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Crest evolution in newts: implications for reconstruction methods, sexual selection, phenotypic plasticity and the origin of novelties

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Abstract

The dorsal crest of newts (Salamandridae) is a novel, phenotypically plastic, sexually selected trait that may evolve in association with complex courtship behaviours. We estimated a near-comprehensive, time-calibrated phylogeny for salamandrids and analysed the evolution of their crests and display behaviour. Different models give conflicting reconstructions for crest evolution, showing that likelihood can estimate incorrect ancestral states with strong statistical support. The best-fitting model suggests that crests evolved once and were lost repeatedly, supporting the hypothesis that sexually selected traits may be frequently lost. We demonstrate the correlated evolution of crests and courtship behaviour and show that species with larger numbers of crest-related traits have larger repertoires of behaviours. We also show that phenotypically plastic morphological traits can be maintained over long macroevolutionary timescales (~25–48 Myr). Finally, we use salamandrids to address how novel structures may arise, and support a model involving the expansion and subdivision of pre-existing structures.

Introduction

Darwin (1871) first suggested that sexual selection is a major driver of morphological diversity among closely related species. Darwin's theory of sexual selection was neglected for more than a century, but in recent decades, an exciting literature has developed on the processes that drive the evolution of elaborate male ornaments and other signal-related traits (reviews in Kirkpatrick & Ryan, 1991; Andersson, 1994; Shuster & Wade, 2003; Mead & Arnold 2004; Kokko *et al.*, 2006). However, large-scale patterns of evolution in these traits remain poorly known. Although important studies of sexually selected traits have been performed in a phylogenetic context (e.g. Basolo, 1990; Ryan & Rand, 1995; Prum, 1997; Arnqvist, 1998), several fundamental questions remain. For example, it is unclear whether most changes involve origins of novel traits or losses of traits with more ancient origins (e.g. Wiens, 2001; Ord & Stuart-Fox, 2006) and how evolution of morphological and behavioural display traits are related (e.g. Prum, 1990; McLennan, 1991, 1996; Wiens, 2000). Here, we address these and related questions through a phylogenetic analysis of crests in newts and related salamanders (family Salamandridae). Analysing crest evolution in newts also creates opportunities to study the evolution of phenotypic plasticity and the origin of novel morphological traits in a phylogenetic context.

Darwin (1871) was also the first to suggest that the mid-dorsal body crests (Fig. 3) of many European newts (e.g. *Triturus*) evolved through sexual selection. Decades later, experimental studies confirmed that females prefer males with taller crests (e.g. in *Triturus carnifex*: Malacarne & Cortassa, 1983; *Triturus cristatus*: Hedlund, 1990; Malmgren & Enghag, 2008; *Lissotriton vulgaris*: Green, 1991; Gabor & Halliday, 1997). These crests have been noted for hundreds of years, and there have been intensive studies of newt phylogeny (e.g. Arntzen & Sparreboom, 1989; Titus & Larson, 1995; Caccone *et al.*, 1997; Weisrock *et al.*, 2006; Steinfartz *et al.*, 2007; Zhang *et al.*, 2008). However, the evolution of crests among species has not been studied.

Phylogenetic patterns of trait evolution in newts may be particularly interesting for several reasons. First, there is considerable variation in crests among species (Fig. 3), with some having low, short, smooth, inconspicuous crests (e.g. *Lissotriton helveticus*; Schlüpmann & van Gelder, 2004) and others having tall, denticulated, distinctly coloured crests that extend from the tip of the

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tail to the tip of the snout and nearly double the height of the individual (e.g. *Ommatotriton ophryticus*; Raxworthy, 1989). Many other sexually selected ornaments may also show extensive variation among species (e.g. plumage in birds; Prum, 1997; Omland & Lanyon, 2000). However, few studies, if any, have tested whether different components of these ornaments are correlated in their evolution or evolve randomly among species.

