

Habitat fragmentation affects movement behavior of migrating juvenile common toads

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Abstract Displacement characteristics in animals are crucial drivers of successful movement decisions in resources acquisition, migration, and dispersal. As landscape structure is modified by human activity, mobility patterns are likely to evolve in response to habitat fragmentation. In species with complex life cycles that involve obligatory migrations between different habitats, one can predict that movement propensity would be promoted by fragmentation as long as it allows to reaching a habitat patch. Here, we compare the movement characteristics of naive toadlets sampled in populations distributed over a fragmentation gradient to test the hypothesis of a positive correlation between fragmentation and mobility levels. We studied toadlet movement in experimental arenas providing small patches of suitable conditions. We recorded the use of these patches (*patch behavior*)

or the absence of their use (*overtaking behavior*). The more fragmented the original landscape, the more prone the toadlets were to combine these two behaviors, thus showing a higher motivation to explore. Moreover, the more fragmented the original landscape, the less the toadlets exhibited *patch behavior*. As the toadlets were reared in a common environment, the behavioral differences detected, relating to the level of fragmentation, resulted from inheritance. Our results thus illustrate that fragmentation is likely to create cross-generational transmittable variations in movement characteristics.

Keywords Evolution · Habitat fragmentation · Movement propensity · Toadlet

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Introduction

Although movement is a key component in almost any ecological or evolutionary process, there is still much to learn about the behavioral mechanisms underlying the efficiency of movement, such as orientation and habitat selection (Angeloni et al. 2008; Nathan et al. 2008). Beyond interspecific variability in mobility patterns, there is an increasing interest in variations between populations, notably because anthropogenic alterations in local conditions can exert a strong selective pressure on animal movements. In particular, landscape fragmentation, through habitat loss and patch isolation, could drive evolutionary changes of movement characteristics, such as the propensity to move, navigation capacity, or moving performance (Baguette and Van Dyck 2007). As outlined in theoretical and empirical studies, the directional selection exerted on movement characteristics in the context of dispersal may vary greatly according to the way fragmentation alters the dispersal

cost–benefit balance (mostly in relation to the spatial and temporal variations in unoccupied fragments; Travis and Dytham 1999; Heino and Hanski 2001; Merckx *et al.* 2003, Schtickzelle *et al.* 2006; Bonte *et al.* 2010). Studying flying insects, Van Dyck and Matthysen (1999) first suggest the possibility that movement pattern and/or morphology could change in response to habitat fragmentation. They argued that subtle changes could be tracked and established results in support of such a continuous evolutionary response. Other studies have indeed shown that different components of movement behavior can be modified under selection pressure resulting from a changing landscape configuration: movement capacity (Taylor and Merriam 1995), movement propensity (willingness to fly and cross boundaries in the butterfly *Pararge aegeria*; Merckx *et al.* 2003), through tiptoe behavior in spiders (*Pardosa monticola*; Bonte *et al.* 2006), and orientation capacities through habitat detection ability (Merckx and Van Dyck 2007).

Besides dispersal, other movements crucial for completing life cycles, such as migrations require habitat shift (i.e., complementation processes; Pope *et al.* 2000) or foraging activity across specific resource patches (i.e., supplementation processes, Dunning *et al.* 1992) are also strongly exposed to the selective forces determined by landscape structure (Joly *et al.* 2001, 2003). Complementary or supplementary processes cannot easily be counterselected by habitat fragmentation since they are not optional at the scale of individual life cycles. In these situations, one should therefore expect fragmentation to promote higher movement efficiency either through navigation ability or moving performance until reaching a threshold above which coadaptation of traits is broken (e.g., when the relative size of locomotive appendages exceeds the limits of the possible). This is especially true for the complementation process, since life cycles impose to pay the cost of migration due to habitat shift (e.g., from breeding habitat to growing habitat).

Our study focuses on a species with a complex life cycle, the common toad *Bufo bufo*, to investigate the responses of populations compelled to move regularly across fragmented landscapes (Janin *et al.* 2009, 2011). By focusing on variations in postmetamorphic migration, we predicted greater movement propensity and exploratory movements in animals inhabiting fragmented landscapes. For this purpose, we compared the landward movement of naive toadlets (i.e., just after metamorphosis) originating from populations sampled along a gradient of landscape fragmentation (defined by the number of forest fragments surrounding the focal pond) in an experimental arena. Within this arena, toadlets could either visit small pieces of forest litter, the target habitat of postmetamorphic migrants, or travel further. We investigated the relationship between toadlet behavior and forest fragmentation in the landscape from which they came.

