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ITALIAN CRESTED NEWTS (*TRITURUS CARNIFEX*) IN THE BASIN OF GENEVA: DISTRIBUTION AND GENETIC INTERACTIONS WITH AUTOCHTHONOUS SPECIES

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ABSTRACT: Three surveys in the Basin of Geneva (Switzerland and adjacent France) reveal a decline of crested newts, from 22 known populations in 1975, to 11 in 1987, and to seven in 1997. Using morphological and genetic markers, 16 out of 18 populations are identified as (allochthonous) *T. carnifex* or mixed populations of *T. carnifex*–*T. cristatus*. Syntopic populations are characterized by the incomplete mixing of genotypes. The presence of the genes of *T. cristatus* in *T. carnifex* demonstrates past hybridization events. Two populations are identified as (autochthonous) *T. cristatus*; its presence is now restricted to the northeastern fringe of the Basin. Identification of preserved material indicates that the introduction of *T. carnifex* took place early this century. The data imply that over 30–40 generations, *T. carnifex* has established itself in the Basin of Geneva at the expense of *T. cristatus*. No significant change in the relative distribution of the two species was observed from 1987–1997, and we conclude that the recent spread of *T. carnifex* has been non-existent or slow.

Key words: Allozymes; Hybridization; France; Introduction; Introgression; Newt; Switzerland; *Triturus carnifex*; *Triturus cristatus*

INTRODUCED organisms are often detrimental to native biota and this is increasingly recognized as an important contributor to the loss of biodiversity world-wide. Introductions may be deliberate (as food stock or pest control agents, or in attempts to increase species diversity), but they can also take place naively (e.g., the release of pets) or accidentally (e.g., escape from enclosures). Cases involving amphibians include the introduction of the giant toad, *Bufo marinus*, into Queensland, Australia to control the sugar cane beetle (Eastal, 1981); the escape from frog farms in Spain of the marsh frog, *Rana ridibunda*, which has hybridized with local *Rana perezi* (Ar-

ano et al., 1995); the release of unwanted pets such as the bullfrog, *Rana catesbeiana* (reviewed in Stumpel, 1992); and the introduction of midwife toads, *Alytes obstetricans*, and other continental European species to increase biodiversity in the United Kingdom (Anonymous, 1997; Lever, 1979, 1994). Burke (1991), Dodd and Seigel (1991), and Reinert (1991) reviewed the translocation of amphibians as a conservation strategy.

Introductions can take place with closely related species being absent or present. For example, the Italian crested newt, *Triturus carnifex*, has been introduced both to São Miguel island, Azores, where amphibians used to be absent (Malkmus, 1995), and to the United Kingdom and Switzerland (Anonymous, 1997; present paper) where a closely related species occurs. The latter category of introductions

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is of special concern. Taxa closely related to the introduced one are more likely to be affected than more distantly related taxa, because ecological and genetic similarities increase the likelihood of competition or hybridization (see examples by Abernethy, 1994; Echelle and Echelle, 1997; Griffiths et al., 1996). Competition between closely related species may have more impact if the native species is less able to compete effectively with the introduced species due to changes in its habitat. This is exemplified by the frog *Eleutherodactylus johnstonei* which has been introduced to several Lesser Antillean islands, but may not expand at the expense of local species if the habitat is not disturbed (reviewed in Malhotra and Thorpe, 1999).

The present study addresses the fate of introduced Italian crested newts, *Triturus carnifex*, in the Geneva Basin, Switzerland and their effect on autochthonous northern crested newts, *T. cristatus*. The natural ranges of these two species are parapatric, or narrowly sympatric in some areas of Germany and Austria (Freytag, 1978; Klepsch, 1994; Schmidler, 1976). In Switzerland, their ranges are widely separated, with *T. cristatus* distributed north and *T. carnifex* south of the Alps. The upper altitudinal limit for both species in Switzerland is around 1000 m (Grossenbacher, 1988). The occurrence of *T. carnifex* in the Basin of Geneva, north of the Alps, is thought to be an introduction (Fig. 1), but documentation regarding the introduction is lacking.

Genetic analysis of *T. carnifex* from Geneva, compared with samples across its geographic range, shows that they belong to the western subspecies *T. c. carnifex* and not to the eastern subspecies *T. c. macedonicus*. [The latter subspecies, originally described by Karaman (1922), will be resurrected as a valid taxonomic unit (Arntzen and Wallis, 1999)]. The most likely geographic origin of the introduced stock is Tuscany, northern Italy (J. W. Arntzen, unpublished data).

This study has two purposes: first, to see if there is historical evidence of expansion of the introduced species (*T. carnifex*) at

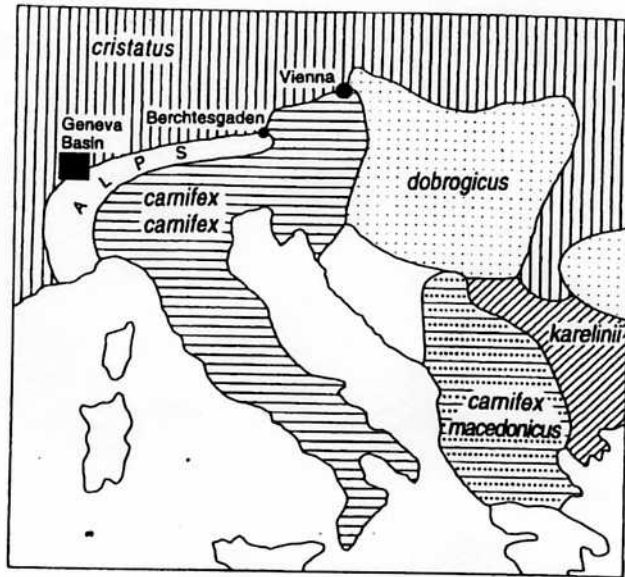


FIG. 1.—Schematic distribution of crested newts (*Triturus cristatus* superspecies) in central and southern Europe. The Geneva Basin study area is detailed in Fig. 2.

the expense of the native species (*T. cristatus*) in the Geneva Basin, characterized by a disturbed habitat; second, to document the nature and extent of genetic interaction between the introduced and native species.

