

# Butterfly dispersal in farmland: a replicated landscape genetics study on the meadow brown butterfly (*Maniola jurtina*)

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## Abstract

**Context** Anthropogenic activities readily result in the fragmentation of habitats such that species persistence increasingly depends on their ability to disperse. However, landscape features that enhance or limit individual dispersal are often poorly understood. Landscape genetics has recently provided innovative solutions to evaluate landscape resistance to dispersal. **Objectives** We studied the dispersal of the common meadow brown butterfly, *Maniola jurtina*, in agricultural landscapes, using a replicated study design and

rigorous statistical analyses. Based on existing behavioral and life history research, we hypothesized that the meadow brown would preferentially disperse through its preferred grassy habitats (meadows and road verges) and avoid dispersing through woodlands and the agricultural matrix.

**Methods** Samples were collected in 18 study landscapes of 5 × 5 km in three contrasting agricultural French regions. Using circuit theory, least cost path and transect-based methods, we analyzed the effect of the landscape on gene flow separately for each sex.

**Results** Analysis of 1681 samples with 6 microsatellites loci revealed that landscape features weakly influence meadow brown butterfly gene flow. Gene

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flow in both sexes appeared to be weakly limited by forests and arable lands, whereas grasslands and grassy linear elements (road verges) were more likely to enhance gene flow.

**Conclusion** Our results are consistent with the hypothesis of greater dispersal through landscape elements that are most similar to suitable habitat. Our spatially replicated landscape genetics study allowed us to detect subtle landscape effects on butterfly gene flow, and these findings were reinforced by consistent results across analytical methods.

**Keywords** Agricultural landscape · Gene flow · Landscape resistance · *Lepidoptera* · Linear mixed-effect model · Movement · Spatial replication

## Introduction

As a consequence of climate and land use change, species have to adapt to increasingly challenging conditions. To do so, they can remain in the same places and modify their phenotypic and genotypic variations to improve their fitness, or/and they can disperse in search of better environmental conditions. In this context, dispersal—the movement of individuals that can sustain gene flow (Ronce 2007)—is of increasing interest. The resistance of the landscape mosaic to dispersal is not homogeneous; some landscape features can enhance dispersal whereas others act as barriers (Taylor et al. 1993). A variety of research approaches have been used to estimate landscape resistance to dispersal, including telemetry or tracking studies, and movement data analysis (Zeller et al. 2012). However, these direct methods are not feasible for all species and are time- and labor-consuming. Furthermore, they quantify short term dispersal and may miss rare long distance events that can be critical to maintaining gene flow among populations (Nowicki et al. 2014). Finally, most direct observations of dispersal focus on the process, but

make no assessment of the outcome (i.e. reproductive success, Baguette et al. 2013).

Indirect method based on gene flow estimates reflects dispersal events across generations accounting for reproductive success, thus quantifying ‘effective dispersal’ (i.e. dispersal plus reproductive success), and giving a more integrative view of landscape effects on movement. Recently, landscape genetics—a research discipline which combines landscape ecology, population genetics and spatial statistics—has begun to provide alternative means to evaluate landscape resistance to multi-generational plant and animal dispersal (Manel and Holderegger 2013). Landscape genetics relies upon the fact that dispersal plus reproduction drive gene flow, so assessing the relationship between genetic patterns and landscape structures can provide insight into how landscape features impede or support dispersal.

To assess the relationship between genetic patterns and landscape structures, landscape modeling is often used in landscape genetics studies to conceptualize and synthesize the interactions between environmental conditions and individual behavior. It is important to recognize the assumptions and limitations inherent to different modeling approaches: least cost path (Adriaensen et al. 2003) assumes a unique path between two locations, hypothesizing that individuals perceive their environment in a way that leads them to “select” the optimal path, whereas circuit theory (McRae et al. 2008) evaluates all possible pathways, assuming that movements follow random walks. Both methods require parameterizing resistance surfaces with cost values reflecting energy cost, mortality risk, or the willingness of the species to traverse landscape features or cross ecotones. Parameterizing resistance surfaces has been highly criticized in previous studies because it often relies on expert opinion (Rayfield et al. 2010; Spear et al. 2010; Koen et al. 2012). A simpler approach is to use a transect-based or strip-based analysis (Pavlacky et al. 2009; Emaresi et al. 2011), which avoids these subjective parameterization issues, but implicitly assumes rectilinear migration routes (Spear et al. 2010). Transect-based methods can be used to quantify not only landscape composition (i.e. the proportion of each land use), but also landscape configuration (e.g. patch density, Coster et al. 2015) between each pair of locations. The width of the transect reflects the distance at which the species perceives its environment—its perceptual range

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(Emaresi et al. 2011). As resistance methods (i.e. circuit theory and least cost path) and transect-based methods rely on different theoretic assumptions and because each method has its own advantages and drawbacks, it is worth comparing them in explaining biodiversity patterns (Zeller et al. 2012).