Second, previous studies have documented extensive variation in male courtship display behaviour among salamandrids, especially the European species (e.g. Arntzen & Sparreboom, 1989; Houck & Arnold, 2003). Although one might expect that the evolution of morphological displays (i.e. the crest) and behavioural displays should be tightly correlated, the only statistical phylogenetic test of this question (Wiens, 2000) found the evolution of these traits to be decoupled, seemingly because species that lost their morphological display trait (belly patches) maintained behavioural displays similar to species retaining this trait. Also, given that a clade may contain a diversity of display morphologies and display behaviours, it is unclear how the repertoires of displays and behaviours are related (e.g. do species with many derived morphological traits also have many derived behavioural traits? Or might species with more diverse behavioural repertoires have fewer morphological traits?). These possible relationships have not been previously tested.

Third, crests may be interesting for understanding how phenotypic plasticity evolves and contributes to morphological diversity (e.g. West-Eberhard, 2003), a topic only rarely addressed from a phylogenetic perspective (e.g. Emlen *et al.*, 2005; Gomez-Mestre & Buchholz, 2006). The dorsal crests of male European newts develop only during the breeding season and are then reabsorbed (e.g. Griffiths, 1996). Crest height is condition dependent, and crests may be reduced or lost entirely when newts are brought into the laboratory or starved (e.g. Green, 1991; Halliday & Arano, 1991). Thus, crests can be phenotypically plastic between seasons and within a season.

Finally, dorsal crests in European newts are largely unique among adult salamanders and amphibians, in general (Duellman & Trueb, 1986). Thus, an analysis of their evolution can potentially shed light on how novel traits arise, a key topic in evolutionary biology (e.g. Futuyma, 2005; Müller & Newman, 2005).

In this study, we apply a comparative phylogenetic approach to analyse the evolution of crests in newts. First, we combine existing data sets to develop a new, time-calibrated phylogeny for Salamandridae. Next, we assemble a data set on the distribution of crests and crest-related characters among salamandrid species (including courtship behaviours). We use the phylogeny to reconstruct the patterns of crest evolution among species and to test whether different crest components (e.g. extent, shape and colour) evolved concordantly or independently. We also test whether crest evolution is related to the evolution of different components of the courtship display and whether the acquisition of more modifications of the display morphology (crest) is related to the evolution of larger repertoires of behavioural display traits. We also use our analysis to address the evolution of phenotypic plasticity and evolutionary novelty in the group.

Materials and methods

Phylogenetic tree

Detailed methods for estimating the time-calibrated phylogeny are described in Appendix S1 in the Supporting Information. In short, we constructed a phylogeny that included most salamandrid species based on mitochondrial DNA data from the literature. We first obtained data from whole mitochondrial genomes for 37 salamandrid species, representing all salamandrid genera (from Zhang *et al.*, 2008). To increase the sampling of species, we next obtained data from Weisrock *et al.* (2006) for the ND1 and ND2 genes, a data set including most salamandrid species (~70 species). We then performed a combined likelihood analysis of these two data sets (using RAXML 7.2; Stamatakis, 2006), incorporating the maximum number of characters and taxa possible. This analysis yielded a generally well-supported, generic-level tree that is highly concordant with mitogenomic phylogenies but includes nearly all salamandrid species.

We then used the ND1–ND2 data alone to estimate divergence times (given that these genes have data for almost all species). We used the Bayesian uncorrelated lognormal approach (Drummond *et al.*, 2006) implemented in BEAST version 1.5.4 (Drummond & Rambaut, 2007), with eight fossil calibration points (see Appendix S1 for details).

Reconstructing character evolution

We obtained data on the distribution of male dorsal crests among salamandrid species based on descriptions and photographs in the literature, observations by the authors on living specimens in the field and photographs (available for almost all species) at AmphibiaWeb (http://amphibiaweb.org/index.html). The data matrix and literature sources for each species are given in Appendix S2. We recorded data on the following five characters (for examples of morphology, see Fig. 3).

1 Presence of male dorsal crests. In many salamandrids (especially *Ichthyosaura*, *Lissotriton*, *Ommatotriton* and *Triturus*), males develop crests on the dorsal midline of the body during the breeding season. These crests are seemingly extensions of the dermis (Halliday, 1975) and are not confined to the tail (but are typically continuous with the tail crest). We coded species as having a dorsal crest (state 1) or lacking one (state 0).