MATERIAL AND METHODS

The study area is the canton of Geneva in Switzerland and adjacent France, together comprising the Geneva Basin (Fig. 2). This basin is dominated by the Rhône and Arve rivers, and bordered by Lac Léman and by mountain ridges associated with the Jura (to the northwest) and the Alps (to the southeast). The region is intensely cultivated and densely populated. The historical aspect of this work is based on material from the Museum of Natural History, Geneva (MHNG) and Jaussi's (1979) survey of the region covering the period 1972–1975 in which he found crested newts in 22 ponds out of 75. All 22 sites that had crested newts in 1975 (Jaussi, 1979) were visited in 1987, as well as new sites that were found by random search or with the help of Swiss and French topographic maps (scale 1/25,000). Ponds were searched for newt eggs, and, where appropriate, newts were captured by dip-netting and light-assisted searches

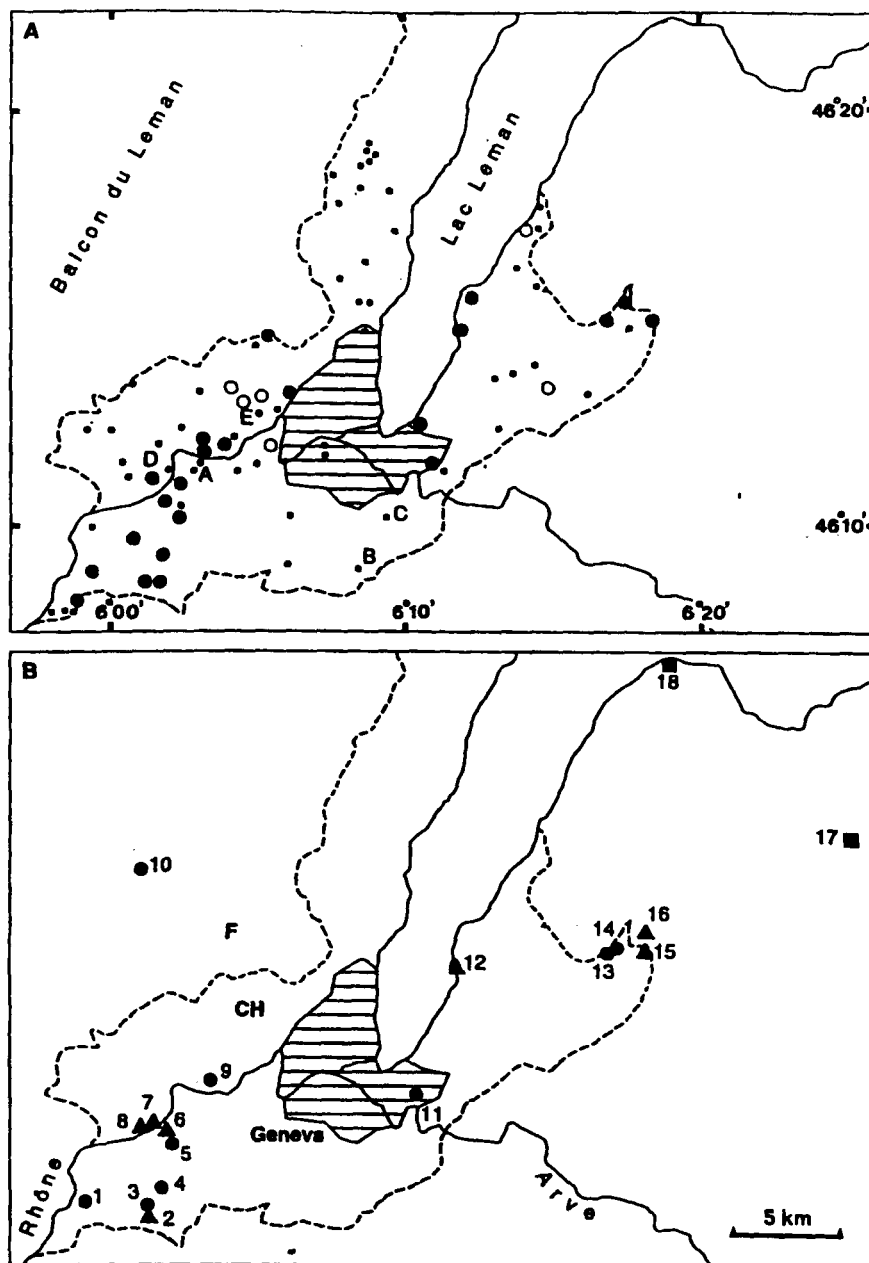


FIG. 2.—Distribution of crested newts in the Basin of Geneva, in (A) 1972–1975, with large dots indicating crested newt sites and small dots indicating ponds and other water bodies with amphibians, but devoid of crested newts (Jaussi, 1979; note that no distinction was made between *Triturus cristatus* and *T. carnifex*). Localities indicated by letters refer to museum records and those reported by Keller et al. (1993) are shown by open dots. (B) Situation in 1987 and 1997, with round, square, and triangular symbols referring to populations of *T. carnifex*, *T. cristatus* and mixed populations, respectively. The interrupted line represents the French (F)–Swiss (CH) border. The numbering and lettering of localities is as in Table 1. The presumed introduction site of *T. carnifex* is number 11.

of the pond shoreline at night. Newts were identified as “typical *T. cristatus*”, “typical *T. carnifex*”, or “intermediate” on the basis of the following features.

