

Do landscape barriers affect functional connectivity of populations of an endangered damselfly?

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SUMMARY

1. Landscape genetic approaches were used to assess functional connectivity of populations of the endangered damselfly *Coenagrion mercuriale* in a fragmented agricultural landscape in Switzerland. Spatial genetic clustering methods combined with interpolation by kriging and landscape genetic corridor analysis were applied to identify landscape elements that enhance or hinder dispersal and gene flow.

2. Spatial genetic clustering analysis divided the sampled populations into a northern and a southern genetic group. The boundary between the two groups coincided with a hill ridge intersecting the study area. Landscape corridor analysis identified five landscape elements that significantly affected gene flow. Elevation change, Euclidian distance, patches of forest and flowing waterbodies acted as barriers, whereas open agricultural land enhanced gene flow between populations of *C. mercuriale*.

3. This study showed that movement of *C. mercuriale* was not restricted to its preferred habitat (i.e. streams). Populations linked via continuous open agricultural land were functionally well connected if they were not more than about 1.5–2 km apart. In contrast, substantial elevation change and larger forest patches separated populations. These findings may serve as a basis to define conservation units and should be considered when planning connectivity measures, such as determining the locations of stepping stones, or the restoration of streams.

Keywords: conservation, dispersal, fragmentation, landscape genetics, Odonata

Introduction

Traditional European agricultural landscapes provided habitats for many insect species, including freshwater species such as dragonflies and damselflies. However, because of management intensification during recent decades (Stoate *et al.*, 2001), many species have become threatened through habitat fragmentation and isolation of remaining populations (Fahrig, 2003). To counteract these negative trends, various conservation strategies have been undertaken that focus on the preservation or restoration of remaining habitat patches as well as measures that enhance connectivity (Reed, 2004). Conservation management of dragonflies and damselflies is mainly focussed on the preservation and restoration of habitats (Wildermuth & Küry, 2009), but little is known about population connectivity. In this study, we assess connectivity in a

rare damselfly inhabiting streams in an agricultural landscape.

Connectivity can be estimated in a structural or a functional way. Whereas structural connectivity is based on the analysis of landscape elements and their spatial configuration, functional connectivity considers the behavioural response of a species to landscape elements by measuring dispersal and gene flow (Tischendorf & Fahrig, 2000a,b). Dispersal in aquatic insects usually consists of movement by flight at the imaginal terrestrial life stage occurring within and between streams as well as movement by drift at the larval aquatic stage within streams (Bilton, Freeland & Okamura, 2001). At both life stages, dispersal can be quantified either directly by observation or mark–recapture studies or indirectly by genetic methods (Bilton *et al.*, 2001; Clobert, 2001). The main disadvantage of mark–recapture studies is that rare,

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long-distance dispersal events are difficult to detect (Allendorf & Luikart, 2007) and that dispersal distances are often underestimated (Van Dyck & Baguette, 2005; Kamm *et al.*, 2009). This is especially the case for the adult life stage of freshwater insects, as has been shown for dragonflies (e.g. Keller *et al.*, 2010).

To overcome these limitations, genetic methods can be used to investigate historical or contemporary dispersal and gene flow (Manel, Gaggiotti & Waples, 2005). For many freshwater insects, population genetic structure has helped uncover species-specific dispersal patterns (Hughes, Schmidt & Finn, 2009). For instance, genetic structure showed widespread adult dispersal in an ephemeropteran species (Hughes *et al.*, 2000). In contrast, limited adult dispersal was detected in a plecopteran species, and larval dispersal seemed to be predominant (Schultheis, Weigt & Hendricks, 2002). Such knowledge on the dispersal potential of species is essential to develop successful conservation strategies. However, it is important to identify those landscape elements that either enhance or hinder dispersal and gene flow in aquatic insects.

The field of landscape genetics provides tools to detect the role of landscape in species' dispersal patterns by correlating population genetic parameters with landscape elements (Segelbacher *et al.*, 2010; Storfer *et al.*, 2010). While the majority of landscape genetic studies has been performed on vertebrates in terrestrial ecosystems (Storfer *et al.*, 2010), few analyses have been carried out on freshwater insects (e.g. Alp *et al.*, 2012). Even fewer studies have considered landscape effects on dispersal and gene flow at the adult life stage (but see Wilcock *et al.*, 2007). Spatial genetic clustering methods, which are based on assignment tests (Manel *et al.*, 2005), have been widely used in studies on freshwater insects (e.g. Chaput-Bardy *et al.*, 2008; Alp *et al.*, 2012) to give a first impression of levels of gene flow. When clustering methods are combined with geostatistical surface interpolation (e.g. spatial kriging; Ripley, 1981), major landscape barriers to gene flow can be located (e.g. Murphy *et al.*, 2008; Row, Blouin-Demers & Loughheed, 2010). Nevertheless, to identify landscape elements that enhance or hinder gene flow, additional landscape genetic methods can be applied, such as least-cost path analysis (Adriaensen *et al.*, 2003). Least-cost path analysis requires expert knowledge on species-specific dispersal behaviour to derive movement costs, which are then translated into resistance surfaces. Subsequently, least-cost paths between pairs of populations are calculated. A study on a black fly (Finn *et al.*, 2006), for instance, tested effective distances based on resistance surfaces, which included

