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Inbreeding and road effect zone in a Ranidae: the case of Agile frog, *Rana dalmatina* Bonaparte, 1840

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Abstract

Inbreeding has often been invoked in the extinction of local populations. In eleven western France populations of Agile frog studied, observed heterozygosity was significantly lower than expected in all cases, giving new evidence of such a depression in small populations. It especially occurred in ponds located near an highway rather than in undisturbed populations ($F_{\text{IS}} = 0.544$ and 0.315, respectively). Thus, our results argue for a "road effect zone". Discussing about road distance and conservation policies, we propose that roads are directly involved in inbreeding and in local extinction. Thus, road construction ought to consider conservation management. ***To cite this article: D. Lesbarrères et al., C. R. Biologies 326 (2003).***

Résumé

Consanguinité et zone d'impact génétique de la route chez un ranidé : le cas de la grenouille agile, *Rana dalmatina* Bonaparte, 1840. Dans la baisse d'effectif des populations, la consanguinité est un phénomène important. Dans les onze populations de grenouilles agiles étudiées, H_{obs} est significativement plus faible que H_{exp}, mettant en évidence la dépression génétique des petites populations. Cela est d'autant plus remarquable dans les populations proches d'une autoroute que dans les populations non-perturbées (F_{IS} respectivement de 0,544 et 0,315). Nos résultats confirment donc l'existence d'une zone d'impact sur la génétique des populations. Dans un contexte de diminution des populations et de gestion conservatoire, nous proposons que la construction de routes joue un rôle direct sur la consanguinité et les extinctions locales. ***Pour citer cet article : D. Lesbarrères et al., C. R. Biologies 326 (2003).***

Keywords: inbreeding; heterozygosity; highway; roadway distance; *Rana dalmatina*

Mots-clés : consanguinité ; hétérozygotie ; autoroute ; éloignement de la chaussée ; *Rana dalmatina*

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1. Introduction

Inbreeding is a major thematic involving researches in conservation biology. Firstly because in captivity, mating among related individuals may decrease viability and fertility and secondly because inbreeding can be strongly involved in the extinction of small local populations [1,2]. However, in natural populations, inbreeding has been considered of little relevance by the engineers and policy administrators, considering that other ecological factors were more important in reducing population size and in extinguishing popula-

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tions [3]. Nowadays, according that isolation of small populations is increasing and population size is a factor involved in genetic depletion, then small populations in fragmented landscapes tend to lose their genetic diversity [4].

Among anthropic modifications of the landscape caused by the increase of human population and its activities (deforestation, regrouping of lands, destruction of wetlands) highways construction presents either a direct risk for the survival of species or contribute to indirect threat through populations fragmentation [5]. Actually, highways are involved in habitat loss and vertebrate mortality [6] and are particularly concerned by integrated conservation and development projects [7]. However, one of the factors limiting the implementation of real conservation policy is the absence of agreement between scientists and engineers. As biological conservation should not lead to the final stop of roads construction, the goal is to progress by studying where to place them according to their need and their impact [8]. Then, studies presenting model and results of biodiversity [9,10] and genetic variability [11,12] in regard of human structures impact, are good values.

Amphibians are particularly sensitive to the modifications of their habitat. Indeed, according to their annual biological cycle, frogs present three different phases: reproduction, summering and hibernation. Actually, it may take place in different locations, highlighting the need of connectivity among sites. Then, it determines as much sites and corridors that have to be protected [13]. The persistence of a barrier effect on seasonal migrations, as involved by highways, gives raise to serious damages on population structure and migration. The road mortality is obviously the most concrete consequence in short-term and this is all the more detrimental with respect to the amphibians [14, 15]. The pollution is also a threat for highway adjacent environments. There are two kinds of pollution: sound, from the noise of the vehicles, and chemical pollution, following the emanations of these same vehicles. These emanations, made up of heavy metals, cause deposits on the ground, in the water and on the plants, contaminating insects, small mammalians and amphibians [16]. However, while lots of biotic parameters are proved to affect heterozygosity and allelic differentiation, specific population genetic effects are expected to occur as a result of highway presence (isolation, genetic substructuring...) and few studies have experienced the influence of road structures on genetic quality and on inbreeding in amphibians [17]. By studying heterozygosity, we are able to estimate inbreeding, to predict populations equilibrium and then to propose adapted conservation plans.

