

Introduction

Differential distribution of asexual species at high altitudes and latitudes has long been noted and coined geographical parthenogenesis. This pattern is thought to arise from the better colonization abilities of asexuals.

Daphnia appear to comply with this as subarctic and arctic species typically reproduce by obligate parthenogenesis (Beaton & Hebert 1988, Dufresne & Hebert 1994). Some *Daphnia* populations from high altitudes are also known to reproduce asexually i.e. those in the Bolivian Andes (Mergeay et al. 2008) and also those in the High Tatra mountains (Dufresne et al. 2011). By contrast, although also inhabiting alpine environments, *D. pulicaria* from the Pyrenees reproduce primarily by cyclic parthenogenesis (Dufresne et al. 2011), suggesting complex glaciation history may play a role in transitions to asexuality.

This study aimed to determine the reproductive mode and clonal diversity patterns of *Daphnia pulicaria* from four lakes in the Italian Alps.

Materials and methods

Four alpine lakes were sampled in Gran Paradiso, National Park (GNP, Western Alps) including Lake Nivolet, Lake Trebecchi Inferiore, Lake Trebecchi Superiore, and Lake Lillet. The lakes are spatially very close to each other but belong to different river catchments: lake Lillet is situated in the Orco river basin, whereas the others three lakes are located within the Dora di Savarenche river catchment.



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Discussion

It is most likely that *Daphnia* inhabiting the four lakes in the Western Alps reproduce by obligate parthenogenesis as there is no clonal diversity and no males were observed in laboratory cultures.

Daphnia from GNP had microsatellite genotypes that clustered in two different clades in the NJ tree. These results are congruent with a previous mtDNA study that showed that *Daphnia* from Lake Nivolet, Lake Trebecchi inferiore and Lake Trebecchi superiore belong to the boreal clade of European *D. pulicaria* whereas those from Lake Lillet were genetically distinct and belong to the alpine clade of European *D. pulicaria*.

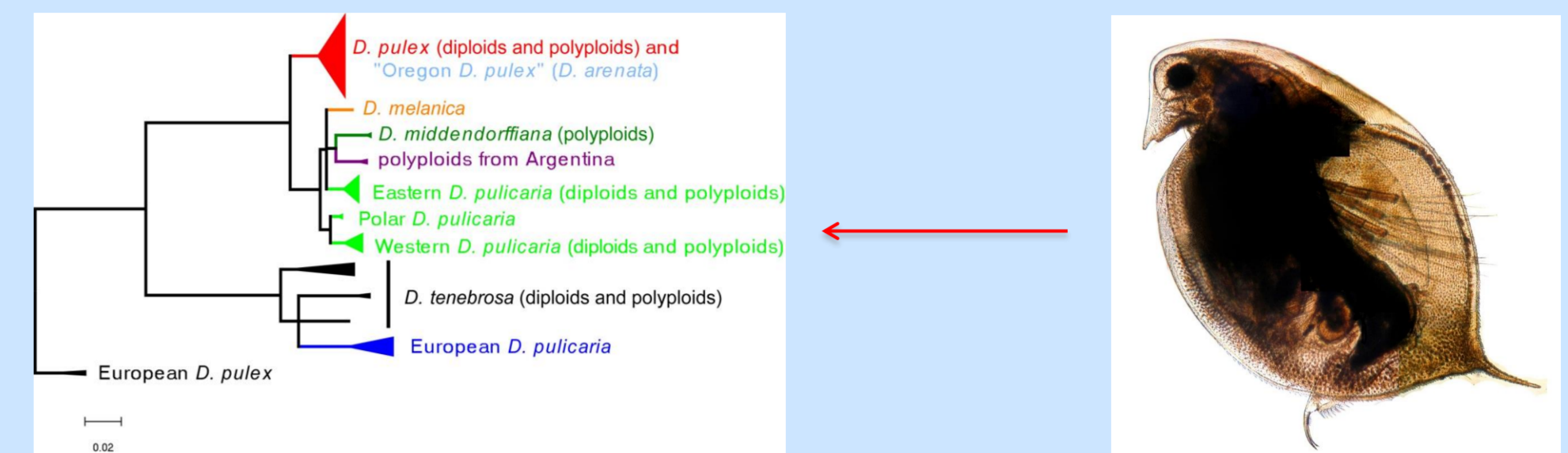
The two clones were diploids as the other alpine clones of the High Tatra mountains.

