Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian

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Summary

1. Heterochrony, the change in the rate or timing of development between ancestors and their descendants, plays a major role in evolution. When heterochrony produces polymorphisms, it offers the possibility to test hypotheses that could explain its success across environments. Amphibians are particularly suitable to exploring these questions because they express complex life cycles (i.e. metamorphosis) that have been disrupted by heterochronic processes (paedomorphosis: retention of larval traits in adults). The large phenotypic variation across populations suggests that more complex processes than expected are operating, but they remain to be investigated through multivariate analyses over a large range of natural populations across time.

2. In this study, we compared the likelihood of multiple potential environmental determinants of heterochrony. We gathered data on the proportion of paedomorphic and metamorphic palmate newts (*Lissotriton helveticus*) across more than 150 populations during 10 years and used an information-theoretic approach to compare the support of multiple potential processes.

3. Six environmental processes jointly explained the proportion of paedomorphs in populations: predation, water availability, dispersal limitation, aquatic breathing, terrestrial habitat suitability and antipredator refuges. Analyses of variation across space and time supported models based on the advantage of paedomorphosis in favourable aquatic habitats.

4. Paedomorphs were favoured in deep ponds, in conditions favourable to aquatic breathing (high oxygen content), with lack of fish and surrounded by suitable terrestrial habitat. Metamorphs were favoured by banks allowing easy dispersal.

5. These results indicate that heterochrony relies on complex processes involving multiple ecological variables and exemplifies why heterochronic patterns occur in contrasted environments. On the other hand, the fast selection of alternative morphs shows that metamorphosis and paedomorphosis developmental modes could be easily disrupted in natural populations

Keywords: ecological processes; long-term survey; metamorphosis; newt; polymorphism; spatio-temporal scale

Introduction

Heterochrony, the change in timing or rate of developmental events, relative to the same events in an ancestor, plays a major role in both micro- and macro-evolutionary processes (Gould 1977; McNamara & McKinney 2005; McNamara 2012). Through the underdevelopment (paedomorphosis) and overdevelopment (peramorphosis) of characters by heterochrony (McKinney & McNamara 1991), large morphological variation can be achieved without deep genetic changes (Voss & Shaffer 1997; Voss & Smith 2005). Heterochronic processes were involved in the evolu-tion of a large number of invertebrate and vertebrate clades, including humans, and are observable both in fossil and living species records (McKinney & McNamara 1991; McNamara 1997; Gao & Shubin 2001).

Complex life cycles are widespread in most phyla in the animal kingdom (Hall & Wake 1999). They are characterized by the occurrence of metamorphosis, an abrupt transformation of a larva into a juvenile or a metamorphosed adult (Laudet 2011). In most cases, metamorphosis is associated with a habitat change, such as from water to land or from the water column to the bottom of wetlands (Laudet 2011). By interfering with this developmental process, heterochrony (specifically, paedomorphosis) produces larval organisms that are able to breed and do not shift habitat (Laudet 2011). This is frequent in caudate amphibians (i.e. newts and salamanders) where the disruption of metamorphosis occurred in nine of ten contemporary families (Wiens, Bonett & Chippindale 2005). Such heterochrony is obligate (i.e. fixed) in all the members of entire families, such as amphiumids, cryptobranchids, proteids and sirenids. In other families, particularly in salamandrids and ambystomatids, paedomorphosis is facultative with two morphs that can coexist: the metamorphs that develop from aquatic larvae to terrestrial adults through metamorphosis and habitat change (Fig. 1a) and the paedomorphs that mature in a larval stage (i.e. in retaining gills) in the aquatic habitat (Fig. 1b) (Denoël, Whiteman & Joly 2005b).

Determining the environmental pressures that could promote heterochrony is a major challenge in evolutionary ecology (Emel & Bonett 2011). When heterochrony acts at the intraspecific level, that is, as a polymorphism, it provides the opportunity to compare morph payoffs across environ-ments (Diz, Páez de la Cadena & Rolán-Alvarez 2012). Facultative paedomorphosis in newts and salamanders is thus a fascinating example to examine the ecological context of the evolution of complex life cycles. The genetic underpinning of paedomorphosis is complex and not yet fully understood (Page et al. 2010; Voss et al. 2012). However, there is evidence that paedomorphosis can be based on a similar genetic architecture at both the intra- (i.e. in polymorphisms) and inter-specific (fixation of novelties during evolution) levels within salamander families, thus linking micro- and macro-evolutionary processes (Voss & Smith 2005; Voss et al. 2012). This similarity confirms inferences from phylogenetic reconstructions that linked multiple recent speciation events in salamander families with intraspecific heterochrony (Shaffer 1984). A likely pathway of the evolution of novel paedomorphic species is via selection of thyroxin-response alleles that delay metamorphic timing (Voss et al. 2012). Rapid speciation through paedomorphosis also found support from artificial selection experiments observing changes in the proportion of paedomorphs at each generation (Semlitsch & Wilbur 1989; Voss & Smith 2005). Wilbur & Collins (1973) proposed a model for the evolution of complex life cycles, suggesting that paedomorphs are selected when aquatic conditions are particularly favourable, while the terrestrial habitats are unsuitable. Empirical

