Absence of heterosis in hybrid crested newts

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We investigated relationships between phylogenetic relatedness, hybrid zone spatial structure, the amount of interspecific gene flow and population demography, with the newt genus *Triturus* as a model system. A bimodal hybrid zone of distantly related species in France combined low gene flow with hybrid sterility and heterosis whereas a suite of unimodal hybrid zones in closely related *Triturus* showed more or less extensive introgressive hybridization with no evidence for heterosis. We here report on population demography and interspecific gene flow in two *Triturus* species (*T. macedonicus* and *T. ivanbureschi* in Serbia) that are moderately related, engage in a heterogeneous uni-/bimodal hybrid zone and hence represent an intermediate situation. The study with 13 diagnostic nuclear genetic markers of a population at the species contact zone showed that all individuals were hybrids, with no parentals detected. Age, size and longevity and the estimated growth curves are as in pure species populations, so that we conclude to the absence of heterosis in *T. macedonicus* - *T. ivanbureschi*. Observations across the genus support the hypothesis that fertile hybrids allocate resources to reproduction and infertile hybrids allocate resources to growth.

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Abstract

~~We investigated~~ relationships between phylogenetic relatedness, hybrid zone spatial structure, the amount of interspecific gene flow and population demography, with the newt genus *Triturus* as a model system. A bimodal hybrid zone of distantly related species in France combined low gene flow with hybrid sterility and heterosis whereas a suite of unimodal hybrid zones in closely related *Triturus* showed more or less extensive introgressive hybridization with no evidence for heterosis. We ~~here~~ report on population demography and interspecific gene flow in two *Triturus* species (*T. macedonicus* and *T. ivanbureschi* in Serbia) that are moderately related, engage in a heterogeneous uni-/bimodal hybrid zone and hence represent an intermediate situation. The study with 13 diagnostic nuclear genetic markers of a population at the species contact zone showed that all individuals were hybrids, with no parentals detected. Age, size and longevity and the estimated growth curves are as in pure species populations, so that we conclude to the absence of heterosis in *T. macedonicus* - *T. ivanbureschi*. Observations across the genus support the hypothesis that fertile hybrids allocate resources to reproduction and infertile hybrids allocate resources to growth.

Introduction

Hybrid zones are regions where genetically distinct populations meet and hybridize (Barton and Hewitt, 1985; Harrison, 1993). They provide natural settings for the study of speciation. In particular they allow research on the consequences of new, previously untested genetic combinations of differentiated genomes in nature. Hybrid zones may take several forms, from long and narrow zones to large areas of overlap and mosaics (Arnold, 1992). The general positive relationship between the degree to which hybridization proceeds and the phylogenetic relatedness of the species involved is well established (Jiggins and Mallet, 2000). In spite of some variation in the strength of reproductive isolation for individuals from spatially isolated populations, or among different species pairs from the same evolutionary lineages (Seehausen et al., 2014; Kearns et al., 2018) evidence accumulates that, in diploid animals, reproductive isolation increases and introgression between lineages decreases with divergence (Singhal and Moritz, 2013; Arntzen et al., 2014; Beysard and Heckel, 2014; Dufresnes et al., 2014; Montanari et al., 2014; Taylor et al. 2014). Unfortunately, much less is known about how species' ecological preferences - and with that species distributions and local spatial configurations - may affect hybridization and *vice versa,* and how phylogenetic relatedness affects the interaction with ecology. Yet, such knowledge is relevant for the understanding of hybrid zones and the evolutionary inferences we draw from them. As Barton and Hewitt (1985) proposed '… as soon as the loss of fitness through hybridization inside the zone becomes small enough … the zone will collapse into broad sympatry'. Accordingly, the more closely related hybridizing species are, the more hybridization will take place, over yet smaller areas. A negative relationship between the level of hybridization and the amount range overlap was indeed noted by Zuiderwijk (1980) in a variety of European amphibian species pairs, but geographical variation and ecological differences remain to be studied.

We propose that a good group to investigate if reproductive isolation accumulates gradually would fulfill the following requirements. It would i) be monophyletic and ii) show more or less contiguous species ranges where iii) closely as well as distantly related species engage in hybrid zones and iv) ecological profiles of the species are different. Finally, v) to reduce the impact of spatial scale a low dispersal capability would be an asset. One group that qualifies is the European newt genus *Triturus*. The genus is diverse and has a pan-European distribution, with outer ranges up to the Atlantic and the Mediterranean and reaching into Scandinavia, Russia, the Caucasus and Iran. The species group inner ranges form a patchwork and all nine species are, in one place or the other, involved in intra-generic hybridization (Arntzen et al., 2014; Wielstra et al., 2014b). The genus *Triturus* is composed of three clades, namely the *T. marmoratus* group (or marbled newts) with two species (clade A), the *T. cristatus* group with four species (clade B) and the *T. karelinii* group with three species (clade C). Clades A and B engage in western Europe and clades B and C meet up in southeastern Europe (Arntzen, 2003; Wielstra et al., 2014b). See Figure 1 for species distributions and phylogenetic relationships.

