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Intra-specific variability of hindlimb length in the palmate newt: an indicator of population isolation induced by habitat fragmentation?

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Habitat fragmentation is one of the main drivers of global amphibian decline. Anthropogenic landscape elements can act as barriers, hindering the dispersal that is essential for maintaining gene flow between populations. Dispersal ability can be influenced by locomotor performance, which in turn can depend on morphological traits, such as hindlimb length (HLL) in amphibians. Here, we tested relationships between HLL and environmental variables—road types, forests and agricultural lands—among 35 sub-populations of palmate newts (*Lissotriton helveticus*) in southwestern France. We expected roads to select for short-legged newts due to a higher mortality of more mobile individuals (long-legged newts) when crossing roads. Accordingly, short-legged newts were found in the vicinity of roads, whereas long-legged newts were found closer to forests and in ponds close geographically to another water body. HLL in newts was hence influenced by habitat types in a heterogeneous landscape, and could therefore be used as an indicator of population isolation in a meta-population system.

1. Introduction

Global amphibian population declines are due to many factors, including the destruction and fragmentation of their habitats [1]. Their bi-phasic life cycle forces them to move between different habitats and renders them extremely vulnerable to the alteration of both aquatic and terrestrial environments.

Dispersal is crucial for maintaining gene flow between populations [2]. The costs associated with dispersal are likely to limit resource allocation, which can cause covariations between dispersal capacities and life-history traits [3]. In particular, because selection for efficient displacement might lead to leg elongation, morphological adaptations to dispersal could be deduced from estimates of leg length [4]. Dispersal abilities may be associated with locomotor performance [5], which is in turn related to hindlimb length (hereafter HLL) through its effects on take-off speed, jump distance [5] and distance moved [6] in anurans and on running burst speed and endurance in salamanders [7]. Thus, longer legs are expected to facilitate more rapid or longer-distance dispersal events [6]. These relationships were demonstrated in frogs [5], but also in walking and running organisms [7,8].
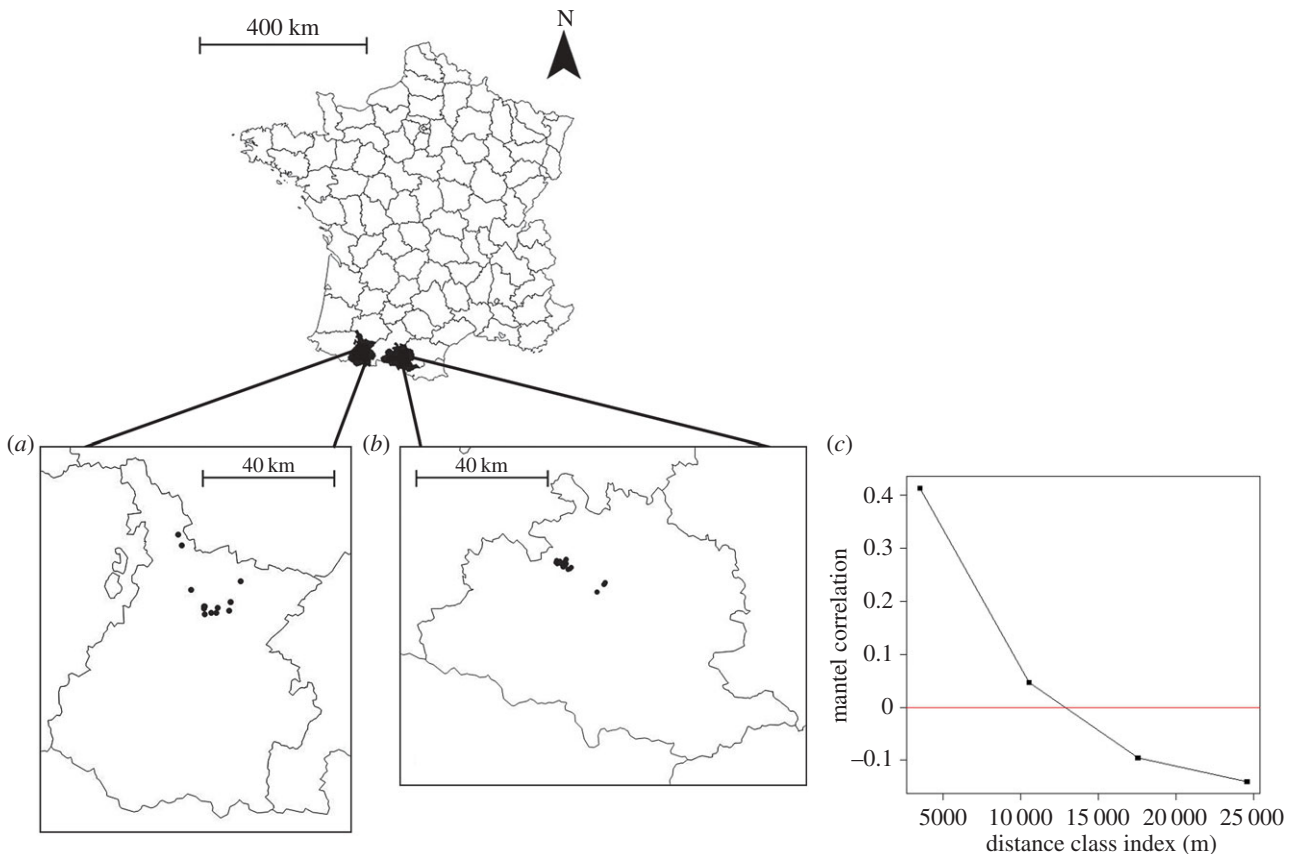
Figure 1. (*a,b*) Study sites where palmate newts were sampled. (*c*) Spatial correlogram between Mantel statistics and distance class index: significant Mantel correlation (black points) indicates spatial correlation.