results from both mesocosm experiments and field studies supported this model (Semlitsch 1987; Denoël 2003; Bonett & Chippindale 2006).
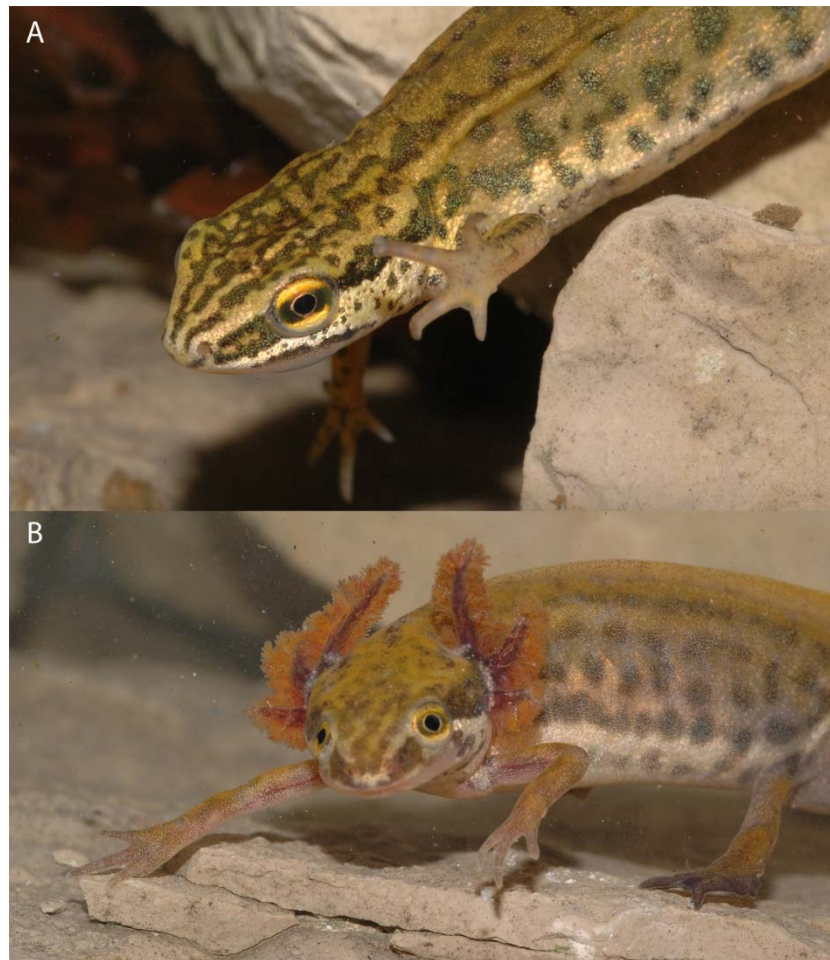

Figure 1. Alternative morphs produced by heterochrony in palmate newts. (a) The metamorph completes metamorphosis and shifts between aquatic and terrestrial habitats. (b) The paedomorph retains larval traits such as gills at the adult stage and remains in water.

Paedomorphosis and metamorphosis rates are affected by ecological features, such as pond drying and fish presence (e.g. Collins 1981; Semlitsch, Harris & Wilbur 1990; Bonett & Chippindale 2006; Denoël et al. 2009). The complexity of environmental features and the presence of paedomorphosis in unexpected habitats suggest that more complex processes than usually expected might be acting in populations (Denoël et al. 2001; Whiteman et al. 2012). Statistical approaches related to the information theory are particularly suitable to evaluating the relative support of alternative hypotheses on evolutionary and ecological processes (McIntire & Fajardo 2009; Ficetola et al. 2010; Denoël et al. 2013). These approaches could be particularly useful to disentangle the varied mechanisms that can explain the relative success of paedomorphosis over metamorphosis.

Within this framework, we aimed to identify potential processes that may determine variation of the proportion of paedomorphs across both space (i.e. among populations living in different habitats) and time (i.e. within a population in different years) in the palmate newt *Lissotriton helveticus*. In this species, both developmental modes (Fig. 1) are commonly expressed in a

high number of populations over a wide variety of habitats, making it an excellent model species to assess multiple alternative scenarios. We considered six specific hypotheses that fit expectations drawn by Wilbur & Collins (1973) and Whiteman (1994) on the advantage of paedomorphosis and allow a priori predictions of relation-ships between the proportion of paedomorphs in populations and environmental variables that change across space where discrete populations live (i.e. among ponds) (Table 1). 1. Water availability hypothesis: larvae opt for paedomorphosis when drying is less likely (Semlitsch 1987); 2. Predator hypothesis: paedomorphs have disadvantages in the presence of aquatic predators or superior competitors, such as fish (Denoël, Džukić & Kalezić 2005a); 3. Refuge hypothesis: there is coexistence between prey and predator, or the competitor is favoured by the presence of shelters (Martin et al. 2012; Winandy & Denoël 2013); 4. Aquatic breathing hypothesis: highly oxygenated water favours gilled organisms (Jonz & Nurse 2006); 5. Dispersal limitation hypothesis: Vertical slopes surrounding wetlands limit emigration of metamorphs, thereby determining disadvantages (Doležalová et al. 2012); and 6. Terrestrial habitat suitability hypothesis: a suitable terrestrial habitat (high wood cover) favours newts (Denoël & Lehmann 2006). Among these hypotheses, only two (water availability and fish presence) were assessed separately in experiments (Semlitsch 1987; Jackson & Semlitsch 1993; Denoël 2003) and also obtained support from field data (Denoël, Džukić & Kalezić 2005a; Bonett & Chippindale 2006; Denoël et al. 2009), but the joint role of multiple processes remains unknown. Furthermore, some of these variables (particularly those related to hypotheses 1, 3 and 4) can show variation along a relatively short time-scale. We therefore assessed whether the processes determining spatial variation of heterochrony can also affect the proportion of paedomorphs within populations across years.