Since all animals were reared under similar controlled conditions in the laboratory from hatching until the experiment (the common garden experimental protocol), any difference in choice behavior would be a consequence of transmissible variations (either genetic/epigenetic heritability or a maternal effect).

Materials and methods

Study design and sampled populations

In April 2008, seven toad clutches were sampled at ten ponds distributed along a gradient of landscape fragmentation. We investigated the landscape structure with special emphasis to forested areas that constitute the toad's usual terrestrial habitat (Denton and Beebee 1994). The oldest maps of the region are those established by Cassini (father and son) (1750, scale 1/86,300, available at the Institut Géographique National (I.G.N.), 69771 St Didier-aux-Mont-d'Or, France). Despite the fact that the forest patches on these documents are suggested by symbols and not precisely delimited, we can consider that the fragmentation gradient of forest already existed at this date. This is confirmed by military maps drawn during the nineteenth century (1848–1889, scale 1/80,000, available at I.G.N.). We also can expect that functional connectivity has progressively decreased for toads because of the intensification of agriculture during the twentieth century. However, maps of land use by agriculture are not available for that period of time. Finally, we can consider that common toad's populations have experienced these fragmentation levels for at least 90 generations. Among the multiple fragmentation metrics (Calabrese and Fagan 2004), we chose the number of forest fragments (or “fragment number”) that reflects the primary effect of fragmentation, i.e., the cutting up of habitats into smaller pieces. When a habitat is broken up, this is very likely to constraint both the success of the first migration and, later, the movements between fragments to supplement resources (food, water, and shelter), which can be very restrictive for toads (Janin *et al.* 2011). As these movements are not optional, we considered that the number of forest fragments was a better integrative variable of fragmentation than the proportion of forest area. Nevertheless, we measured the overall “forest area” as a percentage of the total area under study as a complementary variable. To define a forest fragment, we plotted in a Geographic Information System each pixel or group of pixels of forested ground completely surrounded by another defined land use. The number of fragments within a 500-m radius of the focal pond was then counted (this distance probably reflecting the habitat area used by toads).

Tadpole rearing

Each clutch sample was housed in a 10-L aquarium in a room where temperature varied from 17 to 20 °C. After hatching, the tadpoles were housed until metamorphosis in the same aquariums, at a density of 35 tadpoles per aquarium, and fed ad libitum with boiled spinach. Aquariums were cleaned and the water changed completely twice a week.

Behavioral experiments

The behavioral experiments were performed using a 150-cm radius dodecagonal arena made of inert plywood. The arena was placed in the center of an air-conditioned room with a shadowy light to minimize biases to isotropy. In each of 12 arena sectors, a small patch of forest habitat (15×7.5 cm) was placed midway between the center and the border of the arena in order to form a ring of patches equally spaced and equivalent to half of the ring area (Fig. 1). Each patch of forest habitat was composed of a mixture of forest soil and litter thoroughly dried after sampling to eliminate any potential prey, and rehydrated with tap water just before using. At metamorphosis (stage 46, Gosner 1960), a group of five animals per clutch was randomly selected to participate in behavioral experiments. The five juveniles were released at the center of the arena after a habituation period of 5 min in a release box (10-cm diameter Plexiglas box). Given the spacing of forest patches, we could therefore assume that (1) each toadlet had an equal possibility of reaching the arena border without entering a forest patch, and (2) each toadlet overtaking the ring of patches had detected the presence of

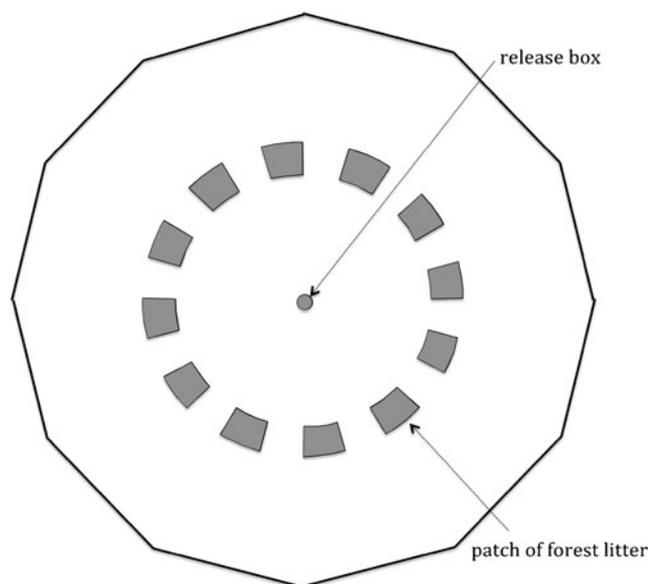


Fig. 1 Schematic view of the experimental arena (3 m in diameter). Gray patches correspond to patches of forest litter. The toadlets were released at the center of the arena