several landscape elements thought to be important for insect flight (e.g. difference in elevation or dispersal along streams). By assessing how well effective distances explain genetic distances, conclusions are drawn about the inhibiting or enhancing effect of the landscape elements in the resistance surface. However, the relative contribution of an individual landscape element in determining the least-cost path is unknown. Additionally, least-cost path analysis requires *a priori* knowledge of species' movement behaviour. This information is often not available. In contrast, an alternative method called strip, transect or corridor analysis (Lindsay *et al.*, 2008; Pavlacky *et al.*, 2009; Angelone, Kienast & Holderegger, 2011; Emaresi *et al.*, 2011) does not require such *a priori* knowledge, and the effect of each landscape element on gene flow can be assessed separately. In this type of analysis, straight lines, connecting pairs of populations, are buffered to form corridors. Subsequently, landscape elements within these corridors are quantified and serve as predictor variables for gene flow estimates. Despite its advantages, transect analysis has rarely been used in studies on freshwater insects. One exception is a study on two caddisflies, which assessed the effect of geological features along transects on genetic differentiation (Wilcock *et al.*, 2007).

In this study, we analysed the effects of a fragmented agricultural landscape on dispersal and gene flow at the adult life stage of the rare damselfly *Coenagrion mercuriale*, Charpentier. This Atlanto-Mediterranean species is mainly distributed in western Europe and north-western Africa (Wildermuth, Gonseth & Maibach, 2005). *Coenagrion mercuriale* is threatened in most of its distribution area and listed in Annex II of the Berne Convention and the EC Habitats and Species Directive. In Switzerland, the species is critically endangered (Gonseth & Monnerat, 2002). In agricultural landscapes, *C. mercuriale* is mainly found along slow-flowing, summer-warm streams and ditches (Sternberg, Buchwald & Röske, 1999). Previous studies have shown that various habitat characteristics have a positive effect on the density of *C. mercuriale* (e.g. Sternberg *et al.*, 1999; Rouquette & Thompson, 2005; Hepenstrick, 2008; Koch, Wildermuth & Walter, 2009). Among these are abundant water vegetation (bankside, emerging and wintergreen submerged vegetation), stream substrate consisting of silt and permanent flowing open water. In contrast, the presence of shading trees and bushes has a negative impact on the density of *C. mercuriale*.

Several mark-recapture studies and genetic analyses have shown that *C. mercuriale* is a sedentary species with low dispersal ability (e.g. Watts *et al.*, 2007). The recorded maximum movement distance of marked individuals is

1790 m (Watts *et al.*, 2004a; Rouquette & Thompson, 2007), while genetic studies indicated dispersal up to 2 km and rather high migration rates (Watts *et al.*, 2006). The latter result is supported by an observation of *C. mercuriale* up to 3 km away from the closest reproductive habitat (Hunger & Röske, 2001). In addition, several landscape elements have been identified that seem to facilitate, hinder or have no effect on the movement of *C. mercuriale*. Over short distances, movement was found to be restricted to reproductive habitat (Hunger & Röske, 2001; Rouquette & Thompson, 2007), and high ground and scrub vegetation seem to act as barriers (Purse *et al.*, 2003; Watts *et al.*, 2006). In contrast, motorways, railways and large rivers have no measurable impact on the dispersal of *C. mercuriale* (Watts *et al.*, 2004a). Overall, however, little is known about the dispersal of *C. mercuriale* at the landscape level, and no detailed landscape genetic study has been performed thus far.

In this study, we conducted a landscape genetic analysis to (i) assess functional connectivity between populations of *C. mercuriale* by examining their spatial genetic structure and to (ii) identify whether landscape elements, in addition to geographic distance, have an inhibitory or enhancing effect on dispersal and gene flow. For this purpose, we combined genetic clustering methods and interpolation by kriging with landscape corridor analysis. The study was carried out in a fragmented agricultural landscape in Switzerland that consisted of several stream systems in which all *C. mercuriale* populations had previously been mapped and monitored.