Table 1. Effect of environmental variables on spatial variation in the proportion of paedomorphs in the palmate newt. Six variables representing distinct ecological processes explain the proportion of paedomorphs vs. metamorphs in natural populations. Estimates show results from the best quasi Akaike's information criterion (qAIC) model

Hypothesis	Prediction	Estimate	d.f.	F	P	Importance	% deviance[a]
Predator	−	−3·42	1,145	5·6	0·020	0·856	6·0
Dispersal limitation	+	2·91	1,145	17·7	<0·001	0·999	19·2
Aquatic breathing	+	1·74	1,145	21·1	<0·001	>0·999	22·8
Terrestrial habitat suitability	−	1·16	1,145	7·7	0·006	0·956	8·3
Antipredator refuge	+	−0·64	1,145	4·2	0·043	0·754	4·5
Water availability	+	0·32	1,145	33·9	<0·001	>0·999	36·7

[a] Percentage of explained deviance that is accounted for by each variable.

Materials and methods

Sampling

We surveyed 152 ponds inhabited by the palmate newt, *L. helveticus*, over 10 years (2002–2011) at the time of breeding (April–June). These ponds represent all the ponds inhabited by newts that could be found in Southern Larzac (France) after analysing local reports, topographic maps, ortho-images and interviews of local owners. Larzac is limestone plateau with traditional farmland agriculture. This is the area with the highest known rate and number of populations of paedomorphic newts (Denoël 2007). The sampling encompassed 68% of known populations of the species (Denoël 2007). Each pond was sampled by dip-netting (40 × 35 cm dip-net with a 1.8 m pole) along transects to cover the entire surface of the ponds several times (Denoël & Lehmann 2006). Deep zones were additionally sampled with large nets (seine nets measuring 5–10 m2). Censuses ended after many unsuccessful netting attempts in varied areas of the ponds. This method has the advantage of sampling all microhabitats and thus gives comparable values across ponds as all ponds were surveyed similarly. Previous studies showed that the detection probability with this type of removal estimate is very high (Wilbur 1997) and that using dip-nets is an adequate method for sampling European newts (Arntzen 2002; Denoël et al. 2013). Ponds were sampled with a similar methodology once a year, with an average of 3.1 times during the 10-year study period, to take into account morph variation across time.

At each pond, we recorded the proportion of paedomorphs in the adult population. Paedomorphs differ from metamorphs by multiple traits, such as the presence of gills and gill slits only present in the paedomorphs (Fig. 1). Their adulthood was established by the presence of a developed cloaca (Denoël 2002).We also measured six habitat variables that may provide advantages or disadvantages to paedomorphs: maximum water depth with a metered pole, proportion of pond area covered by aquatic vegetation (visual estimate), presence of vertical slopes all around the pond, percentage of dissolved oxygen (using a Hach Lange multi HQ40 optic oximeter, Mechelen, Bel-gium) and presence of fish (combining visual observations and seining) at the time the census was taken. All fish present in Larzac are non-native and have been introduced for ornamental reasons or as bait for fishermen in many kinds of ponds, including the deep and shallow ones (Denoël, Džukić & Kalezić 2005a). Three species were found during the study: the goldfish *Carassius auratus*, the common carp *Cyprinus carpio*, and the mosquitofish *Gambusia affinis*. Historical records indicate that only one of the studied ponds contained fish in the seventies (Gabrion 1976). In streams, paedomorphs have been shown to be favoured by loosely associated streambeds (i.e. large gravel) (Bonett & Chippindale 2006), but this substrate was not present in the study ponds, so this variable was not included. Although food availability could affect paedomorphosis (Semlitsch 1987; Denoël & Poncin 2001), we did not include this variable because it was impossible to quantify given our large sample size (n = 477 censuses over 152 ponds). We determined wood cover in a 100-m buffer zone around each pond from infrared ortho-images (Institut Géographique National, France, 0.5-m resolution) in arcgis 9.3. We used this buffer size because radiotracking studies showed that 50% of terrestrial movements occur within this distance (Semlitsch 2008).Statistics

An information-theoretic approach was used, based on Akaike's information criterion (AIC), to evaluate the support of hypotheses explaining spatial and temporal variation of the proportion of paedomorphs in populations (Burnham & Anderson 2002; McIntire & Fajardo 2009). The AIC is a numerical value used to rank competing models in terms of information loss in

approximating the unknowable truth. Parsimonious models explaining more variation have the lowest AIC values and are considered to be the best models (Burnham & Anderson 2002).