at least one forest patch, whatever the direction of its movement. The behavior of toadlets was recorded for 30 min by an observer unaware of the toadlets origin from a mezzanine above the lighting fixture of the arena. After each experiment, we measured the body size and mass of each toadlet. We classified toadlet behavior into three types: (1) *patch behavior* when the animal entered a forest patch without exploring the area beyond the patches, (2) *overtaking behavior* when the animal went beyond the ring of patches without entering any forest patch and reached the arena border, and (3) *combined behavior* when the animal combined visiting a habitat patch with moving beyond it, whatever the order of these behaviors (Table 1). Patch behavior reflects the motivation to experience conditions associated with the cues coming usually from the target habitat (Rothermel 2004). Overtaking behavior reflects a dominance of the motivation to travel over that of experiencing the target habitat. Combined behavior reflects potential conflicts between the two former motivational trends. We excluded from the dataset the toadlets that did not reach the test circle of patches. We monitored the behavior of five toadlets per clutch (i.e., 35 toadlets for each pond, and 350 in total).

Statistical analysis

We used generalized linear models to compare the proportions of toadlets adopting each behavior type according to landscape fragmentation experienced by their population. We have assumed a binomial distribution of errors and a logit link function for behavior data. “Fragment number” was implemented as landscape variable into models (we controlled correlation between forest amount and the number of forest fragments), and body size and the residuals of the linear model between body size and mass as morphometric variables. Clutch was implemented as a random variable. In order to consider the association of two behavior, we used logistic regression of explanatory variables (fixed, landscape and morphology; random, clutch) on condition-dependent response variable corresponding to combined behavior (probability of overtake behavior given patch behavior), and we evaluated the significance of the effect with *z*-tests. To evaluate the effect of explanatory variables on behavioral options in case of single behavior, we also used logistic regression of explanatory variables on a response variable in which the

Table 1 Results of *F* tests applied to logistic regressions on behaviors

Models	Deviance	<i>df</i>	<i>P</i> value
Overtaking (patch=1)~number of fragments	4.5	166	0.034
Single behavior~number of fragments (1=patch behavior, 0=overtaking behavior)	3.9	162	0.050

response variable is composed of the two exclusive variables, one corresponding to success and the alternative to fails (patch behavior corresponds to success and overtake behavior to fail since the one excludes the other). All statistical analyses were performed with R 2.15.0, and we used the *lmer* function to construct generalized linear model (R Development Core Team, Vienna, Austria).

Results

Among the 350 toadlets tested, 224 effectively left the release box and reached the test zone (the circle of patches), but this behavior did not depend on the number of fragments (deviance=2.10, $df=1$, $p=0.15$). Whatever the fragmentation level, the most frequent was the single patch behavior (Fig. 2). The proportions of other behavior types varied according to fragmentation (Fig. 2). Correlations (two coefficient values because of the data sets for each variable slightly differed) between fragmentation level and forest area were high ($t=-7.47$; $df=166$; coefficient of correlation= -0.50 ; $p<0.001$ for combined behavior and $t=-7.4$; $df=162$; coefficient of correlation= -0.50 ; $p<0.001$ for single behavior). We thus only investigated the relationship between fragment number and behavior. For each type of models, neither body size ($z=-0.14$; $p=0.89$ for combined behavior and $z=0.50$; $p=0.62$ for single behavior) nor body condition (the residuals of the linear model between body size and mass) ($z=0.67$; $p=0.50$ for combined behavior and $z=0.48$; $p=0.63$ for single behavior) had a significant influence (Table 2). Combined behavior (overtaking after visiting the patch) is positively and significantly related to the number of forest fragments (Table 2 and Fig. 3a), whereas patch behavior is negatively related to fragment number (Table 2; Fig. 3b).

