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Presumable incipient hybrid speciation of door snails in previously glaciated areas in the Caucasus ☆

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ABSTRACT

Homoploid hybrid speciation, speciation by hybridization without a change in chromosome number, may be the result of an encounter of closely related species in a habitat that is different from that usually occupied by these species. In the northwestern Caucasus the land snail species *Micropontica caucasica* and *M. circassica* form two distinct entities with little admixture at low and intermediate altitudes. However, at higher altitudes in the Lagonaki plateau, which were repeatedly glaciated, *Micropontica* populations with intermediate characters occur. Admixture analyses based on AFLP data demonstrated that the populations from the Lagonaki plateau are homoploid hybrids that now form a cluster separate from the parental species. The Lagonaki populations are characterized by a mtDNA haplotype clade that has been found in the parental species only once. The fixation of this haplotype clade in most hybrid populations suggests that these haplotypes are better adapted to the cooler conditions in high altitude habitats and have replaced the haplotypes of the parental species in a selective sweep. The fixation of a presumably adaptive mitochondrial haplotype clade in the Lagonaki populations is an important step towards speciation under the differential fitness species concept.

1. Introduction

In the two past decades, it became clear that hybridization is much more frequent than previously assumed and that it does often not result in infertile descendants, but in introgression, the exchange of genes between species, or even in hybrid speciation (Mallet, 2005, 2007). The outcomes of hybridization may depend on the circumstances. In a stable environment, recombinant genotypes may remain restricted to a narrow hybrid zone between two species by a balance between dispersal into the tension zone and selection against hybrids (Barton and Hewitt, 1985) and introgressive hybridization may not have a lasting impact on evolution. If hybridization is the result of a loss of environmental heterogeneity that relaxed divergent selection and/or removed ecological barriers to gene flow between divergently adapted species, the species might merge (Seehausen et al., 2008). However, introgressive hybridization may also elicit reinforcement of reproductive isolation between incipient species (Servedio and Noor, 2003). Finally, hybridization may result directly in speciation. Hybrid speciation by chromosomal doubling (allopolyploidy) is known for a long time (Grant, 1981; Mable et al., 2011). However, recent studies of *Rhagoletis* fruit flies (Schwarz et al., 2007), *Lycaeides* (Gompert et al., 2006), *Heliconius* (Mavarez et al., 2006) and *Papilio* (Kunte et al., 2011) butterflies, sculpins (Nolte et al., 2005) and Italian sparrows (Hermansen et al., 2011) showed that gene exchange between species may promote the adaptation of hybrid populations and their evolution into separate species also without a change in chromosome number. Such homoploid hybrid speciation is facilitated by the invasion of novel or extreme habitats that increases ecological isolation and reduces introgression and competition with the parental species (Seehausen, 2004; Gross and Rieseberg, 2005; Gompert et al., 2006; Mallet, 2007; Nolte and Tautz, 2010; Abbott et al., 2013). The frequency of the different outcomes and, thus, the evolutionary importance of hybridization are still insufficiently understood. Therefore, more studies of potential cases of hybridization under various environmental conditions are necessary to evaluate the role of hybridization in evolution.

Although the Caucasus region is one of the most important hotspots of biodiversity (Myers et al., 2000; Zazanashvili et al., 2004), phylogeographic studies investigating speciation patterns within this region are missing. Door snails of the genus *Micropontica* are

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endemic to the northwestern Greater Caucasus and inhabit limestone rock faces (Likharev, 1962; Egorov, 2002, 2008; Kijashko, 2005). *Micropontica closta* lives along the Black Sea coast from Matsesta in Russia to Akhali Atoni in Abkhazia (Kijashko and Tuniev, 2007). The other *Micropontica* species are closely related and are sometimes classified in a separate subgenus, *Baleopsina* (Nordsieck, 1975), which has even been ranked as a distinct genus by Egorov (2008). *Micropontica caucasica* is known from several isolated localities on the northwestern slope of the Greater Caucasus as well as a few localities on the southwestern slope at altitudes between 250 and 800 m (Likharev, 1962; Egorov, 2002). *Micropontica circassica* has been recorded from isolated localities at the northwestern slope of the Greater Caucasus eastwards to Mount Shidzha-Tmas in the upper reaches of the Malka River and also from the upper Kodori valley in Abkhazia (Likharev, 1962; Egorov, 2002), where it lives at altitudes between 1000 and 1800 m. The two species differ in the closing apparatus in the aperture of the shell (Fig. 1–4). *Micropontica circassica* has a principal plica and a spiral lamella, which are absent or rudimentary in *M. caucasica*. The lunella and the clausilium plate, which closes the aperture when the animal withdraws is dorsally or dorso-laterally right situated in the body whorl of *M. circassica* so that it cannot be seen in front view. In contrast, it shifted towards the aperture in *M. caucasica* so that it can be seen in front view. Populations occurring at low and intermediate altitudes can easily be attributed to these two species. However, populations at higher altitudes (1750–2050 m) of the Lagonaki plateau and adjacent Lagonaki ridge show a larger morphological variability. While some of the individuals from the Lagonaki plateau resemble typical *M. caucasica* or *M. circassica*, others combine characters of the two species or show intermediate character states. For example, specimens from the Belorechensk pass in the southern part of the Lagonaki plateau, which have been named *Clausilia caucasica* var. *interjecta* Rosen, 1914, morphologically approach *M. caucasica* by possessing a weak or rudimentary spiral lamella, but resemble *M. circassica* in the presence of a weak principal plica and a dorsal lunella. This form was considered a separate species by Egorov (2002, 2008). Moreover, some specimens from the Lagonaki plateau show features unknown from typical *M. caucasica* or *M. circassica*. For example, the population from the Belorechensk pass has a weak palatal fold. Another population from the Lagonaki plateau with a strongly developed anterior end of a palatal fold has been described as another separate species *M. annae* Kijashko, 2005, but was synonymized with *M. interjecta* by Egorov (2008).