To analyse spatial variation, we built binomial generalized linear models (GLMs) relating the average proportion of paedomorphs in each pond to the six variables representing the hypotheses that show variation among ponds. To take into account overdispersion, models were built using the quasibinomial family. We built GLMs representing all combinations of hypotheses. We then calculated the quasi Akaike's information criterion (qAIC) for each model and ranked models on the basis of qAIC (Burnham & Anderson 2002; Richards 2008). For each candidate model, we calculated the Akaike weight w, which represents the probability that a given model is the best approximating model, given the data and the set of candidate models. We also calculated the importance of variables as the sum of the weight of all models including a given parameter. Variable importance can be interpreted as the probability that a predictor is part of the best model (Burnham & Anderson 2002). Ponds were surveyed a different number of times. The estimate of the proportion of paedomorphs is probably more reliable in ponds monitored several times. We therefore explicitly accounted for variation in the reliability of the proportion of paedomorphs by weighting each case by the number of surveys performed (Gómez-Rodríguez et al. 2012). This approach is similar to the one proposed to integrate detection probability into distribution models, when only some of sites are surveyed multiple times (Gómez-Rodríguez et al. 2012). In ponds monitored several times, we used the average value for environmental variables that show variation among years (water depth, oxygen and vegetation cover).

Data on the proportion of paedomorphs were overdispersed, as the majority (59%) of ponds were without paedomorphs, and a preliminary GLM with binomial error showed significant overdispersion. We followed two approaches to take into account the impact of overdispersion into our analyses. First, as described above, we built global models considering all ponds using quasibinomial errors and ranked models according to quasi-AIC. This is the approach generally recommended when dealing with overdispersion in information-theoretic analyses (Richards 2008). As an alternative approach to overdispersion, we used a two-step approach (Zuur et al. 2009). In the first step, we built models for all ponds with a binary response variable (presence/absence of paedomorphs) (binomial error). Next, we built models with the proportion of paedomorphs as the response variable, but only for ponds that contained paedomorphs. As with this data set overdispersion remained significant, also these models were built using quasibinomial errors.

To analyse temporal variation, we built generalized mixed models (GLMMs) relating the average proportion of paedomorphs per pond and per year to three variables showing interannual variation (oxygen, water depth and aquatic vegetation). This analysis was limited to the 81 ponds in which newts (either metamorphs or paedomorphs) had been detected for at least 2 years. Pond identity was included as a random factor in the models. Overdispersion was also present in these data, but in GLMMs, the quasilikelihood family does not provide consistent results; therefore, we used individual-level random effects to take into account overdispersion (Browne et al. 2005). We then calculated the AIC of each model, ranked the models on the basis of AIC, and calculated the Akaike weight of each model and the importance of each variable. The Durbin–Watson test con-firmed that for no ponds, the residual temporal autocorrelation was significantly greater than zero. In all our analyses, we included only sites where newts had been detected, and the proportion of paedomorphs was the dependent variable. The study took place during the reproductive peak of both morphs, that is, in late spring. To take into account potential temporal variation for the proportion of paedomorphs, we included

the date of surveys and its quadratic term in the preliminary models. Including the survey date generally did not improve model performance (measured using AIC/qAIC) compared with the respective models with no date. For simplicity's sake, in the results, we report the analyses not taking the date into account. The results including the date are qualitatively equivalent and are reported in the supporting information. In our models, we did not use AIC corrected for small sample size (AICc) because our sample size was large compared with the number of predictors and because of concern regarding the application of the AICc to mixed models. The results of the analyses remain unchanged if corrections for small sample size are applied and are reported in the supporting information. We used a permutation test to confirm that the residuals of our models were not affected by spatial autocorrelation ($P > 0.1$ for all the best models) (Lichstein *et al.* 2002). Independent variables were not strongly correlated (Pearson's $r < 0.4$ for all pairwise correlations), and we did not detect multicollinearity issues in any of the models.

In addition to the use of AIC/qAIC, we calculated the significance of variables included in the best model to improve interpretation of the role of environmental variables. For quasibinomial GLMs, significance was assessed using F tests; for GLMMs, significance was assessed using likelihood ratio tests. For GLMs, we also calculated the proportion of deviance explained by the model (R^2_D) and the proportion of explained deviance that is accounted for by each environmental variable included in the best qAIC model. Prior to performing the analyses, proportion data of environmental variables (aquatic vegetation and wood cover) were transformed using the square-root arcsine, while oxygen and water depth were transformed using the square root to reduce skewness. All analyses were performed in r using the lme4 and mumin packages. We used conditional plots to visualize regression model for continuous variables.

Results

Of the 152 ponds inhabited by palmate newts, 58 contained both paedomorphs and metamorphs, three, paedomorphs only and 91, metamorphs only. In ponds where both morphs coexist, the proportion of paedomorphs varied between 2% and 96%.

