

# EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY  
THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. 45

June, 1991

No. 4

*Evolution*, 45(4), 1991, pp. 805–826

## RESTRICTED GENE FLOW IN A MOVING HYBRID ZONE OF THE NEWTS *TRITURUS CRISTATUS* AND *T. MARMORATUS* IN WESTERN FRANCE

J. W. ARNTZEN<sup>1</sup>

*Institute of Taxonomic Zoology, University of Amsterdam,  
Mauritskade 57, P.O. Box 4766, 1009 AT Amsterdam, THE NETHERLANDS*

AND

G. P. WALLIS<sup>2</sup>

*Department of Zoology, University of Leicester, University Road,  
Leicester LE1 7RH, UK and*

*Department of Zoology, University of Western Australia, Nedlands, WA 6009, AUSTRALIA*

*Abstract.*—Two hybridizing species of newts, *Triturus cristatus* and *T. marmoratus*, with overlapping distributions show a parapatric distribution when surveyed in detail. The factors that govern the distribution of *cristatus* vs. *marmoratus* in the département (province) of Mayenne in western France are identified as forestation and relief. The parapatric hybrid zone running through Mayenne is narrow but widens to approximately 20 km in an area with mixed habitat. In this area most breeding sites are shared and F<sub>1</sub> hybrids form about 4% of the total population.

Analysis of survey data collected about 30 years previously also shows an essentially parapatric distribution. Comparison of past and present distribution maps reveals that *cristatus* has superseded *marmoratus* over large areas in the south of Mayenne. An area where *marmoratus* replaced *cristatus* also exists, but it is more limited in size.

Gene flow between *cristatus* and *marmoratus* is analyzed using 10 diagnostic genetic markers [9 protein loci and mitochondrial (mt) DNA]. In syntopic populations nuclear gene flow is bidirectional with a mean frequency of introgressed alleles ( $f$ ) of 0.3%. In allotopic populations of *cristatus* and *marmoratus* gene flow is present in areas of species replacement ( $f = 0.3\%$ ), while gene flow appears to be absent in those areas that have been continuously occupied by a single species. At the biogeographic level, the presence or absence of introgression is paralleled by the persistence or absence, respectively, of pockets of *cristatus*–*marmoratus* syntopy.

All F<sub>1</sub> hybrids possess the *cristatus* type mtDNA. This may be due to asymmetric interspecific mate choice and would explain the observed absence of introgression of the maternally inherited mtDNA genome in areas where *cristatus* replaced *marmoratus*.

The *cristatus*–*marmoratus* hybrid zone bears characteristics of both the clinal (parapatric) hybrid zone model and the mosaic hybrid zone model. Such a mixed model—for which we propose the term “reticulate hybrid zone”—can be appreciated only if studied over a two-dimensional geographic area and also through time.

*Key words.*—Allozymes, Amphibia, gene flow, hybrid zone, mitochondrial DNA, newts, *Triturus*.

Received August 9, 1990. Accepted November 21, 1990.

<sup>1</sup> Present address: Department of Zoology, University of Leicester, University Road, Leicester LE1 7RH, UK.

<sup>2</sup> Present address: Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand.

Hybrid zones between parapatric species pose numerous interesting evolutionary questions. A more thorough understanding of the dynamics of these zones has come about in recent years through a variety of genetic data including allozyme loci, ribosomal genes, and the mitochondrial genome (Hewitt, 1988; Harrison, 1990). Many hybrid zones can be classified as tension zones (Key, 1968) having arisen through secondary contact of partly differentiated populations. The genetic structure of these has been described by diffusion models, where zone width is determined by the opposing forces of migration and disruptive selection, which tend to broaden and narrow the zone, respectively (Barton and Hewitt, 1985). A transect across a zone under this model is expected to reveal smooth concordant sigmoidal transitions (clines) for the genetic characters examined. Selection acting on a locus can change cline shape and position relative to other clines, as well as giving asymmetric introgression, and may be the cause of such observed effects (Hunt and Selander, 1973; Karlin and Guttman, 1981; Gollmann, 1984, 1991; Arnold et al., 1987). When a zone is considered in an ecological and historical context, then temporal and spatial variation cloud the situation further. Many hybrid zones run along ecotones and may be spatially and temporally patchy with respect to physical and biotic factors, as well as convoluted on a fine scale (Harrison, 1990). If the mosaic nature of the environment impinges on genotypes differentially (Harrison, 1986; Harrison et al., 1987; Rand and Harrison, 1989; Harrison and Rand, 1990), then a single transect study relying on one-dimensional information (Blackwell and Bull, 1978; Green, 1983; Szymura and Barton, 1986) is limited in the conclusions that it can make. Hybrid zones merit study over a two-dimensional geographic area, and if possible over the dimension of time also.

Old World newts of the genus *Triturus* are excellent material for studying problems of genetic isolation in a geographic and historical context. The 12 species in the genus show a wide range of natural hybridization intensities and their distributions and ecology have been studied in detail for many years. Genetic interactions range from broad intergradation of races (*T. italicus*, Ragghi-

anti and Wake, 1986; *T. vulgaris*, Schmidtler and Schmidtler, 1983; Raxworthy, 1990), through hybrid zones (*T. vulgaris*-*T. montandoni*, Pecio and Rafinski, 1985; *T. dobrogicus*-*T. karelini* and other taxa within the *T. cristatus* superspecies, Wallis and Arntzen, 1989; *T. cristatus*-*T. marmoratus*, present study), to cases of occasional hybridization among species with widely overlapping ranges (*T. helveticus*-*T. vulgaris*, Griffiths et al., 1987; and possibly *T. karelini*-*T. vittatus*, Schmidtler and Schmidtler, 1967; Raxworthy, 1990). Hybridization phenomena can thus be related to phylogeny, distribution patterns, ecology, and behavior (Arntzen, 1986b; Arntzen and Sparreboom, 1987, 1989; Macgregor et al., 1990). Such a synthesis is interesting from an evolutionary viewpoint and crucial to a better understanding of the widespread phenomena of hybridization and hybrid zones (Mayr and O'Hara, 1986; Hewitt, 1988).

*Triturus cristatus* and its close relatives in the *T. cristatus* superspecies have contiguous distributions over Europe and adjacent Asia, but do not occur in northern Scandinavia, southern France, and the Iberian Peninsula (Wallis and Arntzen, 1989). *Triturus marmoratus* has a southwestern European distribution that overlaps with that of *T. cristatus* over an area up to 300 km wide in central France (Zuiderwijk, 1989). The species display profound differences in morphology and a number of life history parameters, many of which can be regarded as adaptations to different habitats, *T. cristatus* being a more aquatic species than *T. marmoratus* (Table 1). There are also substantial molecular differences in their albumins (Busack et al., 1988; albumin immunological distance 5-17 units), isozymes (Rafinski and Arntzen, 1987; Nei's  $D = 0.86 \pm 0.27$ ), and mtDNA (present study;  $d = 0.075-0.083$ ).

Newts have a long aquatic reproductive period each year with synchronous, multiple matings. Breeding usually takes place in standing water such as field ponds, forest ponds, fens, and reservoirs. In some areas in central France, *T. cristatus* and *T. marmoratus* share breeding sites and hybridize. Heterospecific pairs may give rise to viable offspring, but positive assortive mating reduces hybridization (Zuiderwijk, 1986).

TABLE 1. Summary of selected comparative data for *Triturus cristatus* and *T. marmoratus* in an area of range overlap in western France.

	<i>T. cristatus</i>	<i>T. marmoratus</i>	References
<b>Morphology</b>			
Body form and coloration	Large bodied, moderately slender build, basic color dark brown, ventral surface bright orange with irregular black spots, white stipples laterally	Large bodied, heavy build, basic color black, dorsally thickly mottled or blotched with green, ventrally many white stipples	
Total length, mean	Males 115 mm, females 127 mm	Males 120 mm, females 140 mm	Vallée (1959)
Snout-vent length, mean	Males 63 mm, females 70 mm	Males 62 mm, females 68 mm	Francillon-Vieillot et al. (1990)
Number of presacral vertebrae	17-18	14-15	Vallée (1959)
<b>Life history parameters</b>			
Age of sexual maturation	2-3 years	3-5 years	Francillon-Vieillot et al. (1990)
Modal age	3 years	6 years	Francillon-Vieillot et al. (1990)
Longevity	14 years	14 years	Francillon-Vieillot et al. (1990)
Length of reproductive period, mean	2.5 years	4 years	Arntzen and Hedlund (1990)
Annual female fecundity, mean	190 eggs	420 eggs	Arntzen and Hedlund (1990)
Lifetime female fecundity, mean	500 eggs	1,700 eggs	Arntzen and Hedlund (1990)
<b>Ecological characteristics</b>			
Terrestrial habitat	Relatively flat and open terrain, devoid of much shelter	Hilly and forested terrain with much shelter	Schoorl and Zuiderwijk (1981)
Aquatic habitat	Field ponds, fens, reservoirs	Field ponds, springs, forest ponds	Schoorl and Zuiderwijk (1981)
Length of aquatic phase	March to July-August	March to June-July	Bouton (1986); Arntzen (unpubl.)
<b>Ethological characteristics</b>			
Courtship behavior	"Cat-buckle" and "rocking" frequent, "tail-lash" infrequent in a strategy of sexual interference	"Cat-buckle" and "tail-lash" infrequent, "rocking" negligible in a strategy of sexual defense	Sparreboom (1986); Green (1989); Zuiderwijk (1990)
Juvenile life	Semiaquatic	Terrestrial	Francillon-Vieillot et al. (1990)

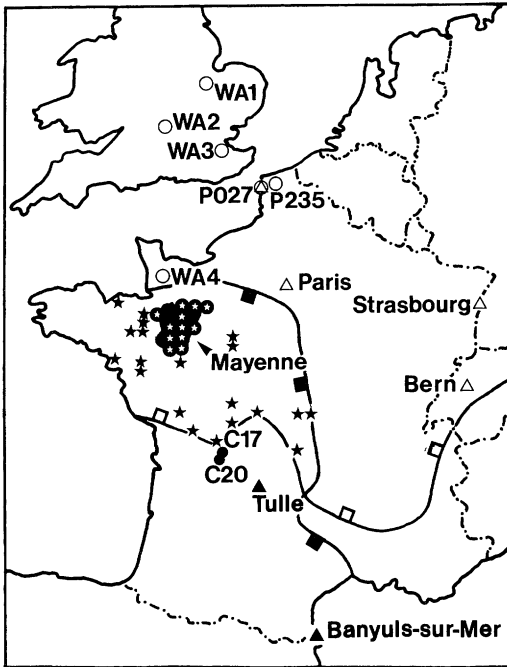


FIG. 1. Approximate range borders of *Triturus cristatus* and *T. marmoratus* in western Europe (Grossenbacher, 1987; Zuiderwijk, 1989). Square symbols are placed at the inner side of the species' range, open symbols for *T. cristatus*, solid symbols for *T. marmoratus*. In black: the département Mayenne, France. Natural hybrids have been recorded in 32 grid blocks (stars; for details see text). Circles and triangles indicate allopatric populations where reference samples were taken for molecular and morphological studies, respectively.

Since the discovery of the nature of the hybrid (once considered a valid species, "*T. blasii*"), many successful laboratory crosses have been undertaken. However, records of naturally occurring hybrids are relatively scarce. From the literature (references #71, 883, 1569, 2466, 2487, 2939, 2941 and 3121 listed in Parent, 1982; Le Garff, 1984; Zuiderwijk, 1989; and present study) 91 independent hybrid sites were traced, of which 59 are located in the département (province) Mayenne. Hybrids are recorded throughout the area of range overlap (Fig. 1).