How did transitions to obligate parthenogenesis occurred in these populations?

Preliminary results indicate that alleles that suppress meiosis in females but not in males as in North American *Daphnia* likely involved (as in Lynch et al. 2008).

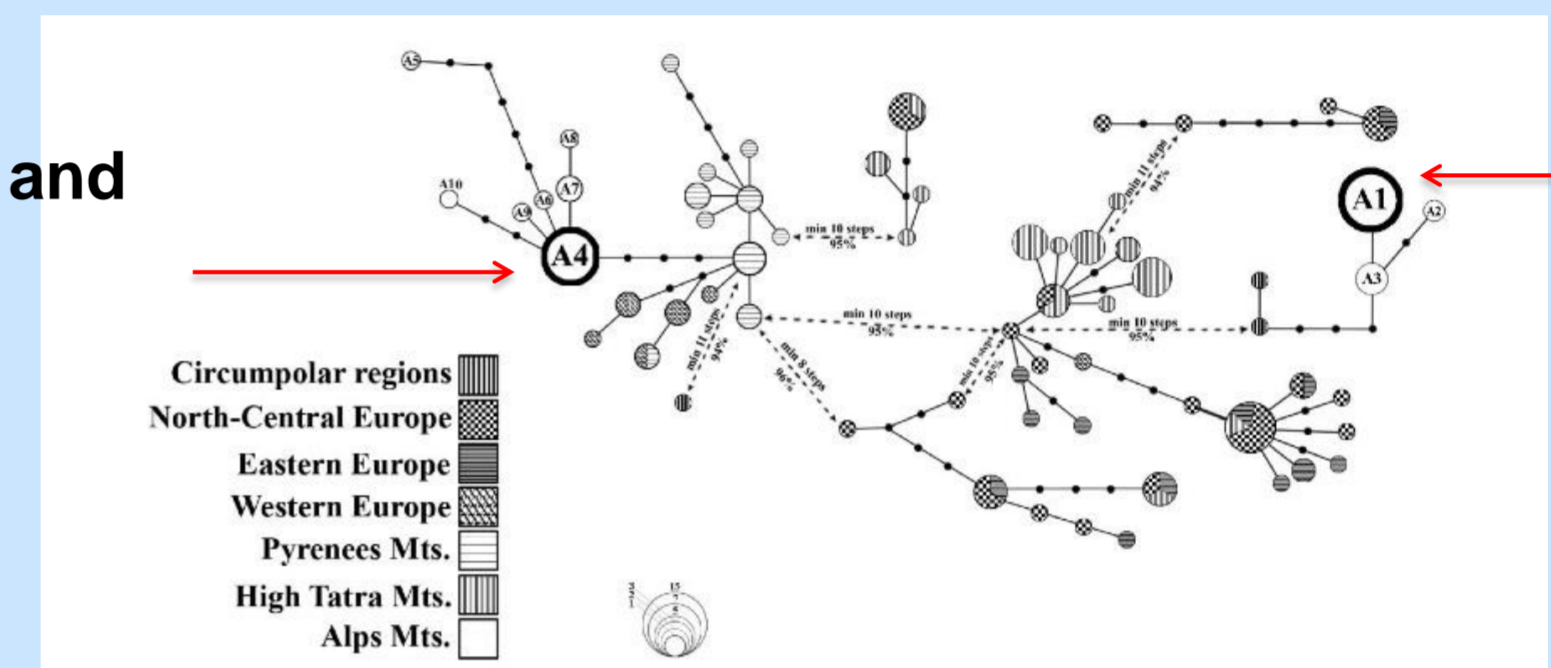
Future studies using genomic tools will help to better assess genetic relationships among alpine and lowland populations of European *D. pulicaria*. As well, gene expression studies comparing lowland and alpine populations are needed to decipher local adaptations to life in high altitude environments. Since impacts of human-related activities and climate changes on mountain species are known to be dramatic, we pose strong issues for the conservation of these extremely localized taxa.