Thus, our study presents two issues. Firstly, it documents genetic heterozygosity in small populations of Agile frog, *Rana dalmatina* Bonaparte, 1840, that may be considered as disturbed or non disturbed by a highway. Secondly, our purpose is to analyse how linear infrastructures may influence this observed natural genetic variability and may be involved in inbreeding.

2. Material and methods

We sampled 11 populations of Agile frog, *Rana dalmatina* in western France. They were considered as belonging to environments with different degrees of road influence.

(i) seven populations were investigated in ponds far from important roads or highways, in meadows between Angers and Cholet, Maine & Loire, France (considered as undisturbed populations).
(ii) four other populations were located close to the A11 highway, between Angers, Maine & Loire and Nantes, Loire Atlantique, France (considered as disturbed (“highway”) populations).

As seasonal migrations were supposed to occur over kilometres, we measured the distance from each pond to a high traffic road ($\geqslant 10\,000$ vehicles per day) to estimate the importance of this human influence on migration axes and summer area.

Population size was estimated by numbering clutches and by estimating the number of calling males. Ponds below 40 adults frogs or 20 clutches were considered as small populations.

In May 1999, genetic sampling was made by collecting a mean of 14.6 spawns for each population. About 10% of the eggs of each clutch of the same population were collected and reared in plastic pans of 5 litres. Then, tadpoles represent a strong sampling of the genetic pool of the population. After emergence, 24 to 36 froglets (mean = 30.4) from each population were preserved at −23°C until genetic analysis

Table 1
Genetic summary statistics for the *Rana dalmatina* undisturbed and highway populations in Western France

Populations	Mean alleles per locus	Mean H_{obs} per locus	Mean H_{exp} per locus	F_{IS}
Undisturbed ($n = 7$)	2.5	0.358	0.522	0.315
Highway ($n = 4$)	2.23	0.151	0.330	0.544

and others were released. Crude protein extracts were used for horizontal starch-gel electrophoresis. Samples were homogenised in equal volume of distilled water and centrifuged at 12 000g for 5 minutes à 4°C. Migration was performed in 12% continuous Tris citrate starch gels, for 3–5 hours at 300 V and 4°C. Slices were stained for revealing specific enzymes using standard procedures [18] and nine enzymes encoded by 12 polymorphic loci were investigated (AK, EC 2.7.4.3; CK-1 and CK-2, EC 2.7.3.2; 6-PGDH, EC 1.1.1.44; GPI, EC 5.3.1.9; αGDH, EC 1.1.1.8; LDH-1 and LDH-2, EC 1.1.1.27; MDH-1 and MDH-2, EC 1.1.1.37; MPI, EC 5.3.1.8; PGM, EC 5.4.2.2).

3. Results

Mean distance between undisturbed ponds and high traffic road was 2173 m ± 1092. It was significantly higher than mean distance between “highway” populations and the roadway (mean = 94.3 m; SD = 62.2; $t_{\mathrm{Welch}} = 4.645$; df = 6; $p = 0.003$). All populations were small; the difference between size of undisturbed populations and highway populations was not significant ($U_{\mathrm{Mann–Whitney}} = 7.5$; $p = 0.23$).

Firstly, *Rana dalmatina* populations of western France present an important allozymic variation. It is related with a high polymorphism and an average of 2.5 alleles per enzyme in undisturbed populations (Table 1). However, in highway populations the number of alleles per enzyme averages 2.23 and was significantly lower ($t_{\mathrm{Welch}} = 4.339$; df = 3; $p = 0.023$).

No significant difference in heterozygosity level was recorded among the seven undisturbed populations ($F = 0.139$; df = 6; $p = 0.991$). In these populations, H_{obs} was significantly lower than H_{exp} ($t_{\mathrm{Welch}} = 14.3$; df = 8; $p < 0.0001$). On the other hand, no significant difference in heterozygosity level was recorded among the four highway populations ($F = 1.124$; df = 3; $p = 0.324$) while H_{obs} was significantly lower than H_{exp} ($t_{\mathrm{Welch}} = 6.504$; df = 3; $p = 0.007$).

Moreover, H_{obs} was significantly lower in highway populations than in undisturbed ones ($t_{\mathrm{Welch}} = 14.18$; df = 8; $p < 0.0001$). Consequently, undisturbed populations present a significantly lower F_{IS} index than highway populations ($F_{\mathrm{IS}} = 0.315$; 0.544, respectively; $t_{\mathrm{Welch}} = 5.605$; df = 3; $p = 0.011$). The difference between H_{obs} and H_{exp} have been also analysed for each locus (Table 2).