The aims of the study were (1) to describe the distribution of the hybridizing newts *Triturus cristatus* and *T. marmoratus* in western France, (2) to relate the local distribution of the newts to the habitat preferences of the species, and (3) to analyze gene flow between these Linnean species in

an ecological, biogeographic, and historical context. Interspecific gene flow was examined using both nuclear (allozyme) and cytoplasmic (mtDNA) markers. A preliminary account covering 30% of the electrophoretic data has been published (Arntzen, 1986a). mtDNA is especially useful for tracing introgression as it is reported to penetrate species boundaries more readily, and it is inherited intact through the female germ line (Harrison, 1989). The study was inspired by a detailed inventory of *T. cristatus* and *T. marmoratus* (Schoorl and Zuiderwijk, 1981) and by Vallée's (1959) suggestion of morphological intergradation on the basis of osteological analyses. The present study shows that interspecific gene flow may be a function of both the degree of sympatry, which is in turn related to local environment, and movement of the (parapatric) hybrid zone, as documented over a 30-year period.

In this study the term hybrid zone will be used in a broad sense as formulated by Harrison (1990): "Hybrid zones are spatially bounded interactions between genetically distinct groups of individuals resulting in at least some offspring of mixed ancestry. Allopatric populations of the two genetically distinct groups are found outside the zone of interaction." In this paper a parapatric hybrid zone is defined as a spatially continuous contact area of potentially hybridizing taxa that have contiguous or slightly overlapping distributions as documented from a detailed inventory.

#### MATERIALS AND METHODS

*Study Area and Ecological Differentiation.*—Newts were studied in the département Mayenne (henceforth Mayenne) in western France. There are three major landscape types (denoted area A, B, and C in Fig. 2), which relate to the distribution of *cristatus* and *marmoratus* (Schoorl and Zuiderwijk, 1981). To confirm their analysis, two ecological parameters, relief and forestation, were studied for 154 newt sites all over Mayenne. High detail topographic maps (1:25,000) were used for this purpose (Institut Géographique National, 1971, 1973, 1975–1978). Measurements were made over a circular area round the pond, with a radius of 4 cm. A neighborhood range

of this size has biological significance as it is of the same order of magnitude as our dispersal rate estimate for these newts (see below).

The parameter relief was summarized from the mapped altitude contours. The parameter "forestation" was quantified by measuring (1) the length of hedgerows in the landscape ( $\text{km km}^{-2}$ ), and (2) the proportion of the area forested. These measures were combined into a single one by setting complete forest cover equal to the highest observed density score for hedges ( $15 \text{ km km}^{-2}$ ).

*Past and Present Distribution.*—The most recent inventory of *crystatus* and *marmoratus* in Mayenne has been carried out in 1979–1980 by Schoorl and Zuiderwijk (1981). Their original dot map is presented here as a continuous distribution map, consisting of a mosaic of contiguous polygons (Fig. 4). Each individual polygon ("Dirichlet cell") represents the area surrounding a site that is closer to it than to any other sample site (Matérn, 1979). This method involves a certain amount of extrapolation, as is the case with all continuous distribution maps. The map is based, however, on a detailed inventory and the amount of extrapolation is small with regard to the overall distribution pattern (mean radius of extrapolation is 3.9 km).

To compare the distribution of *crystatus* and *marmoratus* with the situation 25–35 years earlier, the data of Vallée (1959 Fig. 2, with modifications from Tables 1 and 2) are analyzed in the same way. Like that of Schoorl and Zuiderwijk (1981), the inventory of Vallée (1959) comprises Mayenne almost entirely, but is slightly less detailed (mean radius of extrapolation is 4.4 km). The mean sample sizes for adults are comparable ( $\bar{N} = 9.1$ ,  $SD = 17.6$ , Vallée, 1959 Table 2;  $\bar{N} = 12.6$ ,  $SD = 12.6$ , J. Schoorl, pers. comm.). Regarding these studies, populations from Mayenne qualify as *allotopic* if only one of the species and no hybrids are recorded and *syntopic* if both species are found (or one of the species and/or the hybrid).

For 22 ponds that were sampled for the genetic analysis, the selected environmental parameters were measured in the same way. In contrast to the studies previously men-

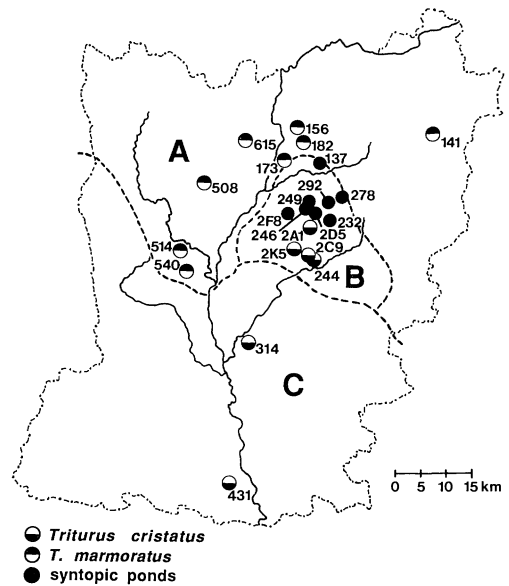


FIG. 2. Schematic representation of landscape types in the département Mayenne, France. A, Wooded and hilly area; B, wooded and flat central area; C, flat area. Circles refer to study ponds.

tioned, large numbers of newts were captured to estimate species composition ( $\bar{N} = 176.0$ ,  $SD = 243.4$ ) and populations were defined as syntopic only if each species comprised 5–95% of the entire population of adult large-bodied newts (excluding hybrids); the remaining populations were classified as allotopic.

*Sampling.*—From 1980 to 1988, 299 allotopic *marmoratus* were sampled from eight ponds in the northern part of Mayenne and 283 allotopic *crystatus* were sampled from six ponds in the southern part of Mayenne and the southern part of the central area. Syntopic *marmoratus* and *crystatus* ( $N = 245$  and  $N = 147$ , respectively) were sampled from eight ponds in the central area. For comparison, allopatric *crystatus* were sampled from southwestern England and northwestern France (département Pas-de-Calais) ( $N = 132$ ), and allopatric *marmoratus* ( $N = 109$ ) from the département Charente in southwestern France (Fig. 1). A number of allopatric and allotopic gravid female *crystatus* ( $N = 5$  and  $N = 40$ , respectively), *marmoratus* ( $N = 5$  and  $N = 17$ , respectively), and hybrids ( $N = 10$ ) were retained for analysis of mtDNA. Localities and partitioned sample sizes are given in

Appendix 1. All putative hybrids were easy to distinguish from *cristatus* and *marmoratus* by virtue of their mixed coloration pattern (Table 1). On the dorsal side a green marbling is invariably present, but less clear than in *marmoratus*. Ventrally an orange coloration is found but less bright and generally less extensive than in *cristatus*; white stipples are found as in *marmoratus*. The coloration pattern of hybrids spans a wide range (cf. Vallée, 1959 Plate II) but does not overlap with that of the parental species.

Hybridization and introgression are evidenced by morphological anomalies of various types, most clearly in digits. Samples from allopatric areas (southwestern Sweden; département Pas-de-Calais; France; Bern, Switzerland; and département Charente, France) were compared with data from Mayenne (Vallée, 1959). Regenerating toes were found to be more subject to malformations (J. W. Arntzen, pers. obs.). Due to long-term population studies involving individual marking by toe clipping, recent data may be biased, and are excluded from the analysis.

**Nuclear Genes.**—Tissue samples for protein electrophoresis were obtained in the field from live newts by clipping tail tips so that many individuals could be sampled without damaging populations. Ten to 50  $\mu$ l of blood was collected from the *arteria caudalis* in heparinized capillary tubes. Tubes and tail tips were transported to the field laboratory on wet ice. Tail tips were ground in homogenizing buffer (100 mM Tris, 1 mM EDTA, and 0.05 mM NADP; pH to 7.0 with HCl) and centrifuged. Cellular fractions were removed from blood samples by brief centrifugation. Supernatants were decanted in each case and stored individually at  $-30^{\circ}\text{C}$  followed by later storage at  $-70^{\circ}\text{C}$  until eventual electrophoresis. Nine loci were selected on the basis of sharing no alleles in allopatric populations of the two species: glucosephosphate isomerase (*Gpi*), lactate dehydrogenase (*Ldh-2*), malate dehydrogenase (*Mdh-1*), 6-phosphogluconate dehydrogenase (*Pgd*), albumin (*Alb*), transferrin (*Trf*), esterase (*Est*), and two muscle proteins (*Prot-1* and *-4*). The enzymes were run on starch gels using muscle tissue (for details see Arntzen, 1986a; Rafinski and Arntzen, 1987). Alternatively, *Est* was assayed on polyacrylamide slab gels

from blood plasma, as were *Alb* and *Trf* (see Rafinski and Arntzen, 1987). Muscle proteins were studied on polyacrylamide slab gels according to Maurer (1971, system 1). In sympatric populations, alleles (electromorphs) that had not been found in allopatric reference populations were sometimes found at low frequencies. If indistinguishable from alleles in the opposite species they are called alien alleles. These are inferred to result from introgressive hybridization. Another source of genetic variation in sympatric populations comes from so-called "rare alleles." They are defined as alleles whose occurrence is associated with an interspecific hybrid zone, and whose products have been named "hybrizymes" (Woodruff, 1989). They are not found in allopatric reference populations of either the same or the opposite species.

**Cytoplasmic Genes.**—mtDNA was isolated from mature ovaries of female newts according to Wallis (1987). All samples were digested with each of the following 10 restriction enzymes (hexanucleotide recognition sites): *Bgl*II (A/GATCT), *Cla*I (AT/CGAT), *Eco*RI (G/AATTC), *Eco*RV (GAT/ATC), *Hind*III (A/AGCTT), *Pst*I (CTGCA/G), *Pvu*II (CAG/CTG), *Sst*II (CCGC/GG), *Xba*I (T/CTAGA), and *Xho*I (C/TCGAG). Fragments were separated by agarose gel electrophoresis and visualized directly using ethidium bromide. The positions of all restriction sites on the mtDNA molecule were determined by means of double and triple enzyme digestion experiments. Nomenclature of mtDNA type is that of Wallis and Arntzen (1989).

