

# Morphological clines in dendritic landscapes

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## SUMMARY

1. In complex landscapes such as river networks, organisms usually face spatio-temporal heterogeneity and gradients in geomorphological, water, ecological or landscape characteristics are often observed at the catchment scale. These environmental variables determine developmental conditions for larval stages of freshwater insects and influence adult phenotypic characteristics. Environmental clines are therefore expected to generate morphological clines. Such a process has the potential to drive gradual geographical change in morphology-dependent life history traits, such as dispersal.

2. We studied the influence of aquatic and terrestrial environmental factors on morphological variations in *Calopteryx splendens* across the Loire drainage. To investigate these effects we took explicitly into account the hierarchical structure of the river network.

3. We analysed eight morphological traits. Results showed significant body size variation between tributaries and the presence of a morphological cline at the drainage scale. We observed an effect of pH and water temperature on body size. Individuals in downstream sites were larger than individuals in upstream sites, and adults whose larval stages were exposed to alkaline pH and high temperatures during summer were larger.

4. Body size affects flight abilities in insects. Thus, our results suggest that morphological clines may generate an asymmetric dispersal pattern along the downstream–upstream axis, downstream populations dispersing farther than upstream ones. Such a process is expected to influence population genetic structure at the drainage scale if larval drift and floods do not balance an asymmetrical dispersal pattern of adults along the downstream–upstream gradient. To assess the influence of environmental gradients on the variation of life history traits it is important to understand the population biology of freshwater insects, and more generally of riverine organisms. It is also essential to integrate such data in conservation or restoration programmes.

*Keywords:* abiotic factors, *Calopteryx splendens*, environmental gradients, odonates, river network

## Introduction

Environmental heterogeneity or ecological gradients can generate phenotypic variations in many organisms (Smith *et al.*, 1997; Smith, Schneider & Holder, 2001; West-Eberhard, 2003). Factors affecting metabolism are crucial because they strongly influence key aspects of the life cycle like development or repro-

duction (Anholt, Marden & Jenkins, 1991; Atkinson, 1994; Angilletta *et al.*, 2003). As a consequence, spatial variation of environmental conditions can induce differentiation of life history traits between populations (dispersal, survival rate, reproductive success). In order to predict population processes from landscape to regional levels, it is therefore necessary to identify gradients or discrete large-scale variations of the environment and to quantify phenotypic responses to heterogeneity.

Large rivers often undergo disturbances associated with flooding or drought, but they are also characterized by other sources of spatio-temporal heterogeneity

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(Ward, 1998; Gomi, Sidle & Richardson, 2002; Tockner *et al.*, 2002; Ward *et al.*, 2002; Wiens, 2002). A river exhibits variation in geomorphological features, landscape structure and seasonal variation (water level, current flow regime, chemical characteristics). On the one hand, a river network is a dynamic element included in a larger system, with riparian zones commonly forming a mosaic of habitats related to flood regime, vegetation, or soil hydromorphology and composition whose variation induces species-specific responses among aquatic organisms (Ward *et al.*, 2002). On the other hand, the geomorphology of a river network produces longitudinal gradients in substrate, soil moisture or water characteristics (Ward, 1998). Lower reaches are characterized by homogeneous substrate, high turbidity and oxygen deficits. In the middle reaches, by contrast, light and nutrient levels are higher, water is clearer, substrate is often patchy and thermal variance is maximal (Ward, 1998).

Spatial heterogeneity in aquatic and terrestrial habitats between upstream and downstream reaches influences both species distribution and community composition (Hawkins *et al.*, 1997; Ward & Tockner, 2001; Benda *et al.*, 2004; Lowe, Likens & Power, 2006). Variations in physicochemical and biological (i.e. food availability) water characteristics and the surrounding terrestrial landscape features affect stream insect populations (Hawkins *et al.*, 1997; Malmqvist, 2002). Oxygen, nitrate and ammonium concentrations, pH, conductivity and summer hydraulic conditions strongly influence the distribution of taxa in a river (Franken, Storey & Williams, 2001; Usseglio-Polatera & Beisel, 2002). The aquatic environment, and especially water temperature, is well known to affect larval development and therefore size of emerging adults of freshwater insects whose adult stage is terrestrial (Atkinson, 1994; Angilletta *et al.*, 2003). As a consequence, any longitudinal gradient of a variable influencing development may differentially affect the characteristics of populations distributed along the river network. On land, the width and composition of riparian vegetation can influence movements of freshwater insects into the riparian zone (Wiens, 2002). Hence, many authors have described a positive correlation between habitat heterogeneity and morphological traits related to flight (Roff, 1990; Wagner & Lieberr, 1992; Roff, 1994a; Taylor & Merriam, 1995). Variation in such traits is expected to change population dispersal characteristics. Among species living in

river network landscapes, freshwater insects are of particular interest because they exploit riverine and riparian landscapes, exposing them to variations in both environments. Insects undergo dramatic changes (metamorphosis) during their life cycle and are prone to population divergence via phenotypic plasticity (West-Eberhard, 2003; Ananthakrishnan & Whitman, 2005). Thus, spatial variation in aquatic and terrestrial environments is likely to generate population phenotypic variation in species exploiting river networks.