From these hypotheses, we expect HLL to vary intra-specifically both within and between habitats, provided that the landscape is heterogeneous [9]. In landscapes affected by fragmentation and/or modification, habitat types vary in their resistance to movement, creating a complex matrix in which movements can either be favoured or limited [10]. In particular, habitat types such as agricultural lands [11] or road infrastructures [12] can strongly influence the dynamics of amphibian meta-populations.

Here, we assessed the relationship between forests, agricultural areas, pond density, road types and HLL in the palmate newt (*Lissotriton helveticus*). We sought to determine if environments where dispersal is limited are associated with variation in HLL. Because several types of roads have been identified as dispersal barriers [12], we could expect that they will select for a particular morphological type. We could predict that roads will select for highly mobile newts (those having long legs) that could avoid car mortality through rapid movements. Alternatively, these individuals could be counter-selected by roads as they should be more likely to encounter cars, which may increase their mortality rate. These two alternative predictions should generate significant relationships between landscape components and HLL in newts. Clarifying these relationships is crucial for understanding population functioning, and hence predicting how amphibians could respond to rapid environmental changes.

2. Material and methods

(a) Sampling methods and sites
Individuals were sampled in southern France during the breeding season. Sampling sites were located in an area dominated by forest;

and in a less forested area with higher road network. Newts were captured with a net, measurements were taken and individuals were immediately released at the capture site. In total, 79 ponds were sampled but only those from which 10 adults were caught were considered for statistical analyses ($N = 35$; figure 1*a,b*). A total of 350 individuals including 185 females (per site: median = 5; range min–max = 4–7) and 165 males (per site: median = 5; range min–max = 3–6) were sampled.

(b) Morphological measurements and environmental features
For each individual, we reported sex and measured the HLL and snout–vent length (SVL) to the nearest millimetre using a caliper. Environmental features were considered within a 1 km buffer zone (estimated dispersal capacities of the newt species [13]) around each sampling site.

We considered different types of environmental features from the Corine Land Cover database (European Environmental Agency) and from the BD TOPO database (Institut Géographique National), for land cover and road networks, respectively (table 1). The geographical spatial analyses were performed using ArcGis 10.0 (ESRI).

(c) Statistical analysis
We built interpolation maps for males and females separately using the spatial interpolation tools from QGIS 2.10.1. We first tested if spatial autocorrelation occurred in the spatial distribution of HLL using a Moran test [14], and we built a spatial correlogram to describe the spatial pattern and spatial correlation distance of HLL variability across sampled sites [15]. To test if variation in HLL was related to habitat type, we used linear mixed-effect models with *site identity* as a categorical random factor, the three habitat types, the density of water bodies and the eight distance

Table 1. Summary of environmental features used in our models to study the influence of habitat types on HLL variability in the palmate newt. CLC code: CORINE land cover nomenclature.

value	environmental variable	CLC code	description	mean (min – max)
surface area within a radius of 1 km	artificial surfaces	112	surface including discontinuous urban fabric	0.24 (0.00 – 1.19) km^2
	agricultural areas	211, 242, 243, 231	surface including non-irrigated arable lands, complex cultivation patterns, lands principally occupied by agriculture and pastures	1.96 (0.50 – 3.14) km^2
	forest areas	311, 312, 313, 321, 324	surface including broad-leaved forests, coniferous forests, mixed forests, natural grasslands and transitional woodland shrub	1.10 (0 – 2.64) km^2
distance from the sampling site	artificial surfaces	112	distance to the closest discontinuous urban fabric	2.07 (0 – 5.32) km
	agricultural areas	211, 242, 243, 231	distance to the closest non-irrigated arable land, complex cultivation pattern, land principally occupied by agriculture or pasture	0.00 (0 – 0.39) km
	forest areas	311, 312, 313, 321, 324	distance to the closest broad-leaved forest, coniferous forest, mixed forest, natural grassland or transitional woodland shrub	0.37 (0 – 1.92) km
	highways	—	distance to the closest highway	16.01 (1.16 – 25.58) km
	primary roads	—	distance to the closest primary road	16.34 (1.50 – 24.49) km
	secondary roads	—	distance to the closest secondary road	0.25 (0.00 – 0.94) km
	unpaved roads	—	distance to the closest unpaved road	1.32 (0.01 – 2.77) km
	water body	—	distance to the closest water body (among all 79 sampled ponds)	0.57 (0.03 – 2.99) km
density within a radius of 1 km	density of water bodies	—	number of water bodies around each sampling pond (even ponds with fewer than 10 sampled newts)	2.43 (0 – 6) ponds