External Morphology

Typical individuals of *T. carnifex* have a stout body, large head, well developed

legs, a yellow–orange belly with few, large, round blackish dots with a diffuse edge, and few white lateral spots. In contrast, typical individuals of *T. cristatus* have a slender build, an orange belly, with many, irregular, deep black dots with a distinct edge, and many white lateral spots (Wallis and Arntzen, 1989; Wolterstorff, 1923).

Vertebral Counts

Triturus cristatus typically has 16 rib-bearing vertebrae while *T. carnifex* typically has 15 (Arntzen and Wallis, 1994). The number of rib-bearing vertebrae (RBV) was counted from X-ray photographs of the preserved material from the collections of the Museum of Natural History, Geneva and the Zoological Museum of the University of Amsterdam.

Wolterstorff Index

As an alternative to the vertebral count, Wolterstorff's morphometric index (WI = $100 \times$ forelimb length/interlimb distance) was taken. WI-values are typically lower than 64 (males) or 54 (females) in *T. cristatus* and higher than that in *T. carnifex* (Arntzen and Wallis, 1994, 1999).

Allozyme Markers

North-Italian individuals of *T. carnifex carnifex* and *T. cristatus* have fixed differences at several protein loci. Species-specific character states were revealed for 6-phosphogluconate dehydrogenase (*6-Pgd*) and malic enzyme (*Me*) from muscle tissue, and for alpha-esterase (α -*Est*) from blood plasma. Tissue samples from post-metamorphic newts were obtained in a non-destructive manner, by cutting the tail-tip (Arntzen and Wallis, 1991). Eggs and young embryos of crested newts were collected from aquatic vegetation over the entire accessible part of the breeding sites, or from plastic strings placed in the pond. Species identity of the egg/embryo was based on the inferred *6-Pgd* genotype. Both pre- and postmetamorphic samples were used for the study of genetic variation at *Gpi*. This locus is of limited diagnostic value; the frequency of its most common allele is 0.97 across the range in *T. cristatus* whereas the same allele has an average frequency of 0.80 in *T. carnifex carnifex* and 0.07 in *T. c. macedonicus* (J. W. Arntzen, unpublished data). Electrophoresis followed established protocols. Buffers used were as in Arntzen and García-París (1995): buffer "C" for GPI and 6-PGD and buffer "E" for α -EST and ME. The staining of enzymes followed Shaw and Prasad (1970). Hardy-Weinberg

equilibrium of alleles and genotypic linkage disequilibrium of diagnostic loci were tested using GENEPOP 3.1 (Raymond and Rousset, 1995).

RESULTS

Historical Material

The first actual record of *T. (cristatus) carnifex* from the Basin of Geneva in the collection of the Museum of Natural History of Geneva dates from 1987 (Table 1) although Jaussi (1979) suspected that they were present earlier than this. Prior to this date, all collected material was identified as *T. cristatus cristatus* or as *T. cristatus* [the latter designation is ambiguous because *T. carnifex* has at times been considered a subspecies of *T. cristatus* (Mertens and Wermuth, 1960)]. Some typical specimens of *T. carnifex* and typical specimens of *T. cristatus*, with RBV counts matching the external morphology, are present in lot MHNG 718.93 from "Geneva" (Fig. 3). The date (and exact locality) of collection of this syntopic sample is unknown, but it was registered in the Museum in 1908 (J. Mariaux, personal communication). We identified lot MHNG 877.89, collected in 1945–1947 at Aire (= Aire-la-Ville; Jaussi, 1979) as *T. carnifex* on the basis of external morphology and low vertebral counts (RBV 14–15, in 14 specimens studied). However, low WI-scores outside the range of *T. carnifex* were observed in two specimens. We assigned all post-war samples from the MHNG to *T. carnifex*, with the exception of one female (MHNG 901.59) from Troinex that has a RBV count of 16 and a morphology of *T. cristatus* (Fig. 3).

1987 Distribution

In 1987, crested newts were found at 11 of the 22 previously reported localities (Jaussi, 1979:Fig. 2). No new crested newt localities were found in Switzerland, but four were found in the adjacent region of France (Fig. 2B). The sample size averaged 20.5 for adults plus juveniles (eight samples, range 1–121) and 114 for eggs plus embryos (11 samples, range 7–465). Based on the aforementioned distinguishing criteria, eight samples were exclusively *T. carnifex*, two samples were *T. cristatus*,

TABLE 1.—Crested newt localities in the Basin of Geneva. Upper panel—surveys of 1987 and 1997; lower panel—museum records. Methods for species identification are: a = external morphology; b = vertebrae count; c = Wolterstorff-index; d = three diagnostic enzyme loci in adults or juveniles; and e = one diagnostic enzyme locus in eggs and young embryos. Country codes are: F = France and CH = Switzerland.