Methods

Study area and sampling

Our study area was located in the Oberaargau region on the Swiss plateau (Fig. 1), which is dominated by intensive agriculture with crop fields, pastures, meadows, settlements and forest patches. Nevertheless, several rare species occur in this area, and it has therefore been designated as a Swiss Smaragd area (Delarze *et al.*, 2003; Hedinger, 2009). Smaragd is the Swiss equivalent of the European Union Natura2000 framework and comprises a national network of designated areas that are established to protect threatened and rare animals, plants and habitats. One of the focal species of the Oberaargau Smaragd area is the southern damselfly *Coenagrion mercuriale* (Odonata: Coenagrionidae). Here, this damselfly species occurs along several stream systems composed of slow-flowing, unshaded streams across an area of about 120 km² (Fig. 1). In 2008, all existing populations of

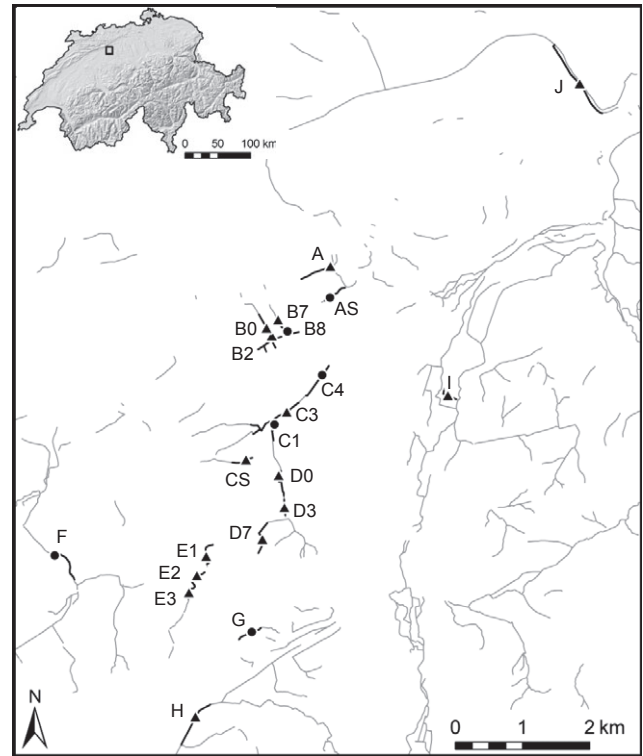


Fig. 1 Occurrences of *Coenagrion mercuriale* in the Oberaargau region of Switzerland (black lines). Grey lines represent above-ground streams. Triangles indicate sampling locations where more than ten individuals could be sampled, circles give locations with less than ten individuals sampled. The inset shows the location of the study area within Switzerland.

C. mercuriale had been recorded, and the local habitat preferences of the species were analysed (Hepenstrick, 2008).

Samples of *C. mercuriale* for genetic analysis were collected from all known populations in the Oberaargau (Hepenstrick, 2008) towards the end of the mating season in 2009 (mid to end of July). Depending on local population size, we sampled up to 45 individuals from each continuous section of stream (i.e. stream section not interrupted by roads, bridges or houses). Coordinates were recorded for the centre of each section. We collected mid-leg tibiae of males only, since the proportion of females at streams was low and handling of females could have damaged their wing surfaces, which might subsequently have hindered underwater respiration during oviposition. Where fewer than 30 individuals were found, all males present were sampled. In total, tibiae of 450 individuals from 19 stream sections were collected (Table 1; Fig. 1). Tibiae were immediately placed in 100% ethanol and subsequently stored at -20 °C in the dark.

Table 1 Sample sizes and genetic diversity measures for populations of *Coenagrion mercuriale* in Switzerland (for sampling locations, see Fig. 1)

Population	Sample size	H_o	H_e	A_r	F_{IS}	HWE test
A	33	0.476	0.456	2.960	-0.043	0.904
AS	4	–	–	–	–	–
B0	36	0.455	0.483	3.096	0.058	0.123
B2	45	0.465	0.505	3.312	0.080	0.316
B7	12	0.596	0.540	3.531	-0.104	0.892
B8	9	–	–	–	–	–
C1	1	–	–	–	–	–
C3	23	0.525	0.517	3.014	-0.016	0.989
C4	4	–	–	–	–	–
CS	15	0.457	0.478	2.747	0.044	0.485
D0	35	0.507	0.497	2.887	-0.021	0.965
D3	15	0.478	0.509	3.193	0.060	0.906
D7	29	0.468	0.496	2.930	0.055	0.700
E1	39	0.526	0.492	2.852	-0.068	0.973
E2	12	0.569	0.505	2.786	-0.128	0.995
E3	33	0.485	0.486	2.873	0.002	0.594
F	6	–	–	–	–	–
G	2	–	–	–	–	–
H	10	0.458	0.479	3.000	0.043	0.893
I	33	0.467	0.460	2.866	-0.016	0.930
J	16	0.453	0.46	2.889	0.015	0.902

For all larger populations (with a sample size ≥ 10), observed (H_o) and expected (H_e) heterozygosity, allelic richness (A_r), inbreeding coefficient (F_{IS}) and the P -value for test of Hardy–Weinberg equilibrium (HWE) are given.

