Spatial genetic variation of a riparian wolf spider *Pardosa agricola* (Thorell, 1856) on lowland river banks: the importance of functional connectivity in linear spatial systems.

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Spatial genetic variation of a riparian wolf spider *Pardosa agricola* (Thorell, 1856) on lowland river banks: the importance of functional connectivity in linear spatial systems.

Abstract

Habitat patches along river systems are often highly isolated and characterised by a high degree of heterogeneity at different spatio-temporal scales. The connectivity between river bank arthropod populations directly adjoining the river channel is often greatly disturbed due to river regulation. While flight-active arthropods easily disperse upstream, less mobile species are expected to show predominant downstream dispersal unless specific upward movements are prevalent. In linear river ecosystems, downstream drift of organisms may therefore prevail with subsequently strong asymmetrical gene flow. We analysed patterns of genetic variation within and among nine spatially structured populations of the highly stenotopic riparian wolf spider (Lycosidae) *Pardosa agricola* (Thorell, 1856) using Amplified Fragment Length Polymorphism (AFLP) markers. No evidence was found for downstream accumulation of genetic diversity. The high genetic diversity is hypothesised to be the result of historical drift processes. Nearby populations on the same river shore were less genetically differentiated compared with populations further away and/or on the opposite shore. This indicates that short-distance dispersal still occurs on river banks during low water flow levels, but at the same time that the river channel constitutes a physical barrier for species exchange between opposite shores. The rehabilitation of the riparian corridor will increase the amount of suitable habitat, the functional connectivity during periods of low flow-discharges and eventually riparian spider population persistence.

***Key-words (5)**:* AFLP; functional connectivity; genetic differentiation; Lycosidae; river regulation

Introduction

The interaction between demographic and genetic processes ultimately affects the persistence of species in spatially structured systems and should therefore underlie conservation efforts (Lande, 1988; Keller and Waller, 2002). While intensive ecological studies that aim to relate environmental parameters with species distribution patterns are a crucial first step, it is clear that insights into the genetic structure of complex populations, and the direction of species exchange and gene flow provide additional information for species conservation (Frankham, 1995; Vernesi et al., 2008; Van Looy et al., 2009a).

Natural stochastic events are important for the persistence and the development of ecosystems, but they also affect population dynamics (Plachter and Reich, 1998; Robinson et al., 2002; Lake et al., 2007). Consequently, disturbance events impact the genetic structure of the species present as well, especially in linear ecosystems where unidirectional drift of organisms (and genes) is expected (Stelter et al., 1997; Arens et al., 1998; Jacquemyn et al., 2006; Honnay et al., 2009). Functional connectivity refers to connectivity from the viewpoint of the organism itself, i.e. dispersal probability between habitat patches as determined to the species' life history and the dispersion of (suitable) habitat patches (Bélisle, 2005), that is. Asymmetric dispersal and reduced functional connectivity may therefore decrease (meta)population viability (Vuillemier and Possingham, 2006) and limit adaptations to local conditions (Riechert, 1993a,b).

Rivers divide the landscape and create highly diverse ecosystems with increased habitat heterogeneity on different spatiotemporal scales (Ward et al., 2002; Wiens, 2002). The spatial distribution of habitat patches along a river's trajectory is often patchy and functional connectivity generally depends on species' habitat affinity and mobility (Dìaz et al., 2007;

66 Paillex et al., 2007). This does not necessarily imply that highly mobile species show a lower
67 degree of genetic differentiation or vice versa that immobile species are highly genetically
68 structured since propagules can be moved by water-mediated dispersal from upstream
69 populations and/or zoochorous transfer (Tero et al., 2003; Pollux et al., 2009), eventually
70 resulting in a bidirectional pattern of gene flow.
71
72 Human practices often interrupt the connectivity between riverine habitat patches (Ward
73 and Stanford, 1995). River regulation (e.g. embankments, weirs) not only disrupts water
74 discharge regimes but also modifies the patchiness of habitats which are directly affected by
75 flooding (e.g. floodplains, river banks), and additionally affects habitat suitability by
76 sedimentation processes (Allan and Castillo, 2008). Moreover, disturbed water level
77 fluctuations may distort dispersal movements of stenotopic riparian species (Plachter and
78 Reich, 1998; Ward et al., 1998; Bates et al., 2006) and thus (re)colonisation events (Collinge
79 et al., 2001). Next to it, the agricultural intensification and degradation of the surrounding
80 catchment area intervenes in the lateral exchanges of organisms and obstructs migration
81 movements from and towards overwintering sites (Lang and Pütz, 1999; Rothenbücher and
82 Schaefer, 2006).
83
84 While flight-active arthropods easily spread upstream (Stelter et al., 1997; MacNeale et al.,
85 2005; Bates et al., 2006), cursorial arthropods are restricted by their limited mobility and the
86 scattering of suitable river bank patches (Ward et al., 1998; Collinge et al., 2001). Lambeets
87 et al. (2008a) showed that larger, cursorial, stenotopic riparian spiders in particular are
88 subject to increased flooding disturbance. Although unidirectional (downstream) dispersal
89 may still be possible (e.g. by water surface locomotion; Stratton et al., 2004; Lambeets and