Spatial Variation

Spatial variation of the proportion of paedomorphs was strongly related to all the environmental variables considered (Table 1). The best model included all six variables representing our *a priori* hypotheses, providing support to four of the six hypotheses on processes determining the proportion of paedomorphs. The best model explained a good amount of variation ($R^2_D = 0.42$) and indicated that the proportion of paedomorphs was lower in ponds with fish and with abundant vegetation, while it was higher in deep ponds and in ponds with vertical slopes, high oxygen content and surrounded by high forest cover (Table 1; Fig. 2). Four hypotheses (water availability, dispersal limitation, predation and aquatic breathing) were supported by this model, whereas two were not (antipredator refuge and terrestrial habitat suitability) as our *a priori* hypotheses predicted opposite relationships. Five additional models showed support at 0.001 or higher (Table S1, Supporting Information). They all were subsets of the best model, and coefficients were always similar to those of the best model. Water depth, oxygen and bank slope were included in all the models with high support and were the variables with the highest support (Tables 1 and S1, Supporting Information). The remaining variables showed support >0·7 (Table S1, Supporting Information). Models including the date of the surveys or ranked using qAICc yielded equivalent results (Tables S2 and S3, Supporting Information).
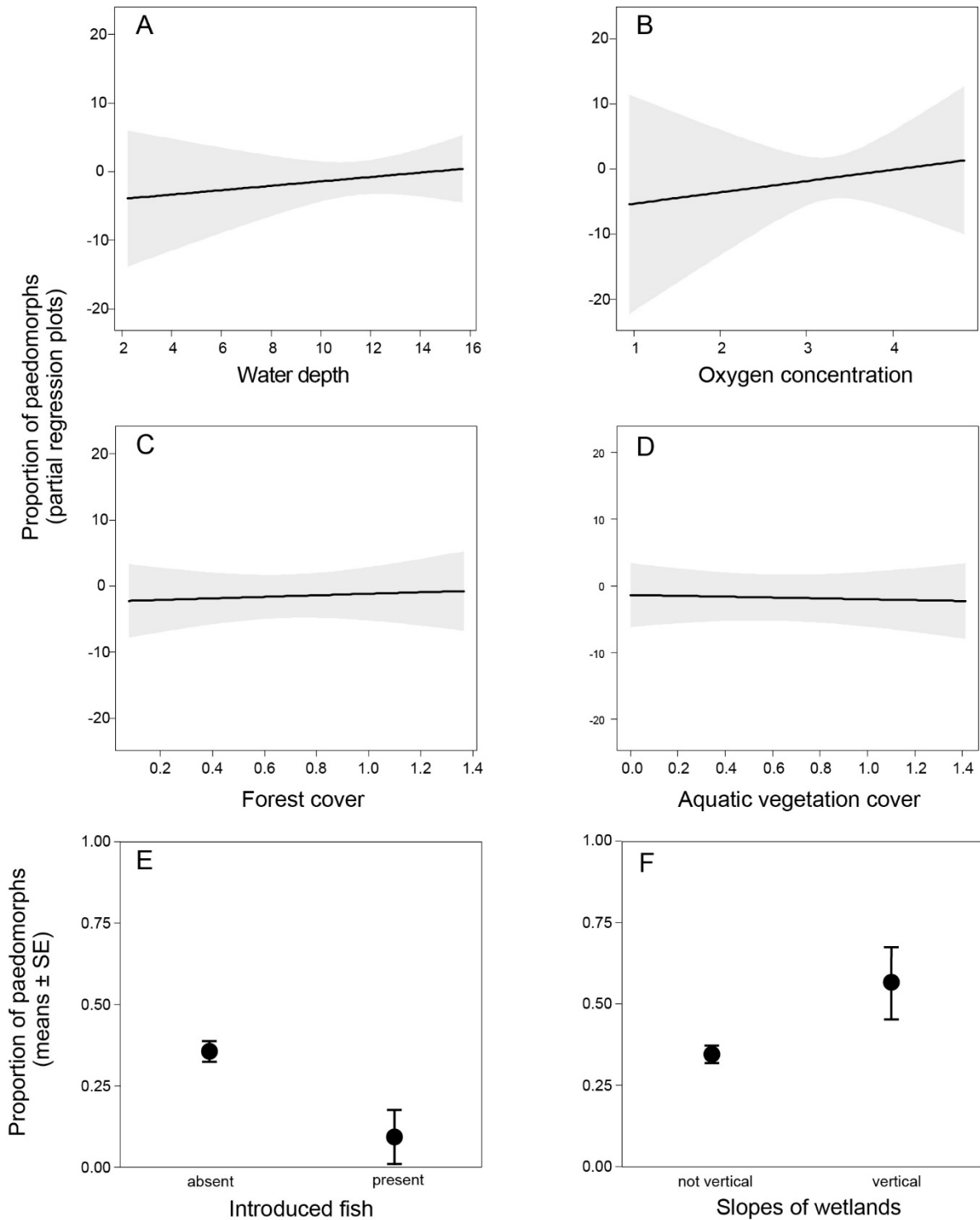
Figure 2. Relationship between environmental variables and the proportion of paedomorphs in natural populations of palmate newts. (a–d): conditional regression plots, relating the proportion of paedomorphs to (a) water depth (squareroot), (b) oxygen concentration (squareroot), (c) forest cover (arcsin squareroot) and (d) aquatic vegetation cover (arcsin squareroot). Shaded areas represent 95% confidence bands. (e–f): proportion of paedomorphs in wetlands differing for the presence of introduced fish (e) and vertical slopes (f). Error bands are standard errors of the mean. See Table 1 for statistical results.

Due to overdispersion, the analysis was repeated using a two-step approach. The first step allowed to identify the parameters that differentiate ponds with at least one paedomorph from ponds without paedomorphs. The best model explained a good amount of variation ($R^2_D = 0.45$)

and indicated that paedomorphs were present in deep ponds, without fish, with vertical slopes and with high oxygen content (Table 2a; Fig. 3), supporting the predictions of four hypotheses (water availability, predation, dispersal limitation and aquatic breathing). Fish presence, pond depth and oxygen content showed the highest importance. Eight additional models showed support at 0.001 or higher (Table S4a, Supporting Information). These models included fish presence, pond depth and oxygen content nearly always.