Biotic factors such as interspecific interactions and resource availability can influence population characteristics too. Low food levels or predator avoidance often induce a smaller size at metamorphosis (Anholt, 1991; Ball & Baker, 1996). Intraspecific interference affects survival and growth rates at high population densities (Gribbin & Thompson, 1990), as well as larval distribution. Similarly, competition between early-instar stonefly larvae increases body size variance even if this effect is attenuated before emergence (Peckarsky & Cowan, 1991).

The larval development of odonates is aquatic and their adult stage is terrestrial. However, the conditions of larval development influence age and size at maturity. Many biotic and abiotic factors affect larval development. Among biotic factors, high larval density (Corbet, 1999), food availability (Anholt, 1991; Mikolajewski *et al.*, 2005) and predator presence (Werner & Anholt, 1993; Mikolajewski *et al.*, 2005) increase development time and decrease size at emergence. Growth and distribution of stream insects are also very sensitive to abiotic factors (Hawkins *et al.*, 1997; Corbet, 1999). For instance, water temperature strongly influences emergence date and can influence body size of adults. The genus *Calopteryx* (Odonata: Zygoptera) is particularly interesting because morphology reflects population response to environmental factors (Taylor & Merriam, 1995; Svensson *et al.*, 2004). For instance, Taylor & Merriam (1995) compared flight abilities of *Calopteryx maculata* between forest and pasture and showed that wing morphology was related to landscape structure.

Investigating how the environment acts on phenotypic traits at the catchment scale is fundamental to predict population processes such as dispersal or adaptation. To our knowledge, no study has explored the influence of ecological gradients and river network structure on morphological variation and few data on population variation across an entire

catchment area are available for semi-aquatic species (Hugues *et al.*, 1999; McGlashan, Hugues & Bunn, 2001; Bohonak & Jenkins, 2003; Svensson *et al.*, 2004). In this context, we investigated the pattern of morphological variation in the banded damselfly, *Calopteryx splendens* (Harris, 1782), across a large dendritic drainage to investigate the effect of clinal environmental factors on adult phenotypic variation. We focused on adults because individuals have accumulated environmental effects at this stage. We applied a dichotomous sampling strategy, reflecting the hierarchical drainage structure, and developed a statistical analysis reflecting the nested structure of river networks, focusing on abiotic factors suspected or known to influence adult morphology.

## Methods

### *Study species and sites*

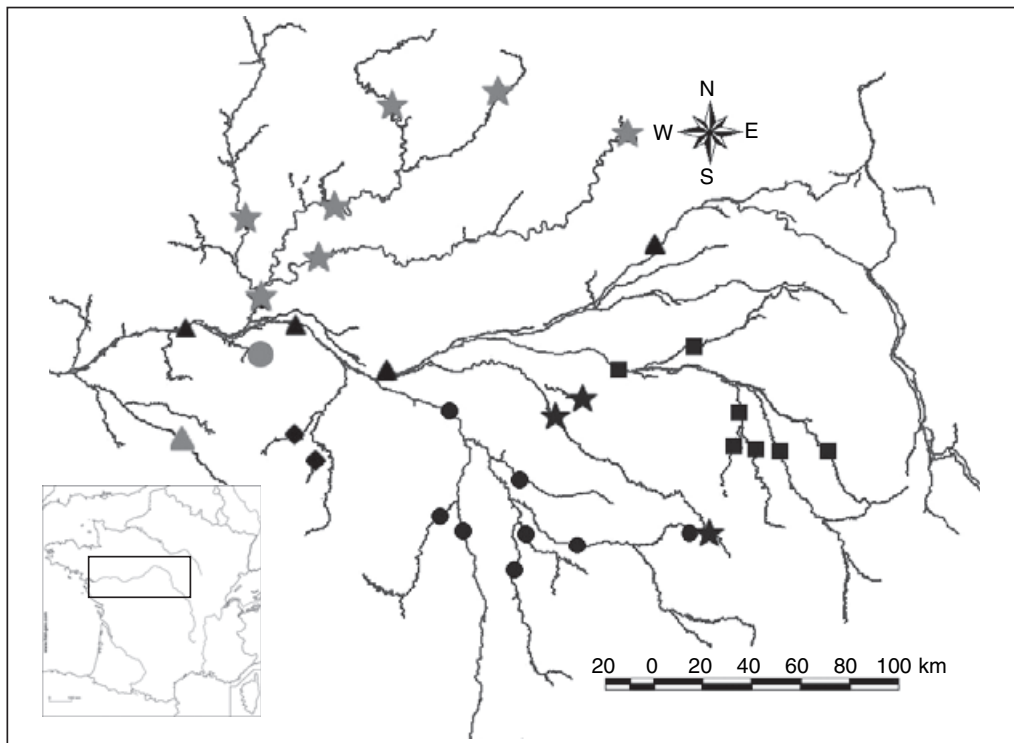
The banded damselfly is common along streams in France. In western Europe, its larvae spend 1 year in the stream (Askew, 2004). Adults, which emerge from May to September, have a lifespan of 3–6 weeks. The

maturation stage lasts 2–3 days. Males (which have dark wing spots) and female damselflies differ in size and weight in their larval and adult life stages, females being larger than males (Corbet, 1999).