90 Bonte, 2009), upstream transport of propagules is expected to be hampered by the high
91 degree of isolation and the low quality of remaining habitat patches along the riparian
92 corridor (cost-distance effects; Fahrig, 2007).
93
94 We investigated patterns of genetic variation within and among spatially structured
95 populations of the riparian wolf spider (Lycosidae) *Pardosa agricola* (Thorell, 1856) along a
96 lowland gravel river using dominant Amplified Fragment Length Polymorphism (AFLP)
97 markers. The Common Meuse, geographically situated between Flanders (Belgium) and The
98 Netherlands, is the less modified, dynamic mid-section of the River Meuse and contains
99 several isolated gravel bars and islands along its trajectory (Fig. 1). AFLP-analysis has proven
100 useful to study genetic diversity in wolf spiders (Fu et al., 2008) and generally to study
101 genetic differentiation with supposed asymmetric dispersal (Arens et al., 1998; Jacquemyn
102 et al., 2006; Honnay et al., 2009). *P. agricola* is considered as a rare stenotopic riparian
103 species (Albert and Albert, 1976; Harvey et al., 2002) that inhabits flood-disturbed river
104 banks once they are exposed and winters in the surrounding alluvial grasslands during
105 annual winter flooding (K. Lambeets, unpubl. data). The species' current distribution is
106 restricted to the most downstream part of the river trajectory but upstream populations
107 were present till the beginning of this century (Lambeets et al., 2008b), suggesting that the
108 combination of extreme hydropeaking (discharges >2,500 m^3s^{-1}), from which the last-one
109 dates back to January 2003 (see Honnay et al., 2009; Van Looy et al., 2009a) have induced
110 extinction and hampered upstream recolonisation events. Additionally, extended low flows
111 might degrade local habitat suitability (Semmerkrot et al., 1997; Liefveld and Schulze, 2005).
112 River restoration projects aim at restoring the lateral connectivity between the river and the
113 hinterland as well as the longitudinal connectivity of the riparian corridor by locally removing

114 embankments and restoring a more natural discharge regime respectively (Van Looy and De
115 Blust, 1995; Van Looy et al., 2009b).
116
117 The aims of this study were to assess whether the population structure of *P. agricola*
118 matches the supposed asymmetric (unidirectional) pattern (i.e. a linear population structure
119 caused by the water current) of downstream habitat colonisation (Kawecki and Holt, 2002).
120 Increased genetic diversity is expected in downstream populations due to the unidirectional
121 influx of genes. Additionally, between-population gene flow is expected to be higher
122 between adjacent (highly connected) populations on the same river shore compared to
123 populations which are further away and/or on opposite shores. Based on the AFLP-derived
124 genetic structure, implications for the rehabilitation of the riparian corridor are provided in
125 the context of further river restoration efforts.
126
127
128 **Material and methods**
129 **Study area, species and sampling**
130 The Common Meuse is situated between Flanders (Belgium) in the West and the
131 Netherlands in the East (Fig. 1). It is the 45 km long non-impounded, non-navigable reach of
132 the River Meuse and starts where the river descends from the rocky primary soils of the
133 Ardennes and enters the lowlands. The high slope is responsible for its fast flowing gravel-
134 bed character. Since it is rain-fed, the Common Meuse is characterised by strong water level
135 fluctuations and a wandering pattern of isolated river banks (Van Looy and De Blust, 1995).
136 Discharge regimes range from 10 m^3s^{-1} during dry periods up to 3,000 m^3s^{-1} in periods of
137 (extremely) heavy rainfall in the catchment area. Due to canalisation and normalisation of

138 the River Meuse, a tendency for prolonged low flows and hydropeaking occurs (Semmerkrot
139 et al., 1997). Currently, parts of the Common Meuse are still heavily buoyed which restrains
140 natural dynamic processes (Van Looy and De Blust, 1995; van Winden et al., 2001). Over 50%
141 of the alluvial plane is still intensively used for agricultural purposes while alluvial grasslands,
142 sand-gravel bars or pioneer vegetation on overbank depositions only occupy 5% of the
143 surface (Van Looy et al., 2009b).
144 Along the Common Meuse the stenotopic riparian wolf spider *Pardosa agricola* is one of the
145 most abundant spider species (Lambeets et al., 2008b; 2009). Controlled lab experiments
146 with *P. agricola* juveniles (n = 340; 20 specimens per test taken from each 17 females)
147 showed no ballooning propensity at all for this wolf spiders species (Bonte and Lambeets,
148 unpubl. data) which agrees with predictions based on the species' habitat affinity (Bonte et
149 al., 2003a). The river banks where *P. agricola* still occurs in high numbers (Fig. 1, rectangular
150 in-set) are flooded after heavy rainfall in spring and early summer but remain inundated
151 throughout late autumn and winter. These river banks differ in their susceptibility to flood
152 events (discharge when flooded ranges from (strongly disturbed) 76 m^3s^{-1} up to (more
153 stable) 247 m^3s^{-1}, mean 172 m^3s^{-1} +/- 16SE), but still provide enough suitable habitat for *P.*
154 *agricola* to establish viable populations (see Lambeets, 2008; Lambeets et al., 2009). On
155 average the inter-patch distance amounts to 2,521 m +/- 261SE (range: 390 m – 3,354 m;
156 Table 2). Furthermore, these banks are characterised by a clear succession of typical riparian
157 plants such as *Rorippa sylvestris, Lythrum salicaria, Artemesia vulgaris, Polygonum aviculare*
158 and *Xanthium orientale* (Peters et al., 2000). Despite extensive survey efforts in the
159 surrounding grassland habitats, no specimens of *P. agricola* were found, even along the river
160 dikes or at distances only a few meters away, except for a small relict population at an
161 erosion channel nearby river bank KE (Fig. 1; Lambeets et al., 2005). All historical upstream

populations of *P. agricola* were extirpated, probably by the extensive flood of 2003 (see Honnay et al., 2009). In May and August 2007, *P. agricola* individuals of the downstream river banks were collected by hand sampling (Fig. 1, rectangular in-set). We were not able to find any individuals at KE. We aimed to collect at least 20 individuals per population, although this was not possible on sparsely populated river banks (Table 1). All wolf spiders were stored in plastic vials with a humid plaster bottom before DNA extraction.