In this study, we used these three analytical methods to test hypotheses concerning how butterflies disperse through the landscape. Butterflies are emblematic of agricultural landscapes but their populations have declined dramatically in recent decades, mainly because of the loss and fragmentation of their habitats (EEA 2013). We chose to focus on the meadow brown butterfly (*Maniola jurtina*), a widespread butterfly capable of dispersing 50–300 m, depending on studies (Schneider 2003; Stevens et al. 2013).

Our first objective with this study was to determine the best analytical approach for modeling landscape effects on butterfly dispersal, as measured by gene flow. Indeed, comparing the models fits obtained with transect-based analysis, least cost path and circuit theory would give us insight into how butterflies perceive and disperse across the landscape. Then, we sought to determine which landscape features enhance and inhibit dispersal in a grassland butterfly in agricultural landscapes.

Following the expectation of greater dispersal through favorable habitats than through non-habitats (Eycott et al. 2012), we hypothesized that suitable habitats for meadow brown butterflies such as grasslands, forest edges and, to a lesser extent, road verges, green lanes and grassy field margins would enhance gene flow, whereas arable lands and forest interiors would limit their movements. We predicted that terrain irregularity would inhibit butterfly effective dispersal (effect detected in other taxa: Dickson et al. 2005; Watts et al. 2015), and that solar exposure would enhance the movements of this ectotherm (Cormont et al. 2011). In addition, as the dispersal behavior of the meadow brown butterfly is known to differ between sexes (Brakefield 1982; Ducatez et al. 2014), we assumed that the response to landscape features would also differ between sexes. Specifically, because males actively and intensively search for females, we expected a lower resistance of all landscape features in males than in females.

To test these hypotheses, we used a replicate landscape-level sampling design with 18 study landscapes of 5 × 5 km located in three contrasting

regions in France, with six replicate landscapes per region. Because the distribution of the meadow brown butterfly is continuous rather than patchy in our study landscapes, making delineation of population boundaries challenging, we utilized an individual-based sampling design (Landguth and Schwartz 2014). This sampling scheme also enhances the power of statistical analyses by increasing the number of sampling points, while keeping sample size constant (Prunier et al. 2013).

## Methods

### Study species

The meadow brown butterfly *M. jurtina* is a univoltine species broadly distributed throughout Europe. This butterfly inhabits meadows, and to a lesser extent, road verges, glades, hedgerows and forest paths, where its caterpillars feed on a variety of grasses (*Festuca* spp., *Agrostis* spp., Brakefield 1982). Although it is an abundant butterfly in France, meadow brown butterfly has been declining over the two last decades (EEA 2013) and is suffering from habitat loss in intensively cultivated landscapes where the patchiness of remnant suitable habitats makes dispersal ability crucial (Delattre et al. 2013a). The meadow brown butterfly is an intermediate disperser with mean dispersal distances reported to range from 50 to 300 m (Öckinger and Smith 2007; Ouin et al. 2008; reviewed in Schneider 2003; Stevens et al. 2013). While capable of dispersing several kilometers (Schneider et al. 2003), most meadow brown butterflies are quite sedentary, spending their whole lifetime in a small area (Grill et al. 2006).

The choice of the study species was a compromise between availability of neutral molecular markers, the abundance of the species in all our agricultural study landscapes and species traits (specialization and dispersal). Indeed, among the few butterfly species for which markers are available, most are either protected, rare/absent in France or infrequent in farmland. The meadow brown butterfly is a model species which has been studied for decades, and its biology (Brakefield 1982) and ecology (Conradt and Roper 2006; Öckinger and Smith 2007) are well known. With this foundation of knowledge, we were able to develop an appropriate and robust sampling

scheme to test hypotheses about meadow brown butterfly movement and landscape resistance.

### Study sites

Our study took place in three contrasting agricultural regions in France (Fig. 1). The northernmost region, Burgundy, is dominated by annual crops (cereals, corn and oilseed); mean elevation is 200 m (range 115–286 m). The second region, Aquitaine, is dominated by vineyards interspersed with woodland and grassland patches; topography is flat [mean elevation: 63 m (4–133 m)]. The southernmost region, the hills and valleys of Gascony, is a crop-livestock farming system with many small woods embedded in a mosaic of crops, grasslands and hedges; elevation ranges from 166 to 400 m (mean: 267 m).