The second step allowed identified parameters that are related to the proportion of paedomorphs in ponds where paedomorphosis was observed. The best model explained 31% of variation and indicated that, within the ponds with paedomorphs, their proportion was higher in deep sites, with vertical slopes, high oxygen content and surrounded by forest. This model supported three hypotheses (water availability, dispersal limitation and aquatic breathing), while it was against the prediction of terrestrial habitat suitability hypothesis. Oxygen content was the variable with the highest importance (Table 2b). However, in this case, there was some uncertainty in model selection, as several models showed support at 0.001 or higher (Table S4b, Supporting Information).

Table 2. Effect of environmental variables on spatial variation of paedomorphosis in the palmate newt: analysis performed using a two-step approach. (a) Variables representing ecological processes, allowing to distinguish ponds with and without paedomorphs [estimated from the best Akaike's information criterion (AIC) model, Table S3a, Supporting Information]. (b) Variables representing processes determining variation in the proportion of paedomorphs in ponds where paedomorphosis was found (estimated from the best quasi Akaike's information criterion (qAIC) model, see Table S3b, Supporting Information)

| Hypothesis	Prediction	Estimate	d.f.	χ^2	P	Importance
a) Presence/absence of paedomorphs						
Predator	−	−4·43	1	74·4	<0·001	>0·999
Dispersal limitation	+	1·27	1	3·8	0·049	0·678
Aquatic breathing	+	1·03	1	12·9	<0·001	0·996
Water availability	+	0·89	1	210·8	<0·001	>0·999
b) Proportions of paedomorphs						
Dispersal limitation	+	1·95	1,56	4·9	0·031	0·773
Aquatic breathing	+	2·02	1,56	15·9	<0·001	0·998
Terrestrial habitat suitability	−	1·26	1,56	5·5	0·023	0·828
Water availability	+	0·16	1,56	3·57	0·063	0·623
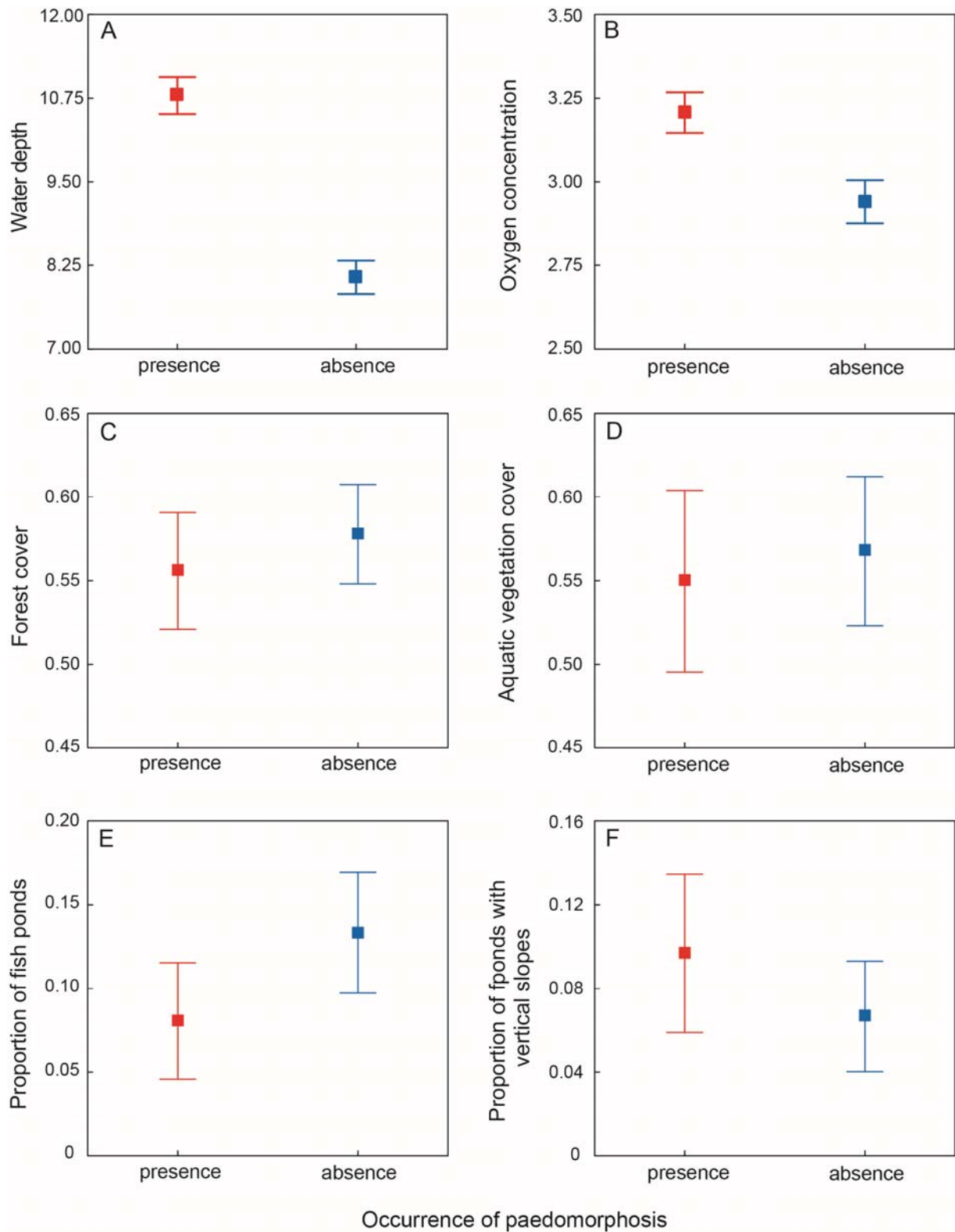
Figure 3. Mean ± SE values of environmental variables in natural populations of palmate newts with and without paedomorphs: (a) water depth (squareroot), (b) oxygen concentration (squareroot), (c) forest cover (arcsin squareroot), (d) aquatic vegetation cover (arcsin squareroot), (e) presence of introduced fish and (f) vertical slopes. See Table 2 for statistical results.