The A and B clades are distantly related with an estimated 27.6 Ma period of lineage independence (Wielstra and Arntzen, 2011). At their hybrid zone in France, *T. marmoratus* (clade A) and *T. cristatus* (clade B) interspecies F_1 hybrids are infrequent (ca. 4 % of the total population) and introgression is rare (< 0.1 %, Arntzen et al., 2009). The F_1 hybrids show hybrid vigour in body size and longevity, that is, they get larger and older than their parents (Francillon-Vieillot et al., 1990; Figure 2). The hybrid zone is bimodal, with a well-understood ecological differentiation. Forested and hilly areas are mostly occupied by *T. marmoratus* and open and flat areas have *T. cristatus* (Schoorl and Zuiderwijk, 1981; Visser et al., 2017). Representatives of clades B and C - together known as crested newts - show an intermediate level of phylogenetic relatedness with 10.4 Ma of lineage independence. The species involved in the contact are *T. cristatus, T. dobrogicus* and *T. macedonicus* in clade B and *T. ivanbureschi* in clade C. Among these four, *T. dobrogicus* stands out as a lowland species (Vörös et al., 2016). The species meet up in the Balkan peninsula along the lower Danube and in a wide zone running from Belgrade (Serbia) to Thessaloniki (Greece) (Figure 1). Species within the B and C clades have 8.8 – 5.3 Ma of lineage independence and engage in a variety of more or less unimodal hybrid zones across Europe and Asia (Arntzen et al., 2014 and Wielstra et al., 2017ab), with no evidence for heterosis (hybrid vigor – for data reviews see Arntzen, 2000; Lukanov and Tzankov, 2016).

Because of the intermediate level of relatedness, the genetic and spatial interactions among species in the B and C clades are of special interest. We here studied hybridization between *T. macedonicus* and *T. ivanbureschi* where they meet in southeastern Serbia. A phenotypically mixed population was examined with a panel of nuclear genetic markers to estimate ancestry and heterozygosity and individual age was estimated by skeletochronology. We searched for heterosis by analyzing hybrids for longevity, body size and growth.

Materials and methods

Post-metamorphic crested newts were collected from March to July 2013 in a pond near the village Vlasi in the southeast of Serbia (43.00 N, 22.64 E, altitude 468 m a.s.l.). The focal newt population we henceforth refer to as ‘Vlasi’. A morphological classification on the basis of external morphology and colouration characteristics (Arntzen and Wallis, 1999) was in five groups as *T. macedonicus*-like (group 1, N=26), leaning towards *T. macedonicus* (group 2, N=48), an intermediate phenotype (group 3, N=146), a phenotype leaning towards *T. ivanbureschi* (group 4, N=83) and *T. ivanbureschi*-like (group 5, N=33). A small part of the newt’s tail at its tip was taken for genetic analyses. Permission for fieldwork and to collect was obtained from the Ministry of Energy, Development and Environmental Protection of the Republic of Serbia (permit no. 353-01- 35/2013-08).

Molecular data were gathered for a panel of nuclear encoded SNP markers (see below). We included reference samples with N=3 for seven populations of *T. ivanbureschi* and seven populations of *T. macedonicus* available from Wielstra et al. (2013) (Table 1). Reference populations were located to the south of the Vlasi population, to stay away from other, more northerly distributed *Triturus* species. Wielstra et al. (2014a) produced sequence data for 52 short (ca. 140 bp) nuclear markers positioned in 3'UTR regions of protein-coding genes for three individuals from four populations positioned throughout the ranges of both *T. ivanbureschi* and *T. macedonicus*. We focussed on the subset of 24 nuclear markers with species diagnostic allele variants for *T. ivanbureschi* and *T. macedonicus*. Additionally an mtDNA SNP (*nd4*) was designed from Sanger sequence data taken from Wielstra et al. (2013). We determined diagnostic SNPs by checking the sequence alignments by eye in MacClade 4.08 (Maddison and Maddison, 2005).

Genotyping was conducted commercially at the SNP genotyping facility of the Institute of Biology, Leiden University, using the Kompetitive Allele-Specific PCR (KASP) genotyping system (LGC KBioscience, UK). This involves fluorescence-based genotyping using SNP-specific primers. We used the program Kraken to design two forward or reverse primers that are specific for (i.e. with a final base complementary to) one of the two potential SNP variants, in addition to a general reverse or forward primer (Semagn et al., 2014). We genotyped 378 crested newts in total: 21 individuals of both parental species (the eight populations on which SNP identification was based plus an additional six populations) and 336 individuals from Vlasi. Sequence alignments and Kraken input for all markers are available in Suppl. Inf. 1. Raw output of the KASP genotyping protocol is in Suppl. Inf. 2. The Ion Torrent next-generation sequence data of Wielstra et al. (2014a) used for SNP discovery are available from Dryad Digital Repository entry http://dx.doi.org/10.5061/dryad.36775. The genetic and morphological data used in the analysis are presented in Suppl. Inf. 3 and 4.

The program GenePop was used to quantify Hardy-Weinberg equilibrium (HW) and linkage disequilibrium (LD) (Raymond and Rousset, 1995). The program NewHybrids (Anderson and Thompson, 2002) was used to estimate the proportion of the Vlasi population consisting of recently formed hybrids. Reference populations were invoked with the 'z-option'. The program HIest was used to estimate heterozygosity and ancestry (Fitzpatrick, 2012).