### Field sampling

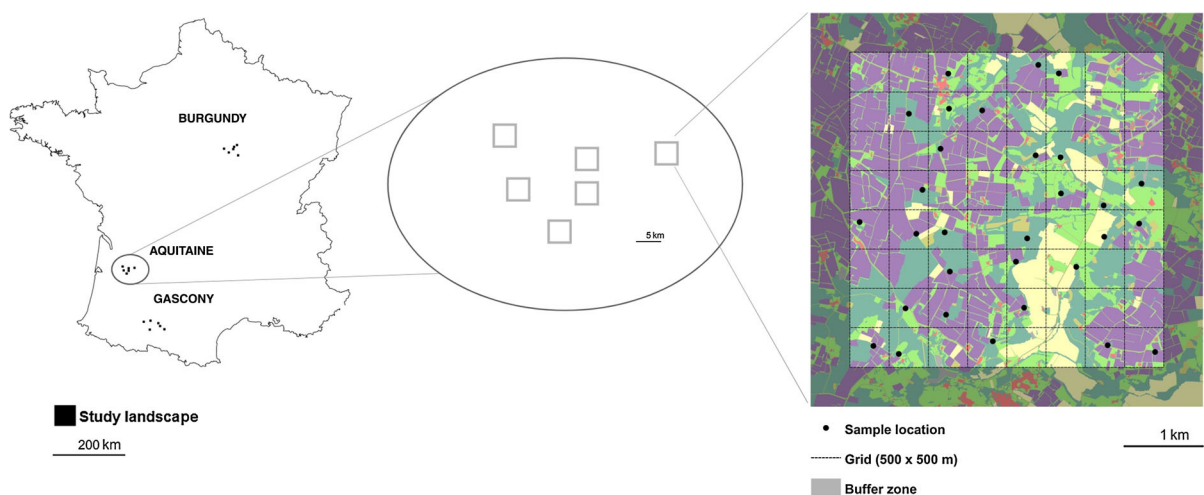
In the three regions, we used a landscape-level sampling design with six replicated  $5 \times 5$  km landscapes per region (see Appendix A1 as Supplementary Material for the methodology applied to select each study landscape and the main characteristics of these landscapes). In each of the 18 landscapes, our objective was to collect tissue from two males and two females at 25 locations randomly selected on a regular  $500 \times 500$  m grid (Fig. 1). The grid was

placed 500 m away from the border of the study landscape (buffer zone, Fig. 1) to limit border bias effect on the estimation of landscape resistance between pairs of locations near the boundaries of the study landscape (Koen et al. 2010). If fewer than 4 samples were collected at the selected locations, additional locations were chosen to obtain 100 genetic samples per study landscape. Genetic sample was collected by removing a leg, which was stored in 95 % EtOH at  $-20$  °C until DNA extraction. To avoid sampling the same individual twice, we always removed the same leg from all butterflies (left leg of the prothorax).

In summer 2013, we collected a total of 1681 samples: an average of 94 meadow brown butterflies at 18–30 locations (mean = 26.1) per study landscape. At each location, we collected from one to seven meadow brown butterflies (mean = 3.7). We obtained 1.9 times more males than females (Table S1 in Supplementary Material), despite our attempt to follow a sex-balanced sampling design.

### Genotyping and genetic analyses

DNA extraction, PCR amplifications and genotyping were performed as described in Richard et al. (2015) using 15 microsatellite loci (Mj0008, Mj5287, Mj5647, Mj5563, Mj7132, Mj0247, Mj3956, Mj5522, Mj7232, Mj0283, Mj2410, Mj3637, Mj4870, Mj5331, Mj0272).



**Fig. 1** Sampling design. Three regions and six  $5 \times 5$  km study landscapes per region were considered. In each study landscape, sample locations were randomly selected on a regular  $500 \times 500$  m grid placed 500 m away from the border

We used Genepop (Rousset 2008) to test for linkage disequilibrium among pairs of loci, and the R package ‘popgenreport’ (Adamack and Gruber 2014) to obtain the number of alleles per marker. We also used Genepop to detect the presence of null alleles, a phenomenon known to be frequent in *Lepidoptera* (Meglecz et al. 2004), and discarded markers with high null allele frequency rates ( $>0.2$ ). To test for possible sex-biased gene flow in the meadow brown butterfly, we analyzed the data for each sex separately.

There was no linkage disequilibrium between pairs of loci, but locus Mj2410 showed sex linkage: all the females were homozygotes. Evidence of frequent null alleles ( $>0.2$ ) was detected at loci: Mj5522, Mj5287, Mj5647, Mj3956, Mj5563, Mj0272, Mj0283 and Mj3637. As a consequence, we only retained loci Mj0008, Mj7132, Mj0247, Mj7232, Mj4870 and Mj5331 in further analyses (see descriptive statistics in Table 1). Only complete genotypes for the six selected microsatellite markers were analyzed, leading to a total sample size of 1526 samples (92 % of the samples collected, 1000 males, 526 females).