The study was carried out in western France along the River Loire (Fig. 1). In order to investigate the effect of dendritic landscape structure on population morphological variation, we captured adult *C. splendens* at thirty-three sites distributed on the main channel and in seven tributaries in the lower part of the Loire drainage. We chose sites based on stream width and stream order, only including streams wider than 15 m from a hydrographical database (BD CARTHAGE © IGN-MATE 2005). Our study area encompassed four stream orders, seven sub-catchments and 22 streams in the lowland section of the river. We observed the presence of mating and laying behaviour, exuvia and teneral at each site.

### *Adult morphometry*

Using insect nets, we captured 998 individuals (about 30 individuals from each site), 662 males



**Fig. 1** Location of the 33 sites on the seven tributaries and on the main channel (black triangles) of the Loire drainage in western France (BD CARTHAGE © IGN-MATE 2005). A different symbol is used for each tributary.

and 336 females, from 26 May to 30 June 2005. We only collected mature damselflies because thoracic mass is fixed at this stage, whereas it increases during the maturation stage after emergence (Anholt *et al.*, 1991). Adults were caught on the riverbank because mating and oviposition take place exclusively on or close to the water surface (Corbet, 1999). We used a digital calliper (precision: 0.01 mm) to measure eight morphological characteristics, associated with flight ability, on the left side of each individual: forewing length (from the base to the tip of the wing), forewing width (measured along a perpendicular line from the nodus), maximal forewing width (longest perpendicular line), thorax length, thorax width and thorax mass, abdomen length and femur length of the hind-leg. Thoraces were stripped of wings, heads and all appendages, dried for 72 h at 50 °C and weighed (Taylor & Merriam, 1995).

#### *Characteristics of the bankside*

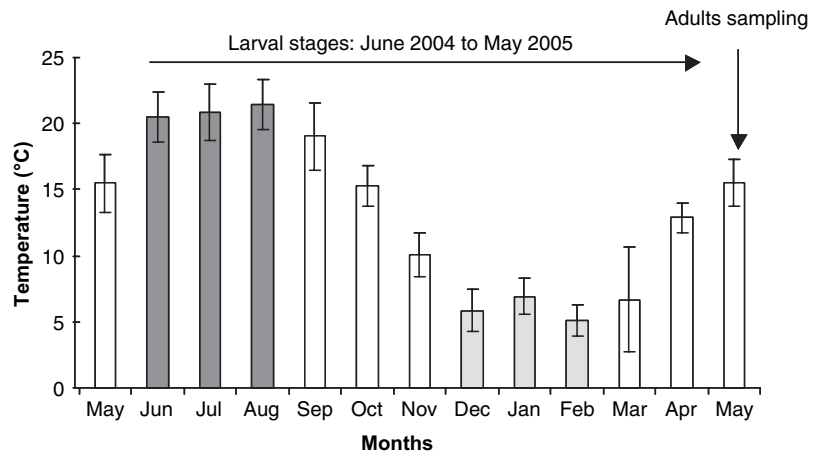
To investigate the influence of riparian habitat heterogeneity on morphology, we carried out 100-m transect surveys at each sampled site. We recorded the percentage cover of vegetation using the point quadrat method (Henderson, 2003). We placed a transect every 10 m along a 100-m section parallel to the stream. All transects were 5-m long and perpendicular to the stream. On each of them, we recorded at 50 cm intervals vegetation height (0–1, 1–2 or >2 m) and vegetation type (monocotyledonous or dicotyledonous). These variables were selected because of their known impact on density and distribution of species like the banded damselfly (Ward & Mill, 2005) or the southern damselfly, *Coenagrion mercuriale* (Rouquette & Thompson, 2005). We used the number of height strata and the percentage cover of each vegetal stratum to calculate the Shannon–Wiener diversity index, providing a measurement of riparian habitat heterogeneity at each site. In the case of *C. maculata*, individuals in pasture are larger, have heavier thoraces, and longer and wider wings than individuals in forest (Taylor & Merriam, 1995); thus, we noted tree coverage according to a two-level classification (<10% or >10%). We also scored bank slope according to a four-level classification (1: 0–25°, 2: 25–50°, 3: 50–75° and 4: >75°) every 10 m along a 100-m section parallel to the stream.