We investigated the hypothesis that the observed morphological diversity in the Lagonaki populations resulted from hybridization between *M. caucasica* and *M. circassica* using AFLP markers and mitochondrial DNA sequences. If the observed pattern is the result of continuous gene flow between *M. caucasica* and *M. circassica*, we would expect that nuclear genotypes of the population from the Lagonaki plateau are composed of variable proportions of the genomes of *M. caucasica* and *M. circassica* and that these populations do not form a distinct cluster. In contrast, the population from the Lagonaki plateau should form a cluster separate from *M. caucasica* and *M. circassica* with a more homogeneous genetic composition, if they resulted from homoploid hybrid speciation.

2. Material and methods

2.1. Sampling

Twelve *Micropontica* populations belonging to the *M. caucasica*/*circassica* group were sampled (Fig. 5). Samples include typical *M. caucasica* populations from the northern and the southern slope of the Greater Caucasus, typical *M. circassica* populations as well as several morphologically variable populations from the Lagonaki plateau including specimens corresponding morphologically to *M. interjecta* and *M. annae*. Each sample was taken from limestone rocks that were less than 100 m apart from each other. Mean annual temperatures of the sampling localities were acquired from the WorldClim database (http://www.worldclim.org; ca. 1 km^2 resolution; Hijmans et al., 2005).

Specimens of *Micropontica closta*, *Quadriplicata* and *Mucronaria* were used as outgroups. The classification, locality data and voucher numbers of the specimens used in this study are compiled in Table 1. The samples were stored in 100% isopropanol at −20 °C.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissue samples of the foot following the protocol proposed by Sokolov (2000) with slight modifications as detailed in Scheel and Hausdorf (2012).

Fragments of 16S rDNA and the cytochrome c oxidase subunit 1 (*cox1*) gene were amplified by polymerase chain reaction (PCR) using the primer pairs 16Sar and 16Sbr (Palumbi, 1996) and LCO1490 (Folmer et al., 1994) and a modified version of HCO2198 (Folmer et al., 1994) as specified by Sauer and Hausdorf (2009), respectively. Amplifications were performed in 25 µl volumes containing 2 µl 10× amplification buffer B (biolabproducts), 4 µl $MgCl_2$ (25 mM), 1 µl dNTP mix (5 mM each; biolabproducts), 1 µl of each primer (10 µM), 0.2 µl Taq DNA polymerase (5 U/µl; biolabproducts) and 1.0 µl template DNA under the following reaction conditions: an initial denaturing at 94 °C for 2 min, 35 PCR cycles (94 °C for 30 s, 48–50 °C for 30 s, 72 °C for 30 s), and a final extension at 72 °C for 5 min.

5 µl of the PCR product were purified with 0.65 µl thermosensitive alkaline phosphatase (1 U/µl; Thermo Scientific) and 0.35 µl exonuclease I (20 U/µl; Thermo Scientific) at 37 °C for 15 min.

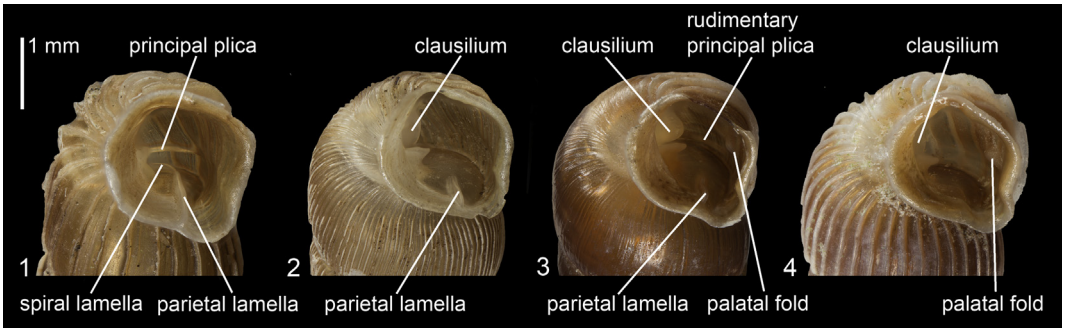

Fig. 1–4. Closing apparatus in the aperture of the shell of *Micropontica* species. (1) *M. circassica* from Azish-Tau Ridge, limestone cliffs near touristic base "Gornaya Kuban" (ZMH 86759); (2) *M. caucasica* from limestone cliffs NW of Dakhovskaya (ZMH 100036); (3 and 4) *Micropontica interjecta* from Lagonaki ridge, limestone cliffs on SW slope of Gora Matuk (ZMH 86987).