With the six retained loci, we estimated inter-individual genetic distances and calculated the mean genetic distances between individuals for each pair of locations within the same landscape. We tested the Kosman (Kosman and Leonard 2005) and Smouse (Smouse and Peakall 1999) genetic distances (both computed with the R package ‘popgenreport’)—two metrics widely used in individual-based landscape genetics studies (Adamack and Gruber 2014).

### Landscape genetics analyses

For each study landscape, we combined available national GIS data to obtain a preliminary land use

map, then corrected and updated this map with aerial photographs and field observations (Appendix A1 as Supplementary Material). Our final maps distinguished eight land uses: woodlands, woodland edges, semi-natural grasslands, arable lands, roads, built-over areas, grassy strips (road verges, grassy field margins and green lanes) and vineyards/orchards. Built-over areas were not retained for subsequent analyses because they represented a very small fraction of the landscapes (mean cover:  $3\% \pm 1$  SD). We used a 30 m digital elevation model and the Geomorphometry and Gradient Metrics Toolbox for ArcGIS 10.2 (Evans et al. 2014) to derive topographic roughness (rough), and heat load index (hli). Topographic roughness is a measurement developed by Riley et al. (1999) to express the elevation difference between adjacent cells of a digital elevation grid. Heat load index takes into account latitude, slope, and aspect to quantify solar exposure (McCune and Keon 2002).

We utilized two different resistance methods, least cost path and circuit theory, to assess the influence of different landscape features on butterfly movement. For both methods, we hypothesized that each land use can either reduce or enhance gene flow (Prunier et al. 2014). To test for both conductance and resistance effects, we created two resistance surfaces for each land use and each topographic feature (i.e. topographic roughness and heat load index). All resistance surfaces had a cell size of 100 x 100 m (a compromise between precision and computation time). We first created a resistance surface to test for resistance to gene flow where pixel values were the proportion of the land use in the cell or mean roughness, and heat load index. We then created the inverse resistance surface ( $1/\text{proportion}$ ) to consider conductance instead of resistance

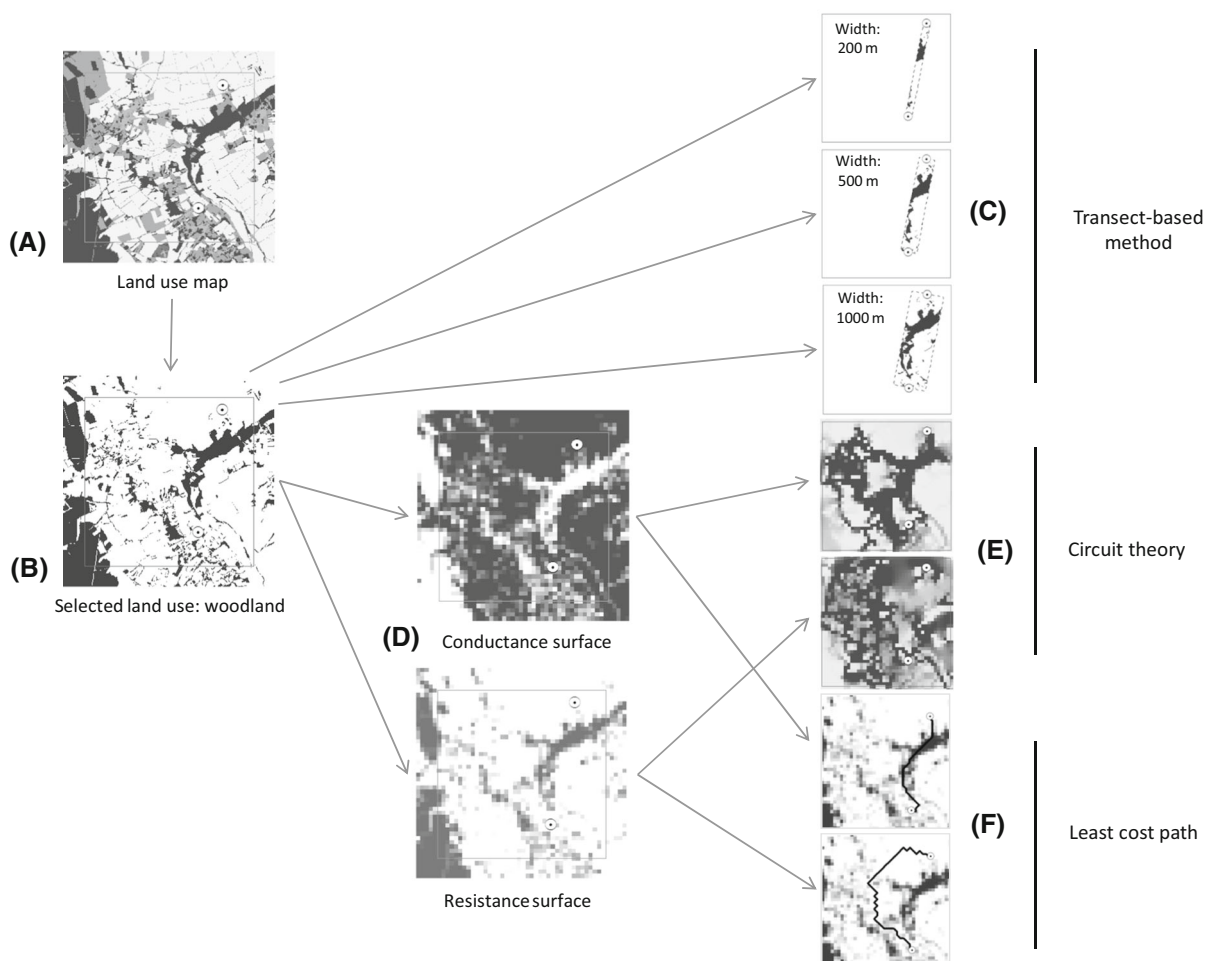
**Table 1** Main characteristics of the six selected microsatellite markers for the meadow brown butterfly