#### *Characteristics of the watercourse*

We recorded three physical or geographical variables at each capture site: dominant substratum (silt or sand), stream order (four classes from 1 to 4) and distance from the sea along the network pathway. We also recorded physical and chemical variables (pH, temperature and nitrate concentration of the water) reflecting the environmental conditions of larval development (from May 2004 to May 2005) experienced by the adults we sampled. Using monthly mean recordings from the database of the National Network of Water Data (RBDE Loire-Bretagne), we could extract data for 16 streams and 20 water sampling stations located on, or next to (mean  $\pm$  SD = 7.29 km  $\pm$  1.48) our sampling sites. We focused our analyses on the periods of the year when environmental conditions are most influential for larval development (i.e. summer and winter). On the River Loire, the water level is usually high and temperature at its lowest during winter. In summer, the water level is low and temperature is high. The maximal (summer) and minimal (winter) monthly water temperatures, recorded during the year before adult sampling in 2005, respectively included June–August 2004 (hereafter referred to as summer) and December–February 2004 (hereafter referred to as winter) periods (Fig. 2). Summer and winter nitrate concentrations [NO<sub>3</sub><sup>-</sup>] and summer and winter pH were also analysed. PH, which provides information about chemical stream conditions, as it may be correlated with salinity or conductivity (Cannings & Cannings, 1987, in Corbet, 1999), also may influence larval development and survival (Corbet, 1999). In contrast, nitrate concentration is related to trophic conditions and anthropogenic pressure on rivers.

#### *Statistical methods*

To investigate the effect of terrestrial and aquatic habitats on morphological variation, we first carried out a normalized Principal Component Analysis (PCA) with the eight morphological variables. We used PC1 and PC2 as new morphological variables (both having eigenvalues >1), to investigate the effect of environmental factors on adult morphology. No transformations of PC1 and PC2 were needed to meet the assumptions of normality and homoscedasticity. We used Linear Mixed-Effects Models (Laird & Ware,



**Fig. 2** Mean monthly water temperature ( $\pm$ SD) in all sampled streams. May 2005 corresponds to adult *Calopteryx splendens* sampling. The period of larval development of captured adults extended from June 2004 to May 2005. Dark shaded bars show the three warmest months (i.e. summer), light shaded bars, the three coldest months (i.e. winter).

1982) with environmental factors and sex as independent variables.

Because of the hierarchical structure of the dataset, three nested effects were included in each model: sampling site, nested in sampling date, overall nested in tributary. These three factors were considered as random effects. Sampling date was included in the model because adult body size tends to decrease with emergence date (Atkinson, 1994).

We categorized independent variables to improve the estimation of the relationship between the dependent (i.e. morphology) and the independent variables (i.e. environmental factors). Using independent variables as continuous variables requires estimation of a single coefficient across the whole variable range, but it assumes a linear relation between independent and dependent variables. Instead, when using categorical environmental variables, each class is considered as an independent level. The Shannon–Wiener index and all aquatic parameters were cate-

gorized (Table 1). For each categorical variable, classes were defined according to constant sample size.

Regarding the large number of independent variables in relation to the 998 individuals and three nested random effects (i.e. 33 sites, 17 dates and eight tributaries including the main channel), we computed three different models for each dependent variable PC1 and PC2. Model 1, which included sex, distance from the sea, habitat heterogeneity  $H'$  (Shannon–Wiener index), tree coverage, bank slope, substrate type and stream order, allowed us to test whether watercourse structure, surrounding terrestrial habitat and sex affect adult morphology. Two other models were performed to test whether aquatic habitat, distinguishing between summer and winter seasons, affects the morphology of damselflies. Model 2 included summer temperature, summer pH and summer nitrate concentration. Model 3 included winter temperature, winter pH and winter nitrate concentration.

From each complete model, we performed a backward selection, removing, at each step, the least significant factor at the threshold  $\alpha = 0.05$ . Statistical analyses were computed using R software (the R Foundation for Statistical Computing, 2005).

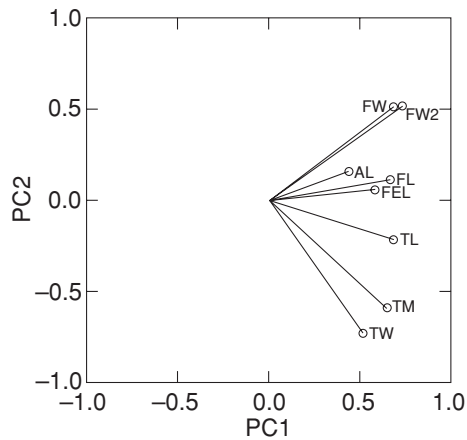
**Table 1** Variables, number of classes, minimum and maximum of fixed variables included in Linear Mixed Effect Models

Fixed variable	Number of classes	Minimum	Maximum
Shannon–Wiener index	5	1.28	2.4
Summer temperature	5	17.95 °C	22.90 °C
Winter temperature	6	4.92 °C	7.43 °C
Summer pH	5	7.30	8.70
Winter pH	6	7.20	8.68
Summer [NO <sub>3</sub> ]	5	1.27 mg L <sup>-1</sup>	56.33 mg L <sup>-1</sup>
Winter [NO <sub>3</sub> ]	5	8.5 mg L <sup>-1</sup>	41.67 mg L <sup>-1</sup>