Genetic analysis

Samples were lyophilised and ground in an MM300 mixer mill (Retsch, Haan, Germany) for 4 min. DNA extraction was performed with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers' instructions with the recommended changes for insects. As tibiae of damselflies are only a few millimetres in length, only half the volume of the recommended quantities of extraction chemicals was used, and DNA was eluted twice with 75 μ L elution buffer.

For genotyping, we used twelve species-specific microsatellite markers published by Watts *et al.* (2004b): LIST4-002, LIST4-023, LIST4-024, LIST4-031, LIST4-034, LIST4-035, LIST4-037, LIST4-042, LIST4-060, LIST4-062, LIST4-063, LIST4-066. PCR was performed in 10 μ L reaction volumes with 2 $\times$ Multiplex Mix (Qiagen, Hilden, Germany) following the protocol described in Keller, Brodbeck & Holderegger (2009). Fragments were analysed on an ABI 3730xl sequencer (Applied Biosystems, Carlsbad, CA, USA) and genotyped with GENEMAPPER 3.7 (Applied Biosystems). Genetic diversity measures were calculated in GENETIX v.4.05 (H_o , H_e ; Belkhir *et al.*, 1996–2004) and FSTAT 2.9.3.2 (A_r , F_{IS} ; Goudet, 1995). Tests for

Table 2 Correlations among different genetic distance measures calculated for populations of *Coenagrion mercuriale*

Genetic distance measure 1	Genetic distance measure 2	Pearson correlation	Spearman correlation
F_{ST}	G'_{ST}	0.991	0.990
F_{ST}	G_{ST}	0.985	0.986
F_{ST}	D_{est}	0.985	0.980
G'_{ST}	G_{ST}	0.972	0.975
G'_{ST}	D_{est}	0.997	0.993
G_{ST}	D_{est}	0.954	0.955

population-based linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) as well as pairwise genetic differentiation (F_{ST} ; Weir & Cockerham, 1984) were assessed with GENEPOP 4.0.10 (Raymond & Rousset, 1995). As most landscape genetic studies (Storfer *et al.*, 2010), we used F_{ST} as a measure of gene flow. Note that F_{ST} -values were highly correlated (Pearson $r \geq 0.95$) to other genetic distance measures (D_{est} , G_{est} , $G_{Hedrick}$; Table 2). As F_{ST} is an effective measure for genetic structure (Whitlock & McCauley, 1999), it can indirectly be interpreted as (past) gene flow (Spear *et al.*, 2005; Emaresi *et al.*, 2011). Slightly negative F_{ST} -values were set to 0. Populations were grouped for global HWE and LD tests, and global F_{ST} was calculated in FSTAT 2.9.3.2 (Goudet, 1995).

Clustering analysis

Individual-based Bayesian genetic clustering methods group individuals into clusters aiming to maximise HWE and minimise linkage disequilibrium within clusters. We performed clustering analysis with TESS v.2.3 (Chen *et al.*, 2007) with the default spatial interaction parameter ($h = 0.6$) and irregular sampling adjustment (including geographic distances). The admixture model (CAR) was run ten times for each potential number of clusters (K_{max}) ranging from $K_{max} = 2$ –6 with a total of 500 000 sweeps and a burn-in of 100 000 sweeps. Model selection using the deviance information criterion (DIC) did not clearly indicate a most likely K_{max} . However, we only found small standard deviations of DIC (Fig. 2) and high and consistent individual cluster membership probabilities for $K_{max} = 2$. For $K_{max} = 3$, for instance, cluster membership probabilities were lower than 0.15 for the third cluster. Therefore, we used the clustering results for $K_{max} = 2$ to calculate the means of all estimated cluster membership coefficients from 20 runs, after correcting for label switching by permuting individuals (Jakobsson & Rosenberg, 2007). With the kriging technique, the membership means of both clusters were interpolated across the study area using the 'Krig' function of the R-package

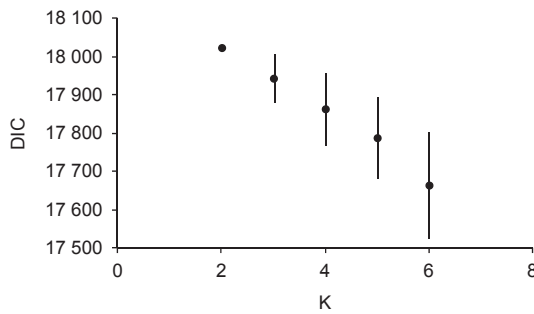


Fig. 2 Estimation of number of clusters ($K_{\max}$) for populations of *Coenagrion mercuriale*: mean deviance information criterion plot (DIC) from TESS analysis.

'fields' (Furrer, Nychka & Sain, 2010) in R (R Development Core Team, 2011). For both clusters, a separate grid was created (ARCGIS 9.3.1; ESRI, Redlands, CA, USA) where each cell represented either the calculated (TESS) or the interpolated cluster membership proportion. Both grids were then combined by assigning to each cell the higher cluster membership value of the two grids. The resulting combined grid was then overlaid on a land-cover and topographic map (i.e. the overlay approach of landscape genetics; Storfer *et al.*, 2010).