Pond number	Locality name	Country	Description	n	Identification method	Inferred species composition
1	1997 Champslong	CH	Large polluted ponds in operational quarry	2	e	<i>carnifex</i>
2	Champs Grillet I	CH	Small pond in operational quarry	334	e	Mixed
3	Champs Grillet II	CH	Shallow pond in operational quarry	15	e	<i>carnifex</i>
4	Laconnex	CH	Large ponds in abandoned quarry, designated nature reserve	142	e	<i>carnifex</i>
5	Petite Grave	CH	Two large, polluted ponds in operational quarry	1	ad	<i>carnifex</i>
6	Moulin de Vert	CH	Shallow pond in abandoned quarry, designated nature reserve	12	ad	Mixed
7	Verbois	CH	Large pond in abandoned quarry, designated nature reserve	586	abcde	Mixed
8	1997 Verbois	CH	Newly dug small pond in abandoned quarry, designated nature reserve	50	acd	Mixed
9	Maisson Carrée	CH	Flooded arable fields (no newts found in quarry pit)	7	e	<i>carnifex</i>
10	Clos de Suet	F	Quarry pond, subject to desiccation	21	e	<i>carnifex</i>
11	Malagnou	CH	Garden pond—presumed introduction site	3	a	<i>carnifex</i>
11	1997			15	acd	<i>carnifex</i>
12	La Pointe à la Bise	CH	Margins of Lac Lemman, designated nature reserve	31	ae	<i>carnifex</i>
12	1997			32	acde	Mixed
13	1997 les Rottes	CH	Two 'etangs' (artificial lake) in nature reserve	8	e	<i>carnifex</i>
14	Les Pres de Vilette	CH	Etang in nature reserve	24	e	<i>carnifex</i>
15	Les Trembles	CH	Two etangs in nature reserve	50	ae	Mixed
15	1997			15	e	Mixed
16	Les Etolles	F	Etang with a set of small satellite ponds	127	abcde	Mixed
16	1997			8	acd	Mixed
17	Lully	F	Etang	63	e	<i>cristatus</i>
18	La Pénatière	F	Garden pond	1	a	<i>cristatus</i>
Pond number	Locality name	Country	MHNG collection number and collection details	n	Identification method	Inferred species composition
—	Geneva	CH	588.91, no date, by E. Pictet	2	ab	<i>cristatus</i>
—	Geneva	CH	718.93, no further data	7	ab	Mixed
—	Geneve	CH	718.95, 1903 by C. Mottaz	1	ab	<i>cristatus</i>
—	Geneve	CH	815.17, 1902 by V. Fatio	2	ab	<i>cristatus</i>
A	Aire	CH	877.89, 1945–1947 by E. Dottrens	26	ab	<i>carnifex</i>
B	Monthoux nr. Troinex	CH	901.59, 1952 by E. Dottrens	1	ab	<i>cristatus</i>
C	Vessy	CH	903.93, 1954 by V. Aellen	1	ab	<i>carnifex</i>
C	Vessy	CH	908.90, 1952 by P. Strinati	2	ab	<i>carnifex</i>
C	Vessy	CH	929.100, 1953 by E. Dottrens	1	ab	<i>carnifex</i>
D (= 7)	Russin	CH	2464.45–49, 1987 by J. W. Amtzen	5	ab	<i>carnifex</i>
E	Vernier	CH	2494.74–76, 1989–1990 by A. Keller	3	ab	<i>carnifex</i>

and five samples were mixed (Table 1). Al-
lotopic individuals of *T. carnifex* were
found in each of the pond clusters, at the
shore of Lac Lemman, in the suburbs of Ge-
neva (including the presumed introduction

site, pond no. 11: see Discussion) and in
the foothills of the "Balcon du Lemman"
mountain range. Syntopic populations of *T.*
carnifex-*T. cristatus* with both species or
individuals of intermediate phenotype (or

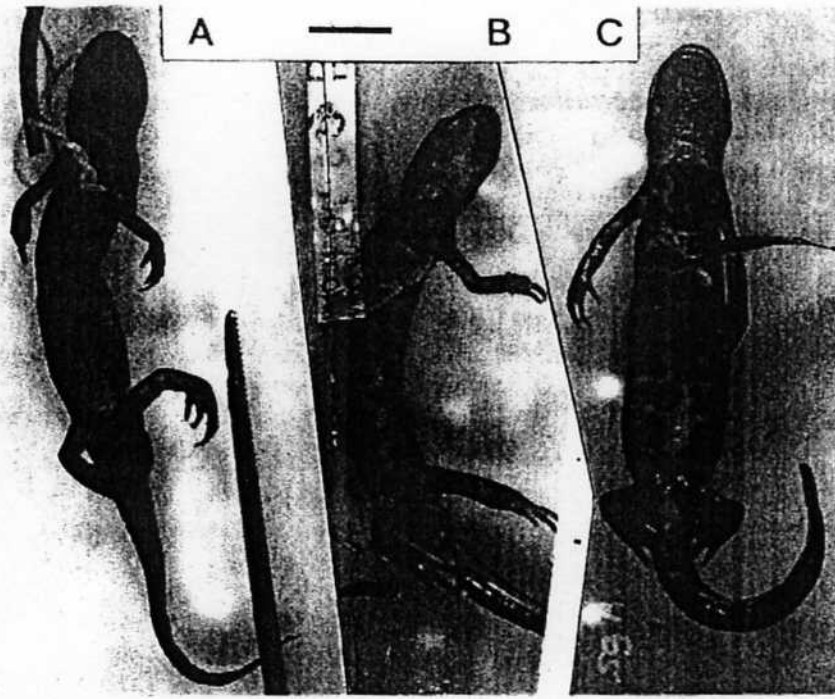


FIG. 3.—Preserved (A) *Triturus carnifex* and (B) *T. cristatus* from Geneva (MHNG 718.93) and *T. cristatus* from Troinex (C, MHNG 901.59). Scale bar represents 1 cm. The specimen to the left has the few, large, rounded black ventral spots with diffuse edge that are typical for *T. carnifex*. The specimens in the middle and to the right possess a ventral pattern typical for *T. cristatus*, with many small black spots [aggregated dots have an irregular shape due to the growth and merger of individual dots—see Arntzen and Teunis (1993) for a documented example]. The ventral coloration could not be determined in these alcohol preserved specimens. For an evaluation of morphometric and meristic data, see text.

both) were found at sites 6, 7, and 16. At sites 2, 7, and 15, most eggs and embryos were identified as belonging to *T. carnifex* and only a few (<5%) could be allocated to *T. cristatus*. Allotopic *T. cristatus* was only found at the northeastern fringe of the study area (Fig. 2B).