## Results

### *Morphological variables: body size and body shape*

The two principal axes of the normalized PCA, PC1 and PC2, respectively explained 39.5% and 18.8% (total 58.3%) of the overall morphological variation. Because all variable loadings were positive, PC1 can be seen as a measure of overall body size. In contrast,



**Fig. 3** Bivariate plot of variable loadings on PC1 (body size) and PC2 (body shape) from a normalized PCA based on 8 morphological measurements taken from adult *Calopteryx splendens*. FW, forewing width; FW2, maximal forewing width; FL, forewing length; FEL, femur length; TL, thorax length; TM, thorax mass; TW, thorax width; AL, abdomen length.

two sets of variables had opposite effects on PC2. The loadings of thorax variables (length, width and mass) were negative whereas the loadings of wing variables were positive. Thus, PC2 can be considered as a measure of general body shape (Fig. 3).

#### *Effect of habitat on individual morphology*

From Model 1, the minimal model retained both sex and distance from the sea (see Table 2 for statistical details). We found no significant effect of habitat heterogeneity  $H'$  (LME model,  $F = 0.164$ , d.f. = 4,  $P = 0.931$ ), substrate type ( $F = 0.0007$ , d.f. = 1,  $P = 0.994$ ), tree coverage ( $F = 0.602$ , d.f. = 1,  $P = 0.467$ ), nor bank slope ( $F = 1.148$ , d.f. = 3,  $P = 0.394$ ), or river order ( $F = 0.582$ , d.f. = 3,  $P = 0.638$ ) on PC1. For PC2, the minimal model included sex only (Table 2). According to the negative coefficient derived for males (Table 2), females were significantly larger than males. The negative coefficient of distance from the sea indicates that downstream individuals were significantly larger than upstream. We detected no effect of habitat heterogeneity  $H'$  ( $F = 0.222$ , d.f. = 4,  $P = 0.899$ ), bank slope ( $F = 1.328$ , d.f. = 3,  $P = 0.364$ ), tree coverage ( $F = 0.339$ , d.f. = 1,  $P = 0.577$ ), stream order ( $F = 1.634$ , d.f. = 3,  $P = 0.249$ ), substrate ( $F = 1.224$ , d.f. = 1,  $P = 0.290$ ), or distance from the sea ( $F = 2.957$ , d.f. = 1,  $P = 0.106$ )

on PC2. The model taking into account the hierarchical structure of the dataset (i.e. three nested random effects: sampling site, nested in sampling date, overall nested in tributary) shows for each sex the relationship between body size (PC1) and distance from the sea for individual (Fig. 4a), site (Fig. 4b) and tributary (Fig. 4c) levels. Figure 4c represents mean body size (PC1) for the seven tributaries and the main channel of the Loire drainage. Body size declined with distance from the sea, following the longitudinal downstream–upstream succession of tributaries (Fig. 4c,d). Mean body size of individuals sampled in the main channel (i.e. letter A, Fig. 4c,d) occupied a central position probably because of its connection with the seven tributaries.

Body shape (PC2) was affected by the sex of individuals only (Table 2). According to the positive coefficient derived for males (coefficient  $\pm$  SE =  $0.142 \pm 0.058$ ), body shape values were larger for males than for females.

#### *Effect of water characteristics on morphology*

The same statistical procedure was used to test for the effect of water characteristics on the morphology of individuals.

From Model 2, including summer water parameters, the minimal model retained temperature and pH. Adults were significantly larger (higher scores of PC1, i.e. body size) when their larval stages were exposed to high temperatures and pHs (Table 3). According to the correlation matrix for the summer period (Table 4), waters were significantly more alkaline at sites closer to the sea. Analyses revealed no effect of nitrate concentration ( $F = 0.094$ , d.f. = 1,  $P = 0.775$ ) on PC1.

For PC2, the minimal model included temperature only (Table 3). In contrast, we detected no effect of nitrate concentration ( $F = 0.158$ , d.f. = 1,  $P = 0.711$ ) or pH ( $F = 3.160$ , d.f. = 1,  $P = 0.136$ ) on PC2. Interestingly, body shape seemed to be positively influenced by summer water temperature.