AFLP analysis

The day after sampling, all wolf spiders were frozen in liquid nitrogen, freeze-dried for 48h and homogenised with a mill (Retsch MM 200) to fine powder. DNA was extracted from the thorax and legs using the DNeasy Blood and Tissue kit (Qiagen). DNA quality and concentration were estimated on 1.5% agarose gels. Hundred ng of DNA was used for AFLP analysis according to Vos et al. (1995). Restriction and ligation was performed in a single step. Amplification of fragments was performed in 2 steps using the primer combinations P*st*I+A/M*se*I+A for preamplification and P*st*I+AGT/M*se*I+ACC, P*st*I+ACT/M*se*I+ACC, P*st*I+ACT/M*se*I+AGA, P*st*I+ACT/M*se*I+AGG for selective amplification. Fragment separation and detection took place on a Nen IR^2 DNA analyzer (Licor) using 36 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50 to 700 bp) were included for sizing of the fragments. Control samples were included in each gel to check for reproducibility between gels. Two percent of the samples were replicated within and between gels. The overall banding patterns within gels were 99% identical while between gels this was more than 95%. Only clear, intense bands were scored. Scoring was done using the SAGAmx software (Licor). We scored the presence or absence of every marker in each individual as 1 or 0 (present or absent) to form a binary data matrix. Scoring of the markers was done twice independently.

186 The scoring results were more than 90% comparable. Markers for which there were too
187 many conflicting scores were excluded from the data set.
188
189 **Data analysis**
190 Based on allele frequencies, within-genetic diversity was estimated by the proportion of
191 polymorphic loci (PPL) and Nei's genetic diversity (expected heterzygosity, H_j and average
192 heterozygosity, H_w) as well as the proportion of total genetic variability within a population
193 compared to the total genetic variability (population differentiation; F_{ST}; 500 permutations)
194 and total metapopulation diversity (H_t) (Lynch and Milligan, 1994). These measures were
195 calculated using AFLP-SURV (Vekemans et al., 2002). To identify whether or not larger
196 populations are genetically more diverse, a linear regression was performed between a
197 surrogate measure for local population size and H_j as well as PPL (SAS 9.1, proc reg).
198 Population size was approximated by using the numbers of individuals caught during a
199 previous pitfall sampling campaign multiplied by the river bank width at that moment (a
200 good relative proxy for the amount of suitable habitat; see Lambeets et al., 2008a; 2009).
201 These numbers accorded very well with the relative differences in search time during this
202 sampling of living spiders (Lambeets & Bonte, unpub. data). As samples were collected
203 during the main activity period of *P. agricola* (May - June) and in the same habitat type, this
204 product represents a comparable proxy for population size (Baars, 1979), notwithstanding
205 pitfall traps rather reflect activity-densities (Maelfait and Baert, 1975).
206 To assess the degree of molecular variation within and among populations, total genetic
207 diversity was partitioned by applying a hierarchical analysis of molecular variance (AMOVA;
208 Table 2) on Euclidean pairwise genetic distances using GENALEX 6.1 (Peakall and Smouse,
209 2005). Significances were determined based on 999 permutations. The $Ø_{ST}$ is an analogue for

210 F_{ST}-values used for dominant markers such as AFLP, and was derived from the Euclidean
211 genetic distances. Its significance was calculated using the Monte Carlo procedure in
212 GENALEX 6.1 (999 permutations).
213 Pairwise genetic distances among the nine investigated populations and their level of
214 significance were obtained from AMOVA. Again 999 permutations were applied. A principal
215 coordinates analysis (PCoA) was performed on this matrix using GENALEX 6.1 and the first
216 two axes were plotted graphically. The relationship between pairwise genetic distances (F_{ST}),
217 derived from AFLP-SURV, and geographic distances (Table 3), respectively along the river
218 channel and Euclidean distances (calculated by ArcGIS 9.1), was assessed with Mantel-tests
219 implemented within the ade4 package of R statistical software v.2.6.0 (R Development Core
220 team; 999 replicates). In order to test for the existence of functional connectivity, we
221 similarly performed a Mantel-test between pairwise genetic distances and pairwise river
222 bank ranking according to their degree of functional connectivity as estimated by previous
223 experimental work (Lambeets and Bonte, 2009). River banks that are connected during
224 summer and reachable by cursorial movement were ranked 1, those that are situated at the
225 same river side, but only reachable by cursorial movement during extremely low water flows
226 as 2, those close together but separated by the river as 3 and those distantly located and on
227 the opposite river side as 4 (Table 3). We additionally performed an analysis of covariance on
228 the pairwise F_{ST} values with river side location as fixed factor (either same of opposite river
229 side), pairwise along-river or Euclidean distance as a covariate to examine whether potential
230 patterns in isolation by distance depended on the river side location.
231 To test the hypothesis of upstream genetic erosion due to downstream drift after being
232 washed away, a linear regression was performed between the distances from the banks
233 along the river channel in a downstream direction and H_j as well as PPL (SAS 9.1; proc reg).

234 Similarly we tested the hypothesis that genetic variation would be negatively related to the
235 magnitude of local flooding disturbance because of frequent colonisation/extinction events.
236 A previously derived compound measure of flooding disturbance (PC_{dyn}; see Lambeets et al.,
237 2008a) was used to test the relation with expected heterzygosity (H_j). Bayesian inference of
238 population structure was performed using BAPS v5.3 (Corander et al., 2008). Mixture
239 clustering of individuals was done using five replicates of K between 9 and 13. Admixture
240 analysis was done using 50 iterations.
241
242
243 **Results**
244 The four AFLP primer combinations resulted in 59 highly reliable polymorphic markers. Our
245 data-set contained information for 153 wolf spider individuals spread over nine populations
246 with different relative population sizes (Table 1).
247 Genetic diversity within populations was high (Table 1), with the percentage of polymorphic
248 loci (PPL) ranging from 91.5% to 100% (mean: 95.84%). Concordantly, the average expected
249 heterozygosity (H_w) was 0.392 (range per population, H_j: 0.370 – 0.413). PLP nor H_j were
250 significantly related to population size (resp. $t = 0.65$, $p = 0.5379$; $t = 0.91$, $p = 0.3921$).
251 Genetic diversity (H_j) was not accumulating downstream ($t = -1.35$, $p = 0.3611$) nor was it
252 affected by flooding disturbance ($t = 0.98$, $p = 0.3611$), ruling out upstream erosion and local
253 colonisation-extinction dynamics.
254
255 Total genetic diversity (H_t) was 0.4043. F_{ST} of the actual (sampled) populations was 0.0316
256 and differed significantly from a random assemblage of individuals (500 permutations; $p <$
257 0.0001). This indicates that actual populations are genetically more differentiated than