## RESULTS

Figure 4 shows the present-day distribution of the newts over Mayenne. In the hilly and wooded northern part *marmoratus* is essentially allotopic, whereas in the flat and open southern part *cristatus* is the dominating species. In the relatively flat and forested, approximately 450 km<sup>2</sup> large central area of Mayenne (the so-called "watery area" of Schoorl and Zuiderwijk, 1981), *cristatus* and *marmoratus* are syntopic in the majority of ponds (cf. Arntzen, 1986b). According to Schoorl and Zuiderwijk (1981), *marmoratus* typically inhabits hilly and wooded areas that provide many terrestrial hiding

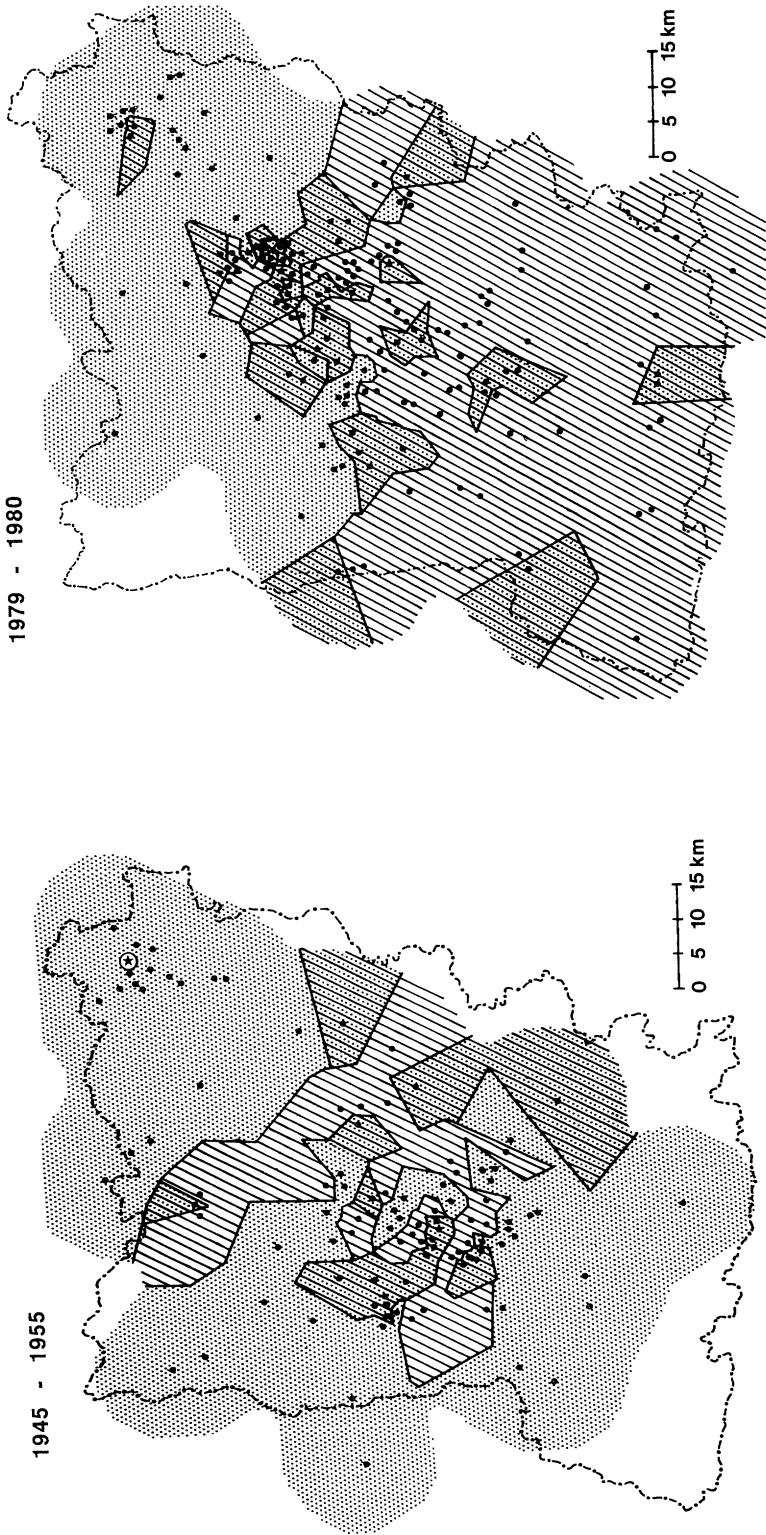


FIG. 3. Distribution of *Triturus cristatus* (hatched) and *T. marmoratus* (shaded) in département Mayenne, France, on the basis of observations from 1945 to 1955 by Vallée (1959) in 104 ponds (indicated by dots). For details on the method of presentation see text. Star refers to an introduced population (cf. Vallée, 1959 footnote to Table 1).

FIG. 4. Distribution of *Triturus cristatus* (hatched) and *T. marmoratus* (shaded) in 154 ponds (indicated by dots). For details on the method of presentation see text.

places, whereas *cristatus* is generally found in flat and open areas with fewer terrestrial hiding sites. Accordingly, the null hypothesis of no association among species composition (allotopic *cristatus*, allotopic *marmoratus*, syntopic *cristatus*-*marmoratus*) and the quantified environmental parameters (relief and forestation) was not corroborated (Kruskal-Wallis,  $P < 0.01$  in each case). Allotopic *marmoratus* sites are characterized by a high density of forest and much relief; *cristatus* and syntopic populations have on average much lower values that are not significantly different from each other (Mann-Whitney  $U$  test). Over the 154 newt localities, relief and forestation are uncorrelated parameters (Spearman rank correlation test,  $r_s = 0.00$ ), which confirms that both are determining factors for the occurrence, and hence distribution, of *cristatus* and *marmoratus*. Strictly speaking these tests may not be valid, since for neighboring ponds the distributional data are not necessarily independent. However, a significant positive correlation was found for the frequency of *marmoratus* and relief ( $r_s = 0.54$ ,  $P < 0.05$ ) over the 22 ponds in which species composition was quantified (Fig. 2, Appendix 1), corroborating the analysis above. The correlation of the frequency of *marmoratus* with forestation is positive but not significant ( $r_s = 0.25$ ). Considering the 12 ponds in the relatively flat central area alone, a positive relationship is found between the frequency of *marmoratus* and forestation ( $r_s = 0.60$ ,  $P < 0.05$ ). As expected, no significant relationship is found with relief ( $r_s = 0.09$ ) in this area.

Electrophoretic comparisons of proteins from allopatric samples of *cristatus* and *marmoratus* confirmed that the nine loci are fixed for alternative, species-specific, alleles. Accordingly, these loci are considered to be diagnostic in sympatric populations. The low level of genetic variation in these species (Rafinski and Arntzen, 1987) is confirmed here with larger sample sizes. Some variation was found at the *Alb* and *Mdh-1* loci in *cristatus* and *marmoratus*, respectively. The corresponding alleles are species specific and do not reduce the diagnostic value of the marker loci. The high number of diagnostic loci permitted reliable distinction between  $F_1$  hybrid and—had they

occurred— $F_2$  hybrid or backcross generations.

In syntopic populations of *cristatus* and *marmoratus*, alleles were occasionally observed that did not correspond to the morphological phenotype of the animal in which they appeared. A total of 10 such alien alleles were observed in 7 individual *cristatus* (allele frequency  $f = 0.4\%$ ) and 12 in 11 individual *marmoratus* ( $f = 0.3\%$ ) (Table 2). These data point to bidirectional nuclear gene flow between syntopic *cristatus* and *marmoratus*.

Introgression of nuclear genes was also observed in allotopic populations of both species. Over the nine loci, 19 alien alleles were found in 17 *cristatus* ( $f = 0.4\%$ ) and 12 in 12 *marmoratus* ( $f = 0.2\%$ ). No significant heterogeneity was found in level of introgression among the four classes of sympatric newts (allotopic/syntopic  $\times$  *cristatus*/*marmoratus*;  $G$  test of independence,  $P > 0.05$ ). The observed level of introgression averaged over the nine loci is low. Assuming these loci to be representative of the genome in terms of introgression, then there is a probability of  $3.0 \times 10^{-3}$  that a randomly chosen allele is alien. Among ponds in which large enough samples were taken to allow statistical analysis of the data (expected frequency of alien alleles  $f_e \geq 5$ ), gene flow is occurring at a similar rate in ponds 173, 278, and 2D5 ( $G$  test for goodness of fit,  $P > 0.05$  in all cases). In pond 2C9 a significantly higher level of introgression than average is found ( $P < 0.01$ ). Among ponds with intermediate sample size ( $2 < f_e < 5$ ) no introgression was found in pond 2F8, and single alien alleles in ponds 431 and 314. In these latter ponds in southern Mayenne, single hybrids (but no *marmoratus*) have been recorded (Schoorl and Zuiderwijk, 1981; and present study).

Rare alleles were found in sympatric populations at five of the nine loci. These are at a similarly low frequency to alien alleles ( $4.1 \times 10^{-3}$  rare alleles locus $^{-1}$ , Table 2). For both classes of genetic variation, loci do not contribute equally to the observed variation ( $G$  test for goodness of fit,  $P < 0.001$  in both cases). In pond 431, rare alleles were found at the *Est* and *Mdh-1* locus, reaching frequencies of 14 and 20%, respectively. Apart from these loci, *Trf* stands

TABLE 2. Interspecific and intraspecific heterozygotes observed in allopatric, allotopic, and syntopic populations of *Triturus cristatus* and *T. marmoratus*. To the right frequency of alien genes (upper part) and rare genes (lower part) in populations and individuals. For sample sizes, and for ponds in which no genetic variation was observed, see Appendix 1. Localities are mapped in Figure 2.

Phenotype	Interspecific heterozygotes at locus									Alien alleles in population (% frequency)	Individuals carrying alien alleles (% frequency)	
	<i>Alb</i>	<i>Est</i>	<i>Gpi</i>	<i>Ldh-2</i>	<i>Mdh-1</i>	<i>Pgd</i>	<i>Prot-1</i>	<i>Prot-4</i>	<i>Trf</i>			
<i>T. cristatus</i>												
Pond 2C9	8	2	1		1					3	15 (7.4)	13 (11.6)
Pond 244		1								1	2 (3.7)	2 (6.7)
Pond 249		1			3						4 (10.1)	3 (13.6)
Pond 292	1	1		1			1	1	1		6 (5.6)	4 (6.7)
Pond 314		1									1 (1.4)	1 (2.4)
Pond 431			1								1 (0.8)	1 (1.4)
Total allopatric	0	0	0	0	0	0	0	0	0	— <sup>1</sup>	— <sup>1</sup>	0 (0)
Total allotopic	8	4	2	0	1	0	0	0	4		19 (3.7)	17 (6.0)
Total syntopic	1	2	0	1	3	0	1	1	1		10 (3.8)	7 (4.8)
<i>T. marmoratus</i>												
Pond 2D5	1			1							2 (1.3)	2 (2.4)
Pond 173				9			2				11 (3.3)	11 (5.9)
Pond 182				1							1 (6.9)	1 (12.5)
Pond 278			4				2				6 (3.3)	5 (4.9)
Pond 292				4							4 (8.9)	4 (16.0)
Total allopatric	0	0	0	0	0	0	0	0	0	— <sup>1</sup>	— <sup>1</sup>	0 (0)
Total allotopic	0	0	0	10	0	2	0	0	0		12 (2.2)	12 (4.0)
Total syntopic	1	0	4	5	0	2	0	0	0		12 (2.7)	11 (4.5)
Phenotype	Intraspecific heterozygotes at locus <sup>2</sup>									Rare alleles <sup>3</sup> in population (% frequency)	Individuals carrying rare alleles (% frequency)	
	<i>Alb</i>	<i>Est</i>	<i>Gpi</i>	<i>Ldh-2</i>	<i>Mdh-1</i>	<i>Pgd</i>	<i>Prot-1</i>	<i>Prot-4</i>	<i>Trf</i>			
<i>T. cristatus</i>												
Pond P027	1										— <sup>1</sup>	0 (0)
Pond 2C9										2	2 (0.1)	2 (1.8)
Pond 2F8										2	2 (2.8)	2 (5.1)
Pond 232										1	1 (7.9)	1 (14.3)
Pond 244					2**					2	4 (7.4)	4 (13.3)
Pond 292										3	3 (2.8)	3 (5.0)
Pond 314										1	1 (1.4)	1 (2.4)
Pond 431		16 [2]	1		16 [6]					9	58 (45.4)	43 (60.6)
Total allopatric	1	0	0	0	0	0	0	0	0	— <sup>1</sup>	— <sup>1</sup>	0 (0)
Total allotopic	0	20	1	0	30	0	0	0	14		65 (12.8)	50 (17.7)
Total syntopic	0	0	0	0	0	0	0	0	6		6 (2.3)	6 (4.1)
<i>T. marmoratus</i>												
Pond C20					5*						— <sup>1</sup>	0 (0)
Pond 173				1							1 (0.3)	1 (0.5)
Total allopatric	0	0	0	0	5	0	0	0	0	— <sup>1</sup>	— <sup>1</sup>	0 (0)
Total allotopic	0	0	0	1	0	0	0	0	0		1 (0.2)	1 (0.3)
Total syntopic	0	0	0	0	0	0	0	0	0		0 (0)	0 (0)

<sup>1</sup> Absent by definition.