variables as continuous fixed effects, and the HLL as response variable. To account for a possible effect of body size on HLL [6], we added SVL as a fixed covariate. We also imposed a Gaussian spatial correlation structure into the fixed predictors in all models to account for spatial autocorrelation [15]. Because newts show a sexual dimorphism [16], we added *sex* as a fixed categorical effect. To standardize the dataset, we scaled and centred all variables to the mean [17] to facilitate the interpretation of the relationships between fixed effects and HLL.

Model selection was performed using the Akaike information criteria (AIC) [18]. The best models (ΔAIC < 2) among all possible models were averaged to determine the relative importance of each selected variable. Two parameters from this averaging procedure were retained to test the importance of each variable in HLL: the confidence interval of the averaged estimated slope of the selected term (high effects had confidence intervals that did not include zero) and the relative weight of the term. All statistical analyses were performed using R v. 3.1.0 (R Development Core Team).

3. Results

The HLL varied from 10 to 17 mm (mean ± s.d. = 1.33 ± 0.12 mm). Significant spatial autocorrelation was detected in HLL variation (Moran test: $I = 0.33$, $p < 0.001$; figure 1c). Four best models were selected and averaged, from which we identified several significant relationships between environmental features and HLL (table 2). Sex was selected

in all best models, with males having longer HLL than females (figure 2). Short-legged newts were found closer to the secondary roads (table 2; figure 2e). Both distances to the closest forest and water body were negatively correlated to HLL, suggesting that long-legged newts were found closer to forests and in ponds close geographically to another water body (table 2; figure 2f). As expected, long-legged newts were the largest individuals.