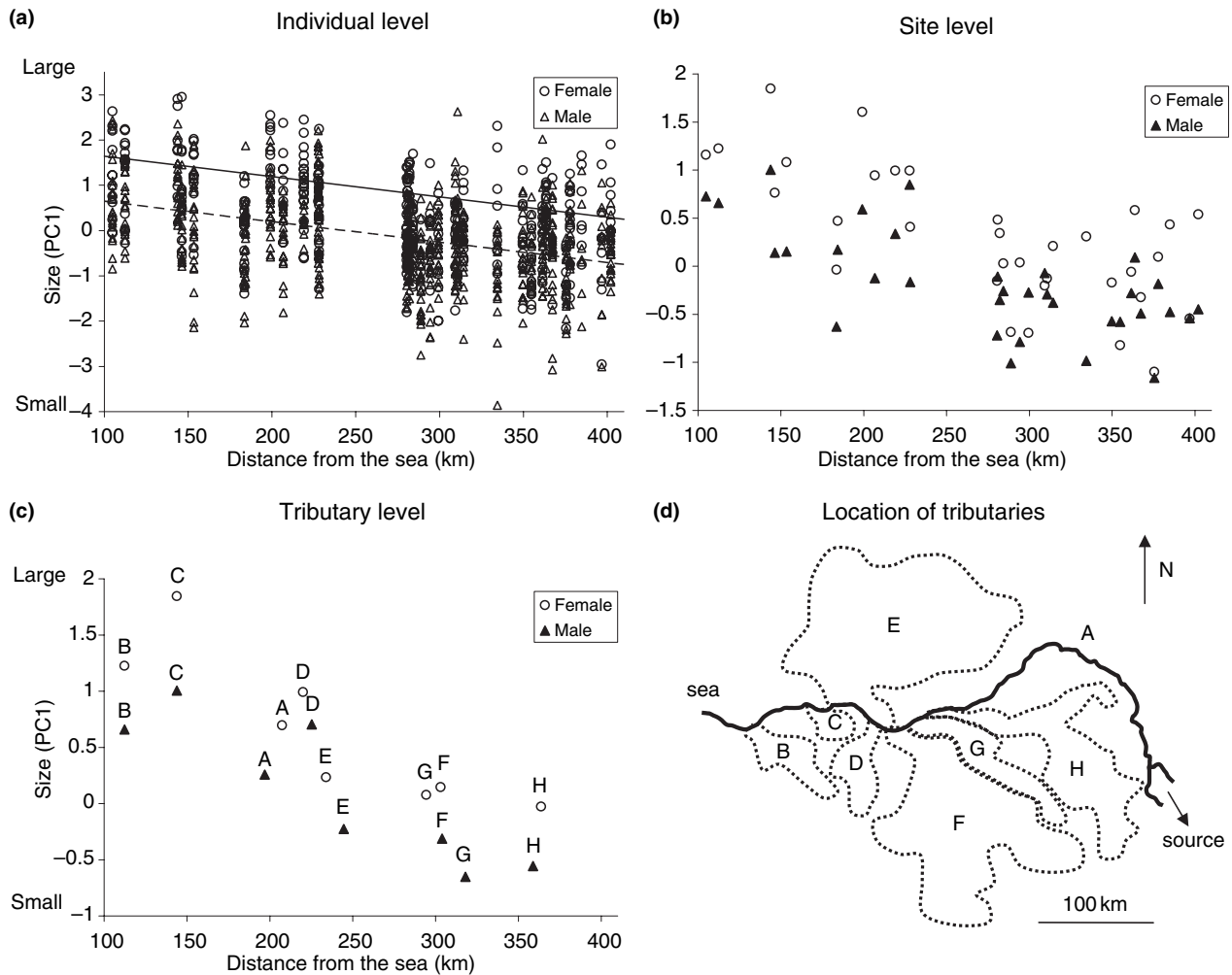
For Model 3, including winter temperature, nitrate and pH, we observed no significant effect on PC1 and PC2 ( $F < 0.26$ ,  $P > 0.63$ ). Therefore, winter temperature, winter nitrate concentration and winter pH during the larval stage, seem to have no effect on adult morphology.

**Table 2** Effect of habitat, terrestrial and aquatic variables, on body size (PC1) and body shape (PC2)

	Value	SE	numDF	denDF	F-value	P-value
PC1						
(Intercept)	1.594	0.257	1	958	38.549	<0.0001
Sex male	-0.500	0.056	1	958	80.135	<0.0001
Distance from the sea	-0.005	0.0009	1	15	25.871	0.0001
PC2						
(Intercept)	-0.146	0.127	1	958	1.324	0.250
Sex male	0.142	0.058	1	958	5.989	0.015

Here is presented the minimal model from the complete Model 1. We used a Linear Mixed-Effects Model with three nested random effects and applied a backward selection procedure (see *Methods*). Females were taken as reference.

NumDF, the numerator degrees of freedom and denDF, the denominator degrees of freedom for the *F*-test.



**Fig. 4** Relationship between distance from the sea and body size (PC1) in adult male ( $\Delta$ ) and female ( $\circ$ ) *Calopteryx splendens*. (a) Effect on individual body size, the continuous line corresponds to females ( $PC1 = 2.094 - 0.005 \times \text{Distance from the sea}$ ) and the dotted line to males ( $PC1 = 1.093 - 0.005 \times \text{Distance from the sea}$ ). Linear regressions were calculated from a Linear Mixed Effects Model including three random nested effects (see Table 3 for statistical results), sampling sites in sampling dates in tributaries. (b) The mean body size for each of 33 sites related to distance from the sea. (c) The mean body size for each seven tributaries and the main channel related to the mean distance from the sea. (d) Schematic location of the seven tributaries, in dotted-line, and the main channel of the Loire drainage in full-line (see Fig. 1 for details).