<sup>2</sup> Homozygotes in square brackets. Alleles at single loci have the same electrophoretic mobilities unless marked by an asterisk (electrophoretic mobility decreasing by increasing number of asterisks).

<sup>3</sup> Hybridzymes, see Woodruff (1989) and text.

out with a rare allele in seven populations of *cristatus*. Of the loci with alien alleles, *Alb* and *Ldh-2* contribute significantly more than average (*G* test for goodness of fit,  $P < 0.01$  in both cases). Alien alleles of *Alb* are found predominantly in *cristatus*, while

alien alleles of the *Trf* locus are found mainly in *marmoratus*. Alleles at these loci appear to have introgressed asymmetrically and in opposite directions. A significant correlation is found between the number of alien and rare alleles as measured over the

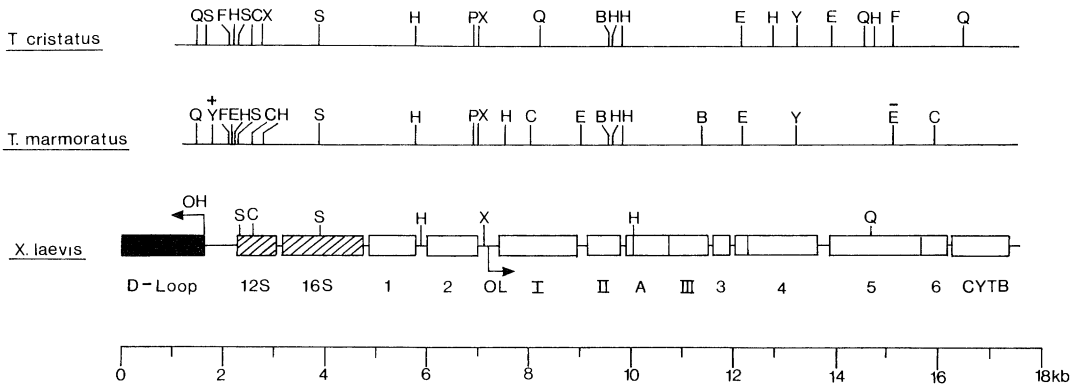


FIG. 5. Restriction maps for the four mtDNA genome classes found in northwestern European *Triturus cristatus* and *T. marmoratus* with the *Xenopus laevis* gene map for comparison (Roe et al., 1985). Restriction sites are represented by vertical lines and labeled using the following code: *Bgl*II, B; *Cla*I, C; *Eco*RI, E; *Eco*RV, F; *Hind*III, H; *Pst*I, P; *Pvu*I, Q; *Sst*II, S; *Xba*I, X; *Xho*I, Y. Probable homologous sites in *Xenopus* used for alignment of maps are also shown. The *Xenopus* map shows the D-loop as shaded, the ribosomal genes (12 S and 16 S) as hatched, and polypeptide coding genes as open boxes. These are interspersed with 22 tRNA genes, and two noncoding regions containing the origins of heavy and light strand replication (OH and OL). The site marked "+" represents a site gain found in genome MAR20; the site marked "-" represents a site loss seen in genome MAR19.

21 ponds in the sympatric area studied for nuclear genes ( $r_s = 0.46$ ,  $P < 0.05$ ).

Four mtDNA genotypes were discovered: a single *cristatus* type (CRI1,  $N = 45$ ), a common *marmoratus* type (MAR18,  $N = 20$ ), and two unique variant *marmoratus* types (MAR19 and MAR20) differing from MAR18 by a single site loss and gain, respectively (Fig. 5, Appendix 2). Of the 32 restriction sites found across all mitochondrial genomes, 14 were present in all samples, 9 were specific to *cristatus*, and 9 were specific to *marmoratus*. The two predominant mtDNA genotypes (CRI1 and MAR18) differ by 18 restriction sites [ $d = 0.079$ ; equation (17), Nei et al., 1985]. In the most parsimonious tree linking all *cristatus* species group mtDNA genomes, restriction site B1 appears as a convergent gain, and thus CRI1 and MAR18 are separated by 19 restriction site changes (Wallis and Arntzen, 1989). No length variation in the mtDNA molecules was observed apart from a 140 bp insertion that was found in three *cristatus* from pond 2C9 (Wallis, 1987). Without exception, the CRI and MAR mtDNA types correspond to their respective phenotypes, so no mitochondrial gene flow has been observed among sympatric populations of *cristatus* and *marmoratus*. This result is not

in line with our expectation of a more pronounced interspecific gene flow at the level of the mitochondrial genome.

Ten individuals identified as hybrids on morphological criteria were heterozygous for all nine protein loci, strongly supporting their morphological identification as  $F_1$  hybrids between *T. cristatus* and *T. marmoratus*. Interestingly, they all possessed *cristatus* mtDNA, with no visually detectable heteroplasmy. Under the null hypothesis of random interspecific mating and no differential viability, the chance of all  $F_1$  possessing the same species for female parent is  $2^{-9}$  ( $P = 0.002$ , two-tailed). If some of the  $F_1$  are sibs, then the significance of this finding is reduced, but two lines of evidence suggest that this is unlikely to be a major explanation. First, with hybrids at a frequency of approximately 4% in syntopic populations (range 1.3–14.3%, Appendix 1), they are more than just an occasional finding, and presumably each year's recruits come from many interspecific matings. Second, hybrids were taken from six different ponds over a wide area. Most hybrids from the same pond differ in age as determined by skeletochronological comparisons (Francillon-Vieillot et al., 1990) and among the 10 hybrids only three are indistinguishably

TABLE 3. Frequency of digital anomalies in *Triturus cristatus*, *T. marmoratus*, and hybrids. Localities are mapped in Figure 1.

	Newts with digital anomalies (sample size)	References
Area of sympatry		
Département Mayenne, France		
<i>T. cristatus</i>	4.6% (701)	} <sup>1</sup> n.s. Vallée (1959)
<i>T. marmoratus</i>	6.3% (571)	
Parental species pooled	5.4% (1,252)	} <sup>1</sup> ** Vallée (1959)
Hybrids	16.9% (65)	
Areas of allopatry		
<i>T. cristatus</i>		
Southwestern Sweden	"Practically absent" (600)	T. Hagström (pers. comm.)
Département Pas-de-Calais, France (e.g., P027)	0.2% (403)	J. W. Arntzen (unpubl.)
Bern, Switzerland	~2% (150)	K. Grossenbacher (pers. comm.)
Paris and Strasbourg, France	0% (76)	Vallée (1959)
<i>T. marmoratus</i>		
Département Charente, France (C17, C20)	9.2% (109)	J. W. Arntzen (unpubl.)
Tulle and Banyuls, France	0% (49)	Vallée (1959)

<sup>1</sup> *G* test of independence, n.s., not significant, \*\* *P* < 0.01.

different in age and from the same pond, and could therefore be sibs. The chance of eight hybrids possessing the same species for female parent is  $2^{-7}$  ( $P = 0.008$ , two-tailed).

Several lines of evidence suggest that there is selection against hybrids: chromosomal incompatibility of the parental species (Lantz, 1947; Lantz and Callan, 1954), low fertility of hybrid males (White, 1946), embryonic mortality (Arntzen and Hedlund, 1990), and morphological aberrations. Digital anomalies are more frequent in hybrids than in sympatric populations of *cristatus* and *marmoratus*, which in turn show anomalies more frequently than most allopatric populations (Table 3).

Comparison of old and recent distribution maps for *cristatus* and *marmoratus* in Mayenne reveals pronounced changes in species distribution (Figs. 3, 4). The southern part of the département has undergone a dramatic change in species composition. Nowadays the area is inhabited almost exclusively by *cristatus*. A few populations were found with a low number of *marmoratus* or occasional hybrids. The reverse pattern is found in the central-northern part of Mayenne, where Vallée (1959) documented *cristatus* only, and now *marmora-*

*tus* is found exclusively. A more exploratory analysis of the biogeographic changes in the light of habitat preferences and the human disturbance that affect them is presented by Schoorl and Zuiderwijk (1981). Over the last 30–40 years the number of hybrids, as counted in syntopic ponds all over Mayenne, has dropped from 15.9% (cf. Vallée, 1959 Table 2) to 6.6% (J. Schoorl, pers. comm.) to 3.8% (present study, data from the "central area" only). Data for the first and second study are collected in a similar way and demonstrate that the change is statistically significant (Mann–Whitney *U* test,  $P < 0.05$ ).

The northwest and the northeast are the only parts in Mayenne that have remained unchanged in terms of species composition, and neither foreign nor rare alleles are observed here. Comparison of populations that are inferred to be located in such stable areas (ponds 141, 508, and 514) with the remaining populations of large-bodied newts, irrespective of species composition, reveals a significant difference in levels of alien alleles [ $P < 0.05$ , *G* test of independence with Williams' correction (Sokal and Rohlf, 1981)] and rare alleles ( $P < 0.01$ ). We conclude that introgression in Mayenne is restricted to (1) areas with high levels of syntopy, and

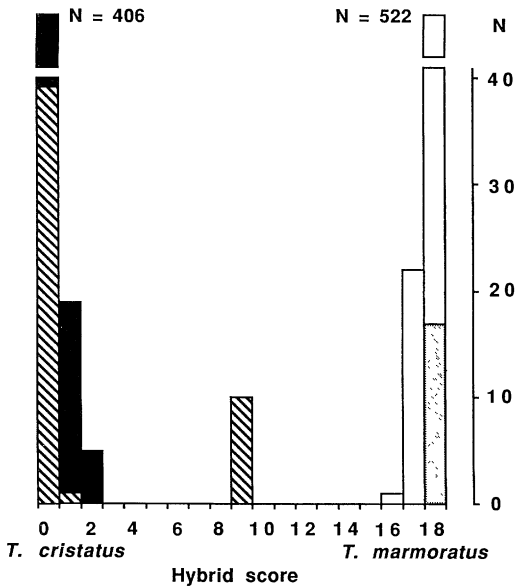


FIG. 6. Histogram showing hybrid scores in a sample of large-bodied newts from département Mayenne, France. Hybrid score is based on nine diagnostic protein loci and runs from 0 (all loci homozygous for *Triturus cristatus* alleles) to 18 (all loci homozygous for *T. marmoratus* alleles). Newts are classified according to morphological phenotype: *T. cristatus* (solid bars,  $N = 430$ ), *T. marmoratus* (open bars,  $N = 544$ ) and  $F_1$  hybrids ( $N = 10$ , all individuals with hybrid score 9). A subsample is studied for mtDNA, which is either of the *cristatus* type (superimposed hatching) or of *marmoratus* type (superimposed shading). Note that the hybrids all possess *cristatus* type mtDNA.