4. Discussion

Our study suggests that habitat features influence HLL variability in the palmate newt. This finding was not driven by indirect environmental effects on body size as (i) we accounted for SVL in our models and (ii) SVL was not statistically related to any of these environmental parameters (only sex significantly affected SVL (as in [16]), not shown). Overall, this strongly suggests that environmental pressures only affect morphological traits potentially related to dispersal as leg length. According to our assumptions, we found that individuals living closer to secondary roads had shorter legs. The presence of roads may constitute an obstacle for many amphibians, even if their influence on dispersal-related traits has been poorly studied [12]. We confirm and extend on previous findings by demonstrating that secondary roads can affect HLL in newts. Roads can increase the mortality risk of the most mobile
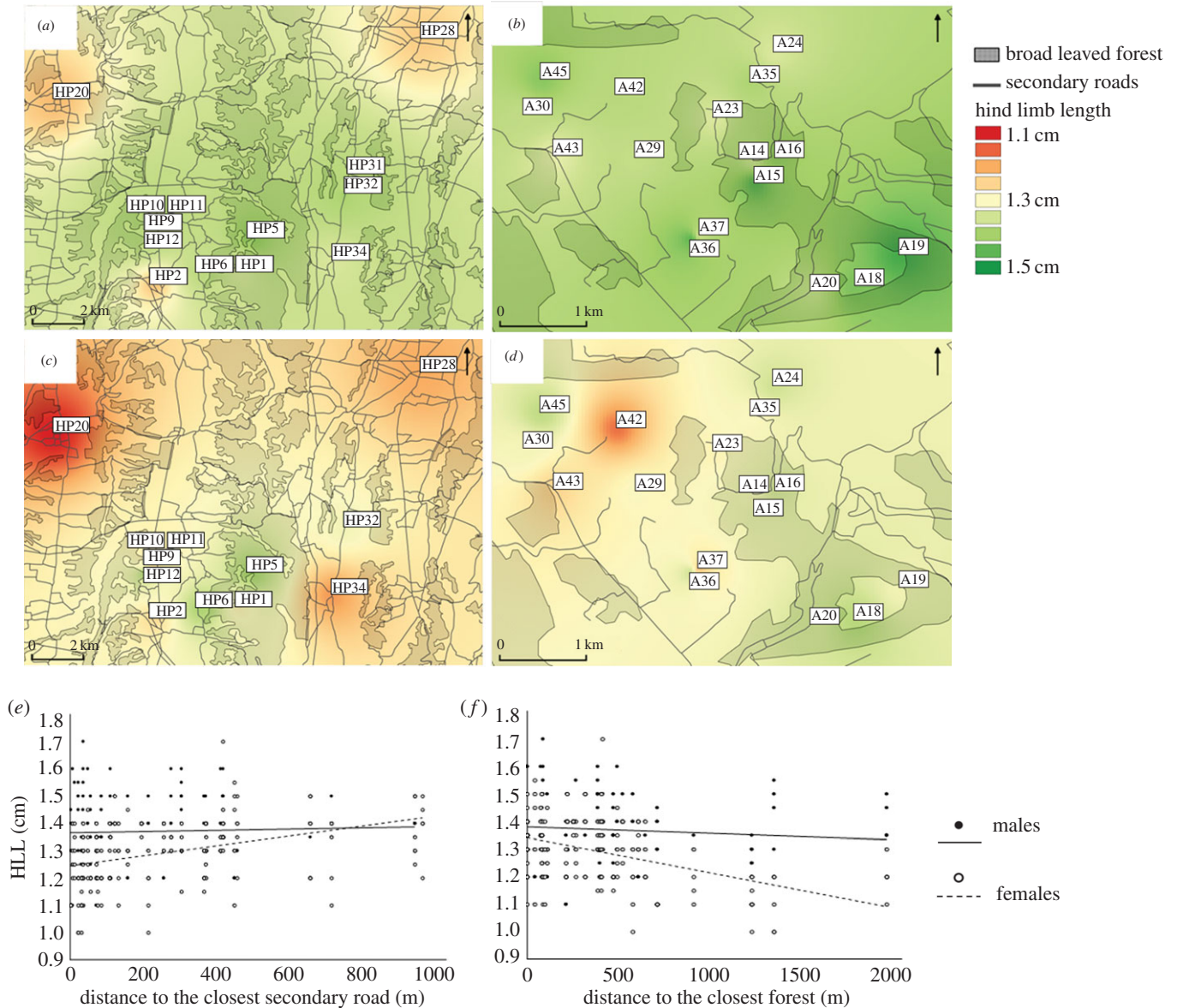
Figure 2. Interpolation maps of the hindlimb length (HLL) in sub-populations of palmate newts for males (*a,b*) and female (*c,d*). The labels represent the sampling ponds. Relationships between HLL with the distance to the closest forest (*e*) and to the closest secondary road (*f*) in the palmate newt. (Online version in colour.)

Table 2. Summary of the model averaging showing the significant variable effects on the HLL variability. Weight: relative Akaike weight of the top-ranked models (ΔAIC < 2) in which the term appeared.

model terms	estimate	95% CI of estimate	weight
intercept	−4.467***	(−5.82; −3.11)	
distance to the closest forest	−0.276**	(−0.45; −0.10)	1.00
sex (male)	0.833***	(0.65; 1.02)	1.00
distance to the closest secondary road	0.193*	(0.04; 0.35)	0.56
distance to the closest water body	−0.164*	(−0.31; −0.01)	0.41
snout − vent length (SVL)	1.095***	(0.74; 1.45)	1.00

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

individuals (the long-legged ones), which in turn might counter-select these individuals, hence decreasing the mean leg length of newts living at the proximity of roads. Consequently, roads—actually car traffic [12]—may be high selective pressures on newts, and particularly on long-legged individuals with a riskier dispersal behaviour.

Newts living in forested areas and in the proximity of another water body tended to have longer HLL. In amphibians, favourable habitats such as forest area [19,20] and proximity to water bodies often indicate for a high abundance of newts, leading to increased intra-specific competition for resource access, which could cause morphological variations

in larval amphibians [19–22]. Given that HLL is associated with dispersal capacities [6], forest areas hence provide suitable conditions for newts, which leads to individuals with a greater propensity for dispersal [23]. Despite the strong relationships between environmental features and HLL that we detected here, others factors can also drive such morphological variations that could be, therefore, subject to natural selection. Pond topology and/or hydroperiod [24] could have strong influences of metacommunity structure by causing variation in population density, and hence shape variability in larval amphibians [21,22]. Complementary studies are still needed to disentangle the potential effect of those last variables on leg length.

To conclude, our results demonstrated that morphological attributes possibly associated with dispersal strongly respond to variation in landscape features. It is noteworthy that other factors may also drive shape variation. We propose that these variations in morphology might mainly arise from natural selection acting against migrant individuals (with long legs), which could suggest micro-geographic adaptation [9]. However, because plasticity and selection are both plausible, further long-term surveys and experimental studies are needed to better tease apart mechanisms behind this result. In heterogeneous landscapes, HLL variability could be used as an indicator of isolated and more at-risk populations. Combined with genetic analyses, these findings could assist conservation management efforts to reduce isolated population extinction risk.

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