	Value	SE	numDF	denDF	F-value	P-value
PC1						
(Intercept)	-10.347	1.977	1	618	27.397	<0.0001
Temperature	0.222	0.038	1	5	33.944	0.002
pH	0.730	0.209	1	5	12.236	0.017
PC2						
(Intercept)	-4.350	1.569	1	618	7.683	0.006
Temperature	0.206	0.076	1	6	7.590	0.033

**Table 3** Effect of summer water characteristics (pH, nitrate and temperature) on body size (PC1) and body shape (PC2) in *Calopteryx splendens*

Here is presented the minimal model from the complete Model 2. We used a Linear Mixed-Effects Model with three nested random effects and applied a backward selection procedure (see *Methods*).

NumDF, the numerator degrees of freedom and denDF, the denominator degrees of freedom for the F-test.

**Table 4** Spearman order correlations for aquatic variables: pH, temperature (*T*) and nitrate concentration ( $\text{NO}_3$ ) in summer (*s*) and winter (*w*)

	pHs	pHw	Ts	Tw	$\text{NO}_3$ s	$\text{NO}_3$ w
pHs						
pHw	0.52*					
Ts	-0.12	-0.33				
Tw	0.07	0.52*	-0.18			
$\text{NO}_3$ s	-0.02	0.57*	-0.37	0.22		
$\text{NO}_3$ w	0.28	0.73*	-0.26	0.48*	0.58*	
Distance from the sea	-0.53*	-0.01	-0.41	-0.03	0.43	-0.03

\* $P < 0.05$ .

## Discussion

### *Environmental factors and body size variation*

We analysed the geographical pattern of morphological variation in *C. splendens* along the Loire drainage and investigated the effect of several environmental factors on adult body size and shape. The main significant factors that influenced the size of individuals were water temperature and pH during summer, and geographical position (distance from the sea). Because adult morphology is fixed after metamorphosis, larval conditions mainly affect the characteristics of the imago. We observed a positive relationship between summer temperature and size of imago at emergence. Similar results have been reported for the stoneflies *Taenionema pacificum* and *Pteronarcella badia*, (Perry, Perry & Stanford, 1987) and *Pteronarcys californica* (Gregory, Beesley & Van Kirk, 2000). In both studies individuals collected in warmer

water were larger. Hemimetabolous aquatic insects, such as odonates, might present an optimal thermal regime at which adult body weight and fecundity are maximized (Vannote & Sweeney, 1980). The maximal water temperature recorded in our study was 22.9 °C, close to the optimal temperature for growth in two species of *Coenagrion* damselflies (Van Doorslaer & Stocks, 2005). This result suggests that summer water temperature plays a key role in larval development of *C. splendens* too. Differences in temperature regime are known to reveal growth plasticity in ectotherms (Atkinson, 1994; Mousseau, 1997; Van Doorslaer & Stocks, 2005). Higher thermal energy can enhance metabolic rates by increasing enzyme activity and can indirectly improve foraging activity (Van Doorslaer & Stocks, 2005), leading to an increase in growth rate. Temperature has been also shown to influence adult wing morphology (Roff, 1994b; Sasaki, Nakasuji & Fujisaki, 2002; Gibert *et al.*, 2004).

Furthermore, we detected an effect of alkaline pH during summer on size at emergence, with larval growth rate presumably increasing with pH. High or low pH values tend to limit the larval development of some odonate species, including *Anax junius* (Punzo, 1988 in Corbet, 1999). However, the interpretation of this result is not straightforward. Odonates usually can survive across a wide range of pH values (Corbet, 1999). Those observed in our dataset on the Loire drainage varied across a limited range, from 7.3 to 8.7. Hence, the relationship between morphological variation and pH might be due to correlation with other factors such as conductivity or the accumulation and slow decomposition of organic matter (Corbet, 1999). An increase in carbon dioxide concentration because



of photosynthetic activity also tends to raise water pH. Photosynthesis itself is driven by solar radiation, nutrient level, temperature and algal biomass. Therefore the effect of pH on body size could actually reflect the relationship between pH and summer photosynthetic activity, a possible indicator of trophic conditions.

Terrestrial habitat can influence the flight morphology of insects, as described in a butterfly species occurring in highly fragmented landscapes (Hill, Thomas & Lewis, 1999). Although this effect is better documented among lepidopterans whose larval development is terrestrial (Thomas, Hill & Lewis, 1998; Norberg & Leimar, 2002), morphological variation between different landscapes has also been observed in species with aquatic larvae such as *C. maculata* (Taylor & Merriam, 1995): these damselflies were larger in pasture than in forest landscapes. In contrast, we observed no significant effect of neighbouring riparian habitat on morphology in *C. splendens*. Morphological characteristics of banded damselflies did not seem to respond to variations in vegetation structure along the river banks. The scale we considered in this study might not have been large enough to capture relevant habitat variation. However, even if vegetation structure, recorded at a fine-scale, varied between our sites, landscape showed a moderate level of land use heterogeneity all over the study area.