(2) areas of species replacement. As gene flow is not otherwise apparent, a geographically stable parapatric hybrid zone of *cristatus* and *marmoratus* would appear to be impermeable to gene flow.

Comparing both distribution maps (Figs. 3, 4) a rough estimate can be made of the dispersal capacity of *cristatus*. Since Vallée's inventory 25–35 years prior to the later study, *cristatus* has extended its territory to the southwest by at least 30 km. Whereas the dispersal that we record is the maximum dispersal achieved, average dispersal may be much less than this. On the other hand, it is not known how much farther south *cristatus* has advanced in the last 30 years. Moreover, dispersal may have been slowed down due to competition with *marmoratus*. An independent estimate of dispersal can be made in the northeast of Mayenne where *cristatus* occurs in a small pocket around the village of St. Aignan de Couptrain. The

presence of *cristatus* in this area contrasts with expectations from the ecological preferences of this species. The local *cristatus* and hybrids may result from an introduction (see Vallée, 1959 footnote to Table 1). In this less favorable terrain, *cristatus* dispersed up to 6 km in approximately 35 years, without fully replacing *marmoratus* (Schoorl and Zuiderwijk, 1981; Arntzen, unpubl.). The potential dispersal rate of *marmoratus* is probably similar to that of *cristatus*, or slightly higher because of its longer generation time and more terrestrial mode of life (Table 1). Taken altogether, a dispersal rate of approximately 2 km per generation for these large-bodied newt species seems a reasonable estimate.

## DISCUSSION

Natural hybrids of *cristatus*–*marmoratus* are relatively scarce and introgression is very limited. The factors accounting for reproductive isolation can be classified as prezygotic and postzygotic. The main factors are (1) the separation of the species on a broad geographic scale (Fig. 1), (2) ecological differentiation separating most *cristatus* and *marmoratus* populations within the area of range overlap (Schoorl and Zuiderwijk, 1981), and (3) positive assortative mating within ponds (Zuiderwijk, 1986). Factors contributing to postmating reproductive isolation include asynapsis at metaphase I and spermatid degeneration in  $F_1$  male hybrids (White, 1946; Lantz and Callan, 1954) and the concomitant sterility. On the basis of their experimental hybridization and back-crossing of *T. marmoratus* and *T. karolini*, Lantz and Callan (1954 pp. 183–184) conclude: "only those gametes which by chance contain reconstituted parental or near-parental chromosome complements are likely to contribute to the future gene pools of the parental species. Hybridization may be presumed to permit some flow of genes from one newt species to another, but certainly not on a large scale." Although we have no evidence for the occurrence of  $F_2$  hybrids (individuals homozygous for 2–3 *cristatus* alleles, 2–3 *marmoratus* alleles, and hybrid at 4–5 loci), this reconstitution of parental types in back-crosses involving hybrid females means that although most of the alien alleles are found singly (or at most doubly), we cannot say much about the

number of generations since hybridization for these individuals. It is indeed conceivable that they are mostly reconstituted  $F_{2-3}$  back-crosses. Maybe the only introgression that we see is the result of a recombination event prior to reconstitution, which would explain the apparent dominance of a single alien allele in each pond. Female  $F_1$  hybrids suffer a 73% reduction in embryo hatching rate compared with the parental species (Arntzen and Hedlund, 1990). The sterility of  $F_1$  hybrid males and low fertility of  $F_1$  hybrid females helps to explain the absence of  $F_2$  hybrids in our samples and the low level of introgression (Fig. 6).

The substantial reproductive isolation of *cristatus* and *marmoratus* is not surprising in the light of the high genetic distance (Nei, 1972) reported for these species ( $D = 0.86$ , Rafinski and Arntzen, 1987). A slightly higher value ( $D = 0.91$ ) is reported for the hybridizing toads *Bufo boreas* and *B. punctatus*, considered one of the most genetically distinct pairs of terrestrial vertebrate species known to hybridize in nature (Feder, 1979). Unlike *T. cristatus*-*T. marmoratus*, however, the toads are probably fully sterile. Introgression between the newts indicates that  $F_1$  hybrids occasionally give rise to viable and fertile offspring, a conclusion circumstantially supported by laboratory crossing experiments involving female hybrids (Lantz and Callan, 1954).

Taking into account the independent evolutionary history of *cristatus* and *marmoratus* that predates the Pleistocene (Rafinski and Arntzen, 1987; Macgregor et al., 1990), it is beyond reasonable doubt that the hybrid zone under consideration is of secondary nature. The parapatric hybrid zone is spatially and temporally complex: it is not simple in shape, its width varies geographically, and its position changes in time. The definitions generally used in analyses of geographic and genetic isolation do not seem appropriate to describing this complex situation. On a broad geographic scale, *cristatus* and *marmoratus* are clearly sympatric, and throughout the sympatric area instances of hybridization have been reported. The transition of *cristatus* in northern France, through the area of overlap to *marmoratus* in the south is, according to Woodruff (1973), properly characterized as localized sympatric hybridization. Surveys

within the area of range overlap reveal, however, that the species generally have mutually exclusive, contiguous ranges: in a given environment generally only one of the species is found. Moreover, this is also true for the survey made 30 years earlier, and although occasionally low levels of syntopy may have been missed due to small sample sizes, it seems to be the usual pattern over the entire area of range overlap (cf. Fig. 1). The spatially continuous contact area of *cristatus*-*marmoratus* may be classified as a temporally stable parapatric hybrid zone. On the other hand, due to the smaller and larger syntopic areas in and close to the main contact zone, the *cristatus*-*marmoratus* overlap shows aspects of a mosaic hybrid zone. Distribution and hybridization of *cristatus* and *marmoratus* in Mayenne fit none of the well-known patterns and point to the complexity of these species interactions. To describe this situation we propose the term "reticulate hybrid zone."

Regarding the high population densities of *cristatus* and *marmoratus* in Mayenne and the high habitat usage (Schoorl and Zuiderwijk, 1981; Arntzen and de Wijer, 1989) the (parapatric) hybrid zone is not located in a density trough as predicted by Barton (1979). The fact that increasing levels of abundance and population density are found toward the area of range overlap in western France (Zuiderwijk, 1980; Schoorl and Zuiderwijk, 1981) does not favor the model in which hybrid zones are explained by hybrid superiority at the potential edge of the species ranges. Hybrid superiority in ecotonal environments is another potential solution (Moore, 1977). Although the newt hybrids may show aspects of heterosis in longevity (Francillon-Vieillot et al., 1990) and female fecundity (Arntzen and Hedlund, 1990), they are less fertile than either parental species. In the "dynamic equilibrium" model of hybrid zone structure, dispersal (a gene flow parameter) and hybrid inferiority (a selection parameter) are the main opposing factors involved in the stability of a hybrid zone (Barton and Hewitt, 1985). According to this model, with strong selection against hybrids, the width of the zone is of the same order of magnitude as the dispersal rate per generation (Barton and Hewitt, 1985). This is in accordance with our measurements of dispersal rate for the newts and with an es-

essentially parapatric hybrid zone between *cristatus* and *marmoratus*. An exception may be found in the "central area" where the parapatric hybrid zone seems much wider, with an estimated width of 20 km. To determine the position and exact width of the zone locally a more detailed inventory is necessary.

In the central area, the two species cooccur and hybridize in most of the ponds (Schoorl and Zuiderwijk, 1981; Arntzen, 1986*b* and unpubl.). Schoorl and Zuiderwijk (1981) found 80% of their hybrids (35/44) in the central area where only 39% (60/154) of their study ponds were situated. We conclude that the central area is characterized by an increased level of syntopy and that the opportunity for hybridization (and introgression) is increased. Nevertheless, the frequency of hybrids averages only 4% and very low levels of introgression are recorded.

Smaller areas of syntopy are found in the south of Mayenne. Comparison of past and present distribution maps (Figs. 3, 4) indicates that these pockets of syntopy probably came into existence as a consequence of the moving of the zone. Males and females of both species are sexually mature at age 5 or earlier (Table 1) so the areas of syntopy may have persisted for a number of generations. Although observed longevity is equal for the species (14 years), the modal age of reproducing individuals is 3 years higher in *marmoratus* than it is in *cristatus* (Table 1). This observation may help to explain the absence of syntopic populations in the *marmoratus* areas that were formerly occupied by *cristatus*. In the pockets of syntopy the rarer species is almost forced to cross with the abundant species (Hubbs, 1955; Avise and Saunders, 1984), which could lead to the extinction of the former. In the short term, however, the chances for (unidirectional) interspecific gene flow may be enhanced and individual alleles of the rare form may persist (i.e., not go extinct). So, the existence of alien alleles in the now allotopic populations of *cristatus* and *marmoratus* is most readily explained by a moving zone leaving a tail of introgression. This explanation is supported by the fact that no introgression is found in areas where the occupying species has not changed. It is not necessary to invoke other explanations such as selection

(Barton and Hewitt, 1985) to account for directed introgression, but they cannot be excluded.

Spatial asymmetry in hybrid zones is commonly reported. Examples include asymmetry of phenotypic characters in frogs (Littlejohn and Roberts, 1975) and birds (Yang and Selander, 1968; Burton and Martin, 1976), genetic characters in grasshoppers, frogs, and mice (Moran et al., 1980; McDonnell et al., 1978; Hunt and Selander, 1973), or both phenotypic and genotypic characters in crabs (Bert and Harrison, 1988). In some cases the asymmetrical pattern is tentatively explained by a positional shift of the zone, whereas in other zones a positional shift is demonstrated but geographic patterns of character distribution are not revealed (Hillis and Simmons, 1986). The present study indicates that an asymmetric distribution of characters, in this case nuclear genes, may indeed result from a zone shift.

The partial fertility of hybrid females should allow introgression of mitochondrial genomes to occur, but this is not observed in the subsample of 40 *cristatus* and 19 *marmoratus* from the region of sympatry (data summarized in Fig. 6). Even if introgression does occur, but has been missed because of restricted sample size, it does not occur on the same scale as that described in *Drosophila* (Powell, 1983), *Mus* (Ferris et al., 1983), and *Rana* (Spolsky and Uzzell, 1984). In the first example, the nuclear genomes of the two species are extremely similar (Takahata and Slatkin, 1984); in Scandinavian *Mus*, there is marked introgression of *domesticus* nuclear genes into *musculus* (Hunt and Selander, 1973) and in the frog example there is the special case of a hybridogenetic system (Spolsky and Uzzell, 1986). Interspecific transfer of mtDNA, which appears to indicate a recent limited episode of interspecific hybridization, has been reported in Scandinavian *Clethrionomys* voles (Tegelström, 1987). Other studies have shown a limited breakdown in the association between mitochondrial and nuclear markers restricted to parapatric contact zones or their near vicinity (Harrison et al., 1987; Baker et al., 1989; Rand and Harrison, 1989; Wallis and Arntzen, 1989). In a study of introgression between two species of Cyprinid fishes (*Notropis*), Dowling et al. (1989) re-

port asymmetric introgression of allozyme markers into the locally rarer species. Introgression of mtDNA was also asymmetric but the direction of the asymmetry varied only with the location and not with the relative frequency of the parental species. Concordance of nuclear and mitochondrial markers across hybrid zones has been demonstrated in *Mus* in Germany (Ferris et al., 1983), *Lepomis* (Avise and Saunders, 1984), *Bombina* (Szymura et al., 1985), and *Pero-myiscus* (Nelson et al., 1987), so the lack of mtDNA introgression in these well-differentiated newt species is not in itself remarkable, except that in this case the zone is moving.