4. Discussion

Agile frog populations of western France evidence a high deficiency in heterozygotes. Indeed, F_{IS} index is high, suggesting an inbreeding process. Several explanations may be invoked for this peculiar result: (1) *Rana dalmatina* populations are small compared with *Rana temporaria* ones that exhibit population sizes superior to hundred individuals [19]. (2) It can reflect a complex reproductive system of this territorial species where all the adult males do not access to reproduction [20]. (3) The high breeding pond fidelity could also result in reducing the genetic variability of populations, especially in amphibians [21]. (4) A selection on a particular stage of life, as eggs for example, may also be regarded as a source of genetic depletion. (5) The heterozygosity deficiency revealed in *Rana dalmatina* populations could be induced by a reduction of reproductive adults number because of road mortality. As heterozygosity deficiency is more pronounced in highway populations than in undisturbed ones, hypothesis 1, 2 and 3 may not be sufficient to explain such a difference, because they rely on species characteristics whatever the population. A selection on eggs could not be evoked too because all of the clutches were sampled in the same generation, and adult heterozygosity analysis confirmed our results [22]. These hypothesese might only result in the heterozygotes deficiency. In contrast, we propose that highways consist in a threat for frogs during their reproductive movements, with a negative

Table 2
Observed and expected heterozygosities within loci for the *Rana dalmatina* undisturbed and highway populations in Western France

Populations	Undisturbed ($n = 7$)		Highway ($n = 4$)	
Locus	H_{obs}	H_{exp}	H_{obs}	H_{exp}
AK	0.275	0.492	0.190	0.467
CK-1	0.276	0.494	0.107	0.394
CK-2	0.332	0.494	0.182	0.498
6-PGDH	0.416	0.628	0.246	0.621
GPI	0.186	0.376	0.138	0.170
αGDH	0.348	0.498	0.096	0.283
LDH-1	0.565	0.737	0.333	0.693
LDH-2	0.413	0.594	0.081	0.500
MDH-1	0.442	0.744	0.213	0.635
MDH-2	0.300	0.490	0.131	0.501
MPI	0.406	0.498	0.018	0.065
PGM	0.328	0.499	0.077	0.192

influence on dispersal [23], reducing population size and involving an inbreeding process.

In general, Agile frog populations size is not considerable. Highway populations seem to be slightly smaller than undisturbed ones (10 adults versus 26 on average, n.s.). Whatever the cause of such a difference (pollution, noise, road mortality...) the low number of reproductive adults may have genetic consequences. Indeed, population size is a factor involved in genetic depletion and small populations loose more rapidly their genetic diversity. In regard to our results, roadway proximity appears as one major cause of this lower genetic variability, thus strongly suggesting that such linear structures increase extinction risk for species. While genetic flows and biological diversity trace broad patterns across the landscape, transportation planning for human mobility traditionally focuses on a narrow strip close to a road or highway. Forman and Deblinger [24] evidenced that populations close to a high traffic road (distance < 600 m) exhibited several deficiencies (biological diversity, genetic depletion) arguing they are located in a “road effect zone”. In the four highway populations of our study, the mean distance between pond and high traffic road is significantly lower than in undisturbed populations. Moreover, highway ponds are close to the roadside, in a distance less than 100 meters and the results on genetic depletion in highway populations, that are within the “road effect zone”, partly validate this model. Forman & Deblinger [24] advised a protected zone of about 600 m to prevent perturbation of mammal and amphibian roadside populations. This distance has been chosen according to noise reduction and gene flow preservation. Indeed, many species are injured by roadway, especially during migration, like snails [25], amphibians [14], birds [26,27] and mammals [28]. Thus, by mapping such a zone, we might learn to predict how future roads will influence environments and where conservation projects (underpasses, noise barriers, ...) have to be built [29,30].

The majority of natural populations extinction results from environment modification, especially in the urbanised landscapes [9]. Extension of human activities and roads construction are ineluctable issues in the twenty-first century. Within the general decline of amphibian populations, it raises considerable issues for species conservation. Then, preserving genetically distinct populations is a fundamental concern for biodiversity [31], considering that small populations are more exposed to extinction than others [3,32]. Thus, we conclude that busy roads and natural areas, like breeding ponds, should be well separated, and that future transportation systems across landscapes can provide for ecological flows and biological diversity in addition to safe and efficient human mobility.

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