A large number of eggs and 121 adults with a phenotype of *T. carnifex* were observed in pond 7. The minimum size of the population was estimated as \hat{n} is approximately 1900 using Edwards' (1974) modification of Bell's (1974) nil-recapture method for two samples (lower two-unit support limit of the maximum likelihood function).

1997 Distribution

In 1997, crested newts were recorded at seven localities. The mean sample sizes were 19.0 for adults plus juveniles (four samples, range 3–50) and 13.4 for eggs plus embryos (four samples, range 2–29). Four of these sites had newts in 1987, two sites had newts recorded by Jaussi (1979)

but not in 1987, and one new site was found (Table 1). No substantial temporal differences were observed in species composition of populations or in the relative distributions of the two species, with the exception of pond 12 where, in 1987, *T. carnifex* was found exclusively, whereas in 1997, a 6-*Pgd* allele indicative of *T. cristatus* was observed in one egg and in one adult. Because the results of both surveys (1987 and 1997) largely coincide, they can be considered jointly (Table 1, Fig. 2). Eleven newt localities were grouped in three clusters with small distances (<1.5 km) between ponds. The other seven localities were more or less isolated with an average distance of 8 km to the nearest known crested newt locality. However, some sites were unoccupied in either 1987 or 1997.

Genetic Analysis

Eggs and embryos were studied in 15 samples from 13 populations. Ten samples were monomorphic for 6-*Pgd*, showing ei-

TABLE 2.—Genetic variation at four enzyme loci in crested newts from the Basin of Geneva with the observed frequency of the most common allele. Upper panel—egg and embryo samples; lower panel—postmetamorphic newts. Reference samples indicated that alleles at the loci *6-Pgd*, *α-Est* and *Me* have diagnostic properties, while in *Gpi* the most frequent allele has a higher frequency in *T. carnifex* than in *T. cristatus* (J. W. Arntzen, unpublished). HW is the test result for Hardy-Weinberg equilibrium using the Fisher exact test as implemented in GENEPOP 3.1 (Raymond and Rousset, 1995). Samples are from 1987, unless indicated otherwise.

Locality	n	<i>6-Pgd</i>	HW	<i>Gpi</i>	HW	<i>α-Est</i>	HW	<i>Me</i>	HW
1	1997	2	1.00						
2		334	0.980	***	0.94	**			
3		15	1.00		0.87	NS			
4		142	1.00		0.92	NS			
7		465	0.998	***	0.82	NS			
9		7	1.00		0.79	NS			
10		21	1.00		0.95	NS			
12		30	1.00		0.70	NS			
12	1997	29	0.98	NS	0.72	NS			
13	1997	8	1.00		0.75	NS			
14		24	1.00		0.69	NS			
15		48	0.96	***	0.83	NS			
15	1997	15	0.97	NS	0.83	NS			
16		104	1.00		0.78	*			
17		63	0.00		0.99	NS			
6		12	0.75	NS	0.83	NS	1.00	0.79	*
7		71	0.94	***	0.76	NS	0.98	NS	1.00
8	1997	50	1.00		0.77	NS	0.95	NS	1.00
11	1997	15	1.00		0.83	NS	0.97	NS	1.00
12	1997	3	0.83	NS	0.67	NS	1.00		1.00
16		23	1.00		0.70	NS	1.00		1.00
16	1997	8	1.00		0.75	NS	0.81	NS	1.00

NS = no significant deviation from expectations under Hardy-Weinberg equilibrium, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

ther the character state typical for *T. carnifex* ($n = 9$) or *T. cristatus* ($n = 1$); a polymorphism was observed in five samples (Table 2). Among the latter samples, a significant deviation from Hardy-Weinberg equilibrium was observed in three, in each case involving a deficit of heterozygotes ($P < 0.001$). All but one of the egg/embryo samples were polymorphic at the *Gpi* locus. A significant deficit of heterozygotes was found in population 2 ($P < 0.01$) and population 16 ($P < 0.05$). A rare allele at the *Gpi* locus was found in populations 2 (frequency 2.1%), 8 (1.0%), 11 (3.3%), and 16 (1.2%).

Hybridization of postmetamorphic newts was studied in seven samples from six localities using three species diagnostic enzyme loci: *6-Pgd*, *α-Est*, and *Me* (Table 2). Five specimens from pond 6 (frequency 42%), eight specimens from pond 7 (11%), a single specimen from pond 12, and three specimens from pond 16 (10%) possessed one or two alleles typical for *T. cristatus* at either locus. A significant def-

icit of heterozygotes was observed at the *Me* locus in population 6 ($P < 0.05$) and at the *6-Pgd* locus in population 7 ($P < 0.001$). A rare allele at the *α-Est* locus was found in population 8 (frequency 0.5%). Tests for linkage disequilibrium between *6-Pgd* and *Me* (population 6) and between *6-Pgd* and *α-Est* (population 7) yielded non-significant results ($P > 0.05$ in both cases).