Corridor analysis

To assess the potential role of landscape elements that may be impeding or enhancing gene flow between populations of *C. mercuriale*, we performed a corridor analysis (e.g. Emaresi *et al.*, 2011). We chose straight-line corridors because the monotonic correlation between pairwise F_{ST} -values and straight-line distances was markedly higher (Spearman's $\rho = 0.479$) than the correlation between F_{ST} and distance along streams ($\rho = 0.316$), the latter being a potential movement habitat of *C. mercuriale*. We limited our analysis to the 15

populations that consisted of at least ten analysed individuals (Fig. 1). Between all pairs of these 15 populations (105 pairs), corridors with widths of 50, 100, 200 and 300 m were drawn. Within each corridor, several landscape elements potentially influencing dispersal and gene flow were quantified. We chose landscape elements encountered by imaginal damselflies, as larval dispersal restricted to streams has been previously reported to be of minor importance (Watts *et al.*, 2004a). Areas of flowing water, forest, buildings, roads and agricultural land within the corridors were derived from a vectorised land-cover map (vector25, resolution = 10 m; Swisstopo, Wabern, Switzerland; Table 3). We used the proportion of the area of each of these landscape elements per total corridor area ($\text{m}^2 \text{m}^{-2}$). Cumulative elevation change (i.e. the total vertical distance along the central line of each corridor) was calculated from a digital terrain model (resolution = 2.5 m; Swisstopo) using 'Surface Tools' (Jenness, 2008) in ARCVIEW 3.x (ESRI). All landscape measures as well as Euclidian distance were specified as predictor variables in regression models. Measures of proportions were arcsin-transformed and distances were log-transformed (Table 3). Pairwise genetic distance between populations (F_{ST}) was specified as the response variable.

We first determined the most representative corridor width using multiple linear regression analysis on distance matrices (Lichstein, 2007), which is rarely used in landscape genetic studies although its application is highly recommended (Storfer *et al.*, 2007; Balkenhol, Waits & Dezzani, 2009; Jaquierey *et al.*, 2011). The 'MRM' function implemented in the R-package 'ecodist' (Goslee & Urban, 2007; Lichstein, 2007) was used to test for significance of regression coefficients and R^2 -values by permuting the response genetic distance matrix to take the non-independence of observations in distance matrices into account. We calculated regression models

Table 3 Landscape elements used as predictor variables in multiple linear regression on distance matrices on 15 populations of *Coenagrion mercuriale* in Switzerland

Element	Description	Input format	Transformation
Flowing water	Above-ground flowing water, without large rivers (buffer: 1 m)	Area of flowing water/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Roads	Busy traffic roads, width ≥ 4 m (buffer: 2 m)	Area of roads/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Buildings	All buildings	Area of buildings/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Forest	All forests	Area of forest/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Agriculture	Agricultural land (e.g. meadows, pastures, crop fields)	Area of agriculture/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Elevation	Cumulative elevation change along straight-line	Elevation change/area of corridor ($\text{m}^2 \text{m}^{-2}$)	arcsin
Distance	Straight-line distance	Length (m)	log

For each landscape element, description, input format and type of transformation is given.

for each corridor width, each with 10 000 permutations. The most representative corridor width was determined by the best fitting model (i.e. the one with the highest R^2 -value).

After having selected the full model with the most representative corridor width, we additionally considered reduced regression models and selected the most supported model using Akaike's information criterion (AIC; Burnham & Andersson, 2002). AIC is a useful indicator to determine the likelihood of models with varying numbers of predictor variables. Note that AIC values are not corrected for the non-independence of observations and interpretation has therefore to be done with care (Burnham & Andersson, 2002). We performed automated model selection using the 'dredge' function of the R-package 'MuMIn' (Barton, 2011), which calculates AIC values for models with all possible combinations of predictor variables and ranks them based on AIC. The relative importance (Burnham & Andersson, 2002) of each predictor variable was then calculated based on AIC weights ('importance' function in 'MuMIn').

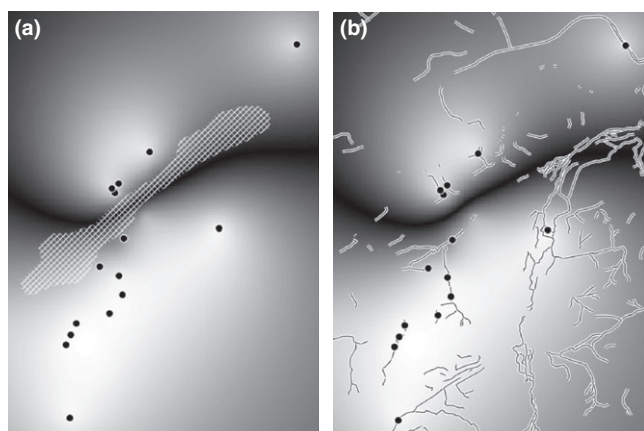


Fig. 3 Results of spatial clustering and overlay analysis with a hill ridge and streams for populations of *Coenagrion mercuriale* (circles) in the Oberaargau region of Switzerland (Fig. 1). (a) Overlay of two genetic clusters with a hill ridge (crosshatched). (b) Overlay of genetic clusters with above-ground flowing streams. The size of the area is 120 km².