	No. of alleles		Null allele rate		He		Ho	
	Males	Females	Males	Females	Males	Females	Males	Females
Mj0008	10	7	0.01	0.01	0.101	0.099	0.091	0.088
Mj7132	20	17	0.06	0.06	0.856	0.866	0.731	0.735
Mj0247	59	53	0.10	0.09	0.955	0.951	0.765	0.771
Mj7232	17	17	0.14	0.14	0.843	0.843	0.615	0.616
Mj4870	13	13	0.16	0.20	0.690	0.711	0.453	0.435
Mj5331	29	27	0.02	0.03	0.903	0.904	0.852	0.835

Number of alleles, null allele frequencies, expected and observed heterozygosity (He and Ho) are given for males (N = 1000) and females (N = 526)

(Fig. 2). This method, based on raw maps, reduces subjectivity in assigning cost values. In addition, to test for isolation by distance (IBD), we created a homogeneous resistance surface where all pixel values were equal to 1. To summarize, we created  $2 \times 9 + 1 = 19$  resistance surfaces (where 2 = one surface to test for resistance + one to test for conductance; 9 = seven land uses + two topographic features; 1 = the IBD resistance surface). For all resistance surfaces, values were rescaled between 0.01 and 100 before least cost path and circuit theory calculations. We computed least cost paths with the R package ‘gdistance’ (van Etten 2014), and used Circuitscape (McRae et al. 2008) to calculate current flow across each landscape

(Fig. 2). We obtained least cost path and electric current values between each pair of locations for the 19 resistance surfaces, then each of these values was used as an explanatory variable in separate regression analyses (after center-scale transformation). Specifically, for each region, sex, method (i.e. circuit theory and least cost path) and resistance surface, the R package ‘nlme’ was used to fit linear mixed-effect models to the data (Pinheiro et al. 2015), with the R function `corMLPE` (<https://github.com/nspope/corMLPE>) to account for multiple memberships with maximum likelihood population effects parameterization (MLPE, Clarke et al. 2002). In our case, the term “population” in MLPE referred to sample location.



**Fig. 2** Flow-chart of methods used to assess landscape effect on gene flow; example with two locations. **a** Land use map of the study landscape. **b** Selection of a particular land use class, here woodland (in *black*). **c** Illustration of the transect-based method. **d** Creation of the two inverse resistance surfaces reflecting

woodland resistance and conductance hypotheses (*black* high resistance value, *white* low resistance value). **e** Electric current if we consider woodland as a dispersal enhancer (above) or as a barrier (below). **f** Least cost paths if we consider woodland as a dispersal enhancer (above) or as a barrier (below)

Indeed, all the locations were present in different pairs, causing some statistical dependency among pairwise distances when they shared a location (e.g. pairs  $i-j$  and  $i-k$ , sharing the location  $i$ , Clarke et al. 2002). The covariate structures of the models incorporated a parameter  $\rho$ , which is the proportion of the variance corresponding to that correlation between pairwise distances. In all the models, pairwise genetic distance was used as the dependent variable, and resistance distance between locations (electric current or least cost path) as the independent variable. In addition, we included study landscape as a random factor. Data from each region and sex were analyzed separately. We compared the models for each resistance surface (including the IBD surface) to the null model (no fixed effect) with the same random effect structure. We estimated model fit with the marginal coefficient of determination for generalized mixed-effect models with Nakagawa & Schielzeth's  $R^2$  (Nakagawa and Schielzeth 2013) from the R package 'MuMIn' (Bartoń 2013).