The anticipated close association between genetic profile at three loci (specimens with or without alleles of *cristatus*) with external morphology class membership (*cristatus* and intermediate versus *carnifex*), vertebral counts (a score of 16 or 15), and morphometric description (low versus high WI-values) was not confirmed (Table 3). Using external morphology and Wolterstorff index, specimens possessing one or more alleles of *T. cristatus* were not identified as *T. cristatus* or intermediate more frequently than could be expected by chance alone (G -test for goodness-of-fit for morphological identification: $G = 0.48$,

TABLE 3.—Correspondence between methods of identification—enzyme markers, external morphology, and Wolterstorff index—in crested newts from the Geneva Basin. Vertebral counts are tightly correlated with the Wolterstorff index (Arntzen and Wallis, 1994) and not here considered.

Pond (year)	<i>T. cristatus</i> allele(s) absent			<i>T. cristatus</i> allele(s) present		
	n	External morphology	Wolterstorff index	n	External morphology	Wolterstorff index
6 (1987)	7	29%	¶	5	100%	¶
7 (1987)	63	100%	§	8	60%	§
8 (1997)	47	100%	79%	3	0%	67%
16 (1987)	23	57%	86%	0	—	—
16 (1997)	5	100%	80%	3	100%	67%
Mean		90%	81%		42%	67%

¶ Criterion not available for juveniles.

§ No data.

$P > 0.05$). Specimens without alien alleles (i.e., observed "pure" *T. carnifex*) were identified by phenotype as *T. carnifex* more frequently than could be expected by chance alone ($G > 30$ and $P < 0.001$ in both cases). The hypothesis that samples with *T. cristatus* would be less polymorphic at the *Gpi* locus than samples without *T. cristatus* (because heterozygosity at the *Gpi* locus on average is lower in *T. cristatus* than in *T. carnifex*) was not supported (Mann-Whitney *U*-test, $Z = 0.74$, $P > 0.05$).

The four categories in which Geneva Basin populations of crested newts were categorized (*T. carnifex* exclusively, populations of *T. carnifex* with some *T. cristatus* or influences of *T. cristatus*, syntopic populations with or without indications for interspecific gene flow, and *T. cristatus* exclusively) show a spatial pattern in which the component of *T. carnifex* decreases in the direction away of the presumed introduction site (Fig. 2).

DISCUSSION

A significant heterozygote deficit at marker loci was observed five times, indicating the existence of some non-panmictic populations. No linkage disequilibrium was identified in two samples appropriate for testing. The most straightforward explanation for deviation from Hardy-Weinberg equilibrium would be the assortative mating of newts with similar genetic backgrounds, but selection against heterozygotes (i.e., offspring from *T. carnifex* and *T. cristatus*) or atypical patterns of gene

expression (such as observed in hybrids between the newts *T. helveticus* and *T. vulgaris*: Arntzen et al., 1998) cannot be ruled out. The observations indicate a decoupling among the various classes of species markers in mixed newt populations of *T. cristatus*-*T. carnifex*. Indeed, methods of identification yielded poorly congruent results. Hybridization and backcrossing in ancestral populations and breaking up of the original gene complexes of *T. carnifex* and *T. cristatus* by recombination, may account for these observations. A comprehensive survey would require recognition of parental types and backcrosses and would encompass the analysis of linkage disequilibria (Abernethy, 1994; Shapiro, 1998). This approach would require more genetic and phenotypic markers than are currently available. However, it is clear that the close evolutionary relationship of the introduced species to the native species has given this introduction a genetic dimension that would have been lacking in unrelated species.

Crested newt populations that have been recorded have steadily decreased in the Geneva Basin, from 22 known sites in the mid-seventies to 15 in 1987, to seven in 1997. Consequently, the average distance between ponds has increased to perhaps beyond the maximum lifetime dispersal distance of a crested newt, and may have decreased chances for the survival and recolonization of local populations (Halley et al., 1996). Surprisingly, the ponds closest to one another (ponds 6 and 7 with a 500-m distance between them)

had very different species compositions. One would expect the small, mixed population 6 to have been rapidly "swamped" by dispersal from the very large (in 1987) and successfully reproducing population of *T. carnifex* in pond 7 (now extinct, probably due to the introduction of predatory fish). The Rhône river may act as a barrier to dispersal between these two populations. The fate of the site 6 population is difficult to determine. The breeding pond was located in a quarry that was recently remodelled, including creation of a lake for fish and wildfowl that replaced a small pond suitable for newt reproduction. Quarries with little or no vegetation constitute a habitat in which *T. carnifex* may thrive, but which is marginal for *T. cristatus* (J. W. Arntzen, unpublished observations). The introduction of plastic oviposition strings in quarry ponds revealed the presence of eggs of *T. carnifex*, sometimes in large numbers. One does not normally find *T. cristatus* in large numbers in such, often polluted, ponds with no or little aquatic vegetation. Consequently, this change in habitat may have rendered the native species less able to compete with the introduced *T. carnifex*.

Museum records demonstrated the presence of *T. carnifex* in the Geneva Basin from early in this century. This contradicts anecdotal information indicating the origin of non-native newts from an introduction at site 11, the garden grounds of the Biological Station at Malagnou, Chêne-Bourg (together with introduced *Triturus vulgaris meridionalis*, also from southern Europe) by E. Guyénot during the post-war period (Prof. Krimbas, personal communication, 1989). The first publication on amphibians by Prof. Guyénot, based on "Zoological Record" citations, is from 1922, which differs from the crucial museum record by at least 14 years. Lack of reliable information renders reconstruction of the historical biogeography of introduced *T. carnifex* difficult. However, the general pattern is unequivocal. In the 20th century [i.e., over 30–40 generations (Francillon-Vieillot et al., 1990; Cvetković et al., 1996)], *T. carnifex* has established itself in the Geneva Basin, presumably at

the expense of *T. cristatus*. The process has been relatively slow, much slower than the 1 km a year spread of *T. cristatus* superseding *T. marmoratus* in a similar contact zone in western France (Arntzen and Wallis, 1991). Indeed, no marked change in species composition was observed at the mixed site 16 from 1987 to 1997, even though a rapid turnover from *T. cristatus* to *T. carnifex* was described in a population at the German–Austrian border north of Salzburg (Umlandt, 1985). Modelling the spread of an invading organism indicates that even an inferior, but established, competitor can significantly reduce the invasion spread (Hart and Gardner, 1997).