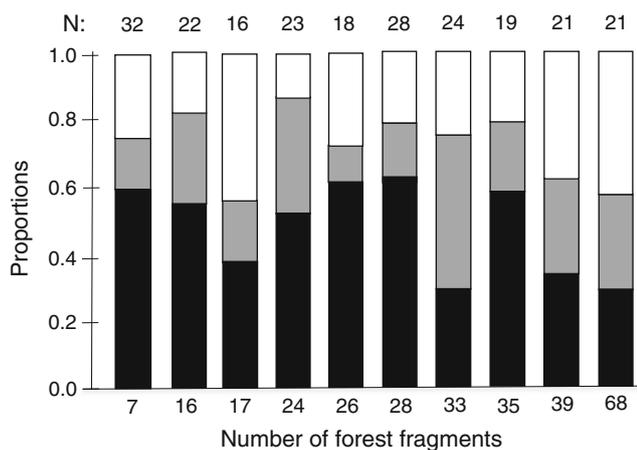


Fig. 2 Proportions of behavior types according to the number of forest fragments in the landscape where the population came from. *White segment* combination of patch and overtake behaviors, *gray segment* overtaking behavior, *black segment* patch behavior, *N* number of tested toadlets

Table 2 Results of *z* tests applied to logistic regressions on behavior during runs

Models	Estimate	Standard error	Z value	P value
Overtaking (patch=1)~number of fragments+random(clutch)				
Intercept	-1.35	0.35	-3.81	<0.001
Number of fragments	0.03	0.01	2.40	0.016
Single behavior~number of fragments+random(clutch) (1=patch behavior, 0=overtaking behavior)				
Intercept	2.56	0.99	2.59	0.010
Number of fragments	-0.04	0.02	-2.48	0.013

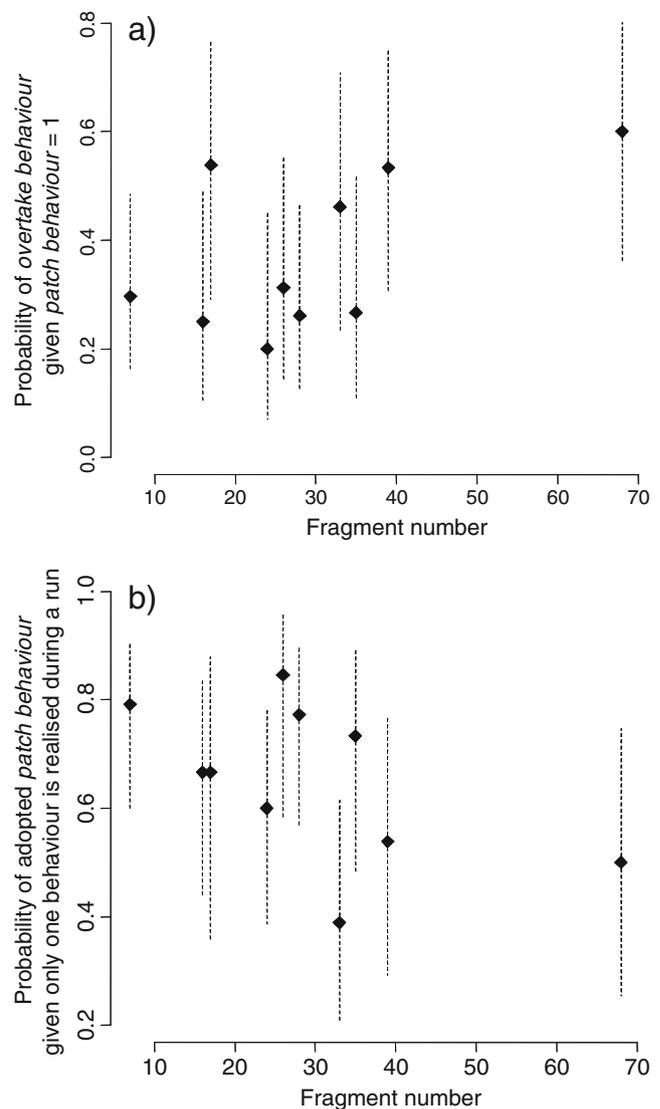


Fig. 3 Observed probabilities for the different behaviors and their binomial confidence intervals (95 %) for **a** overtaking behavior, given patch behavior has occurred and **b** patch behavior, when it is the only behavior observed