European *D. pulicaria*



ND5 tree of members of the *Daphnia pulex* complex

Lakes Trebecchi and Nivolet

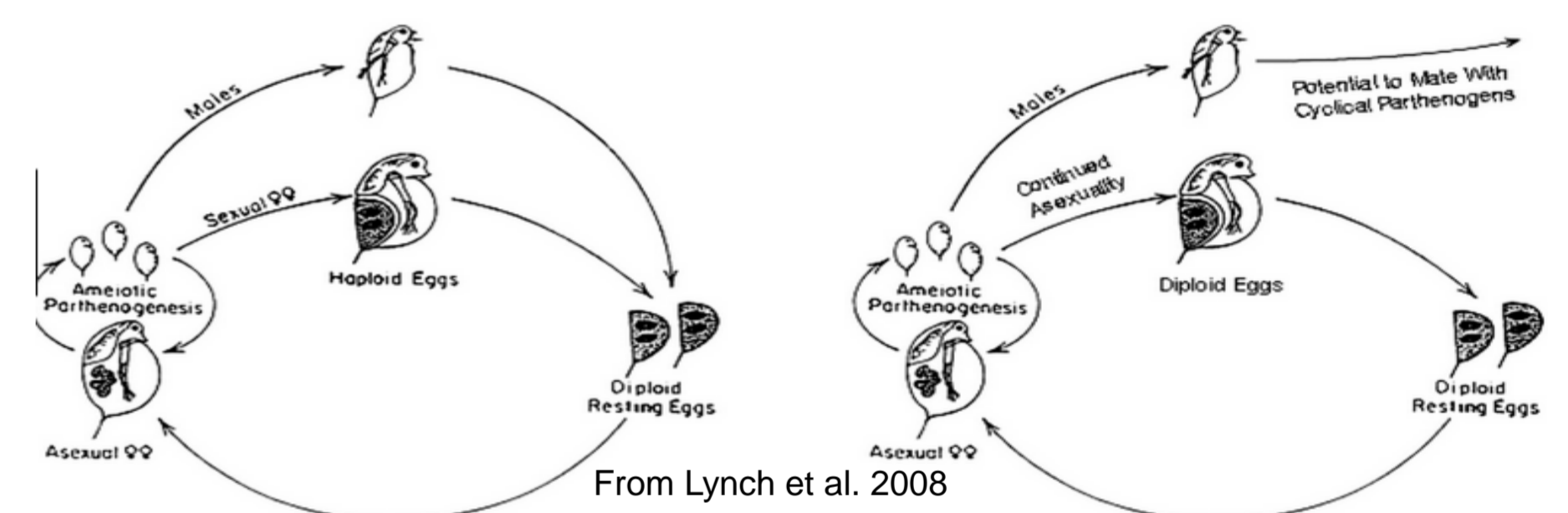


Lake Lillet

Haplotype network based on ND5 sequences (Bellati et al. 2014)