There are several possible explanations for the apparent absence of *marmoratus* mtDNA in  $F_1$  hybrids. The one that we favor is prezygotic and obviously the most straightforward solution. Matings of *cris-tatus* male  $\times$  *marmoratus* female do not occur, or at least are greatly outnumbered by the combination of *marmoratus* male  $\times$  *cris-tatus* female. Lamb and Avise (1986) find just such a pattern in hybrid frogs in Alabama, where 20  $F_1$  hybrids between *Hyla cinerea* and *H. gratiosa* all have *gratiosa* mtDNA, supporting an a priori expectation. In the case of the newts, a posteriori supporting evidence comes from observations made by Zuiderwijk and Sparreboom (1986) of newts in a syntopic pond. They describe *marmoratus* males as being more aggressive and more likely to interrupt matings than *cris-tatus* males. They did not, however, report any interspecific interactions of this nature, or any *marmoratus* male  $\times$  *cris-tatus* female matings, so the evidence is circumstantial. It does though explain the pattern that we see rather well, and may also help to account for the lack of introgression of mtDNA in the south of Mayenne, where we would have most expected to see it through *marmoratus* genomes being incorporated into the *cris-tatus* population spreading north. Consequently, one would be more likely to see introgression of *cris-tatus* mtDNA into a spreading *marmoratus* population, but this applies only to a small north-central region, from which only eight of our specimens come. To have seen introgression of *cris-tatus* mtDNA into *marmoratus* in the northwest or northeast (nine individuals) would have required mtDNA to permeate

through and ahead of the moving zone, which might require a selective mechanism when hybrid breakdown is so strong. Selection is the other main possible explanation for the lack of introgression; incompatibility of *marmoratus* mtDNA with the *cris-tatus* nuclear genome may render hybrids from *marmoratus* mothers inviable. This is in contrast to the finding that mtDNA from one species seems to function effectively in another species (Lansman et al., 1983; Gyllensten et al., 1985). Gametic selection could be another explanation for the lack of *marmoratus* type mtDNA in  $F_1$  hybrids. Heterospecific sperm could have lower success, and sperm of *cris-tatus* may be inviable in *marmoratus* females. A more intensive study in, and immediately adjacent to, the central area of syntopy might help to explain the observed absence of mtDNA introgression.

Rare alleles are found in most hybrid zones that have been studied using enzyme electrophoresis (Barton et al., 1983; Kocher and Sage, 1986; Woodruff, 1989). In the newts from Mayenne, the number of rare alleles is found to be correlated with the number of introgressed alleles. Similarly, a correlation of rare alleles with heterozygosity has been described in *Drosophila* (Koehn and Eanes, 1975). As in most other examples, rare alleles in the *cris-tatus*-*marmoratus* zone seem to be restricted to certain populations and loci. As the rare alleles seem to be induced by hybridization, the resulting allozymes have been termed "hybrizymes" (Woodruff, 1989). Their occurrence has been variously attributed to intragenic recombination, increased mutation, and relaxed selection. Analogous to the increased variation provided by hybrizymes is the increased level of morphological variation in large-bodied newts from Mayenne as expressed by digital anomalies. Such limb malformations are especially found in hybrids. It is likely that the digital anomalies have a direct relation to hybridization and they may well have a genetic basis. In a more detailed study on basipodial variation, using a selected sample of *cris-tatus*, *marmoratus*, and hybrids from Mayenne, Rienesl and Wagner (1989) reach a similar conclusion. The observed increase in the level of variation may result from a breakdown of genetic homeostasis. A relatively high proportion of

animals with malformed digits was found in our samples from Charente (locality C17 and C20, Fig. 2). These localities are close to the zone of range overlap of *cristatus* and *marmoratus* and the observations could be caused by gene flow at loci other than the ones studied. On the other hand, similar limb malformation has been reported in newts with no apparent explanation available (Malkmus, 1980). Morphological characters such as these are generally polygenically controlled and often have an environmental component to the observed variability.

Several factors may be causing the decline of *marmoratus* in syntopic populations. First, directional introgression could destabilize the *marmoratus* genotype and contribute to its decline. As we have found some evidence for asymmetric introgression at the nuclear genome in both the direction of *cristatus* (*Alb*) and *marmoratus* (*Ldh-2*), this explanation seems inadequate. In the absence of directionality, introgression could contribute to the decline of *marmoratus* if *cristatus* increased more rapidly than *marmoratus* for other reasons. Stabilizing selection within the *cristatus* population may eliminate impure genotypes thereby restoring its expansive powers. This explanation is unlikely to account for the observed decline of *marmoratus* as introgression is very limited. Moreover, in syntopic populations *marmoratus*, not *cristatus*, is the more fecund species (Arntzen and Hedlund, 1990). Second, predation on eggs and larvae could lead to displacement of *marmoratus* by *cristatus* (Arntzen, 1988; Arntzen and Hedlund, 1990). Third, it is possible that *cristatus* actually outcompetes and replaces *marmoratus*. As the decrease of one species is associated with the increase of the other, there are reasons to believe that the species are competing, although there is no direct evidence for competition, and it is unknown what vital resources they might be competing for. The fast tempo of species replacement of approximately 30 km in 30 years, relative to the width of the zone of overlap (300 km), indicates that the process may not have a long history and it suggests that *marmoratus* declines because of recent changes in its specialized habitat requirements. Shelter seems especially important to *marmoratus* with its relatively long juvenile pe-

riod and the long annual terrestrial phase of adults (Schoorl and Zuiderwijk, 1981; Francillon-Vieillot et al., 1990). The two ecological parameters that figure in our ecological analysis, relief and forestation, may well reflect this single key parameter affecting directly the relative fitness of *cristatus* and *marmoratus*. Indeed, the decreasing range of *marmoratus* (cf. Figs. 3, 4) is explained in a straightforward way in terms of human mediated changes to the environment that result in a reduction of terrestrial hiding sites. With the introduction of more large-scale farming methods since 1945, many hedges have been removed, especially in the flat southern part of Mayenne.

Although the process of environmental change is continuing, there are reasons to believe that the present day distribution pattern is more stable than the situation 30 years ago, since the parapatric hybrid zone has become located along an environmental (ecotonal) gradient of which the main descriptive parameter is relief. A similar gradient cannot be discerned from Vallée's map. That the number of hybrids, as counted in syntopic ponds, has dropped significantly over the last 30–40 years is in line with this explanation.

The parapatric hybrid zone runs through Mayenne, separating northern *marmoratus* from *cristatus* to the south. From the ecological preferences displayed by the species and their mutually exclusive pattern of distribution, it can be assumed that *marmoratus*, not *cristatus*, is distributed all over the "Collines de Normandie" mountain range that extends over the north of Mayenne. Geographically isolated from the main *marmoratus* distribution and surrounded by *cristatus* populations this patch forms an "enclave" of *marmoratus*. Many more, smaller or larger, enclaves of *marmoratus* may exist in central and northwestern France. Enclaves may have come into existence due to the advance of *cristatus* superseding *marmoratus*. The areas of syntopy and past syntopy in southern Mayenne are noteworthy in this respect.

A very similar scenario is proposed to account for enclaves in the distribution of the mountain dwelling yellow-bellied toad, *Bombina variegata*, which in mountainous areas in central Europe is surrounded by a typical lowland species, the fire-bellied toad,

*Bombina bombina* (Arntzen, 1978). Similar to the situation in the newts, subsequent to initial contact the toad parapatric hybrid zone has become located along an environmental gradient (i.e., relief), although the process probably occurred over a much longer time scale (Arntzen, 1978; Szymura and Barton, 1986). In detailed studies the *Bombina* hybrid zone conforms almost perfectly to the clinal (parapatric) model (Szymura and Barton, 1986), but on a larger geographic scale can be looked on as confirming the reticulate hybrid zone model. The *Bombina* and *Triturus* studies illustrate that the two hybrid zone models that we think appropriate do not necessarily contradict one another, but rather reflect the grain of observation. These examples show that hybrid zones merit study over a two-dimensional geographic area, and if possible over the dimension of time also.

Natural hybridization calls into question the species status of the taxa involved under the biological species concept (Mayr, 1963). In ephemeral zones, the problem can be resolved with relative ease, time permitting. If the taxa undergo fusion with little or no hybrid breakdown, they are generally considered a single species; if gene flow reduces to negligible amounts with time they are considered full species. Ephemeral hybrid zones of the first type may well exist, but are rarely documented, probably because of their transient nature (Hewitt, 1988). Reinforcement of genetic isolating mechanisms leading to speciation was integral to the modern synthesis (Dobzhansky, 1937), but Butlin (1989) suggested a lack of well-substantiated examples. Indeed, most hybrid zones appear to be temporally stable (Hewitt, 1988), not precluding fusion or fission, and may be seen as conforming to the dynamic equilibrium model (Slatkin, 1973; Barton and Hewitt, 1985). Reproductive isolation is taken by most authors to mean that the taxa involved maintain their genetic (and evolutionary) integrity (Häuser, 1987). By some it is taken in a strict sense to mean complete infertility of hybrids and subsequent absence of gene flow (Key, 1981; Barton and Hewitt, 1985). Alternatively, gene flow estimates can be used to address questions of species-level classification (Porter, 1990). According to Key (1981 p. 455), fertility of any hybrid offspring is in

itself sufficient to downgrade taxa to subspecies. As hybrid zones are generally not impermeable to gene flow, the corollary of the strict definition would be that taxa involved in natural hybridization are conspecific. This clearly is an extreme view, and not one that we favor for these species. Often this view is in contrast with opinions held by specialist taxonomists. A particularly clear example of a conflict of this type is reported by Szymura and Barton (1986). Indeed, the strict interpretation was rejected in one of the earliest genetic analyses of introgression through a hybrid zone (Hall and Selander, 1973).

#### ACKNOWLEDGMENTS

Thanks are due to the Ministère de l'Environnement, Direction de la Protection de la Nature, Neuilly, France, for permission to collect newts (permits No. 85/1017 and 88/193 to H. C. Macgregor and No. 81/2519 to J.W.A.), to J. Schoorl and A. Zuijderwijk for precise locality information, to W. van Ginkel for assistance in the laboratory, and to T. Burke, J. Gardner, and R. Harrison for helpful suggestions regarding an earlier version of the manuscript. G.P.W. acknowledges the support of NERC Grant GR3/5445, a Royal Society Overseas Field Research Grant and the University of Western Australia for provision of a Postdoctoral Fellowship.

#### LITERATURE CITED

- ARNOLD, M. L., D. D. SHAW, AND N. CONTRERAS. 1987. Ribosomal RNA-encoding DNA introgression across a narrow hybrid zone between two subspecies of grasshopper. *Proc. Natl. Acad. Sci. U.S.A.* 84:3946-3950.
- ARNTZEN, J. W. 1978. Some hypotheses on postglacial migrations of the fire-bellied toad, *Bombina bombina* (Linnaeus) and the yellow-bellied toad, *Bombina variegata* (Linnaeus). *J. Biogeogr.* 5:339-345.
- . 1986a. Aspects of introgressive hybridization in crested and marbled newts (Amphibia: Caudata). Preliminary results, pp. 155-158. *In* Z. Roček (ed.), *Studies in Herpetology*. Charles Univ., Prague, Czechoslovakia.
- . 1986b. Note sur la coexistence d'espèces sympatriques de tritons du genre *Triturus*. *Bull. Soc. Herp. Fr.* 37:1-8.
- . 1988. Twee opmerkelijke predatoren op salamander-eieren. *Lacerta* 46:161-165.
- ARNTZEN, J. W., AND L. HEDLUND. 1990. Fecundity of the newts *Triturus cristatus*, *T. marmoratus* and their natural hybrids in relation to species coexistence. *Holarctic Ecol.* 13:325-332.