258 random assemblages of individuals. The AMOVA-derived $Ø_{ST}$ value was 0.056 (99
259 permutations; p = 0.01) and very similar to F_{ST}. Nonetheless, only 6% of the genetic variation
260 could be attributed to variation between populations, whereas 94% was explained by intra-
261 population diversity (Table 2).
262 The first two principal components (PCoA; Fig. 2) explained 64.6% and 19.7% of the total
263 variance in genetic variation. Remarkably, this pattern deviates from our expectancy of
264 unidirectional gene flow. The Mantel-tests showed no significant relationship between
265 pairwise F_{ST}-values and pairwise geographic distances (i.e. distance along the river channel; r
266 = $2x10^{-6}$, p = 0.1931) or Euclidean distances (r = $4x10^{-6}$, p = 0.1708), respectively (Fig. 3a,b;
267 Table 3), which indicates the lack of isolation-by-distance. However, a significant positive
268 correlation was found according to the functional connectivity ranking between river banks
269 (r = 0.0067, p = 0.0096; Fig. 3c). This demonstrates the isolation of river banks along the
270 same shore versus on opposite shores (Table 3), just as was revealed by the PCoA. This result
271 was confirmed by the ANCOVA. The distance measures still explained some of the variation
272 (both $F_{1,33} > 3.2$; $0.09 > p > 0.05$) and were retained in the final model. F_{ST}-values, however,
273 clearly differed according to the position along the river (same or opposite shore; $F_{1,33}$ =
274 5.82; p = 0.0216 for model with along-river distance as covariate; $F_{1,33}$ = 5.34; p = 0.0273 for
275 model with Euclidean distance as covariate)
276 The ten best partitions after BAPS analysis were nine or ten clusters (five each) with log(ml)
277 values between -4819 and -4821 and probabilities for the number of clusters 0.35 and 0.60,
278 respectively. As shown in table 4, cluster 1 contains the majority (almost 30%) of the
279 individuals divided over all populations except HE1 and HE2. This can be mainly attributed to
280 the populations VW1 and VW2 with respectively 73% and 86% of the individuals which were
281 assigned to this cluster. The second biggest cluster (5) contains the majority of the

individuals of HE1 and HE2. Apart from these two clusters, cluster 2 and 4 contain individuals spread over most of the populations. The remaining clusters are restricted to fewer populations and only contain a minority of the individuals, and thus of the genetic diversity. Interestingly, all three populations from the left river shore (EL, HE1 and HE2) contain one or more individuals that belong to a unique cluster not appearing in the other populations. Consequently, admixture analysis did not provide evidence for downstream accumulation of genetic diversity.

Discussion

Genetic diversity and differentiation in a wolf spider population

The spatial genetic structure of *P. agricola* populations along the Common Meuse suggests that river regulation and the isolation of the river banks did not strongly affect the species' current genetic diversity. Our results, however, point at decreasing functional connectivity since populations from opposite river shores were more genetically differentiated.

The within-population genetic diversity found for *P. agricola* (H_w: 0.392; PPL: 95.8) was considerably higher than for a recent AFLP-based study of Fu et al. (2008) involving widely dispersed populations of the wolf spider *Pardosa pseudoannulata* (H_w: 0.2554; PPL: 72.99). The high levels of genetic diversity also indicate that the populations of *P. agricola* are likely to be of a common ancestry since it takes time for diversity to decrease (Keller and Waller, 2002). Moreover, our results suggest that river regulation and the resulting fragmentation of populations or the disappearance of upstream populations did not strongly pauperise genetic diversity in *P. agricola* yet, but of course, we lack any reference to the historical

306 situation. Genetic diversity did not increase downstream along the remaining populations of
307 *P. agricola*. However, we cannot reject the hypothesis of unidirectional, asymmetric
308 dispersal (Tero et al., 2003; Vuillemier and Possingham, 2006), because our observed high
309 genetic diversity might be the result of historical downstream drift at larger spatial scales (cf.
310 Pollux et al., 2009) with upstream populations being extinct at contemporary time scales.
311
312 Because our study was implemented along a 9 km-long river section, a low degree of genetic
313 differentiation between *P. agricola* populations was in accordance with expectations (F_{ST}:
314 0.0316; $Ø_{ST}$: 0.056). In comparison, riparian plant populations occurring along the Common
315 Meuse generally show higher differentiation, such as the perennial pioneer *Sisymbrium*
316 *austriacum* (F_{ST}: 0.097; $Ø_{ST}$: 0.091; Jacquemyn et al., 2006) and the dry grassland species
317 *Origanum vulgare* ($Ø_{ST}$: 0.24; Van Looy et al., 2009a). Honnay et al. (2009) found genetic
318 differentiation of the pioneer plant *Erysimum cheiranthoides* to increase over three years of
319 sampling (F_{ST}: 0.06, 0.11, 0.17; $Ø_{ST}$: 0.06, 0.08, 0.18). Studies considering wolf spider
320 populations in other dynamic, but non-linear systems showed similar low levels of genetic
321 differentiation, even at much larger scales: coastal dunes - *P. monticola*, allozyme-based F_{ST}:
322 0.011 (Bonte et al., 2003b) and *Geolycosa pikei*, allozyme-based F_{ST}: 0.020 (Boulton et al.,
323 1998); pine woodlands within an agricultural matrix – morphospecies “Wirra”, mitochondrial
324 DNA-based F_{ST}: 0.086 (Colgan et al., 2002).
325
326 Effects of fragmentation on metapopulation structure depend on the mobility and thus
327 dispersal rate of the species (Casagrandi and Gatto, 2002). Therefore, arthropods capable of
328 active flight (cf. ballooning in spiders) may encounter little difficulty maintaining gene flow
329 among populations surrounded by an unfavourable matrix (Ramirez and Haakonsen, 1999;