#### *Morphological clines and population structure across catchments*

To investigate the morphological variation pattern across the Loire drainage we explicitly included the hierarchical structure of the river network in our analyses. We detected no effect of stream order. However, results clearly supported an overall effect of location in the network on body size. The size of individuals decreased with the distance of the capture site from the sea. On the other hand, body shape was not affected by the geographical location of the populations. Supporting these results, Loh & Bitner-Mathé (2005) also found a greater influence of environmental variation on wing size of drosophilid flies than on wing shape.

According to Bergmann's rule, temperature is the main factor responsible for geographical clines in body size (Gibert *et al.*, 2004). Although this rule

applied originally to endotherms, it has also been investigated in ectotherms and insects (Hawkins & Lawton, 1995; Karan *et al.*, 1998; Arnett & Gotelli, 1999). Several species of *Drosophila* followed Bergmann's rule, because their body size increased with latitude (Karan *et al.*, 1998). Typically, ectotherms are supposed to grow more slowly at low temperatures (Atkinson, 1994). By prolonging growth and delaying maturation, insects can increase body size at emergence (Atkinson, 1994). However, another study of ectotherms reported the opposite pattern, and questioned the generality of the negative relationship between insect body size and temperature (Mousseau, 1997). Our results for *C. splendens* are consistent with this latter study as larger individuals were found in the warmer parts of the catchment. Aquatic insects have a thermal optimum that balances growth with respiration losses (Sweeney & Vannote, 1978). The thermal regime on the west side of the drainage may be closer to the optimum for *C. splendens* than on the east side. However, according to an experimental study of Schütte & Schrimpf (2002), *C. splendens* had similar respiration rates at 12, 18 and 24 °C. This suggests that temperature on its own cannot explain the longitudinal morphological variation of individuals on the Loire river.

The morphological cline in the banded damselfly could therefore reflect a plastic response to the gradual variation of abiotic factors along the river network. Environmental factors can strongly affect the ecology and behaviour of odonates (Wright, 1943 in Corbet, 1999), but the precise action of separate factors is difficult to characterize because they interact with one another and have long-term as well as short-term effects (Corbet, 1999). For instance waters with low pH are generally fishless, and temperature, by controlling metabolism, affects almost every activity, including foraging. Interestingly, these two variables were not correlated in our study.

Biotic factors such as predation and food availability can affect body size too. Predation risk can induce morphological phenotypic plasticity. For instance, smaller larvae were less susceptible to fish predation than larger larvae in *Ischnura verticalis* (Dixon & Baker, 1988). The scale of our study did not allow us to take into account these factors and their distribution on the drainage, but further study of their influence on *C. splendens* are needed.

*Expected consequences of morphological variation on dispersal*

A river network constitutes a system of spatially separated, repeated units that are seldom directly connected; thus, the network represents a decision tree for dispersers (Power & Dietrich, 2002). The connectivity pattern of the network can influence dispersal (Fagan, 2002) in such a way that within-stream population genetic diversity is expected to decline upstream and among-stream diversity to increase (Hernandez-Martich & Smith, 1997). We included in our analyses several morphological traits associated with flight (wing and thorax variables). Flight morphology and flight performance are associated, so that morphology provides a useful indicator of dispersal ability (Berwaerts, Van Dyck & Aerts, 2002). Assuming that large individuals, in terms of wings, thorax and abdomen, disperse farther than small individuals, our data suggest that the body size cline along the drainage could create a parallel gradient in population dispersal capacity and generate an asymmetric gene flow. This would tend to reduce genetic differentiation between streams if downstream populations send migrants farther than their upstream counterparts, and if larval drift and floods do not compensate for adult dispersal. Indeed, odonates can be vulnerable to larval drift (Anholt, 1995; Beukema, 2002). This hypothesis will be tested in a further work.

Predicting the population consequences of environmental variation at the drainage scale is essential to improve our understanding of river ecosystems and the efficiency of protection and restoration policies (Ward *et al.*, 2002). Adult characteristics in insects are strongly influenced by larval conditions, especially developmental temperature. To determine how environmental factors influence variation in phenotypic traits associated with life history traits in insects is necessary to predict population processes in river ecology. In this regard, our study suggests that ecological gradients occurring along river networks and network topology ought to be considered because of their potential effect on gene flow and overall population structure in large catchments.

**Acknowledgments**

This study was funded by Région Pays-de-la-Loire. We thank the students who helped with fieldwork

and particularly Virginie Dupont, Jean Christophe Bain, Line Fillonneau, Constance Xhaard, Delphine Lorber and Aude Houssaye. We gratefully acknowledge Michel Goulard (Laboratoire de Biométrie et d'Intelligence Artificielle, INRA, Castanet-Tolosan), for his insights into statistical analyses, and Damien Picard and Adeline Loyau, for their helpful comments on early versions of this manuscript.

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(Manuscript accepted 12 April 2007)