In a subsample of individuals (77 males, 85 females plus eight juveniles) that represented the five morphological groups, a phalanx of the third toe was taken for age determination by skeletochronology. Lines of arrested growh (LAGs) were counted as in Francillon-Viellot et al. (1990) and we determined age at maturity from 'rapprochement'. This is the tightening of LAGs that is associated with the shift of resources from growth to reproduction. Rapprochement was not observed in 13 individuals (8.0 %). Body size was measured in mm from the tip of the snout up to the posterior end of the cloaca (snout vent length, SVl). Von Bertalanffy growth curves were determined with the function 'growthmodels' in the R package

FSA (FSA, 2017). The few juveniles in the sample remained unsexed and were excluded from the analyses. Confidence intervals were estimated by bootstrapping with nlstools in 2000 bootstrap iterations (Nlstools, 2015). Other statistical analyses were with SAS software (SAS, 2011).

Results

Out of the candidate nuclear markers two (*cnppd* and *kdm3*) were dropped because a diagnostic SNP could not be identified and for four (*ace*, *eif4ebp2*, *ssh2* and *syncrip*) the Kraken software could not design a suitable set of primers. For the 18 remaining diagnostic nuclear markers for which assays could be designed, PCR amplification for one (*amot*) failed. The locus *slc25* yielded a high frequency of missing data in the reference populations, suggesting it did not amplify the *T. ivanbureschi*-allele well and results for this marker were also discarded. For the remainder missing data amounted to 1.8%. The four loci *ahe*, *ddx17*, *dnaj* and *sre* showed highly significant LD for all six pairwise combinations. This result was interpreted as tight physical linkage in a single linkage group. To increase data independence results for the locus *ahe* (with the fewest missing data) were retained and the others dismissed. Subsequent tests for HW and LD yielded no significances under Bonferroni correction. The KASP genotyping output for the remaining 13 markers is summarized in Suppl. Inf. 3. One nuclear SNP marker (*col18*) showed a single instance of heterozygosity in a parental individual, suggesting either a genotyping error or a marker that is not fully diagnostic. With N=82 (21.7 %) of missing data the mtDNA marker (*nd4*) performed relatively poorly. All newts from the Vlasi population that could be genotyped possessed the haplotype typical for *T. ivanbureschi*, as expected for this marker in this system (Wielstra et al., 2017a).

In NewHybrids none of the individuals from the Vlasi population were allocated to either of the parental species, to the F_1 hybrid class or to the class of backcrosses towards *T. macedonicus*. Several individuals were allocated to the ‘backcross to *T. ivanbureshi* class’ (n=8, 2.4%). The far majority of individuals was classified as F_2 hybrids (n=328, 97.6%). When NewHybrids was instructed to take not two but three generations into account, the majority (99.7%) was allocated to that third generation. HIest grouped all Vlasi individuals in the middle of the ancestry times heterozygosity bivariate plot, somewhat off-center in the direction of *T. ivanbureschi* (Figure 3), with the reference populations in the lower left (pure *T. macedonicus*) and lower right corners (pure *T. ivanbureschi*). ANOVA was used to test for differences in H and S over five (1-5) or three morphological groups (1, 2-4 pooled, 5) with no statistically significant results ($P > 0.05$). Pearson correlation coefficients between SVl and the two HIest parameters were not significant, for males as well as for females.

The body size distribution showed that females were significantly larger than males (SVl males 63.9 mm, SVl females 67.5 mm; Student's *t*-test $P < 0.0001$). The age distribution for males and females showed no significant difference (Mann-Whitney U-test, $P > 0.05$; Table 2). In both sexes > 70% of the individuals had an estimated age of 5, 6 or 7 years. Age at maturity estimated by rapprochement was mostly 2, 3 or 4 years in both sexes (average males 3.1, n=75; average for males 3.0, n=74; Suppl. Inf. 4.). No observable rapprochement was more frequent in females (14.9 %) than in males (2.6 %) ($P < 0.05$, G-test of independence), perhaps suggesting a more regular transition from growth to reproduction in females. Longevity was 13 years in males and 11 years in females. The parameters that describe the Von Bertalanffy growth curves and the confidence intervals are presented in Table 3. Results for the Vlasi populations were compared with data from the literature (Cvetković et al., 1996 and A. Ivanović, unpublished data on *T. macedonicus* and Üzüm, 2006 and Üzüm and Olgun, 2009 on *T. ivanbureschi*). In three out of four populations females are larger than males of the same age. The other growth curves for populations and species are similar, with no more than 10 mm difference in SVl among the most different groups (Figure 4). Unlike *T. cristatus* x *T. marmoratus* hybrids, the *T. macedonicus* x *T. ivanbureschi* hybrids showed no heterosis. Longevity increased over species as *T. cristatus, T. marmoratus, T. ivanbureschi* and *T. macedonicus* (Figures 2 and 4).

Discussion

We encountered a population of crested newts near Vlasi in southeastern Serbia that showed large phenotypic variation and - being close to the territory of both *T. macedonicus* and *T. ivanbureschi* - a hybrid nature was assumed. We were unable to determine the extent of hybridization from morphology and yet wanted to find out if this hybrid zone population has a unimodal character (with a majority of hybrids and few or no parentals), a bimodal character (with predominantly the parental species and few hybrids), or something in between. We were also interested in fitness consequences that hybridization might have on the demography of the population, in particular if heterosis would be combined with hybrid sterility.