MacNeale et al., 2005). Cursorial species with restricted mobility might experience more hindrance of impassable boundaries (Bowler and Benton, 2005), with a decreased genetic exchange as result (e.g. Vignieri, 2005). Functional connectivity is therefore suggested to affect *P. agricola*'s genetic structure more than geographic isolation, obviously because the species will not spontaneously cross the current river channel (Lambeets and Bonte, 2009; Lambeets et al., in press). Studies showing isolation-by-distance or downstream (unidirectional) accumulation of genetic diversity often consider much longer river trajectories (Imbert and Lefèvre, 2003; Van Looy et al., 2009a). But even for plants this does not necessarily imply the presence of isolation-by-distance effects (Tero et al., 2003; Jacquemyn et al., 2006; Honnay et al., 2009; Pollux et al., 2009). Unfortunately, evidence of isolation-by-distance for arthropods inhabiting linear ecosystems with a unidirectional gradient could not be found in recent literature (but see Peterson et al., 2001; Bonte et al., 2003b; Desender et al., 2005 for other habitat types).

We attribute the low genetic differentiation and high levels of genetic diversity more to historical than to recent gene flow, and hence to recent colonisation of a highly dynamic ecosystem (Honnay et al., 2009; Van Looy et al., 2009a). Geologically, the location of the River Meuse became relatively stable only 10.000 years ago (Broothaers, 1996). Consequently, the riparian zone along its trajectory could only be colonised during post-glacial range expansion, which may result in high genetic variation and between-population differentiation (cf. Pederschen and Loeschke, 2001). Besides the relatively young geological history of the River Meuse, river banks along the Common Meuse only became highly isolated after the intensification of gravel mining practices and the exploitation of the

353 hinterland (ca. 150 years ago; Van Looy and De Blust, 1995). Hence, the currently observed
354 low levels of genetic differentiation might stem from higher levels of gene flow in the past.
355
356 Changes in river management and the surrounding landscape, however, already result in
357 genetic substructuring. Nearby populations were less genetically differentiated, which
358 suggests short-distance exchange of individuals during periods of low flow still occurs.
359 Although unlikely in our model system, nearby river banks may also have been colonised by
360 the same founding group (Van Looy et al., 2009a). The higher pairwise F_{ST}-values, e.g.
361 between HE and the other river banks (Table 3) and the assignment of individuals of these
362 populations to unique clusters, indicate that founder effects might have caused the genetic
363 variation in the study system (Frankham, 1995; Boulton et al., 1998), yet no relationship
364 between flooding disturbance and genetic diversity was found. Moreover, higher genetic
365 differentiation of populations from opposite river shores confirms the validity of our
366 functional connectivity approach which is based on the flood-avoiding behaviour of *P.*
367 *agricola* (cf. Riechert, 1993a,b). Although *P. agricola* is capable of active water dispersal,
368 landing offshore is costly and the probability of effectively relocating suitable patches
369 downstream might be extremely low due to the fragmented character of the river banks.
370 Colonisation success will in addition depend on the local conditions permitting retention of
371 drifting propagules (Riis and Sand-Jensen, 2006) and stream size (Bang et al., 2007).
372
373 **Riparian corridor connectivity and species conservation**
374 Along with the extreme flood event of 2003 an impressive influx of plant species was
375 recorded and several newly formed gravel bars occurred along the river corridor (Van Looy
376 et al., 2009b). Still, cursorial arthropods such as wolf spiders are disadvantaged by extreme

and sudden floods (Lambeets et al., 2008a) as they might not be able to escape flooding in time or when suitable flood refuges become unreachable (Rothenbücher and Schaefer, 2006; Lambeets et al., in press). Smaller, flight-active arthropods such as carabid beetles, however, may re-establish viable populations quickly (Lang and Putz, 1999; Bonn et al., 2002; Hering et al., 2004) since they are able to escape sudden floods efficiently or they may even extend their distribution when more suitable patches become available (MacNeale et al., 2005; Bates et al., 2006). Short-lasting low flows in summer, however, will prove beneficial particularly for cursorial riparian arthropods as it allows them to migrate along and across the riparian corridor when gravel bars are exposed and thus (re)colonise available habitat patches. But even low flow conditions during summer are highly disturbed as a consequence of upstream weirs and the draining of large amounts of water by, for instance, upstream hydropower plants (Semmerkrot et al., 1997). As demonstrated by Van Looy et al. (2008), sudden changes in water discharge are more adverse for carabid beetle assemblages directly downstream of such power plants. In addition, this may explain why *P. agricola* and other large riparian wolf spiders such as *Arctosa cinerea* (Fabricius, 1777), disappeared from upstream river banks (Lambeets, 2008). By weighing the costs and benefits of ecologically sustainable discharge regimes, however, a minimal water level and discharge should always remain to preserve rheophilic species (Geilen et al., 2004; Liefveld and Schulze, 2005). As dispersal is assumed to be asymmetric in river ecosystems (mainly downstream; Pollux et al., 2009; Van Looy et al., 2009a; but see MacNeale et al., 2005; Honnay et al., 2009), reconnecting patches by the reestablishment of an extensive riparian corridor is unlikely to lead to an increased metapopulation persistence (Veullemier and Possingham, 2006), unless species show a tendency to predominantly disperse along the river shoreline in an upstream direction.