Temporal Variation

For 81 ponds, newts were detected in at least 2 years (average: 4.9 years; range: 2–8). Within these ponds, the interannual variation in the proportion of paedomorphs was significantly related to the three variables representing our *a priori* hypotheses on temporal variation (depth, oxygen and aquatic vegetation). The best model included all these variables (Table 3). In a given pond, the proportion of paedomorphs increased in years in which the water was deepest and the most oxygenated, and when vegetation cover was low (Table 3). These results are in strong agreement with the results of the analysis of spatial variation and support at the temporal level two of the three *a priori* hypotheses assessed, that is, water availability and aquatic breathing, but not antipredator refuge. Four additional models showed weight >0.001. These models included fewer variables, but the coefficients were in agreement with the best model (Table S5, Supporting Information). Oxygen was the variable with the greatest support; water depth and vegetation showed support >0.8. Models taking into account the date of surveys or ranked using AICc yielded equivalent results (Table S6, Supporting Information).

Table 3. Effect of environmental variables on temporal variation in the proportion of paedomorphs in the palmate newt. Three variables representing distinct ecological processes explain the change of proportion of paedomorphs vs. metamorphs in natural populations. Estimates show results from the best Akaike's information criterion (AIC) model

Hypothesis	Prediction	Estimate	d.f.	χ^2	*P*	Importance
Aquatic breathing	+	0·25	1	11·2	<0·001	0·918
Antipredator refuge	+	−0·54	1	71·0	<0·001	0·813
Water availability	+	0·20	1	51·0	<0·001	0·848

Discussion

This multifactorial approach on an amphibian model species gave important insights for the understanding of the success of heterochrony across both space and time: six different processes jointly explained the distribution of paedomorphosis and metamorphosis. The dependence of paedomorphosis on multiple ecological factors suggests that the evolution of heterochrony is based on a large set of favourable conditions and is thus a more complex process than previously thought. Organisms can use metamorphosis to escape from detrimental aquatic conditions, mak-ing the persistence of complex life cycles the most widespread developmental mode, whereas paedomorphosis remains geographically clustered (Semlitsch, Harris & Wilbur 1990; Voss & Smith 2005; Denoël 2007; Denoël et al. 2009).

Due to overdispersion, we analysed the spatial variation in two different approaches: first, we considered the variation in the proportion of newt paedomorphs across all ponds. Subsequently, we repeated the analysis using a two-step approach: the first step aimed at identifying processes determining occurrence of paedomorphs across ponds, while the second one identified processes determining variation in the proportion of paedomorphs if paedomorphosis was present. The approaches gave highly congruent results, as nearly all processes identified by the global analysis were confirmed by the two-step analysis. Aquatic vegetation (antipredator

refuge hypothesis) was the only variable identified by the global analysis that did not emerge in the two-step analysis. However, in the global analysis, aquatic vegetation was clearly the least important one, as showed by its limited weight. Furthermore, the two methods provided complementary results, as the global model allowed to evaluate the overall variation, while the two-step analysis (Table 2) helped to clarify the relative role of variables. For instance, predator presence has a very strong negative effect on paedomorph occurrence, as in the presence of fish, paedomorphs are nearly always lacking (Figs 2e and 3e). Conversely, dispersal limitation, aquatic breathing and water availability influence both the occurrence of paedomorphs and their proportion in populations, suggesting that they play a really major role.

The temporal scale is a key feature of evolution by heterochrony, as heterochronic processes imply changes in rate and developmental processes. Artificial selection experiments showed that paedomorphosis expression can be altered in only a few generations (Semlitsch & Wilbur 1989). Here, we showed that temporal changes of environmental features quickly affected the proportion of paedomorphs and metamorphs in natural populations. The pattern observed at a temporal scale was in accordance with our results at the spatial scale, thus suggesting that the patterns observed are not idiosyncratic of a specific situation, and allowing general insights on how poly-morphism and heterochrony could evolve in natural populations. The processes studied involve environmental features that change rapidly, such as water depth, vegetation and chemical features of ponds. This indicates that short episodes of environmental change could determine selective pressures leading to rapid changes. Longer time series may also be useful to determine the effects of the variables that remained stable across our samplings, particularly fish presence (Denoël, Džukić & Kalezić 2005a). Forest cover is also expected to change over time (e.g. after abandonment of farmlands), but on a time-scale larger than in the present survey. Polyphenisms may also promote diversification by facilitating ecological speciation processes (Pfennig & McGee 2010), but this requires the persistence of favourable ecological conditions. This is in full accordance with genetic models that predict that fast changes in developmental thresholds may lead to speciation, but also to the disappearance of paedomorphosis or metamorphosis (Denoël, Džukić & Kalezić 2005a; Voss & Smith 2005).

