Additional results and discussion to the paper

ASYMMETRIC VIABILITY OF RECIPROCAL-CROSS HYBRIDS BETWEEN CRESTED AND MARBLED NEWTS (*TRITURUS CRISTATUS* AND *T. MARMORATUS*)

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Results

DATA INDEPENDENCE

The new data presented in this paper have been collected in five separate lab studies since 1987. The data derive from four different developmental stages of newt over 58 ponds, involving two categories of hybrid offspring (four categories for adult F_1). To address specific hypotheses and interpretations drawing on evidence from all datasets, it is important to establish independence of samples. The four age classes are completely independent from each other since larvae, hatchlings and embryos were all lethally sampled.

Adult F_1 hybrids from different ponds are always considered independent. Radio-tracking of the two species showed that 64% of newts remained within 20 m of their pond when they leave for the terrestrial phase, with a maximum movement of 146 m (Jehle and Arntzen 2000). In a tagging experiment carried out over three seasons, *Triturus* newts in

pond 278 were labeled while pond 222 (just 275 m away) was monitored. Only one tagged individual was found in pond 222 in up to 5 years since the start of tagging (N_{2006} = 127, N_{2008} = 120; Arntzen unpubl.). Of ponds that had adult F_1 hybrids of the same mitotype, two were 625 m apart (246 and 249) and two were 750 m apart (232 and 2C8). All the rest were over 1 km apart.

Within ponds, ages of some newts were determined skeletochronologically (Francillon-Viellot et al. 1990). F_1 hybrid newts of different ages from the same pond come from different matings so were regarded as independent. We also had to take into account the possibility of capture of sibs across years, which would represent the same mating. Only newts caught ≥4 years apart were considered independent. Survival rates from one year to the next are 67%, 71% and 74% for *T. cristatus, T. marmoratus* and hybrids, respectively (Francillon-Viellot et al. 1990). This temporal argument only applies to ponds 232 and 278, which both had estimated population sizes of *ca* 220 (Jehle et al. 2005) and individual detection probabilities of 53% and 56% within a season, respectively. It is unlikely that hybrid sibs will be captured more than three years apart (<3%).

Discussion

ASSUMPTIONS AND ALTERNATIVE EXPLANATIONS

There are a number of assumptions that we make in considering these data. First, that mtDNA is wholly maternally inherited. Slight paternal leakage could lead to chance amplification of a sequence of paternal origin. There is as yet little if any evidence of substantial paternal inheritance or leakage of mtDNA in chordates (Gyllensten et al. 1991; Ballard and Whitlock 2004; Wolff et al. 2008). As the 10 original hybrids (Arntzen and Wallis 1991) were examined using total mtDNA isolation, purification and RFLP analysis

(Wallis 1987) and all were pure *cristatus*-type we do not view paternal leakage as a likely confounding factor for either F_1 hybrid class. Additionally, RFLP analysis of PCR products gave no evidence of mixed profiles. Second, we assume that yolk GPI activity indicates the maternal origin of embryos. Since embryos possessed either one electromorph or the other (except for one embryo that had both in equal quantities, indicating a heterozygous mother, probably herself an F_1), we are confident that we are scoring the maternally derived phenotype. Third, we assume that all F_1 hybrids are detectable. In both fish and amphibian embryos, the expression of one parental allele (usually the paternal copy) is frequently delayed for some enzymes (Whitt et al. 1977; Vonwyl and Fischberg 1980; Vonwyl 1983; Gasser and Ferrier 1984; Whitt 1984). If one class of F_1 hybrid hatchling tended to show delayed gene expression from one of the parental species, that class may be under-represented. However, all our F_1 hybrid embryos showed clear co-dominance for both *Ldh* and *Mdh*, and no parental type showed "leakage" of a fainter electromorph. Consequently, delayed paternal gene expression does not seem a likely reason for the lower proportion of *cristatus*-mothered F_1 hybrid hatchlings compared to F_1 hybrid adults. As F_1 hybrid adults are morphologically distinctive, intermediate to the parental species (Fig. 1) (Vallée 1959; Arntzen 1996), it is implausible that our reliance (in part) on external morphology to identify hybrids for genetic analyses might have somehow biased our collecting towards one of the two reciprocal hybrids. We excluded just one unusual newt that we suspected to be a backcross. Fourth, we assume that at least 32 of our adult F_1 hybrids were independent samples based on age and pond. If some sort of unusual genetic system existed in which F_1 hybrids tended to exhibit premeiotic exclusion of one parental genome, as in the hybridogenetic frog *Pelophylax lessonae* (Spolsky and Uzzell 1986), then apparent F_1

hybrids might in fact be later-generation hybrids. Under this system, a group of apparent F_1 hybrids could in reality share a single common ancestor representing a single hybridization event several generations ago, and therefore be non-independent. Such a scenario would require most hybrids to have a hybrid parent, but the yolk analysis tells us that in only one case was the mother heterozygous. This explanation would therefore require a system perpetuated by hybridogenetic males crossing with a parental female. As males will not transmit their mtDNA, the scenario would specifically require that hybridogens usually derive from male hybridogens transmitting a wholly *T. marmoratus* nuclear genome while backcrossing to female *T. cristatus*. This scenario is again inconsistent with the yolk analysis, and male hybrids are known to be sterile (Bataillon and Tcherniakofsky 1932; White 1946; Lantz 1947; Lantz and Callan 1954), in accordance with Haldane's Rule (although some of these observations were based on hybrids involving either *T. cristatus carnifex* or *T. c. karelinii,* both now elevated to species).

EXPLANATORY GENETIC MODELS

Maternal effects are another possible factor. If provisioning of the embryo is much greater in one species than the other, then F_1 hybrid embryos from the lower-provisioning mother may have lower viability. In our case, there is no obvious difference in egg size. If anything, the heavier *T. marmoratus* produces a larger yolk sac, which should favor the category of F_1 offspring that are largely lacking in our data. In another way, these effects are rather unexpected. *Triturus* sex chromosomes (chromosome 4) show little heteromorphism; the Y has a larger sub-terminal C-band on the long arm (Sims et al. 1984). On the face of it, therefore, incompatibility asymmetries involving the sex

chromosome in *Triturus* should be less likely than in *Drosophila* (Turelli and Orr 2000). Crossing over is, however, rarely seen on the long arm of chromosome 4 in *Triturus*; when it is, it always involves the terminal heterochromatic blocks (Sims et al. 1984). Since the long arm encompasses at least 5% of a very large genome (24.5 pg; Horner and Macgregor 1983), the scope for independent evolution of the X and Y is actually enormous.

Asymmetries of this type could imply the involvement of a sex distorting endosymbiont microorganism of the sort found in many arthropods (Hurst and Jiggins 2005; Perlman et al. 2006) rather than an intrinsic cytonuclear incompatibility. The endosymbiont may be adapted to a particular host species' nuclear genome. When different taxa are crossed this co-adaptation may break down, with the result that certain crosses are no longer fertile. Ovary samples of four *T. cristatus* from two ponds and three *T. marmoratus* from three ponds were screened for the presence of *Wolbachia* with primers specific for *ftsZ*. No *Wolbachia* could be detected in any of the samples (M. Schilthuizen, pers. comm.), and its vertical transmission has not been demonstrated in any chordate.

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