Results

Tests for global linkage disequilibrium were significant ($P < 0.05$) for four pairs of loci. However, there was no consistent pattern in tests within populations, and we therefore assumed loci to be unlinked. HWE conditions were met both globally and in all populations (F_{IS} ; $P > 0.05$; Table 1). Global genetic differentiation among populations was low ($F_{ST} = 0.043$, range: 0.00–0.13).

Clustering analysis detected two distinct genetic groups in our study area, a northern and a southern group. When interpolating the two clusters by kriging (Fig. 3) and combining and overlaying them on a land-cover and topographic map, the boundary between the two groups coincided spatially with a continuous hill ridge intersecting the study area from west to east (Fig. 3a). Furthermore, as a result of this hill ridge, there was no above-ground stream connecting the southern and the northern genetic groups (Fig. 3b). There were no other landscape elements (e.g. forest) obviously coinciding with the boundary between the two groups.

Multiple regression analysis on distance matrices with permutation testing showed relatively high goodness of fit for all full models with different corridor widths with R^2 -values ranging from 0.440 to 0.542 (Table 4). The best fit was found for the model with a corridor width of 300 m. In this best model, straight-line distance proved to be a highly significant predictor of genetic differentiation ($P \leq 0.001$). The positive regression coefficient denoted an increase of genetic differentiation (i.e. a decrease in gene flow) with increasing straight-line distance (i.e. isolation by distance). The proportion of flowing water and cumulative elevation change were also significantly and positively correlated with F_{ST} -values. Hence, elevation was suggested as an important element influencing gene flow as it already was in the clustering and overlay analysis (see above; Fig. 3a). The only negative, but less significant regression coefficient ($P \leq 0.05$) was found for the proportion of agricultural land. Note, however, that this latter predictor had a high negative monotonic correlation with the proportion of forest (Spearman's

Table 4 Multiple linear regression results of corridor analysis with different widths on *Coenagrion mercuriale* in Switzerland. Model fit (R^2), significance and the sign of regression coefficients for each landscape element are given for four different corridor widths

Corridor width	R^2	Flowing water	Roads	Buildings	Forest	Agriculture	Elevation	Distance
300 m	0.542	(+) ***	–	–	–	(–) *	(+) ***	(+) ***
200 m	0.512	(+) ***	–	–	–	(–) *	(+) ***	(+) ***
100 m	0.464	(+) *	–	–	–	(–) *	(+) ***	(+) ***
50 m	0.442	–	–	–	–	(–) *	(+) **	(+) ***

Models are ranked according to goodness of fit. P -values: *** ≤ 0.001 , ** ≤ 0.01 , * ≤ 0.05 .

$\rho = -0.651$). There were no strong correlations between any other predictor variables ($\rho < 0.6$ in all cases).

With automated model selection based on multiple regression, all subsets of the above full landscape corridor model with 300 m width were calculated and ranked by AIC. The ten most likely models are summarised in Table 5 (i.e. models within $\Delta\text{AIC} \leq 10$). All 10 models were highly significant ($P \leq 0.0001$; multiple regression analysis with permutation testing), and all included the variables flowing water, cumulative elevation change and straight-line distance. These landscape variables were all positively correlated with genetic differentiation, indicating an increase of F_{ST} with increasing proportion of each landscape element. Open agricultural land, which was negatively correlated to F_{ST} , was represented in eight of the ten most likely models ($\Delta\text{AIC} \leq 4$). The relative importance of these four landscape variables (flowing water, cumulative elevation change, straight-line distance and open agricultural land) was high (≥ 0.947 in all cases; Table 5). While the proportion of buildings was also relatively important and consistently positively correlated with genetic differentiation in the most likely models (importance = 0.718), roads and forest were less important (importance ≤ 0.5) and either positively or negatively correlated with F_{ST} -values, varying in different models. For the predictor forest, highly negatively correlated with the variable agriculture, regression coefficients only had a positive sign when the variable agriculture was not included in the same model (models 7, 9; Table 5).

Overall, models that included landscape elements in addition to distance outperformed the simple isolation by distance model, where F_{ST} -values were only regressed

against straight-line distance [$F_{\text{ST}} \sim \log(\text{Distance})$]. Model fit was markedly lower in this latter model ($R^2 = 0.32$) than in all full corridor models ($R^2 = 0.44\text{--}0.54$) in all cases. Furthermore, the ΔAIC of the isolation by distance model was high at 31.6, showing that this model was much less likely than the best model.