The 'Paedomorphic advantage' evolutionary model predicts the evolution of paedomorphosis in favourable aquatic conditions (Wilbur & Collins 1973; Whiteman 1994). Our results are in agreement with this hypothesis, in that four hypotheses on processes determining the variation in the proportion of paedomorphs were strongly supported by the data. First, in favour of the water availability hypothesis, paedomorphs were favoured over metamorphs in deeper wetlands (Figs 2a and 3a). Experimental work has provided consistent results as paedomorphosis is selected in permanent waters and metamorphosis in drying waters (Semlitsch 1987; Denoël 2003). Secondly, paedomorphs, through their aquatic habits, their less cornified skin, and the presence of external gills, could rely more on dissolved oxygen than do metamorphic individuals (Seliskar & Pehani 1935; Andreone et al. 1993; Denoël 2005; Wells 2007). Our models showed the importance of a high oxygen content in allowing the existence of paedomorphs (Figs 2b and 3b). Thirdly, the presence of fish negatively affects paedomorphs more than metamorphs. Fish introduction often contributes to amphibian decline (McGeoch et al. 2010), but its effects on intraspecific diversity remain poorly understood (Jackson & Semlitsch 1993; Denoël, Džukić & Kalezić 2005a; Denoël et al. 2009). The greater effect of fish on the more aquatic morph (Figs 2e and 3e) supports the hypothesis that the evolution of amphibians with complex life cycles could have been strongly driven by interactions with other species, such as aquatic predators or competitors. Fourth, the presence of vertical slopes surrounding wetlands could limit escape from them, that is, preventing a part of the metamorphs from dispersing. We consistently found a higher proportion of paedomorphs in such

environments (Figs 2f and 3f). Rather than favouring paedomorphosis, this probably limits the advantages of metamorphs, which are less well adapted to the water environment. These results suggest also that more research is needed on the advantages of dispersal to under-stand the success of paedomorphosis vs. metamorphosis.

Although all habitat features considered were important in explaining the distribution of paedomorphs, for two variables, the direction of the relationships was unexpected. We expected a higher proportion of paedomorphs in ponds with abundant aquatic vegetation, given that it can pro-vide shelter to them, but we observed the opposite pattern (Fig. 2d). A possible explanation of this unexpected relationship could be in the open water available to paedomorphs in ponds with limited aquatic vegetation. In support of this hypothesis, previous studies showed that paedomorphs are able to better exploit the deep parts and water column of ponds and lakes than meta-morphs (Denoël & Joly 2001; Denoël 2005). It should also be noted that the availability of aquatic vegetation did not explain the occurrence of paedomorphs. Secondly, the presence of suitable terrestrial habitats, which is predicted to provide greater fitness to metamorphosed individuals (Wilbur & Collins 1973), did not favour metamorphosis. Previous work has indicated that pal-mate newts are favoured by forests (Denoël & Lehmann 2006), but we observed a higher proportion of paedomorphs when surrounding forests were abundant (Figs 2c and 3c). In contrast to metamorphs, paedomorphs cannot disperse on land. They thus rely on the alternative phenotype that could colonize new ponds and give birth there to future potential paedomorphs. It is also possible that selective pressures on paedomorphosis in our study system are acting more particularly on the aquatic stage.

Conclusions

Understanding the success of heterochrony in varied landscapes requires the integration of multiple processes occurring across both space and time (Whiteman et al. 2012). Polymorphic extant species proved their usefulness as the results of environmental pressures can be directly observed (Semlitsch 1987; Roff 1996). With large sampling across environmental gradients and replicated measures across time, it becomes possible to identify ecological processes at the basis of transition between major developmental modes such as paedomorphosis and metamorphosis. The identification of varied environmental processes occurring in present-day landscapes can also help design laboratory experiments to study evolution under controlled conditions. The difficulty of carrying out such long-term studies over large spatial scales makes it a difficult task (Whiteman et al. 2012), but the complexity of ecological processes involved in the distribution of alternative morphs makes it a necessary step.

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Author contributions

MD initiated and designed the study, MD collected data, GFF and MD analysed data, MD and GFF wrote the manuscript. All authors contributed significantly to the manuscript.

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Supporting Information

Table S1. Models (with support ≥0·001) explaining spatial variation for the proportion of paedomorphs in palmate newt populations.

Table S2. Models (with support ≥0·001) explaining spatial variation for the proportion of paedomorphs in palmate newt populations.

Table S3. Models (with support ≥0·001) explaining spatial variation for the proportion of paedomorphs in palmate newt populations.

Table S4. Two step models: a) first step. Models (with support ≥0·001) explaining spatial variation for the presence/absence of paedomorphs in palmate newt populations. b) Models explaining spatial variation for the proportion of paedomorphs in palmate newts, considering populations with paedomorphs only. For each model, coefficients of included variables are reported. Models are ranked following increasing AIC/qAIC values. *K*: number of parameters in the model.

Table S5. Models (with support ≥0·001) explaining temporal variation for the proportion of paedomorphs in palmate newt populations.

Table S6. Models (with support ≥0·001) explaining temporal variation for the proportion of paedomorphs in palmate newt populations.