- ARNTZEN, J. W., AND M. SPARREBOOM. 1987. The use of biochemical and behavioural data for the phylogeny of the Old World newts, genus *Triturus*, pp. 25–28. In J. J. van Gelder, H. Strijbosch, and P. J. M. Bergers (eds.), Proceedings of the Fourth Ordinary General Meeting Societas Europaea Herpetologica. Katholieke Universiteit Nijmegen, Nijmegen, Netherlands.
- . 1989. A phylogeny for the Old World newts, genus *Triturus*: Biochemical and behavioural data. *J. Zool.* 219:645–664.
- ARNTZEN, J. W., AND P. DE WIJER. 1989. On the distribution of the Palearctic newts (genus *Triturus*) including the description of a five species pond in western France. *Br. Herpetol. Soc. Bull.* 30:6–11.
- AVISE, J. C., AND N. C. SAUNDERS. 1984. Hybridization and introgression among species of sunfish (Lepomis): Analysis by mitochondrial DNA and allozyme markers. *Genetics* 108:237–255.
- BAKER, R. J., S. J. DAVIS, R. D. BRADLEY, M. J. HAMILTON, AND R. A. VAN DEN BUSSCHE. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket-gopher, *Geomys*. *Evolution* 43:63–75.
- BARTON, N. H. 1979. The dynamics of hybrid zones. *Heredity* 43:341–359.
- BARTON, N. H., R. B. HALLIDAY, AND G. M. HEWITT. 1983. Rare electrophoretic variants in a hybrid zone. *Heredity* 50:139–146.
- BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* 16:113–148.
- BERT, T. M., AND R. G. HARRISON. 1988. Hybridization in western Atlantic stone crabs (genus *Menippe*): Evolutionary history and ecological context influence species interactions. *Evolution* 42:528–544.
- BLACKWELL, J. M., AND C. M. BULL. 1978. A narrow hybrid zone between two western Australian frog species *Ranidella insignifera* and *R. pseudinsignifera*: The extent of introgression. *Heredity* 40:13–25.
- BOUTON, N. 1986. Données sur la migration de *Triturus cristatus* et *T. marmoratus* (Urodela, Salamandridae) dans le département de la Mayenne (France). *Bull. Soc. Herp. Fr.* 40:43–51.
- BURTON, T. C., AND A. A. MARTIN. 1976. Analysis of hybridization between black-backed and white-backed magpies in south-eastern Australia. *Emu* 76:30–36.
- BUSACK, S. D., B. G. JERICO, L. R. MAXSON, AND T. UZZELL. 1988. Evolutionary relationships of salamanders in the genus *Triturus*: The view from immunology. *Herpetologica* 44:307–316.
- BUTLIN, R. K. 1989. Reinforcement of premating isolation, pp. 158–179. In D. Otte and J. A. Endler (eds.), Speciation and Its Consequences. Sinauer Associates, Sunderland, MA.
- DOBZHANSKY, TH. 1937. Genetics and the Origin of Species. Columbia Univ. Press, N.Y.
- DOWLING, T. E., G. R. SMITH, AND W. M. BROWN. 1989. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (family Cyprinidae): Comparison of morphology, allozymes, and mitochondrial DNA. *Evolution* 43:620–634.
- FEDER, J. H. 1979. Natural hybridization and genetic divergence between the toads *Bufo boreas* and *Bufo punctatus*. *Evolution* 33:1089–1097.
- FERRIS, S. D., R. D. SAGE, C.-M. HUANG, J. T. NIELSEN, U. RITTE, AND A. C. WILSON. 1983. Flow of mitochondrial DNA across a species boundary. *Proc. Natl. Acad. Sci. U.S.A.* 80:2290–2294.
- FRANCILLON-VIEILLOT, H., J. W. ARNTZEN, AND J. GERAUDIE. 1990. Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela): A skeletochronological comparison. *J. Herpetol.* 24:13–22.
- GOLLMANN, G. 1984. Allozymic and morphological variation in the hybrid zone between *Bombina bombina* and *Bombina variegata* (Anura, Discoglossidae) in northeastern Austria. *Z. Zool. Syst. Evol.-forsch.* 22:51–64.
- . 1991. Population structure of Australian frogs (*Geocrinia laevis* complex) in a hybrid zone. *Copeia*. In press.
- GREEN, A. M. 1989. The sexual behaviour of the Great Crested newt, *Triturus cristatus* (Amphibia: Salamandridae). *Ethology* 83:129–153.
- GREEN, D. M. 1983. Allozyme variation through a clinal hybrid zone between the toads *Bufo americanus* and *B. hemiophysys* in southeastern Manitoba. *Herpetologica* 39:28–40.
- GRIFFITHS, R. A., J. M. ROBERTS, AND S. SIMS. 1987. A natural hybrid newt, *Triturus helveticus* × *T. vulgaris*, from a pond in mid-Wales. *J. Zool.* 213:133–140.
- GROSSENBACHER, K. 1987. Atlas on amphibians in Switzerland, pp. 171–180. In J. J. van Gelder, H. Strijbosch, and P. J. M. Bergers (eds.), Proceedings of the Fourth Ordinary General Meeting Societas Europaea Herpetologica. Katholieke Universiteit Nijmegen, Nijmegen, Netherlands.
- GYLLENSTEN, U., D. WHARTON, AND A. C. WILSON. 1985. Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. *J. Hered.* 76:321–324.
- HALL, W. P., AND R. K. SELANDER. 1973. Hybridization of karyotypically differentiated populations in the *Sceloporus grammicus* complex (Iguanidae). *Evolution* 27:226–242.
- HARRISON, R. G. 1986. Pattern and process in a narrow hybrid zone. *Heredity* 56:337–349.
- . 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends Ecol. Evol.* 4:6–11.
- . 1991. Hybrid zones: Windows on evolutionary process. In D. J. Futuyma and J. Antonovics (eds.), Oxford Surveys in Evolutionary Biology. Oxford University Press, UK.
- HARRISON, R. G., AND D. M. RAND. 1990. Mosaic hybrid zones and the nature of species boundaries, pp. 111–133. In D. Otte and J. A. Endler (eds.), Speciation and Its Consequences. Sinauer Associates, Sunderland, MA.
- HARRISON, R. G., D. M. RAND, AND W. C. WHEELER. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Mol. Biol. Evol.* 4:144–158.
- HÄUSER, C. L. 1987. The debate about the biological species concept—A review. *Z. Zool. Syst. Evol.-forsch.* 25:241–257.
- HEWITT, G. M. 1988. Hybrid zones—Natural labo-

- ratories for evolutionary studies. *Trends Ecol. Evol.* 3:158-167.
- HILLIS, D. M., AND J. E. SIMMONS. 1986. Dynamic change of a zone of parapatry between two species of *Pholidobolus* (Sauria: Gymnophthalmidae). *J. Herpetol.* 20:85-87.
- HUBBS, C. L. 1955. Hybridization between fish species in nature. *Syst. Zool.* 4:1-20.
- HUNT, W. G., AND R. K. SELANDER. 1973. Biochemical genetics of hybridization in European house mice. *Heredity* 31:11-33.
- INSTITUT GÉOGRAPHIQUE NATIONAL. 1971, 1973, 1975-1978. Cartes 1:25000. Série bleue: 1320 est, Châteaubriand (1976); 1416 #3, 4, 7, 8, Landivy (1975); 1417 ouest and est, Ernée (1977); 1418 ouest and est, Laval (1977); 1419 ouest and est, Cossé-le-Vivien (1977); 1420 ouest and est, Craon (1977); 1516 #1-8, Domfront (1973); 1517 ouest and est, Mayenne (1978); 1518 ouest, Montsûrs (1978); 1518 est, Evron (1978); 1519 ouest and est, Meslay-du-Maine (1977); 1520 ouest and est, Château-Gontier (1977); 1616 #1, 2, 5, 6, LaFerté-Macé (1971); 1617 #1, 2, 5, 6, Villaines-la-Juhel (1975).
- KARLIN, A. A., AND S. I. GUTTMAN. 1981. Hybridization between *Desmognathus fuscus* and *Desmognathus ochrophaeus* (Amphibia: Urodela: Plethodontidae) in northeastern Ohio and northwestern Pennsylvania. *Copeia* 1981:371-377.
- KEY, K. H. L. 1968. The concept of stasipatric speciation. *Syst. Zool.* 17:14-22.
- . 1981. Species, parapatry, and the Morabine grasshoppers. *Syst. Zool.* 30:425-458.
- KOCHER, T. D., AND R. D. SAGE. 1986. Further genetic analyses of a hybrid zone between leopard frogs (*Rana pipiens* complex) in central Texas. *Evolution* 40:21-33.
- KOEHN, R. K., AND W. F. EANES. 1975. The analysis of allelic diversity in natural populations of *Drosophila*: The correlation of rare alleles with heterozygosity, pp. 377-390. In S. Karlin and E. Nevo (eds.), *Population Genetics and Ecology*. Academic Press, N.Y.
- LAMB, T., AND J. C. AVISE. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: The influence of mating behavior. *Proc. Natl. Acad. Sci. U.S.A.* 83:2526-2530.
- LANSMAN, R. A., J. C. AVISE, AND M. D. HUETTEL. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 80:1969-1971.
- LANTZ, L. A. 1947. Hybrids between *Triturus cristatus* Laur. and *Triturus marmoratus* Latr. *Proc. Zool. Soc. London* 117:247-258.
- LANTZ, L. A., AND H. G. CALLAN. 1954. Phenotypes and spermatogenesis of interspecific hybrids between *Triturus cristatus* and *T. marmoratus*. *J. Genet.* 52:165-185.
- LE GARFF, B. 1984. Amphibiens et reptiles de Bretagne. *Penn ar Bedd* 115:190-205.
- LITTLEJOHN, M. J., AND J. D. ROBERTS. 1975. Acoustic analysis of an intergrade zone between two call races of the *Limnodynastes tasmaniensis* complex (Anura: Leptodactylidae) in south-eastern Australia. *Aust. J. Zool.* 23:113-122.
- MACGREGOR, H. C., S. K. SESSIONS, AND J. W. ARNTZEN. 1990. An integrative analysis of phylogenetic relationships among newts of the genus *Triturus* (family Salamandridae), using comparative biochemistry, cytogenetics and reproductive interactions. *J. Evol. Biol.* 3:329-373.
- MALKMUS, R. 1980. Bemerkungen zu einer *Triturus boscai*-Population in einem Brunnenbecken der Serra de Sintra. *Bolm. Soc. port. Ciênc. Nat.* 20:25-40.
- MATÉRN, B. 1979. The analysis of ecological maps as mosaics, pp. 271-287. In R. M. Cormack and J. K. Ord (eds.), *Spatial and Temporal Analysis in Ecology*. International Co-operative Publishing House, Fairland, MD.
- MAURER, H. R. 1971. Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis. De Gruyter, N.Y.
- MAYR, E. 1963. *Animal Species and Evolution*. Belknap, Cambridge, MA.
- MAYR, E., AND R. J. O'HARA. 1986. The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution* 40:55-67.
- MCDONNELL, L. J., D. F. GARTSIDE, AND M. J. LITTLEJOHN. 1978. Analysis of a narrow hybrid zone between two species of *Pseudophryne* (Anura: Leptodactylidae) in south-eastern Australia. *Evolution* 32:602-612.
- MOORE, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. *Qt. Rev. Biol.* 52:263-277.
- MORAN, C., P. WILKINSON, AND D. D. SHAW. 1980. Allozyme variation across a narrow hybrid zone in the grasshopper, *Caledia captiva*. *Heredity* 44:69-81.
- NEI, M. 1972. Genetic distance between populations. *Am. Natur.* 106:283-292.
- NEI, M., J. C. STEVENS, AND N. SAITOU. 1985. Methods for computing standard errors of branching points in an evolutionary tree and their application to molecular data from humans and apes. *Mol. Biol. Evol.* 2:66-85.
- NELSON, K., R. J. BAKER, AND R. L. HONEYCUTT. 1987. Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus*. *Evolution* 41:864-872.
- PARENT, G. H. 1982. Bibliographie de l'Herpetofaune française. Inventaires de Faune et de Flore 17, 18. Secrétariat de la Faune et de la Flore, Paris.
- PECIO, A., AND J. RAFINSKI. 1985. Sexual behaviour of the Montandon's newt, *Triturus montandoni* (Boulenger) (Caudata: Salamandridae). *Amphibia-Reptilia* 6:11-22.
- PORTER, A. H. 1990. Testing nominal species boundaries using gene flow statistics: The taxonomy of two hybridizing admiral butterflies (*Limenitis*: Nymphalidae). *Syst. Zool.* 39:131-147.
- POWELL, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: Evidence from *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 80:492-495.
- RAFINSKI, J., AND J. W. ARNTZEN. 1987. Biochemical systematics of the Old World newts, genus *Triturus*: Allozyme data. *Herpetologica* 43:446-457.
- RAGGHIANI, M., AND D. B. WAKE. 1986. Genic variation and its evolutionary implications in the Italian newt, *Triturus italicus*. *Herpetologica* 42:206-214.
- RAND, D. M., AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: Mitochondrial,