All individuals from the focal population were allocated to the second generation of hybrids, indicating strong admixture along with the absence of recent crossings between the parental species. When a third generation was an option, individuals where allocated to that third generation, further supporting the absence of recent hybrids. Looking yet deeper into the coalescence would require more genetic markers. Ancestry versus heterozygosity plots further support that genotypes in this Vlasi population result from many generations of admixture. In the absence of selection and banning physical linkage LD halves every next generation and will barely be measurable after a few generations. Accordingly, the absence of admixture LD also suggests that hybridization between *T. macedonicus* and *T. ivanbureschi* has been going on for more than a couple of generations.

We conclude that Vlasi is a population with just hybrids and the parental species absent. This finding suggest that *T. macedonicus* and *T. ivanbureschi* engage in a unimodal hybrid zone. The genetic affiliation of the Vlasi population is somewhat closer to *T. ivanbureschi* than to *T. macedonicus* (Figure 3). This is in line with documented distributions of the two species where the Vlasi population is geographically closer to *T. ivanbureschi* than to *T. macedonicus* (Wielstra et al., 2014b). However, the exact position of the center and the width of the cline remain to be documented. This result contrasts with the situation to the northwest, where the two species engage over a wide area of species replacement, including a *T. ivanbureschi* enclave that was cut off from the main distribution by superseding *T. macedonicus* (Arntzen, 2003; Arntzen & Wallis 1999, cf. Figure 1). The sole transmission of the mitochondrial haplotype typical for *T. ivanbureschi*, that we here confirmed for the Vlasi hybrid population, helped to reconstruct the scenario of species replacement (Wielstra et al., 2017a).

Some *Triturus* species engage in smooth clinal transitions with rampant hybridization confined to a narrow zone and others overlap in a spatial mosaic with hybridization infrequent. An example of a mosaic (or ‘bimodal’) hybrid zone is found for the distantly related *T. cristatus* and *T. marmoratus* in France. Narrow zones with rampant hybridization are found at contacts between crested newts species belonging to clades B and C (Arntzen et al., 2014; Wielstra et al., 2017b, cf. Figure 1). A relationship appears to exist with species relatedness, so that closely related species form clines and more distant species engage in a mosaic distribution. The presence of a unimodal (clinal) as well as bimodal (mosaic) hybrid zone structure in the *T. macedonicus* - *T. ivanbureschi* contact is in line with an intermediate level of relatedness.

Conclusion

As here demonstrated, the *T. macedonicus* - *T. ivanbureschi* hybrids are fertile, producing new genetic combinations for testing in nature. Conversely, hybridization in *T. cristatus* and *T. marmoratus* is an evolutionary dead-end because their hybrids are largely sterile and introgression is near-absent. We propose that in the genus *Triturus* heterosis is restricted to hybrids that are infertile and that - even as adults - direct their resources more to growth than to reproduction. This is more likely to be the case for genetically incompatible than for genetically compatible species. The demonstration of heterosis in any of the six or seven hybrid zones of closely related *Triturus* species would contradict this explanation.

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Suppl. Inf. 1 - Input consensus sequences for Kraken.

Suppl. Inf. 2 - Raw output of the KASP genotyping protocol.

Suppl. Inf. 3 - Genetic data in GenePop format.

Suppl. Inf. 4 - Skeletochronology results along with morphometric data and individual Structure scores.

Figure 1. Distribution of nine *Triturus* species over Europe and the Near East (after Wielstra et al., 2011). Major clades are A, the marbled newts, B, the *T. cristatus* species group of crested newts and C, the *T. karelinii* species group of crested newts. Note that the spatial contact of clades is limited to central France (clades A and B) and the southern Balkans (B and C). Heterosis in size and longevity was observed in A x B hybrids (Mayenne, France, white dot, Francillon-Vieillot et al., 1990) and not in B x C hybrids (Vlasi, Serbia, asterisk, present paper). The insert shows the species phylogeny.

Figure 2. Von Bertalanffy growth curves for males and females of the newts *Triturus cristatus* (red)., *T. marmoratus* (green). and *Triturus cristatus - T. marmoratus* F1 hybrids (black) from Mayenne, France. Females are larger than males of the same age. The hybrids are larger and in general older than the parental species. Data are from Francillon-Vieillot et al. (1990).

Figure 3. Ancestry versus heterozygosity plot based on 13 species diagnostic nuclear genetic markers. Individuals from the Vlasi population are shown by open round symbols (N=336). The left corner of the

triangle corresponds to *Triturus macedonicus* (solid square symbols, N=21), the right corner to *T. ivanbureschi* (solid triangle symbol, N=21) and the upper corner to (non-observed) F1 hybrids.

Figure 4. Von Bertalanffy growth curves for males and females of the *Triturus macedonicus* x *T. ivanbureschi* hybrid population from Vlasi in southeastern Serbia (black lines). Other colour codes are pink for *T. macedonicus* and blue for *T. ivanbureschi* populations at localities Keşan and Klaros. Growth curves for the parental species are constructed from published data (see Table 3). Females are larger than males of the same age in three out of four populations. The Vlasi populations of hybrids shows no evidence for hybrid vigour in size, growth or longevity.

Figure 1

Distribution of nine *Triturus* species over Europe and the Near East.