For the transect-based method, we delineated a rectangular area in a straight line between each pair of sample locations. In this transect, we estimated the proportion and patch density of each intervening land use and centered-scaled these proportions by region (Fig. 2). Mean topographic roughness and heat load index were also estimated in each transect. Dispersal strategies depend on the perceptual range of the species under study (Zeller et al. 2012); we therefore tested different transect widths to determine the corridor width between pairs of locations that best fit our data. Consequently, we applied the transect-based method with 200, 500 and 1000 m wide transects (Fig. 2). We used a model selection method similar to that described for resistance methods: for each sex, region and transect width, we used linear mixed-effect models with an MLPE correlation structure. To test for IBD, we compared the null model (with intercept only) with a model with the distances between each pair of locations. Contrary to the two resistance methods, the transect-based approach allowed us to test for multiple effects without subjective assumptions (van Strien et al. 2012). We determined the best model by sequentially adding explanatory variables to the null model. We continued this stepwise procedure until the next variable to be added failed to reduce AIC by 2 points or more (Burnham and Anderson 2002). Collinearity between explanatory variables was tested

and only uncorrelated variables (correlation threshold  $<0.5$ ) were included in the same model. All statistical analyses were performed in R 3.0.2 (R Core Team 2013).

To determine the best analytical approach to model butterfly dispersal in farmland, we compared models fits obtained with least cost path, circuit theory and transect-based analysis.

## Results

The Smouse and Kosman metrics were highly correlated (Pearson = 0.86,  $p < 0.001$ ) and led to similar conclusions (Table 2 and Table S2 in Supplementary Material). Thus, we only present below the results from the Kosman metrics (see Table S2 in Supplementary Material for the results obtained with the Smouse metric). Models that were lower than the null model by 2 AIC points are displayed in Table 2.

The null model was often the best one (Table 2) and environmental factors only explained a very small proportion of the genetic differentiation between pairs of male and female meadow brown butterflies in the three study regions (all  $R_m^2 < 5\%$ , Table 2).

Our analyses revealed some significant and consistent trends across regions: (1) grasslands enhanced gene flow in males in Gascony (transect-based method 500 m:  $p < 0.05$ ) and in females in Aquitaine (transect-based method 200 m:  $p < 0.05$ , transect-based method 500 m:  $p < 0.001$ ); (2) roads—a proxy for grassy road verges—conducted females in Burgundy (least cost path:  $p < 0.05$ ) and males in Gascony (transect-based method 1000 m:  $p < 0.05$ ); (3) arable lands limited males (transect-based method 100, 250 and 500 m:  $p < 0.05$ ) and females dispersal in Aquitaine (circuit theory:  $p < 0.05$ , least cost path:  $p < 0.001$ ), despite an opposite effect detected for females in Aquitaine (transect-based method 500 m:  $p < 0.05$ ); (4) woodland and woodland edges seemed to impede gene flow for females in Gascony (least cost path:  $p < 0.05$ ) and males in Burgundy (circuit theory:  $p < 0.05$ , least cost path:  $p < 0.05$ ), (5) IBD was never detected, and (6) topographical features—roughness and heat load index—were never selected.

In summary, among sexes and regions, none of the three analytical methods seemed to outperform the other ones, but the analytical approaches converged to identify grasslands and, to a lesser extent, grassland-

**Table 2** Models with the Kosman distance for each analytical approach, region and sex. Models 2 AIC points lower than the null model are displayed