- nuclear and reproductive differentiation of crickets by soil type. *Evolution* 43:432-449.
- RAXWORTHY, C. J. 1990. A review and revision of the smooth newt (*Triturus vulgaris*) subspecies, including an identification key. *Herpetol. J.* 1:481-492.
- RIENESL, J., AND G. P. WAGNER. 1989. The basipodial variation patterns in sympatric crested and marbled newts (*Triturus c. cristatus*, *Triturus m. marmoratus*) and their natural hybrids. *Verh. Dtsch. Zool. Ges.* 82:264.
- ROE, B. A., D.-P. MA, R. K. WILSON, AND J. F.-H. WONG. 1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J. Biol. Chem.* 260:9759-9774.
- SCHMIDTLER, J. J., AND J. F. SCHMIDTLER. 1967. Über die Verbreitung der Molchgattung *Triturus* in Kleinasien. *Salamandra* 3:15-36.
- . 1983. Verbreitung, Ökologie und innerartliche Gliederung von *Triturus vulgaris* in den adriatischen Küstengebieten (Amphibia, Salamandridae). *Spixiana* 6:229-249.
- SCHOORL, J., AND A. ZUIDERWIJK. 1981. Ecological isolation in *Triturus cristatus* and *Triturus marmoratus* (Amphibia: Salamandridae). *Amphibia-Reptila* 1:235-252.
- SLATKIN, M. 1973. Gene flow and selection in a cline. *Genetics* 75:733-756.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*, 2nd ed. Freeman, San Francisco.
- SPARREBOOM, M. 1986. Note préliminaire sur le comportement sexuel du triton crêté (*Triturus cristatus*) et du triton marbré (*Triturus marmoratus*) dans des conditions expérimentales. *Bull. Soc. Herp. Fr.* 40:36-42.
- SPOLSKY, C., AND T. UZZELL. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. *Proc. Natl. Acad. Sci. U.S.A.* 81:5802-5805.
- . 1986. Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. *Mol. Biol. Evol.* 3:44-56.
- SZYMURA, J. M., AND N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. *Evolution* 40:1141-1159.
- SZYMURA, J. M., C. SPOLSKY, AND T. UZZELL. 1985. Concordant change in mitochondrial and nuclear genes in a hybrid zone between two frog species (genus *Bombina*). *Experientia* 41:1469-1470.
- TAKAHATA, N., AND M. SLATKIN. 1984. Mitochondrial gene flow. *Proc. Natl. Acad. Sci. U.S.A.* 81:1764-1767.
- TEGELSTRÖM, H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). *J. Mol. Evol.* 24:218-227.
- VALLÉE, L. 1959. Recherches sur *Triturus blasii* de l'Isle, hybride naturel de *Triturus cristatus* Laur. × *Triturus marmoratus* Latr. *Mém. Soc. Zool. Fr.* 31:1-95.
- WALLIS, G. P. 1987. Mitochondrial DNA insertion polymorphism and germ line heteroplasmy in the *Triturus cristatus* complex. *Heredity* 58:229-238.
- 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.
- WHITE, M. J. D. 1946. The spermatogenesis of hybrids between *Triturus cristatus* and *T. marmoratus* (Urodela). *J. Exp. Zool.* 102:179-207.
- WOODRUFF, D. S. 1973. Natural hybridization and hybrid zones. *Syst. Zool.* 22:213-218.
- . 1989. Genetic anomalies associated with *Cerion* hybrid zones: The origin and maintenance of new electrophoretic variants called hybridzymes. *Biol. J. Linn. Soc.* 36:281-294.
- YANG, S. Y., AND R. K. SELANDER. 1968. Hybridization in the grackle *Quiscalus quiscula* in Louisiana. *Syst. Zool.* 17:107-143.
- ZUIDERWIJK, A. 1980. Amphibian distribution patterns in western Europe. *Bijdr. Dierk.* 50:52-72.
- . 1986. Interaction et accouplement du triton crêté et du triton marbré (Urodela, Salamandridae): Méthodes d'observation. *Bull. Soc. Herp. Fr.* 40:28-35.
- . 1989. *Triturus cristatus*, *Triturus marmoratus*, Hybride *Triturus cristatus*. *Triturus marmoratus*, pp. 44-49. In J. Castanet and R. Guyétant (eds.), *Atlas de Répartition des Amphibiens et Reptiles de France*. Société Herpétologique de France, Paris, France.
- . 1990. Sexual strategies in the newts *Triturus cristatus* and *Triturus marmoratus*. *Bijdr. Dierk.* 60:51-64.
- ZUIDERWIJK, A., AND M. SPARREBOOM. 1986. Territorial behaviour in crested newt *Triturus cristatus* and marbled newt *T. marmoratus* (Amphibia, Urodela). *Bijdr. Dierk.* 56:205-213.

Corresponding Editor: G. Hewitt

APPENDIX 1

Locality information, sample size, and species composition in electrophoretically studied allopatric, allotopic, and syntopic populations of *Triturus cristatus* and *T. marmoratus*. Sample sizes for mitochondrial DNA in parentheses. Species composition is determined over the years 1980–1988, without reference to hybrid individuals.

Allopatric populations									
<i>T. cristatus</i> (England)			<i>T. cristatus</i> (depts. Pas-de-Calais and Manche)			<i>T. marmoratus</i> (dept. Charente)			
Pond	Place near by	N	Pond	Place near by	N	Pond	Place near by	N	
WA1	Peterborough	1 (1)	P237, 498, 811	Marquise	112	C17	Confolens	37 (1)	
WA2	Oxford	1 (1)	P027	Boulogne-sur-Mer	15	C20	Rochechouart	72 (4)	
WA3	Maidstone	2 (2)	WA4	St.-Lô	1 (1)				
			Total		132 (5)	Total		109 (5)	
Sympatric area (dept. Mayenne), populations allotopic at the 0.95 level									
<i>T. cristatus</i>					<i>T. marmoratus</i>				
Pond	Neighboring farmhouse <sup>1</sup>	N	Species composition		Pond	Neighboring farmhouse <sup>1</sup>	N	Species composition	
			% <i>T. cristatus</i>	N				% <i>T. marmoratus</i>	N
2A1	la Grande Métairie	24	98	390	540	le Pavillon	26 (1)	97	38
2K5	la Lanfrière	5 (4)	100	10	514	le Moulin de Chavin	17 (2)	100	17
2C9	la Roche Pichemer	112 (10)	97	148	508	la Tuilerie	2 (2)	100	7
244	la Blanchardière	30	100	30	615	le Plantis	29 (1)	100	40
314	la Saulaie	41 (11)	100	75	173	la Merrière	187 (4)	100	193
431	le Pressoir	71 (15)	100	105	156	l'Aunay des Landes	3 (3)	100	37
					182	la Chénais	8	100	12
					141	Launay	27 (4)	100	67
Total		283 (40)			Total		299 (17)		
Sympatric area (dept. Mayenne), syntopic populations									
Pond	Neighboring farmhouse <sup>1</sup>	N			% <i>T. cristatus</i>	N	Species composition		
		<i>T. cristatus</i>	<i>T. marmoratus</i>	Hybrids <sup>2</sup>			% hybrids <sup>3</sup>		
2D5	le Vieux Four	11	85	1 (1)	16	977		1.3	
278	le Petit Planchallais	0	102		16	669		4.6	
232	les Teinières	7	10	5 (5)	20	125		14.3	
137	la Chauvinais	8	6		55	94		4.9	
292	la Sussotière	60	25	1 (1)	70	436		2.6	
249	la Viennière	22	11		80	157		4.6	
246	l'Ecottière	0	0	1 (1)	85	34		5.6	
2F8	la Chaîne	39	6		93	211		1.5	
Total		147	245	8 (8)				Mean: 3.8	

<sup>1</sup> See maps 1:25,000 of the Institut Géographique National.

<sup>2</sup> Two more hybrids were studied, one from St. Ouen des Toits (la Croix du Bouquet, pond 545) and one from St. Aignan de Couptrain (les Plaines, pond 184).

<sup>3</sup> Relative to total number of large-bodied newts; determined in 1980 and 1981.

## APPENDIX 2

Restriction fragment profiles for the four mtDNA genomes found in northwest European *Triturus cristatus* (CRI) and *T. marmoratus* (MAR). Asterisks indicate fragments in which a 140-bp insertion was detectable (Wallis, 1987). Sizes given for larger fragments are obtained by subtraction of the smaller fragments from the total genome size estimate derived from mapping experiments.

	CRI1	MAR18	MAR19	MAR20
<i>Bgl</i> II	16,470	14,505 1,825	14,505 1,825	14,505 1,825
<i>Cla</i> I	16,470	7,850 5,480 3,000	7,850 5,480 3,000	7,850 5,480 3,000
<i>Eco</i> RI	14,730 1,740	6,830 3,420 3,050 3,030	6,830 6,450 3,050	6,830 3,420 3,050 3,030
<i>Eco</i> RV	13,000 3,470*	16,330	16,330	16,330
<i>Hind</i> III	3,940* 3,840 3,540 2,950 1,980 220	8,730 3,000 2,090 1,750 540 220	8,730 3,000 2,090 1,750 540 220	8,730 3,000 2,090 1,750 540 220
<i>Pst</i> I	16,470	16,330	16,330	16,330
<i>Pvu</i> II	6,740 6,430 1,850 1,450*	16,330	16,330	16,330
<i>Sst</i> II	14,250 1,620 600	14,710 1,620	14,710 1,620	14,710 1,620
<i>Xba</i> II	12,220 4,250	16,330	16,330	16,330
<i>Xho</i> I	16,470	16,330	16,330	11,430 4,900