Major clades are A, the marbled newts, B, the *T. cristatus* species group of crested newts and C, the *T. karelinii* species group of crested newts. Note that the spatial contact of clades is limited to central France (clades A and B) and the southern Balkans (B and C). Heterosis in size and longevity was observed in A x B hybrids (Mayenne, France, white dot, Francillon-Vieillot et al., 1990) and not in B x C hybrids (Vlasi, Serbia, asterisk, present paper). The insert shows the species phylogeny. Source: Wielstra et al. (2011).

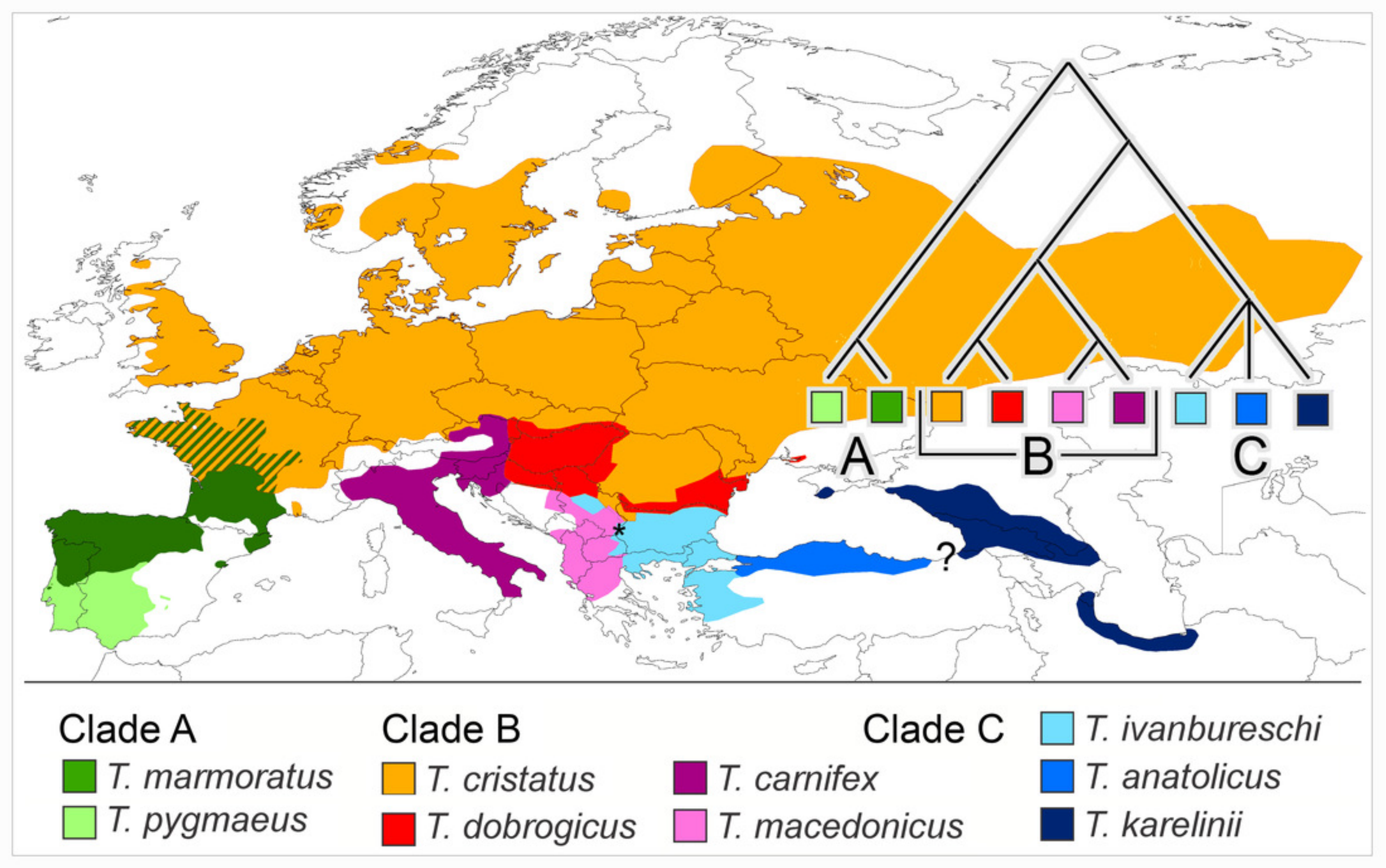

Figure 2

Von Bertalanffy growth curves for males and females of the newts *Triturus cristatus* (red), *T. marmoratus* (green) and *Triturus cristatus - T. marmoratus* F1 hybrids (black) from Mayenne, France.

Females are larger than males of the same age. The hybrids are larger and in general older than the parental species. Data are from Francillon-Vieillot et al. (1990).

