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PRELIMINARY RESULTS ON THE GENETIC CONTROL OF DISPERSAL IN COMMON FROG *Rana temporaria* FROGLETTS

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Post-metamorphic dispersal in the common frog *Rana temporaria* (Amphibia, Anura) was studied with a combination of field (pit-fall traps) and laboratory (arena, artificial crossing) experiments. In the first studied population, the breeding place was surrounded by lines of fence-pitfall traps allowing capture of dispersing froglets. Dispersal was at random on the edge of the pond, but oriented in the most favorable terrestrial habitat at 10 m from the edge. Froglets of this population were then tested in orientation arena built on the University campus, where they also dispersed at random. The two other studied populations reproduced at each side (north and south) of a lake. Froglets from each population were tested in similar orientation arena, where they did not disperse at random but to the north and south direction respectively. In the laboratory we crossed males and females originated from these two populations. Resulting crossed froglets exhibited variable dispersal patterns, which significantly differed from those observed with their respective parents. These results argued for an at least partly genetic control of emigration direction in these two frog populations, that we interpreted as the result of directional selection due to landscape change during the XXth century.

Keywords: dispersal, juveniles, artificial crossing, orientation, local adaptation, amphibian.

INTRODUCTION

Dispersal is a major behavioral trait of many organisms (Clobert et al., 2001). A wide literature has been devoted to orientation in Amphibians, especially during breeding migration, e.g., between terrestrial and aquatic sites (Sinsch, 1992). Both Anura and Urodela can use numerous environmental cues, e.g., water odor to migrate from one place to another (e.g., Sinsch, 1990; Joly and Miaud, 1992 and references inside). These studies concern mainly adult stage and information on juvenile dispersal is particularly scarce.

The aim of this work was to use a combination of field and laboratory experiments to assess the post-metamorphic dispersal in the common frog *Rana temporaria* (Amphibia, Anura).

The first experiment was to study the direction of dispersal in post-metamorphic individuals (called froglets in this paper) from a breeding pond in the field. The second experiment was to test dispersal direction of the same froglets in an arena outside the pond environment. We also tested froglets from two other breeding populations: adults breed at opposite sides of a small lake and landscape structure (road, crops) imposed two obligatory migratory routes for frogs (north and south) in the terrestrial environment. Our first hypothesis was to test if froglet dispersal

followed direction used by adults. In the case of population-specific dispersal, the second hypothesis was to test the existence of a genetic basis of migratory behavior in froglets. We thus experimentally produced hybrids of “north” and “south” frog populations and compared froglets dispersal direction with those of the two parental populations.

MATERIAL AND METHODS

Dispersal in the Field

A peat bog pond (30 × 10 m), situated near “St. François de Sales” (Savoie Department, Southeast of France) at 1350 m a.s.l., is occupied by a large population of common frog. We placed 3 lines of fence-pitfall traps around the pond in 2001. Each line was composed of a fence made of fine plastic netting (1 mm mesh) 3 m long and 50 cm high. Three pitfall traps (diameter of the hole: 12 cm) equipped each system (one at each extremity and one in the middle of the fence). We installed 8 fence-pitfall traps in a circle around the pond (Fig. 1) at 1, 5, and 50 m from the edge of the pond. The traps were protected against the sun by an opaque wooden screen. The traps were visited from 1 to 3 times a week from the start of metamorphosis (June 23) until August 5.

Dispersal in Arena

Arenas were circular enclosures (4 m in diameter), similar to previously described fence-pit fall traps system

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(Fig. 2). The bottom was the natural ground with herbaceous coverage. Orientation of the froglets was estimated from their capture in 12 pitfall traps (diameter of the hole 20 cm) regularly placed along the net. The release site at the center of each arena consisted of a $30 \times 20 \times 10$ -cm plastic box with 2 cm depth of water. Tadpoles (about 30 at each experiment) were placed in this release site when the tail started to regress and froglets can leave it and disperse in the arena over the following days. Traps were controlled twice every 4 days from the beginning of each experiment. Five arenas were built on the campus of the University of Savoie, in an isolated piece of fallow land.

Tested froglets came from three populations: the peat bog pond of “St. François de Sales” where field dispersal



Fig. 1. Fence and pitfall traps used to study froglet dispersal around a natural breeding site.

was tested in 2001 and 2 populations spawning on each edge of Lake Aiguebelette (near the town of Chambéry, Savoie Department). This lake is about 4 km long and 1 km wide and common frog only breed in two breeding place on the north and south edges of the lake. Parts of several spawns were collected in 2002 in each of these populations and eggs and tadpoles were reared in similar conditions until orientation experiments started. Two other pools of froglets were obtained: Adults were caught on land during the breeding migration in the two lake populations. Four males and 4 females of each population were taken to the laboratory and anaesthetized. Ova were obtained by pressing lightly female abdomen, and were immediately fertilized with sperm obtained by male dissection. Each female of the north population was crossed with a male of the south population and reciprocally, to obtain two pools of crossed froglets. Eggs and larvae were reared in similar conditions as those from each parental population.

