

It is now warranted to repeat the experiment described in this paper on several populations in order to quantify the variation of movement and dispersal behavior among populations and hence assess the intraspecific variability in functional connectivity. Indeed, intraspecific variation in dispersal was shown to be equal or even larger than differences among species in butterflies (Stevens, Pavoine, et al. 2010). These results should allow us to develop functional connectivity estimates based on either a most probable path analysis or an individual-based models on both species and sexes and for several populations to see how much they differ with structural, commonly used connectivity estimates and how much they fit the patterns of dispersal flows.

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