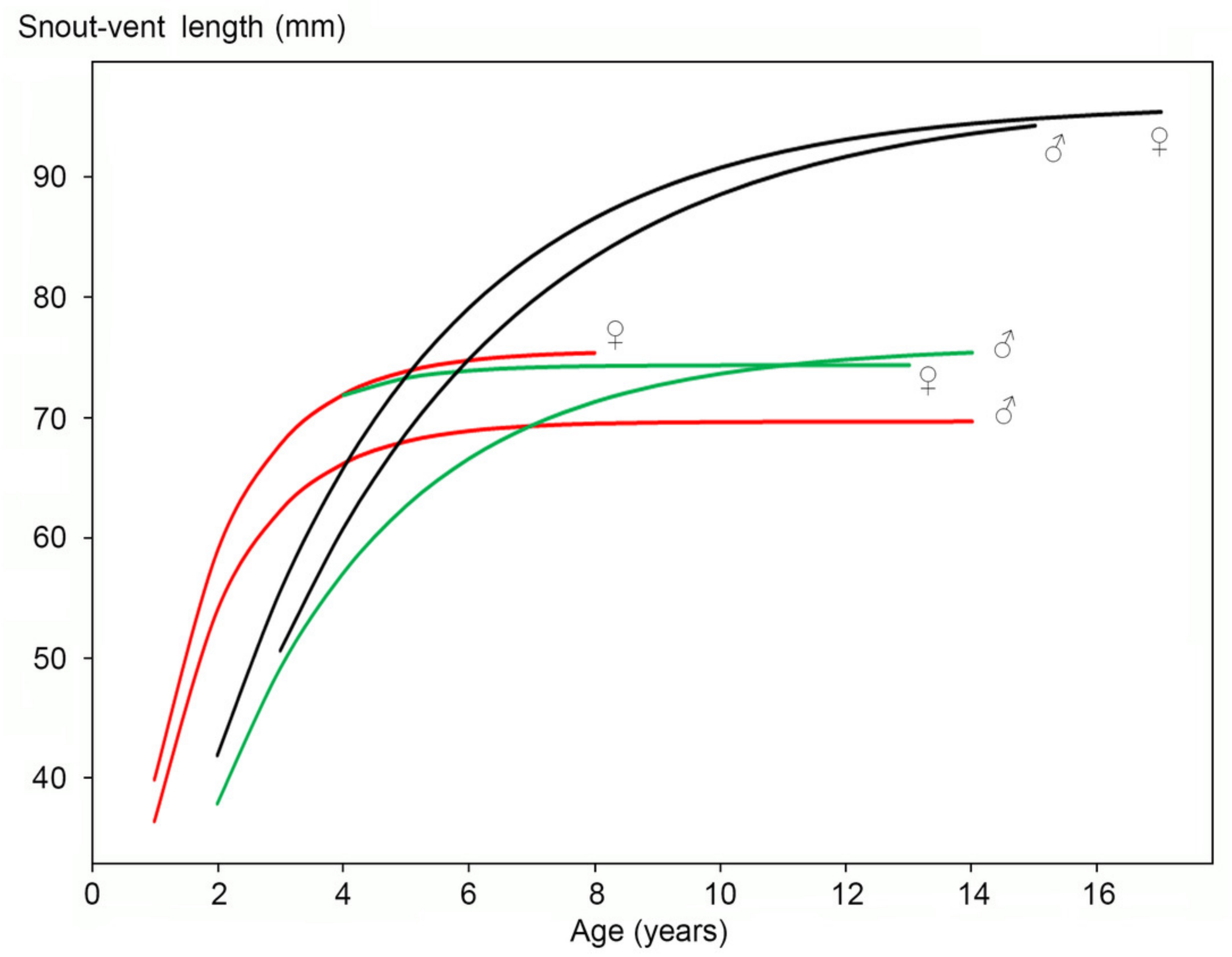

Figure 3

Ancestry versus heterozygosity plot based on 13 species diagnostic nuclear genetic markers.

Individuals from the Vlasi population are shown by open round symbols (N=336). The left corner of the triangle corresponds to *Triturus macedonicus* (solid square symbols, N=21), the right corner to *T. ivanbureschi* (solid triangle symbol, N=21) and the upper corner to (non-observed) F1 hybrids.