Genetic diversity is still very high in all populations, yet we found evidence that isolation, even on this small spatial scale and by the physical presence of the river, affects the genetic structure of a cursorial stenotopic wolf spider. Therefore, rehabilitation of the riparian corridor is needed, by which existing populations must be functionally reconnected on a landscape scale and cursorial species will again get the chance to disperse along-stream or to cross the river at low costs. Current river restoration projects implemented by dike removal and a widening of the current channel will prove beneficial in that opinion, certainly when they would, effectively, integrate the requirements of stenotopic riparian arthropods. Additionally, conservation and restoration efforts should integrate spatial and temporal patterns of water discharges. Allowing for a more natural flooding frequency and magnitude within a less intensively used landscape will increase heterogeneity along different spatiotemporal scales and consequently re-establish and re-connect suitable habitat patches along-stream.

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591

Captions

Fig.1 - Locations of the nine sampled populations of *Pardosa agricola* along the Common Meuse. All sampled river banks (rectangular in-set) were situated in the most downstream part of this reach of the River Meuse, just before the embanked Sand Meuse which lacks gravel bars along its trajectory. Sample data from 1998 yet confirmed the presence of *P. agricola* upstream (arrows); some historical populations are not shown as these river banks have disappeared after the extreme flood event of 2003.

Fig. 2 - Principal coordinates (PCoA) plot of first two axes calculated based on 59 polymorphic markers and using Nei's genetic distances between populations. River banks at the left river shore (Flanders, Belgium) are indicated by a square, banks at the right river shore (the Netherlands) by a circle. The codes referring to the populations are described in Table 1.

Fig. 3 - Relationship between pairwise genetic distances (F_{ST}) and (a) pairwise geographic distances (along the river channel), (b) Euclidean distances and (c) functional connectivity (ranking over river banks), representing isolation-by-distance relations for a metapopulation of 173 *Pardosa agricola* individuals.

Table 1 - Characteristics of the nine *Pardosa agricola* populations on river banks along the Common Meuse. *n*: number of individuals; PopSize: proxy for relative population size; H_j: expected heterozygosity; *PPL*: percentage polymorphic loci; *x* and *y* coordinates according to Belgian Lambert grid; NL: shore at the Dutch river side (the Netherlands) and VL: shore at the Flemish side (Belgium).

Table 2 - Hierarchical analysis of molecular variance (AMOVA) based on 59 AFLP loci in nine populations of *Pardosa agricola*.

Table 3 - Pairwise genetic (F_{ST}; below the diagonal) and geographic distances (m; above the diagonal, [functional connectivity ranking]) for nine populations of *Pardosa agricola* along the Common Meuse (* $p < 0.05$; ** $p < 0.01$; *ns* not significant).

Table 4 – Bayesian inference of population structure of *Pardosa agricola* using mixture clustering and an admixture analysis in BAPS v5.3. Percentages per cluster and total percentages of the individuals included in the AFLP analysis (153, see table 1) are provided. EL, HE1 and HE2 are situated at the left iver shore, the other populations (KW1, KW2, RO1, RO2, VW1, VW2) at the opposite shore. Cluster 3, 7, 8 and 10 are considered unique clusters as only one populations contributes to these clusters.

Fig.1 - Locations of the nine sampled populations of *Pardosa agricola* along the Common Meuse. All sampled river banks (rectangular in-set) were situated in the most downstream part of this reach of the River Meuse, just before the embanked Sand Meuse which lacks gravel bars along its trajectory. Sample data from 1998 yet confirmed the presence of *P. agricola* upstream (arrows); some historical populations are not shown as these river banks have disappeared after the extreme flood event of 2003.