Froglet distribution around the arenas was summarized by a mean vector (data were grouped because animals were caught by trapping: Batschelet, 1981). Randomly distributed captures were tested by Raleigh test. Orientation towards an expected target was accepted if the target is included in the limits of the confidence interval of the mean vector. Circular statistics were from Batschelet (1981).

RESULTS

Dispersal in the Field

The common frog spawn in April in the studied peat bog pond of “St. François de Sales” (Savoie Department,



Fig. 2. Arena used to study froglet orientation on the University campus.

Southeast of France) at 1350 m a.s.l. The froglets started to metamorphose at the end of June. Dispersal from the pond was recorded with fence-pitfall traps. At the edge of the pond, froglets left the breeding site without a preferred direction (mean vector length $r = 0.082$, Raleigh test $P > 0.05$, $N = 408$, Fig. 3a).

At 10 m from the pond edge, froglets were significantly oriented toward a preferred direction (mean vector length $r = 0.652$, Raleigh test $P < 0.001$, $N = 93$, Fig. 3b).

Dispersal in Arena

Froglets of the bog pond were tested in the orientation arena on the University campus. Dispersal direction did not differ from random (mean vector length $r = 0.061$, Raleigh test $P > 0.05$, $N = 285$, Fig. 4a).

Froglets from populations at both north and south sides of the lake were tested in the arena. Dispersal directions of froglets originating from the “north” population significantly differed from random (mean vector length $r = 0.322$, Raleigh test $P < 0.001$, $N = 111$, Fig. 4b). The

mean vector angle $\Phi_m = -11.5 \pm 32^\circ$ (mean \pm confidence interval at $\alpha = 0.05$) did not differ significantly from the north ($\Phi = 0^\circ$) direction.

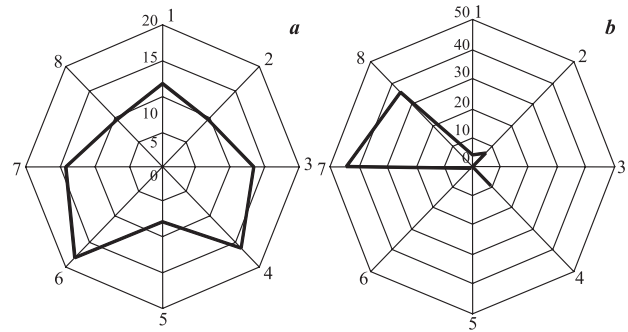


Fig. 3. Froglet dispersal from the pond recorded with fence-pitfall traps: (a) at the edge of the pond ($r = 0.082$, $N = 408$, no preferred direction); (b) at 10 m from the pond edge ($r = 0.652$, $N = 93$, froglets were significantly oriented toward one direction); 1 to 8, number of pit-fall traps with trap No. 1 orientated to the north, sample sizes in each direction expressed in %, scale on the vertical axis.