The presence of genes of *T. cristatus* in *T. carnifex* demonstrates past hybridization events and can be interpreted as "genetic footprints", sensu Scribner and Avise (1993). It is difficult to protect *T. cristatus* from this hybridization, because individuals of *T. carnifex* are themselves protected by law because they are native elsewhere in Switzerland. However, the main threat to the autochthonous *T. cristatus* may not be that its genetic integrity is compromised, but that loss or modification of its habitat renders it unable to compete with the introduced species. These multiple factors demonstrate how diverse and complex are human influences on amphibian populations.

The genetic mixing of *T. cristatus* and *T. carnifex* in the Basin of Geneva is incomplete. Nevertheless, the genetic compatibility of the taxa and the inferred fertility of *T. carnifex* × *T. cristatus* hybrids constitute an argument against their recognition as species under the biological species concept. To gain further insight into this problem, data should be collected from their natural geographic contact. Unfortunately this will be difficult, because the contact zone of *T. carnifex* and *T. cristatus* is relatively short (running from approximately Berchtesgaden in the west to Vienna in the east: see Fig. 1) and in most parts heavily disturbed by large scale environmental change.

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LITERATURE CITED

- ABERNETHY, K. 1994. The establishment of a hybrid zone between red and sika deer (genus *Cervus*). *Molecular Ecology* 3:551–562.
- ANONYMOUS. 1997. Exotic Reptiles and Amphibians in the Wild. FrogLife Advice Sheet 8. Triton House, Halesworth, U.K.
- ARANO, B., G. LLORENTE, M. GARCÍA-PARÍS, AND P. HERRERO. 1995. Species translocation menaces Iberian waterfrogs. *Conservation Biology* 9:196–198.
- ARNTZEN, J. W., AND M. GARCÍA-PARÍS. 1995. Morphological and allozyme studies of midwife toads (genus *Alytes*), including the description of two new taxa from Spain. *Contributions to Zoology* 65: 5–34.
- ARNTZEN, J. W., AND S. F. M. TEUNIS. 1993. A six year study on the population dynamics of the crested newt (*Triturus cristatus*) following the colonization of a newly created pond. *Herpetological Journal* 3:99–110.
- ARNTZEN, J. W., AND G. P. WALLIS. 1991. Restricted gene flow in a moving hybrid zone of the newts *Triturus cristatus* and *T. marmoratus* in western France. *Evolution* 45:805–826.
- . 1994. The 'Wolterstorff-index' and its value to the taxonomy of the crested newt superspecies. *Abhandlungen und Berichte für Naturkunde, Magdeburg* 17:57–66.
- . 1999. Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): data from morphology and mitochondrial DNA. *Contributions to Zoology*: In press.
- ARNTZEN, J. W., P. DE WIJER, R. JEHLE, E. SMIT, AND J. SMIT. 1998. Rare hybridization and introgression in Smooth and Palmate newts (Salamandridae: *Triturus vulgaris* and *T. helveticus*). *Journal for Zoological Systematics and Evolutionary Research* 36: 111–122.
- BELL, G. 1974. Population estimates from recapture studies in which no recaptures have been made. *Nature* 248:616.
- BURKE, R. L. 1991. Relocations, repatriations, and translocations of amphibians and reptiles: taking a broader view. *Herpetologica* 47:350–357.
- CVETKOVIĆ, D., M. L. KALEZIĆ, A. DJOROVIĆ, AND G. DŽUKIĆ. 1996. The crested newt (*Triturus cristatus*) in the Submediterranean: reproductive biology, body size, and age. *Italian Journal of Zoology* 63:107–111.
- DODD, C. K., AND R. A. SEIGEL. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? *Herpetologica* 47:336–350.
- EASTAL, S. 1981. The history of introductions of *Bufo marinus* (Amphibia: Anura): a natural experiment in evolution. *Biological Journal of the Linnean Society* 16:93–113.
- ECHELLE, A. A., AND A. F. ECHELLE. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and Sheepshead minnow. *Conservation Biology* 11:153–161.
- EDWARDS, A. W. F. 1974. Population estimates from recapture studies. *Nature* 252:509–510.
- FRANCILLON-VIEILLOT, H., J. W. ARNTZEN, AND J. GÉRAUDIE. 1990. Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela): a skeletochronological comparison. *Journal of Herpetology* 24:13–22.
- FREYTAG, G. E. 1978. Über *Triturus cristatus* bei Salzburg (Amphibia: Caudata: Salamandridae). *Salamandra* 14:45–46.
- GRIFFITHS, H. I., A. DAVISON, AND J. BIRKS. 1996. Species reintroductions. *Conservation Biology* 10: 923.
- GROSSENBACHER, K. 1988. Verbreitungsatlas der Amphibien der Schweiz. *Documenta faunistica Helvetiae* 7:1–207.
- HALLEY, J. M., R. S. OLDHAM, AND J. W. ARNTZEN. 1996. Predicting the persistence of amphibian populations with the help of a spatial model. *Journal of Applied Ecology* 33:455–470.
- HART, D. R., AND R. H. GARDNER. 1997. A spatial model for the spread of invading organisms subject to competition. *Journal of Mathematical Biology* 35:935–948.
- JAUSSI, M. 1979. Contribution à l'étude des Batraciens du Bassin genevois. *Travail de Diplôme, Université de Genève, Geneva, Switzerland*.
- KARAMAN, S. 1922. Beiträge zur Herpetologie von Mazedonien. *Glasnik der Kroatischen Naturwissenschaftliche Gesellschaft, Zagreb* 34:591–612.
- KELLER, A., V. AELLEN, AND V. MAHNERT. 1993. Atlas de répartition des amphibiens et reptiles du canton de Genève. *Museum Histoire Naturelle de Genève, Geneva, Switzerland*.
- KLEPSCH, L. 1994. Zur Artdifferenzierung der Kammolche (*Triturus cristatus*—Artenkreis) im Waldviertel: morphometrische und molekulargenetische Untersuchungen. *Diplomarbeit, Universität Wien, Vienna, Austria*.
- LEVER, C. 1979. *The Naturalized Animals of the British Isles*. Paladin Granada, London, U.K.
- . 1994. *Naturalized Animals: the Ecology of Successfully Introduced Species*. Poyser Natural History, London, U.K.
- MALHOTRA, A., AND R. S. THORPE. 1999. *Reptiles and Amphibians of the Eastern Caribbean*. MacMillan, London, U.K.
- MALKMUS, R. 1995. *Die Amphibien und Reptilien Portugals, Madeiras und der Azoren—Verbreitung, Ökologie, Schutz*. Die Neue Brehm Bücherei 621. Westarp Wissenschaften, Magdeburg, Germany.
- MERTENS, R., AND H. WERMUTH. 1960. *Die Amphibien und Reptilien Europas*. Waldemar Kramer, Frankfurt am Main, Germany.
- REINERT, H. K. 1991. Translocation as a conservation strategy for amphibians and reptiles: some com-