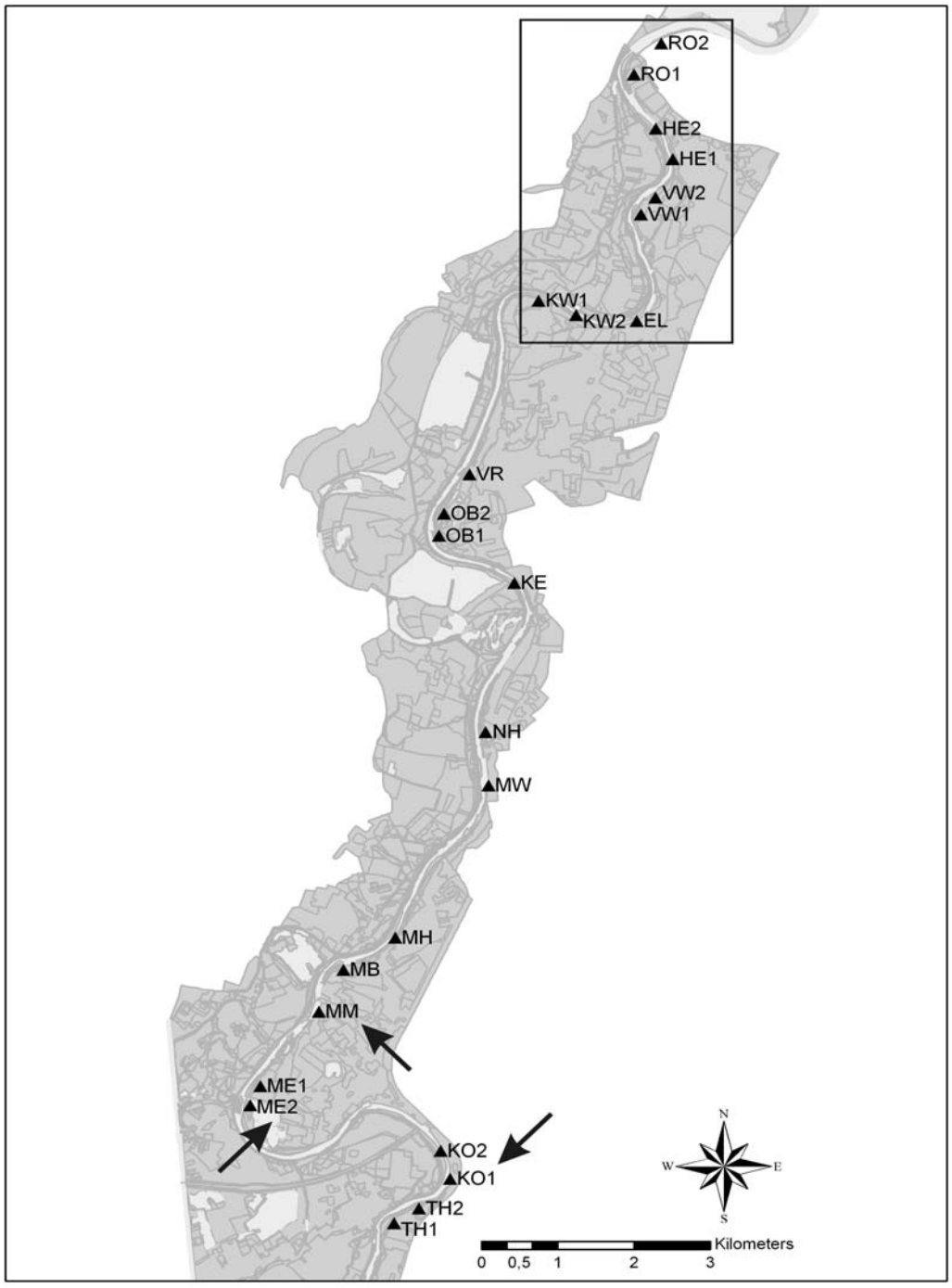


Fig.2 - Principal coordinates (PCoA) plot of first two axes calculated based on 59 polymorphic markers and using Nei's genetic distances between populations. River banks at the left river shore (Flanders, Belgium) are indicated by a square, banks at the right river shore (the Netherlands) by a circle. The codes referring to the populations are described in Table 1.

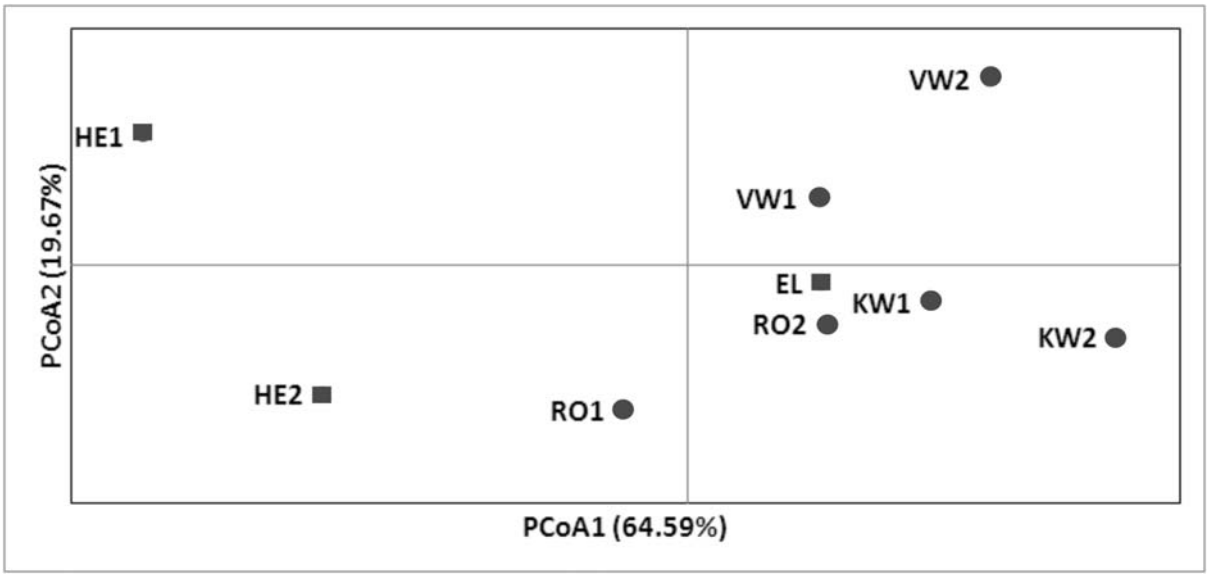
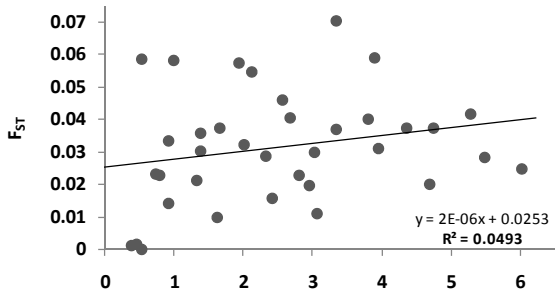
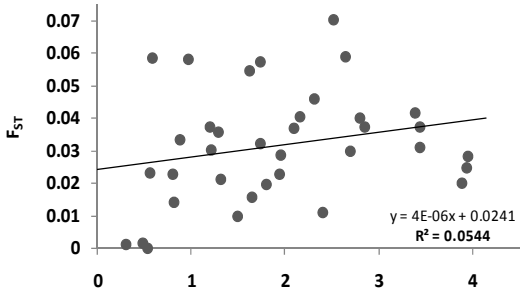


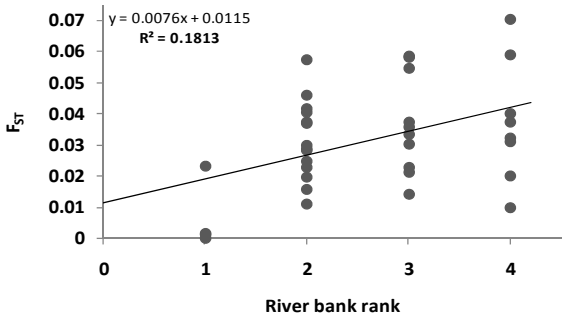
Fig.3 - Relationship between pairwise genetic distances (F_{ST}) and (a) pairwise geographic distances (along the river channel), (b) Euclidean distances and (c) functional connectivity (ranking over river banks), representing isolation-by-distance relations for a metapopulation of 173 *Pardosa agricola* individuals.