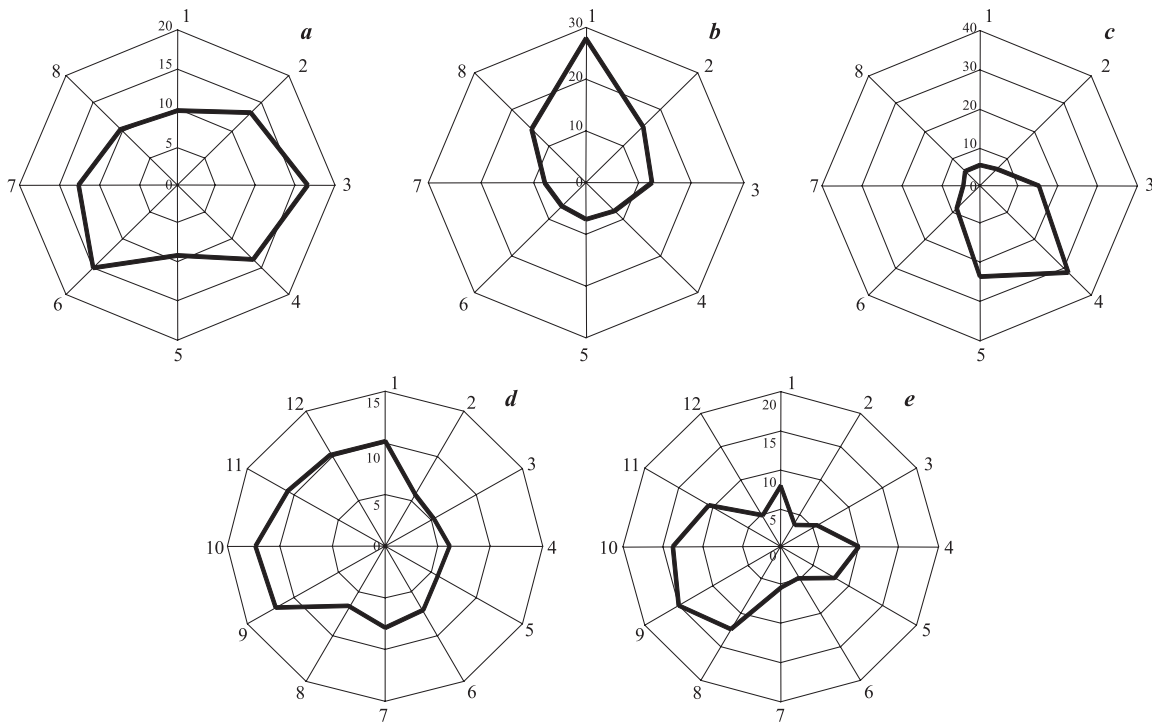


Fig. 4. Froglet dispersal in the orientation arenas: (a) Froglets originating from the bog pond of “St. François de Sales.” Mean vector length $r = 0.061$ ($N = 285$). There is no preferred direction; (b) froglets originating from the north population of the lake. Mean vector length $r = 0.322$, $N = 111$. The mean vector angle ($\Phi_m = -11.5 \pm 32^\circ$) did not differ significantly from the north ($\Phi = 0^\circ$) direction; (c) froglets originated from the south population of the lake. Mean vector length $r = 0.475$, $N = 112$. The mean vector angle ($\Phi_m = 126.7 \pm 24^\circ$) significantly differed from the south ($\Phi = 180^\circ$) direction; (d) froglets originated from artificial crosses [males (north) \times females (south)]. Mean vector length $r = 0.184$, $N = 226$). The mean vector angle ($\Phi_m = 280.1 \pm 35^\circ$) significantly differed from dispersal directions of froglets from parent populations (both north and south); (e) froglets originated from artificial crosses [males (south) \times females (north)]. Mean vector length $r = 0.212$, $N = 153$). The mean vector angle ($\Phi_m = 294.8 \pm 35^\circ$) significantly differed from dispersal directions of froglets from parent populations (both north and south).

Dispersal directions of froglets from the “south” population significantly differed from random (mean vector length $r = 0.475$, Raleigh test $P < 0.001$, $N = 112$, Fig. 4c). The mean vector angle ($\Phi_m = 126.7 \pm 24^\circ$) significantly differed from the south ($\Phi = 180^\circ$) direction.

Adults from the north and south populations were artificially crossed in the laboratory and the obtained froglets were tested in the arena. Froglets [males (north) \times females (south)] did not disperse at random (mean vector length $r = 0.184$, Raleigh test $P < 0.001$, $N = 226$, Fig. 4d). The mean vector angle ($\Phi_m = 280.1 \pm 35^\circ$) significantly differed from dispersal directions of froglets originating from parent populations (both north and south).

Froglets [males (south) \times females (north)] did not disperse at random (mean vector length $r = 0.212$, Raleigh test $P < 0.001$, $N = 153$, Fig. 4e). The mean vector angle ($\Phi_m = 294.8 \pm 35^\circ$) significantly differed from dispersal directions of froglets originating from parent populations (both north and south).

DISCUSSION

Froglet Dispersal

Following metamorphosis, froglets have to disperse in the terrestrial habitat surrounding the breeding and larval development aquatic area. Eggs and tadpoles are exposed to numerous predators and survival is low (e.g., Biek et al., 2002) give 0.06 and 0.34 as mean values of tadpole and metamorphosis survival in *Rana temporaria*). The post-metamorphic life is also risky for such small vertebrates (about 15 mm body length). Froglets left the peat bog pond of this study without preferred directions. However, at 10 m from the edge of the pond, they were caught in only one direction. The pond surroundings are composed of a small mixed forest and grassland. The preferred direction is towards the mixed forest habitat (this result is also obtained with pit-fall traps at 50 and 100 m from the pond, unpublished data). Froglets tested in the arena far from environmental cues of the pond surroundings also dispersed at random. We make the assumption that, at this breeding site, froglets dispersed at random, and those which survived were by chance in a favorable habitat. Adults, equipped over two successive years with transmitters, exhibited strong fidelity to routes in this habitat (Miaud and Martin, unpublished data). Therefore, it seems that adults migrated where they were successful as froglets, after a random emigration at the first breeding place.