Discussion

Genetic clusters

Bayesian spatial clustering identified two distinct genetic clusters in our study area despite low global genetic differentiation among the populations of *Coenagrion mercuriale* ($F_{\text{ST}} = 0.043$). The separation of the populations into a northern and a southern cluster was probably caused by a hill ridge. We also considered the discontinuity of streams, caused by this hill ridge, as the causal barrier to gene flow, but this was not supported in the corridor analysis, where the absence of streams actually seems to have facilitated dispersal and gene flow.

Clustering algorithms sometimes overestimate the number of clusters when there is, as in our case, an isolation by distance pattern (Guillot *et al.*, 2009). Finding two clusters with a distinct spatial genetic pattern, however, implied that isolation by distance did not have a strong influence on the number of clusters in our study system. In general, clustering methods are useful tools to provide a first insight into spatial genetic structure, but they are also thought to be only suitable to identify strong barriers with clear impacts on gene flow (Holderegger *et al.*, 2010).

Table 5 Automated model selection based on multiple regression analysis on all subsets of a full landscape genetic corridor model (width = 300 m) for Swiss populations of *Coenagrion mercuriale*

Model	Flowing water	Roads	Buildings	Forest	Agriculture	Elevation	Distance	AIC	ΔAIC	AIC weight
1	+	n.i.	+	n.i.	–	+	+	–496.482	0	0.308
2	+	n.i.	n.i.	–	–	+	+	–495.358	1.125	0.175
3	+	–	+	n.i.	–	+	+	–495.261	1.222	0.167
4	+	n.i.	+	–	–	+	+	–494.677	1.806	0.125
5	+	–	n.i.	–	–	+	+	–493.432	3.050	0.067
6	+	–	+	–	–	+	+	–493.377	3.105	0.065
7	+	n.i.	+	+	n.i.	+	+	–491.945	4.538	0.032
8	+	n.i.	n.i.	n.i.	–	+	+	–491.620	4.862	0.027
9	+	–	+	+	n.i.	+	+	–491.103	5.380	0.021
10	+	+	n.i.	n.i.	–	+	+	–490.069	6.414	0.012
Importance	1.000	0.333	0.718	0.485	0.947	1.000	1.000			

Models are ranked according to Akaike's information criterion (AIC). Best models ($\Delta\text{AIC} \leq 10$) are shown in detail, including the sign of regression coefficients, AIC, ΔAIC and AIC weight. Additionally, relative variable importance of each landscape element in the most likely models ($\Delta\text{AIC} \leq 10$) based on Akaike weights is shown.

n.i.: landscape variable not included in model.

Dispersal paths

Whether straight-line corridors with fixed widths incorporate the real movement paths of individuals and whether the recorded landscape is relevant for gene flow is often unknown (Spear *et al.*, 2010). However, in our case, straight-line distance better represented pairwise F_{ST} than distance along streams (see Methods section), and we therefore discarded the assumption of dispersal being restricted to streams (Rouquette & Thompson, 2007). Similarly, abundant overland dispersal has been found at the adult life stage of other freshwater insects (e.g. Schmidt, Hughes & Bunn, 1995; Hughes *et al.*, 1998). Also for another damselfly, *Calopteryx splendens*, a combination of overland dispersal and dispersal along watercourses has been detected (Chaput-Bardy *et al.*, 2008). In fact, a mark-recapture study with *C. mercuriale* (Purse *et al.*, 2003) found that short-distance movement only occurs along streams (<300 m), whereas movement over larger distances (300–600 m) is equally likely along streams and across mires and wet heath vegetation. Thus, we assume that *C. mercuriale* actually uses different dispersal habitats for short- and long-distance dispersal (Purse *et al.*, 2003). Straight-line corridors should therefore provide a good representation for the movement of this species across larger distances.

Landscape elements hindering or facilitating gene flow

From the landscape genetic corridor analysis, we conclude that not only the distance but also the landscape between populations of *C. mercuriale* has a significant effect on the species' genetic structure in our study area. However, whether these findings also apply to other landscapes remains to be tested. Both the statistical analysis on the full regression model and the automated model selection on all subsets of this model detected four landscape elements that consistently and significantly affected gene flow in *C. mercuriale*: straight-line Euclidian distance, cumulative elevation change, proportion of agricultural land and proportion of flowing water. As *C. mercuriale* is known to be sedentary and only disperses across short distances (<2 km; Rouquette & Thompson, 2007), an isolation by distance pattern was expected across our 120 km² study area. Isolation by distance has already been reported in previous studies on *C. mercuriale* (Watts *et al.*, 2004a, 2006). Watts *et al.* (2006) also found that higher ground was avoided by this damselfly. In this study, we detected elevation as a dispersal barrier in the genetic clustering and overlay approach as well as in the corridor analysis. Damselflies,

in contrast to many dragonfly species, generally fly at low heights (Sternberg & Buchwald, 1999) and might therefore be susceptible to landscape obstacles, such as hill ridges.