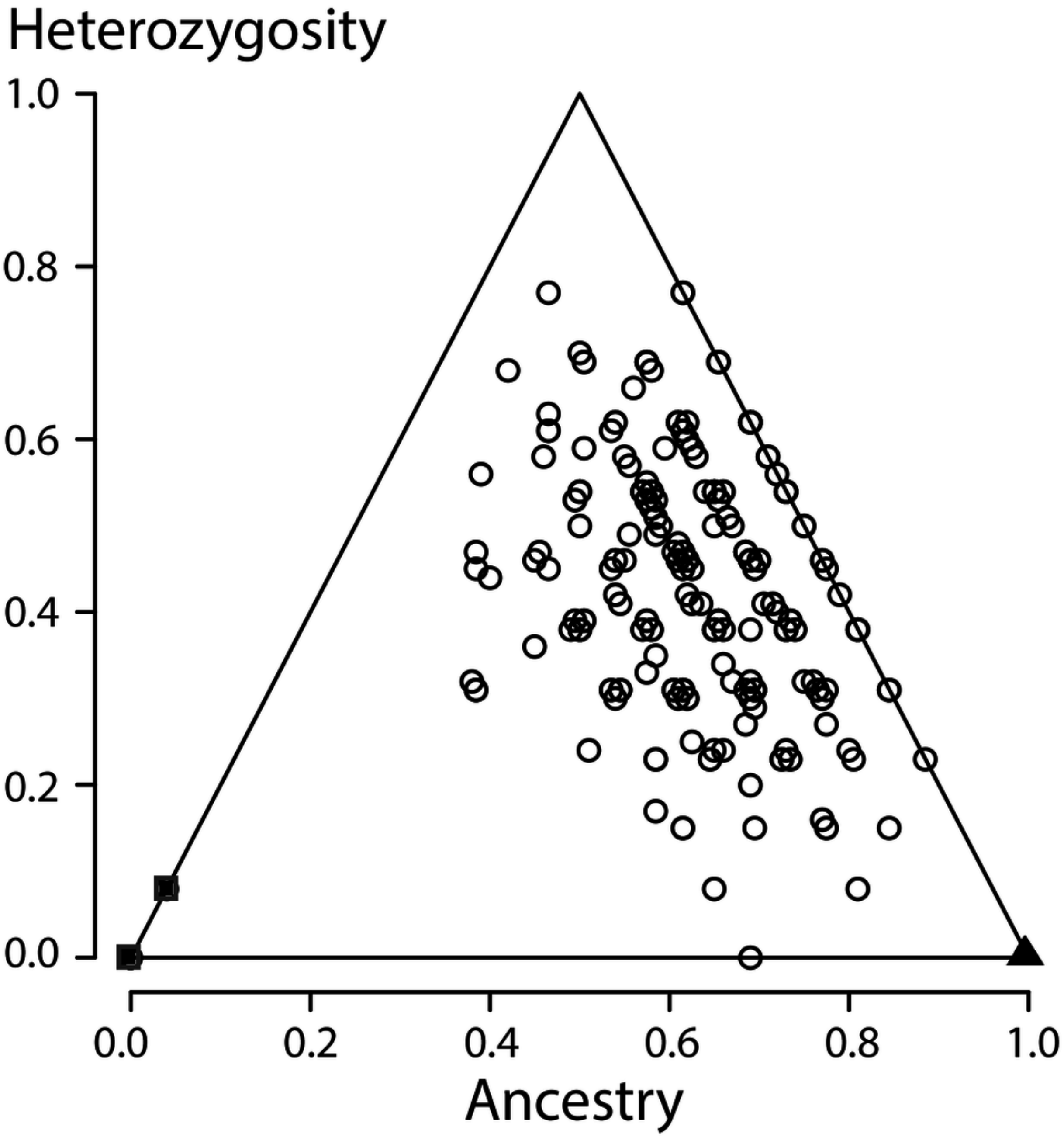
Heterozygosity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Ancestry

Figure 4

Von Bertalanffy growth curves for males and females of the *Triturus macedonicus* x *T. ivanbureschi* hybrid population from Vlasi in southeastern Serbia (black lines).

Other colour codes are pink for *T. macedonicus* and blue for *T. ivanbureschi* populations at localities Keşan and Klaros. Growth curves for the parental species are constructed from published data (see Table 3). Females are larger than males of the same age in three out of four populations. The Vlasi populations of hybrids shows no evidence for hybrid vigour in size, growth or longevity.