Landscape structure is often shaped by human activities and amphibians are well known to be highly sensitive to landscape alteration. Agriculture, urbanism and road

network permitted only two obligatory migratory routes for the common frogs around the small lake of this study. Froglets from the “north” and “south” populations (tested in arena) dispersed in two opposite directions that corresponded to adult migratory routes. Distinction between genetic variation and environmentally induced phenotypic variation can be made using reciprocal transplant and common garden experiments (Mousseau et al., 2000). Our experiments in arena corresponded to this common garden design. Another approach is to obtain hybrids with individuals from populations where the studied traits vary. Our results argue for a genetic basis to froglet dispersal direction: “north” population froglets dispersed to the north while those from the “south” population dispersed to the south in the arena and hybrids exhibited variable dispersal patterns, mostly different from those observed with their respective parents. These results — which have to be considered as preliminary — lead to question which selective pressures act to generate evolutionary change in migration direction. Genetic determination in migratory direction is documented in arthropods (sandhoppers: Scapini and Fasinella, 1990), fishes (Salmon and trout: Raleigh, 1971), and birds (European blackcap: Helbig, 1991). In this last example, evolutionary changes in migratory direction occur relatively rapidly: 7 – 11% of the breeding population migrated to NW direction in 1990 whereas no birds were observed in this direction before 1960. The new NW migratory direction has a genetic basis and must have evolved through rapid microevolution (Helbig et al., 1994). We interpreted our results in the common frog as follow: 1) post-metamorphic dispersal is random and froglets survive in favorable environments. This strategy is successful because recruitment varies greatly from one year to another and favorable habitat move spatially in the landscape matrix. Specific situations can lead to directional selection which, under extreme conditions tends to favor local adaptation over plasticity (Piliucci, 2001); 2) Froglets dispersed on each side of the lake and survived if they reach the favorable habitat (relict forest patch). Old maps (beginning of the XXth century) show that the lake was almost completely surrounded by forest and one can imagine that frogs bred in numerous places around the lake. In less than 100 frog generations, landscape changes greatly and frogs can now reproduce in only two breeding places. Exchange of migrants between them was interrupted, favoring local adaptation in migratory direction.

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REFERENCES

- Batschelet E.** (1981), *Circular Statistics in Biology*, Acad. Press, London.
- Biek R., Funk W. C., Maxell B. A., and Mills L. S.** (2002), "What is missing in Amphibian decline research: insights from ecological sensitivity analysis," *Conserv. Biol.*, **16**, 728 – 734.
- Clobert J., Danchin E., Dhondt A. A., and Nichols J. D.** (eds.) (2001), *Dispersal*, Oxford Univ. Press, New York.
- Helbig A. J.** (1991), "Inheritance of migratory direction in a bird species: a cross-breeding experiment with SE- and SW-migrating blackcaps (*Sylvia atricapilla*)," *Behav. Ecol. Sociobiol.*, **28**, 9 – 12.
- Helbig A. J., Berthold P., Mohr G., and Querner** (1994), "Inheritance of a novel migratory direction in central European blackcaps," *Naturwissenschaften*, **81**, 184 – 186.
- Joly P. and Miaud C.** (1993), "How does a newt find its pond? The role of chemical cues in migrating newts (*Triturus alpestris*)," *Ethol. Ecol. Evol.*, **5**, 447 – 455.
- Mousseau T. A., Sinervo B., and Endler J.** (eds) (2000), *Adaptive Genetic Variation in the Wild*, Oxford Univ. Press, Oxford, USA.
- Piliucci M.** (2001), *Phenotypic Plasticity. Beyond Nature and Nurture*, The John Hopkins Univ. Press, Baltimore, MD.
- Raleigh R. F.** (1971), "Innate control of migrations of salmon and trout fry from natal gravels to rearing areas," *Ecology*, **52**, 291 – 297.
- Scapini F. and Fasinella D.** (1990), "Genetic determination and plasticity in the sun orientation of natural populations of *Talistrus saltator*," *Marine Biol.*, **107**, 141 – 145.
- Sinsch U.** (1990), "Migration and orientation in anuran amphibians," *Ethol. Ecol. Evol.*, **2**, 65 – 79.
- Sinsch U.** (1992), "Amphibians," in: Papi F. (ed.), *Animal Homing*, Chapman and Hall, London.