- ments, concerns, and observations. *Herpetologica* 47:357–363.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249. [version 3.1b, 1997].
- SCHMIDTLER, J. F. 1976. Die bemerkenswerten Kammolche (*Triturus cristatus*) des Berchtesgadener Landes. *Salamandra* 12:32–36.
- SHAW, C. R., AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochemical Genetics* 4:297–320.
- SCRIBNER, K. T., AND J. C. AVISE. 1993. Cytonuclear genetic architecture in mosquitofish populations and the possible roles of introgressive hybridization. *Molecular Ecology* 2:139–149.
- SHAPIRO, L. H. 1998. Hybridization and geographic variation in two meadow katydid contact zones. *Evolution* 52:784–796.
- STUMPEL, A. H. P. 1992. Successful reproduction of introduced bullfrogs *Rana catesbeiana* in northwestern Europe: a potential threat to indigenous amphibians. *Biological Conservation* 60:61–62.
- UMLANDT, G. 1985. Neuer Fundort von *Triturus cristatus carnifex*. *Sauria* 7:27–28.
- WALLIS, G. P., AND J. W. ARNTZEN. 1989. Mitochondrial-DNA variation in the crested newt superspecies: limited cytoplasmic gene flow among species. *Evolution* 43:88–104.
- WOLTERSTORFF, W. 1923. Übersicht der Unterarten und Formen des *Triton cristatus* Laur. *Blätter für Aquarien- und Terrarienkunde*, Stuttgart 34:120–126.

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A NEW SPECIES OF *OSTEOCEPHALUS* (ANURA: HYLIDAE) FROM AMAZONIAN ECUADOR AND PERU

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ABSTRACT: A new species of the hylid frog genus *Osteocephalus* from the upper Amazon Basin of Ecuador and Peru is described. It most closely resembles *O. planiceps*, but it differs in the absence of pale stripes on the heels and above the vent, in being smaller, and lacking brown spots on the flanks. The new species, *O. yasuni*, is unique within *Osteocephalus* in having extensive yellow ventral coloration.

Key words: Anura; Hylidae; *Osteocephalus yasuni*; New species; Ecuador; Peru

TRUEB and Duellman (1971) reviewed the genus *Osteocephalus* and recognized five species: *O. buckleyi* (Boulenger, 1882), *O. leprieurii* (Duméril and Bibron, 1841), *O. pearsoni* (Gage, 1929), *O. taurinus* Steindachner, 1862, and *O. verruciger* (Werner, 1901). Subsequently, Duellman (1974) transferred *Hyla langsdorffii* Duméril and Bibron, 1841, into *Osteocephalus*, and three new species were described—*O. elkejungingerae* (Henle, 1981), *O. subtilis* Martins and Cardoso, 1987, and *O. oophagus* Jungfer and Schie-

sari, 1995. Duellman and Hoogmoed (1992) transferred *Hyla rodriguezii* Rivero, 1968 to *Osteocephalus*, and five additional species belonging to the *O. rodriguezii* group were described by Ayarzagüena et al. (1992). Ayarzagüena (1992) transferred all the species of the *O. rodriguezii* group to the new genus *Tepuihyla*. Duellman and Mendelson (1995) resurrected two species: *O. planiceps* (Cope, 1874) from the synonymy with *O. taurinus*, and *O. cabrerai* (Cochran and Goin, 1970) from the synonymy with *O. buckleyi*. At present, the genus contains 11 species.

Many unresolved alpha-level taxonomic problems persist within *Osteocephalus*. There are at least three undescribed spe-

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