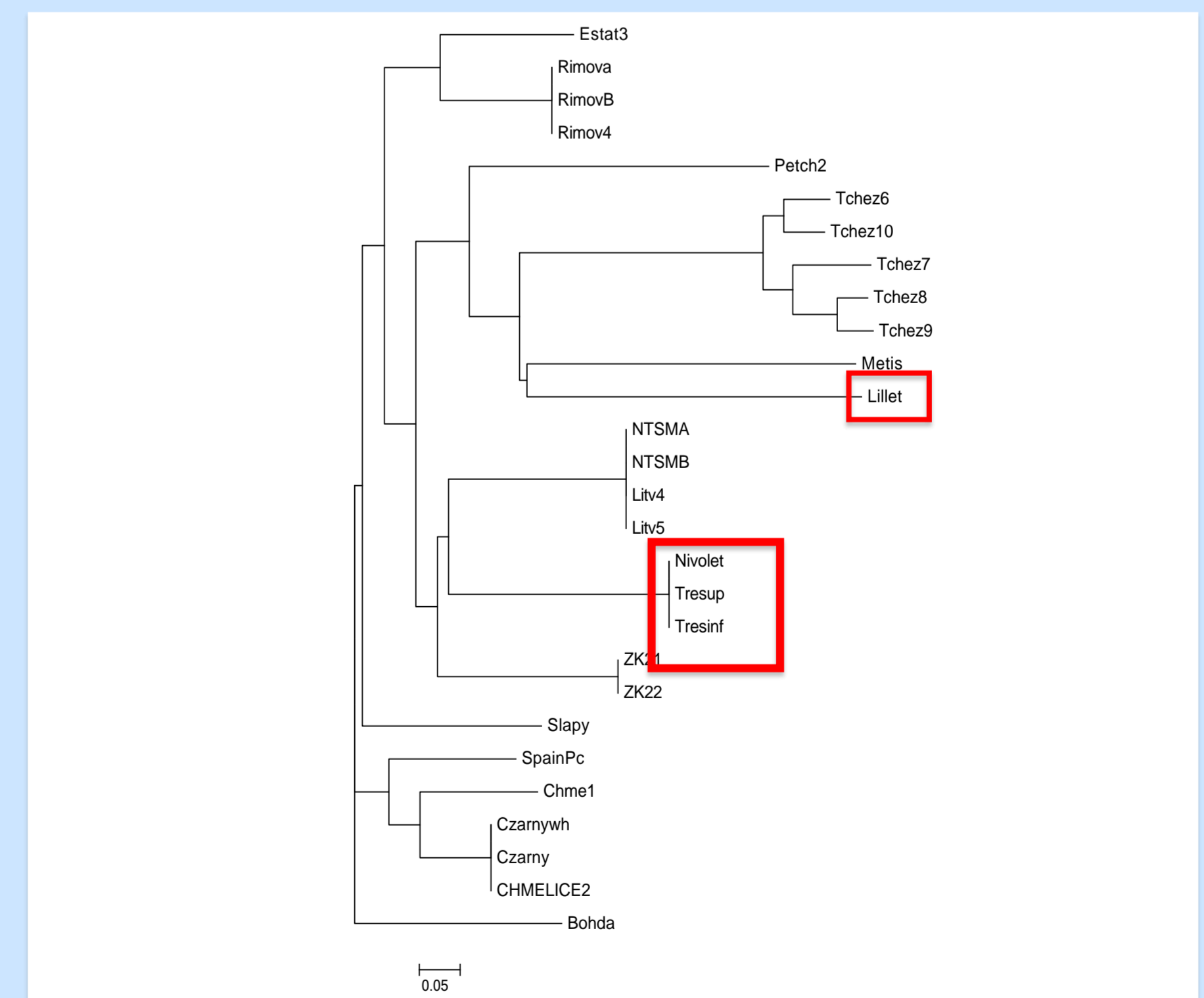
Cyclical Parthenogenesis

Obligate Asexuality



Results

A single multilocus genotype among the 72 individuals genotyped in Lakes Nivolet, Trebecchi Inferiore and Trebecchi Superiore! Another multilocus genotype among the 24 individuals from Lillet



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References: Beaton MJ, Hebert PDN. (1988) Geographical Parthenogenesis and Polyploidy in *Daphnia pulex*. *Am. Nat.* Vol. 132(6): 837-845; Bellati A, Tiberti R et al (2014) A dark shell hiding great variability: A molecular insight into the evolution and conservation of melanistic *Daphnia* populations in the Alps. *Zool J Linnean Soc.* 171(4). Dufresne F, Hebert PDN. (1994) Hybridization and the origins of polyploidy. *Proc. Roy Soc. Ser. B.* Vol. 258: 141-146; Dufresne F, Markova S, et al. Diversity in the Reproductive Modes of European *Daphnia pulicaria* Deviates from the Geographical Parthenogenesis. *PLoS One* 6(5); Lynch M, Seyferth A et al. (2008) Localization of the Genetic Determinants of Meiosis Suppression in *Daphnia pulex* Genetics 180:317-327; Mergeay J, Aguilera X et al. (2008) The genetic legacy of polyloid Bolivian *Daphnia*: the tropical Andes as a source for the North and South American *D. pulicaria* complex. *Molecular Ecology* 17:1789-1800.