Agricultural land was detected to enhance gene flow. However, this variable was highly correlated with the variable forest ($\rho = -0.651$). Indeed, the removal of the predictor variable agriculture from the full regression model led to a model in which the predictor variable forest was significantly and positively correlated with F_{ST} -values ($P < 0.05$). Additionally, in all subset models that excluded the predictor variable agriculture, the regression coefficient of forest had a positive sign. This indicates that forests may be potential barriers to gene flow in *C. mercuriale*. Thus, the effects of the two landscape elements agricultural land and forests on gene flow cannot be separated with certainty, and similar results have to be interpreted with care (Angelone *et al.*, 2011). Nevertheless, the fact that shading trees are known to be avoided by *C. mercuriale* (e.g. Koch *et al.*, 2009) indicates that dispersal is more likely across open agricultural land than through forests.

Surprisingly, we also found a positive correlation between genetic differentiation and the proportion of streams within corridors, suggesting that gene flow is reduced if more streams are present. A possible explanation of this counterintuitive result could be that dispersing individuals of *C. mercuriale* are more likely to stay at encountered streams than at any other landscape type, because streams represent potentially suitable reproductive habitat. Adriaensen *et al.* (2003) previously proposed the idea of suitable habitats hindering or blocking dispersal. The predictor variable buildings only showed a barrier effect in the analysis based on automated model selection. As towns and villages in the study area are small and interspersed with green areas and open water, they might have a smaller impact on damselfly dispersal than larger towns or cities. In some studies, urban areas have been detected as barriers to insect dispersal (e.g. for a solitary bee; Davis *et al.*, 2010) while in other studies, urban areas had no impact (e.g. for a butterfly species; Leidner & Haddad, 2010). Busy roads had no effect on genetic differentiation in *C. mercuriale* in our study. The low impact of roads may be due to the absence of highways in the study area. Highways have repeatedly been shown to have an impact on the genetic structure of animals (e.g. Balkenhol & Waits, 2009; Corlatti, Hacklander & Frey-Roos, 2009; Holderegger & Di Giulio, 2010). Nevertheless, a previous study also found no effect of motorways on gene flow in *C. mercuriale* (Watts *et al.*, 2004a).

Implications for conservation

In our study area, several conservation measures have been applied since 2009 to maintain and enlarge existing populations of *C. mercuriale*. Extensively managed meadow strips of at least 6 m width have been implemented on either side of streams. Furthermore, weed control is carried out within streambeds, and stream banks are mown regularly using basket mowers. These measures mainly focus on habitat protection and restoration, a main general goal of Odonate conservation (Wildermuth & Küry, 2009). However, in fragmented landscapes, where conservation strategies aim to enhance connectivity, knowledge about species-specific dispersal characteristics is essential.

Our study showed that the dispersal habitat of *C. mercuriale* is not restricted to its reproductive habitat and that dispersal is possible across agricultural land. Therefore, connectivity among populations from different stream systems is not necessarily dependent on connections via streams, and populations are not *a priori* genetically isolated from each other if they are separated by agricultural land. Thus, the maintenance and restoration of streams may aid the maintenance and increase the densities of populations of *C. mercuriale*, but is not decisive for the establishment of functional connectivity.

We found that landscape elements and, above all, geographic distance have a significant effect on gene flow in *C. mercuriale*, identifying Euclidian distance as an inhibitor to gene flow. Nevertheless, the low global genetic differentiation in our study area ($F_{ST} = 0.043$) and the fact that pairwise F_{ST} -values among adjacent stream systems were mostly low ($F_{ST} < 0.045$; data not shown) indicated recurrent gene flow even among populations of different stream systems (<4.5 km). However, it remains unknown whether such long-distance dispersal occurs regularly or only sporadically and whether genetic structure is mainly the result of past gene flow or also characterises the present dispersal patterns in the studied landscape. Rouquette & Thompson (2007) observed dispersal up to 1.8 km in a mark-recapture study and, in our study area, a (re)colonisation event of a stream about 1.2 km away from all resident populations has recently been observed (C. Forrer, Büro Kappeler, Berne; unpubl. data). We conclude, therefore, that dispersal can be expected between populations up to 1.5–2 km apart. Probably rare dispersal events may even occur over larger distances (<4.5 km). Nevertheless, the implementation of stepping stone habitats (i.e. the restoration of streams located between existing populations) would considerably enhance connectivity over larger distances (>2 km). Furthermore, when planning the locations of

stepping stones, the barrier effects of substantial elevation change, as well as larger forest patches, should be considered, as these landscape elements will rarely be crossed by *C. mercuriale*.

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