Discussion

This study provides new insights into the relationship between landscape fragmentation and the movement behavior of migrating toadlets. First, whatever the number of forest fragments, the most frequently taken option by a toadlet was the patch behavior, which corresponded to a risk-adverse choice. Juveniles are thus clearly sensitive to favorable microconditions (shelter and humidity) provided by the patch. Our results thus match the findings of other authors (Rothermel 2004, Janin et al. 2011). Nonetheless, the results also show that toadlets differ in movement behavior according to the original terrestrial landscape. Indeed, the more fragmented the terrestrial habitat, the more frequent the expression of combined behavior. This result suggests that the motivation to move and explore, i.e., the propensity to shift from one behavior to another in a limited time increases with habitat fragmentation. Besides, when only one behavior is expressed, the frequency of overtaking behavior is positively related to the fragmentation level. Toadlets from the more fragmented landscapes are thus less prone to stop moving for experiencing suitable conditions than toadlets from the less fragmented landscapes. Juveniles from more uniform landscapes were more inclined to opt for a risk-adverse behavior and were more frequently satisfied with this choice since they rarely associated the entrance of a patch with risk-prone behavior. Although we expected an impact on movement behavior of both “forest area” and “fragment number” and their interaction (corresponding to the mean size of forest fragments), only the “fragment number” was identified as a significant explanatory variable. Rather than the average habitat area, and/or its variability, it is the intensity of the fragmentation that appears as a determinant of population movement characteristics: A single small forest fragment would not elicit a change in population behavior, whereas a large but strongly divided forest area would promote the motivation to move. Thus, movement behavior is not modified in response to habitat availability but to fragmentation per se. A higher motivation to explore may be adaptive in fragmented landscapes in which the resource (forested habitat) is broken up, i.e., if multiple fragments increase the average length of migrations and provide opportunities for multiple migration routes from pond and/or promote resource supplementation (Dunning et al. 1992). Modeling could help us to verify that a strong motivation to explore helps enhance fitness outcomes in landscapes with highly fragmented habitats.

Although our data are not sufficiently numerous to investigate the organization of behavior sequences, we assume that the choice order (first entering the patch or first overtaking it) supports information about the dominant motivation (to move or to stay and consume under suitable conditions), which could be analyzed by other experiments. Moreover,

additional experiments should be carried out to fully investigate variations in movement characteristics relating to landscape fragmentation. Speed, ability to cope with ground heterogeneity, or willingness to cross boundaries between habitat types could be indicators of evolutionary responses to the level of landscape fragmentation (Merckx et al. 2003, Stevens et al. 2004, 2006). Movement-related morphology should also be investigated since this may be influenced by landscape structure (Taylor and Merriam 1995, Merckx and Van Dyck 2006 for flying species) or in response to invasion (Phillips et al. 2006 for amphibians, Merckx and Van Dyck 2002 for butterflies). We strongly suggest that changes in behavior, particularly those promoting movement (distance covered without stopping), propensity to move or to explore, or to cross boundaries must be associated with morphological and/or physiological modifications allowing greater resistance to exigent movement conditions.

Movement success across a fragmented landscape depends on the perception of its structure by the individual that is function on the one hand of the size of each landscape element and on the other hand of the perceptual capacities. The combination of these two characteristics determines the functional grain of the landscape (Baguette and Van Dyck 2007). Assuming a high level of stochasticity in landscape modifications, the evolutionary response of a population to landscape dynamics could result in heritable phenotypic plasticity (Merckx and Van Dyck 2006). In our study, however, the common garden experimental protocol excludes this hypothesis. The observed relationships between behavior and habitat fragmentation are thus expected to emerge from cross-generationally transmitted traits acquired in response to fragmentation. According to Danchin and Wagner (2010), different sources of variation, regrouped under the term inclusive heritability, are likely to be responsible for cross-generationally transmitted traits in this case study: genetic variance but also epigenetic variance and parental effects. Based on these different types of transmitted variance, the number of generations or the time required for such a behavioral change to occur in response to landscape fragmentation could be low.

The present results have important implications for our understanding of population response to landscape degradation through adaptations in movement behavior. Mechanisms of trans-generational transmission of information could exist, even in species with long generation times. Nonetheless, the cost of risk-prone behavior in terms of higher predation pressure during movement, the exhaustion of energy reserves, or faster desiccation has to be balanced against maintaining population viability. Further investigations are necessary to fully understand the balance between costs and benefits and the resulting trade-offs that determine the long-term outcomes of these evolutionary pathways (Hanski et al. 2006).

Ethical standards

Authorizations for the capture of toads were obtained from the Ain and Isère Prefectures after the request has been examined by the French Ministry of Environment and an ad hoc committee. Concerning ethical rules, A. Janin and J.-P. Léna are accredited for performing experiments with living animals (French diploma “Experimentation animale” first level for researchers). This diploma is required to perform behavioral experiments with living animals without any surgical procedure. All the manipulations were performed with great respect of animal welfare.

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