Method	Region	Sex	Effect(s) of the variable(s) selected					$\rho$	p value	$R_m^2$	$R_c^2$
			Wood	W.edge	Arable	Road	Grassld				
Circuit theory	Aquitaine	Males						0.21		0.000	0.000
		Females			–			0.14	*	0.011	0.092
	Burgundy	Males	–					0.22	*	0.004	0.012
		Females						0.21		0.000	0.000
	Gascony	Males						0.20		0.000	0.000
		Females						0.11		0.000	0.000
Least cost path	Aquitaine	Males						0.21		0.000	0.000
		Females			–			0.14	**	0.038	0.115
	Burgundy	Males	–					0.21	*	0.003	0.017
		Females				+		0.20	*	0.006	0.006
	Gascony	Males						0.20		0.000	0.000
		Females		–				0.11	*	0.018	0.018
Transect-based method 200 m	Aquitaine	Males			–			0.21	*	0.003	0.003
		Females					+	0.14	*	0.007	0.085
	Burgundy	Males						0.22		0.000	0.009
		Females						0.21		0.000	0.000
	Gascony	Males						0.20		0.000	0.000
		Females						0.11		0.000	0.000
Transect-based method 500 m	Aquitaine	Males			–			0.21	*	0.004	0.004
		Females			+		+ <sup>a</sup>	0.15	* and **	0.018	0.114
	Burgundy	Males						0.22		0.000	0.009
		Females						0.21		0.000	0.000
	Gascony	Males					+ <sup>a</sup>	0.20	*	0.003	0.003
		Females						0.11		0.000	0.000
Transect-based method 1000 m	Aquitaine	Males			–			0.21	*	0.006	0.006
		Females						0.15		0.000	0.086
	Burgundy	Males						0.22		0.000	0.009
		Females						0.21		0.000	0.000
	Gascony	Males					+	0.20	*	0.004	0.004
		Females						0.11		0.000	0.000

Effect(s) of the variable(s) selected (–: limits gene flow, +: enhances gene flow),  $\rho$ ,  $p$  value(s),  $R_m^2$  and  $R_c^2$  are given. If no ‘+’ or ‘–’ is indicated, it means that the null model is the best supported. For least cost path and circuit theory, we tested simple mixed effects regression models. In the transect-based analyses, we tested additive mixed effects regression models; if more than one variable was selected,  $R_m^2$ ,  $R_c^2$  and  $\rho$  correspond to the best additive model

*Arable* proportion of arable lands, *grassld* proportion of grasslands, *g.strip* proportion of grassy strips, *road* proportion of roads, *wood* proportion of woodlands, *w.edge* proportion of woodland edges.  $R_m^2$ ,  $R_c^2$  marginal and conditional coefficients of determination of the generalized mixed-effect model (Nakagawa and Schielzeth 2013),  $\rho$  proportion of the total variance that results from a correlation between two pairwise observations involving a common location, the maximum value of 0.5 occurring when the individual inter-location effect is large

Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$

<sup>a</sup> Patch density of the respective habitat was selected instead of proportion



like linear habitats as enhancing gene flow; whereas arable lands, woodlands and woodland edges generally impeded gene flow (Table 2).

## Discussion

Our sampling design combined three regions, 18 study landscapes, randomly selected sampling sites and individual-based analyses. We compared three analytical approaches relying on different assumptions of how animals perceive and navigate in the landscape during dispersal (straight-line path, least-cost-path or random walk). We carried out statistical analyses that explicitly take into account the non-independence of pairwise genetics data and consider multiple study landscapes. Results showed that the analytical methods used and the explanatory variables tested had limited ability to explain the pairwise genetic differentiation of the meadow brown butterfly in our studied landscapes. However, our replicated sampling design combined with multiple analytical approaches gave us the ability to detect and corroborate subtle effects of landscape features on dispersal. Indeed, we found that woodlands and arable lands limited meadow brown butterfly gene flow, whereas grasslands and grassy linear elements tended to enhance effective dispersal.

### Landscape weakly impacts meadow brown butterfly genetic structure

In the three study regions, for both sexes and all analytical approaches, landscape features had a small but measurable effect on gene flow. This result is not rare in landscape genetics studies (Broquet et al. 2006; Harrison et al. 2012; Hahn et al. 2013; Coster et al. 2015; Meyer et al. 2015) and can result from different factors (see below).

There was no IBD effect between locations up to 4 km apart, nor even between study landscapes separated from one another by up to 60 km (results not shown). The absence of a relationship between genetic and geographic distances is expected when either gene flow or genetic drift are dominant in shaping population structure (Phillipsen et al. 2015). Here, we suspect that gene flow is high across the regions because inter-landscape  $F_{st}$  values are very low (0.005 on average, Table S3 as Supplementary Material). In addition, an allozyme study showed that genetic differentiation

among the meadow brown butterfly populations is rather weak (Schmitt et al. 2005).

The type and magnitude of landscape effects on gene flow depend on species' biological characteristics (Engler et al. 2014) and landscape context (Jaquiéry et al. 2011; Graves et al. 2012; Cushman et al. 2013). High abundance, low specialization, and high dispersal ability are traits often linked with low genetic differentiation in butterflies (Habel et al. 2013). The meadow brown butterfly is abundant and can utilize resources in the arable mosaic, which can make dispersal easier by reducing energy costs. Small, grassland-like, sub-optimal habitats such as grassy field margins and road verges can provide temporary shelter and feeding opportunities, and facilitate butterfly movements between suitable patches. Thus, the weak effect of landscape features on the genetic structure of the meadow brown butterfly could reflect ecological characteristics and/or disequilibrium between genetic drift and gene flow.

