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Small effective population size and fragmentation in Alpine populations of *Bombina variegata*: the combined effects of recent bottlenecks and postglacial recolonization

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Amphibians are experiencing population declines in all continents due to anthropogenic factors. Evidence of demographic reduction and local extinction have been also reported for the yellow-bellied toad, *Bombina variegata*, along all its distributional range, which includes the Italian Alps. Here we genotyped at the mtDNA *cytb* and at 11 nuclear microsatellites 200 individuals of *B. variegata* from 9 populations sampled in Trentino (north-eastern Italy.). We investigated the fine-scale population structure and we tested for genetic traces of population decline using different methods. We found that all populations showed low level of genetic diversity in comparison with other studies, low estimates of effective population size, and clear evidence of demographic decline. When the age of the decline is estimated, contrasting results are found. Some methods suggest a recent reduction of population size possibly associated with anthropogenic environmental changes, and others support a more ancient bottleneck dating back to the postglacial recolonization of the Alps. We suggest that both demographic processes occurred in the evolutionary history of the yellow-bellied toad populations, and we are now testing this hypothesis by simulations.

Microevolution due to pollution in Amphibians

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Introduction: Genetic variability is important for biological diversity, thus requires conservation measures. Population's genetics is shaped by: 1) Historical events (e.g. postglacial migration); 2) Present environmental stresses (e.g. pollution). Conservation procedures try to mitigate/eliminate nowadays environmental pressures. For example, pollution exerts a great impact through the elimination of sensitive genotypes: contaminant-driven genetic erosion. A population exposed to contaminants show an increase in frequency of resistant genotypes, due to loss of sensitive ones. This can be explained by: 1) physiological alterations: acclimation; 2) appearance of new gene/s underlying resistance mechanisms; 3) loss of sensitive individuals and reduction of the genetic variability through genetic erosion. Only scenarios 2 and 3 lead to a microevolutionary outcome. To distinguish between the two scenarios, researchers should establish if: resistant genotypes are present at reference and impacted site but sensitive genotypes are absent at the latter. Therefore microevolution took place through genetic erosion and not through the arising of new resistance-genes. Focus on the amphibians: Pollution-induced genetic erosion is a central issue for species preservation. This is particularly true for amphibians: their small populations are highly susceptible to genetic drift, inbreeding and erosion. Amphibians are the most globally threatened vertebrates group. Habitat destruction, climate change, increasing UV, diseases, introduction of allochthonous species, and pollution are some causes of amphibians global decline. Furthermore decreased genetic variation can reduce fitness and adaptability to these challenging environments. Little is known about contaminant-driven genetic erosion in amphibians. Nevertheless there is some evidence of its detrimental effects through: 1) reduced fitness, 2) reduced environmental plasticity, 3) absence of protective co-tolerance effects, 4) arising of fitness/resistance trade-offs, 5) increased susceptibility to pathogens. -FitnessGenetic variability should be positively correlated with a higher fitness: genetic-fitness-correlations (GFCs). Amphibians show reliable fitness proxies (developmental rate, growth rate and survival) used to investigate GFCs. Indeed in some species heterozygous individuals show increased fitness in comparison to homozygous conspecifics-environmental plasticity. It is the ability of a genotype to generate different phenotypes in stressed environments. Genetic erosion may be harmful for populations' viability because phenotypic plasticity has a genetic basis and genetic variation for plasticity is generally observed. Cotolerance mechanisms arise from the use of the same metabolic detoxification pathway facing different stressors. It is generally accepted that decreasing genetic variability lowers the tolerance towards other stressors. -Fitness trade-off costs Responses to pollution-induced stresses can be metabolically costly, arising trade-offs. Some studies show how microevolution due to pollution can bear fitness costs associated with the altered physiological processes enabling resistance. -Resistance to pathogens Genetic variability has a role in resistance to pathogens: inbred populations, possibly tolerant to one pathogen, are likely to be susceptible to most other unrelated pathogens. Heterozygosity is very important for the functioning of the immune system. Heterosis improves immune function against *Batrachochytrium dendrobatidis*, a widespread amphibians' parasite.