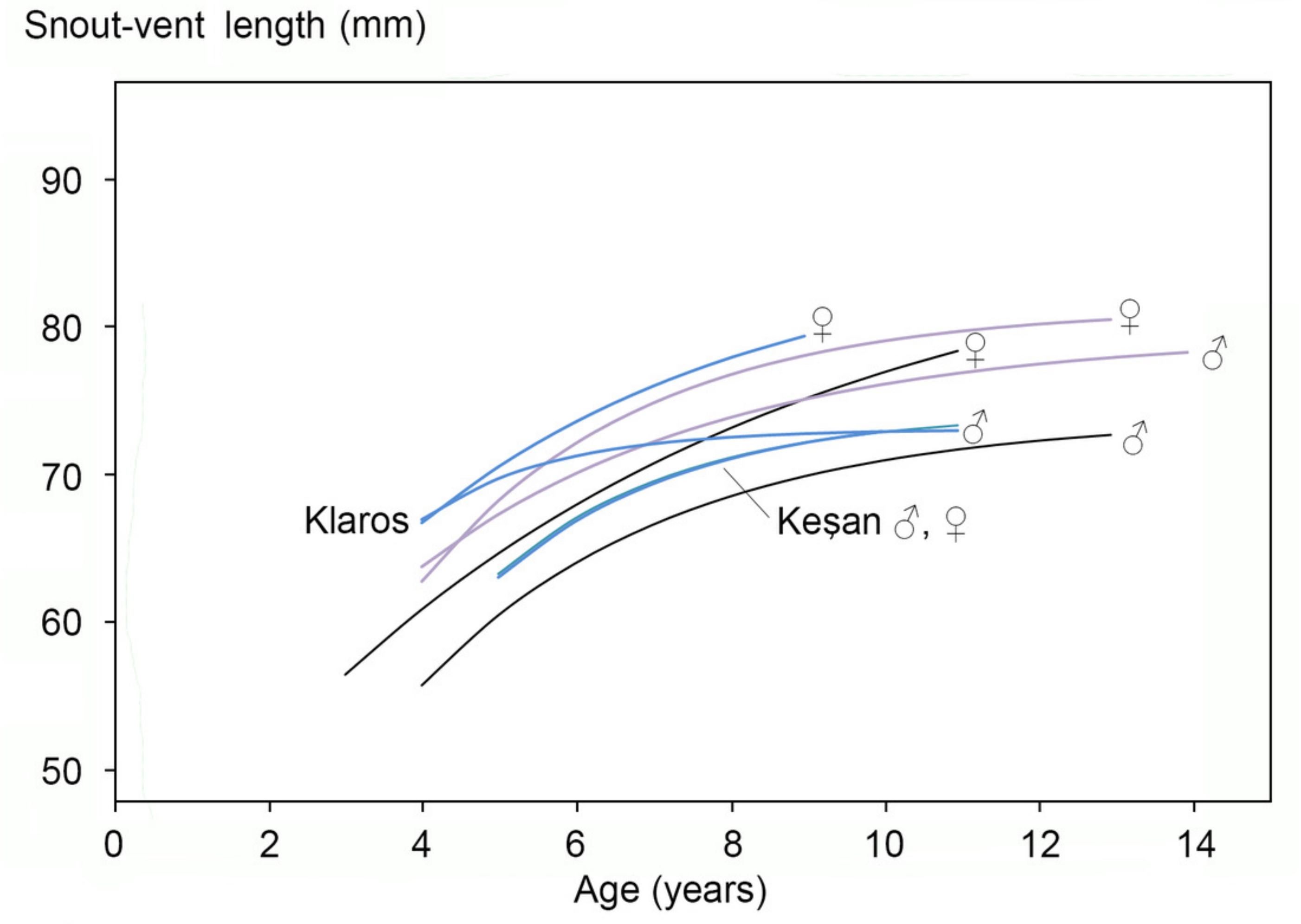

Table 1(on next page)

Populations of *Triturus macedonicus*, *T. ivanbureschi* and the mixed focal population from Vlasi, Serbia, genotyped with a panel of nuclear genetic markers.

Table 1

Populations of *Triturus macedonicus, T. ivanbureschi* and the mixed focal population from Vlasi, Serbia, genotyped with a panel of nuclear genetic markers.

Taxon, locality, country	Coordinates Latitude	 Longitude	Population used for marker design	Sample size	Tissue collection number
Triturus macedonicus					
Paliambela, Greece	38.909	20.970		3	2815-7
Kerameia, Greece	39.562	22.081	yes	3	3775-7
Kounoupena, Greece	39.683	19.764	yes	3	2820-2
Lushnjë, Albania	41.000	19.664		3	3472-4
Vrbjani, Macedonia	41.413	20.816		3	3327, 3583-84
Gostivar, Macedonia	41.817	20.899	yes	3	3601-3
Bjeloši, Montenegro	42.374	18.907	yes	3	3245-7
Triturus ivanbureschi					
Shumnatitsa, Bulgaria	42.297	23.626		3	2779-81
Saint Kosmas, Greece	41.084	24.669		3	2846-8
Alexandrovo, Bulgaria	42.601	25.093	yes	3	2492-4
Keşan, Turkey	40.917	26.633	yes	3	2360-2
Salihler, Turkey	39.181	26.826		3	1808-9, 1812
Alepu, Bulgaria	42.348	27.714	yes	3	2602-4
Kocabey, Turkey	39.352	28.217	yes	3	1879-81
Focal population					
Vlasi, Serbia	42.998	22.637		336	6284-6620

Table 2(on next page)

Ages of juveniles, males and females in the admixed *Triturus macedonicus* – *T. ivanbureschi* population from Vlasi, Serbia. Ages are estimated by skeletochronology.

Table 2

Ages of juveniles, males and females in the admixed *Triturus macedonicus – T. ivanbureschi* population from Vlasi, Serbia. Ages are estimated by skeletochronology.

Age	Juveniles	Males	Females
0			
1			
2	2		
3	5		3
4	1	6	5
5		18	16
6		20	24
7		18	20
8		9	11
9		4	3
10		1	
11			3
12			
13		1	
Total	8	77	85

Table 3(on next page)

Parameter values in the Von Bertalanffy growth curves for populations in the southeast of the *Triturus* range. The focal Vlasi population is of mixed *T. macedonicus - T. ivanbureschi* ancestry.

Table 3

Parameter values in the Von Bertalanffy growth curves for populations in the southeast of the *Triturus* range. The focal Vlasi population is of mixed *T. macedonicus* - *T. ivanbureschi* ancestry.

	Males			Females		
	SVl	k	t_0	SVl	k	t_0
Vlasi (85 males and 93 females)						
Median	73.00	0.36	0.24	78.72	0.34	0.12
CI lower bound	68.99	0.23	-0.94	74.33	0.21	-1.01
CI upper bound	80.33	0.50	0.93	86.95	0.47	0.80
Triturus macedonicus #						
Ceklin, Lokanj and Rid (51 males and 50 females)						
Median	79.56	0.25	-2.34	81.36	0.35	-0.22
CI lower bound	74.25	0.04	-18.46	76.11	0.06	-14.54
CI upper bound	109.65	0.96	2.63	108.77	1.06	2.78
Triturus ivanbureschi						
Keşan (42 males and 34 females) $						
Median	74.23	0.42	0.45	74.46	0.40	0.34
CI lower bound	70.92	0.07	-10.50	70.76	0.07	-10.51
CI upper bound	96.15	1.12	3.19	95.18	1.17	3.25
Klaros (45 males and 39 females) &						
Median	73.88	0.45	-1.32	85.17	0.23	-2.58
CI lower bound	70.64	0.22	-3.99	78.11	0.09	-7.03
CI upper bound	80.16	0.93	0.02	108.99	0.40	-1.01

Data from Cvetković et al. (1996) and A. Ivanović (unpublished).

$ Data from Üzüm (2006)

& Data from Üzüm and Olgun (2009)