### Meadow brown butterfly dispersal in farmland

In general, animals disperse more through favorable habitats than through non-habitats (Eycott et al. 2012), but counterintuitive results showing an increase in movement through sub-optimal or low quality habitats have also been evidenced (Peterman et al. 2014; Prunier et al. 2014). Even though we only detected weak effects of environmental conditions on butterfly gene flow, our findings are mainly consistent with the hypothesis that dispersal is greater through landscape features similar in structure to the preferred habitat. Indeed, we found that grasslands and grassy linear landscape elements enhanced gene flow, which can be due to higher resource availability and population density of the species in such environments (Eycott et al. 2012). The negative effect of woodlands on dispersal detected in this study can be linked to the lack of sunlight butterflies need to fly in forest interior, but also to the physical resistance of the thick vegetation structure in forest that can limit movements. Arable lands support fewer resources and shelters. Dispersal through such environments can be costlier—reducing reproductive success—and riskier—decreasing survival rate—explaining the resistance to gene flow we observed.

The patterns we detected are concordant with mark-recapture studies on this species: Kindlmann et al.

(2004) and Ouin et al. (2008) found that woodlands and crops had a negative effect on movements, whereas grassland cover could enhance movements depending on the study site. Moreover, the positive effect of linear landscape elements we detected concurs with Delattre et al. (2013b): through direct movement monitoring, they showed that grassy field margins support a corridor function by enhancing meadow brown butterfly effective dispersal. So, even if meadow brown butterfly abundance and survival rates are lower in linear elements (Öckinger and Smith 2007), these landscape features can still play a role in their dispersal.

#### Analytical approaches in landscape genetics

Each analytical approach relies on specific assumptions of how animals perceive and navigate the landscape during dispersal (transect-based method: straight-line path, least-cost-path: optimal path selection, circuit theory: random walk). If one of these methods had outperformed another, this could have provided insight into meadow brown butterfly dispersal behavior in farmland. Unfortunately, the low fit of our models prevented comparisons among the analytical approaches and among sexes.

In the future, it could be promising to compare our results to those we would obtain applying individual-based models which simulate individual dispersal strategies. Such models can incorporate stochasticity and model movement behaviors in more details and with more ‘realism’ (Palmer et al. 2014). Some models already exist for the meadow brown butterfly (Kindlmann et al. 2005; Aviron et al. 2007) and their adaptation to our study contexts could improve our understanding of the genetic patterns we observed.

Notwithstanding the assessment of alternative hypotheses about how butterflies perceive and disperse through the landscape, using multiple approaches and replicating study landscapes (Short Bull et al. 2011) can help increase the ability to detect subtle effects. Despite the limited amount of variance explained in our study, some signals are consistent among regions, sexes, analytical approaches, and genetic metrics, thus providing confidence in our inferences.

Although the different analytical methods we used provided complementary information and robustness, we note that the transect-based method produces models which are both easy to compute and easy to

interpret. Moreover, it allows us to test for multiple effects (additive effects of landscape variables) without subjective assumptions. This is not currently possible with resistance methods, even though the simultaneous optimization of multiple surfaces is a promising avenue (Peterman 2014; Peterman et al. 2014).

#### Study limitations

Similar to Richard et al. (2015), we detected a high frequency of null alleles in many loci. Null alleles are known to be an issue in insect genetics, particularly in butterflies, because of high mutation rates which can affect flanking regions of microsatellites (Meglecz et al. 2004). Null alleles are particularly troublesome with individual-based sampling scheme because methods to correct for the presence of null alleles are only available at the population level (Chapuis and Estoup 2007). As a consequence, we used relatively few markers which limited our ability to make rigorous statistical inference. In addition, given the weak effects of landscape features we detected, our results have to be taken with caution.

Further studies including more microsatellite loci and/or other genetic markers (mtDNA, Single Nucleotide Polymorphism, Amplified Fragment Length Polymorphism) on the same species are therefore needed to verify the patterns we detected. Moreover, an exciting alternative lies on the development of genomic approaches to have a greater power to assess dispersal processes (Petren 2013).

#### Implications for conservation

In conclusion, this study demonstrates that the landscape features in our three study regions had little impact on meadow brown butterfly gene flow. Habitat connectivity at the landscape level does not seem to limit gene flow in this species. We can, however, suppose that the negative effects of woodlands and arable lands would impact meadow brown butterfly dispersal in more heavily fragmented landscapes. These conclusions would likely extend to other grassland species with stricter ecological demands and lower dispersal abilities, thus making habitat connectivity crucial to ensure dispersal and gene flow across the landscape. Future research should seek to further explore this hypothesis.

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