(a)



(b)



(c)

651 **Table 1 -** Characteristics of the nine *Pardosa agricola* populations on river banks along the Common
652 Meuse. *n*: number of individuals; *PopSize*: proxy for relative population size; H_j: expected
653 heterozygosity; *PPL*: percentage polymorphic loci; *x* and *y* coordinates according to Belgian Lambert
654 grid; NL: shore at the Dutch river side (the Netherlands) and VL: shore at the Flemish side (Belgium).

river bank	river shore	x	y	n	PopSize	H_j	PPL
KW1	right (NL)	248896	195570	**23**	**6703**	**0.3952**	**96.6**
KW2	right (NL)	249387	195369	**13**	**15517**	**0.3962**	**96.6**
EL	left (VL)	250184	195286	**25**	**21050**	**0.4073**	**100**
VW1	right (NL)	250239	196771	**15**	**4873**	**0.3899**	**93.2**
VW2	right (NL)	250428	197006	**14**	**136**	**0.3768**	**93.2**
HE1	left (VL)	250655	197545	**11**	**347**	**0.3873**	**96.6**
HE2	left (VL)	250432	197967	**9**	**760**	**0.4101**	**96.6**
RO1	right (NL)	250145	198723	**18**	**3589**	**0.3919**	**98.3**
RO2	right (NL)	250505	199156	**23**	**11223**	**0.3693**	**91.5**
			Mean (SE)	**16.8 (1.9)**	**7133 (2455)**	**0.3915 (0.0043)**	**95.8 (0.9)**

655

656

Table 2 - Hierarchical analysis of molecular variance (AMOVA) based on 59 AFLP loci in nine populations of *Pardosa agricola*.

Source of variation	df	SS	MS	Est. Var.	%
Among populations	8	157.864	19.733	0.585	6%
Within populations	144	1430.403	9.933	9.933	94%
Total	**152**	**1588.267**		**10.518**	**100%**

Table 3 - Pairwise genetic (F_{ST}; below the diagonal) and geographic distances (m; above the diagonal, [functional connectivity ranking]) for nine populations of *Pardosa agricola* along the Common Meuse (* $p < 0.05$; ** $p < 0.01$; *ns* not significant).

Population	KW1	KW2	EL	VW1	VW2	HE1	HE2	RO1	RO2
KW1	0	542 [1]	1333 [3]	2963 [2]	3354 [2]	3898 [4]	4361 [4]	5296 [2]	6030 [2]
KW2	0^{ns}	0	791 [3]	2421 [2]	2812 [2]	3356 [4]	3819 [4]	4753 [2]	5488 [2]
EL	0.0211*	0.0228*	0	1630 [4]	2021 [4]	2565 [2]	3028 [2]	3962 [4]	4697 [4]
VW1	0.0195**	0.0159*	0.0099**	0	390 [1]	934 [3]	1397 [3]	2332 [2]	3067 [2]
VW2	0.0371**	0.0226**	0.0321**	0.001^{ns}	0	544 [3]	1007 [3]	1941 [2]	2676 [2]
HE1	0.0591**	0.0703**	0.046**	0.0332**	0.0587**	0	463 [1]	1397 [3]	2132 [3]
HE2	0.0375**	0.0402**	0.0298**	0.0303**	0.0583**	0.0015^{ns}	0	934 [3]	1669 [3]
RO1	0.0415**	0.0373**	0.0309**	0.0285**	0.0574**	0.0359**	0.0143^{ns}	0	734 [1]
RO2	0.0247**	0.0283**	0.0199**	0.011**	0.0406**	0.0546**	0.0372**	0.0231**	0

668

669 **Table 4** – Bayesian inference of population structure of *Pardosa agricola* using mixture clustering and an admixture analysis in BAPS v5.3. Percentages per
670 cluster and total percentages of the individuals included in the AFLP analysis (153, see table 1) are provided. EL, HE1 and HE2 are situated at the left iver
671 shore, the other populations (KW1, KW2, RO1, RO2, VW1, VW2) at the opposite shore. Cluster 3, 7, 8 and 10 are considered unique clusters as only one
672 populations contributes to these clusters.

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	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7	cluster 8	cluster 9	cluster 10
KW1	0.13	0.39	0	0.17	0.04	0.22	0	0	0.04	0
KW2	0.23	0.23	0	0.15	0	0.38	0	0	0	0
EL	0.2	0.15	0.04	0.04	0.2	0	0.12	0	0.27	0
VW1	0.73	0.07	0	0	0.13	0.07	0	0	0	0
VW2	0.86	0	0	0.07	0.07	0	0	0	0	0
HE1	0	0	0	0.09	0.82	0	0	0	0	0.09
HE2	0	0	0	0.11	0.55	0.22	0	0.11	0	0
RO1	0.17	0.28	0	0.28	0.28	0	0	0	0	0
RO2	0.33	0.04	0	0.33	0.04	0	0	0	0.25	0
total	0.29	0.15	0.006	0.15	0.19	0.08	0.02	0.006	0.09	0.006

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