Some Asian newts have a low, nondimorphic ridge on the dorsal midline (e.g. *Cynops*), which does not appear to be homologous to the ornamentation seen in the males of many European newts.

Treating crest height quantitatively might have been desirable but proved problematic. First (and most importantly), we lacked quantitative data on crest height for all relevant species. In addition, it would be difficult to combine this quantitative trait with the other discrete variables for many of the tests.

2 Margination of dorsal crests. The dorsal margins of the crests may be smooth (state 0) or may be irregular or denticulated (1).

3 Anterior extent of dorsal crest. In some species, the crest may extend only from the tail onto the body but not onto the head (state 0). In others, the crest extends onto the head (state 1).

4 Separation of dorsal and tail crests. In some species, the dorsal crest extends continuously from the tail onto the body (state 0), whereas in others, the crest is distinctly reduced in height (or absent) near the sacrum, such that the crests on the tail and body appear to be distinct (state 1).

5 Coloration of crests. In some species, the coloration of the crest is indistinguishable from the adjacent dorsum (state 0), whereas in others, the crests may be distinctly different from the adjacent dorsum, typically because of distinctive dark vertical stripes (e.g. *Ommatotriton* and *Triturus marmoratus* species group) or spots (e.g. *Ichthyosaura alpestris*).

We analysed the evolution of these traits on the time-calibrated phylogeny (from BEAST) using maximum likelihood, as implemented in Mesquite version 2.73 (Maddison & Maddison, 2010). Maximum-likelihood estimation requires a model of trait evolution. We compared the fit of a one-rate model (single rate for both gains and losses of crests) and two different two-rate models. Under the first two-rate model (standard), different rates were estimated for gains and losses. Under the second two-rate model (no losses), a rate was estimated for gains and the rate of crest loss was fixed at zero. For the latter models, root frequencies were set to be equal. We also evaluated the consequences of using equilibrium root frequencies, fixing the root state to zero and incorporating rate heterogeneity among branches using the gamma parameter. The latter two approaches were implemented in BayesTraits (http://www.evolution.rdg.ac.uk/BayesTraits.html; by M. Pagel and A. Meade). However, we found that adding gamma led to only minor changes in likelihood (with little change in support for ancestral reconstructions), and this added parameter led to increased AIC (Akaike information criterion; Akaike, 1983) scores such that models incorporating this parameter had overall poorer fit. Therefore, we do not present results incorporating gamma.

To determine the best-fitting model for each trait, we calculated the AIC for each model. We used the formula $\text{AIC} = 2k{-}2\ \ln\ (\text{likelihood})$, where k is the number of parameters in each model (following Goldberg & Igic, 2008). The best-fitting model had the lowest AIC score. Differences in AIC between competing models were considered negligible if they were < 3, very strong if they were > 10 and moderately strong if they were between 4 and 7 (following Burnham & Anderson, 2002). The no-loss two-rate model was considered to have one parameter, given that the rate for losses (zero) was fixed and not estimated.

We then mapped the evolution of each trait on the phylogeny using the best-fitting model to estimate on which branches gains and losses have occurred. A branch was considered to be unambiguously reconstructed for a given trait and model only if there was significant likelihood support for the reconstruction (i.e. using the standard likelihood decision threshold of 2.0, when the difference between the log likelihoods with and without the state present at that node is 2 or more, or a proportional likelihood of 0.88 or higher).

Our main results focused on the presence and absence of dorsal crests. We estimated the robustness of these reconstructions to two potential sources of error: first, variation in branch lengths and topology and, second, possible effects of the character state on diversification and concomitant effects of diversification on reconstruction (e.g. Maddison, 2006). To address the first source of error, we reconstructed the evolution of crests on 5400 trees (using Mesquite) and compared the likelihood of the one-rate and two-rate models (standard and no-loss, both using equal root frequencies). These 5400 trees were from the 54 million post-burnin trees from the BEAST analysis, sampled every 10 000 generations. To address the second source of error, we performed analyses using the BiSSE approach (Binary State Speciation-Extinction; Maddison *et al.,* 2007) in Mesquite. This method estimates a likelihood value for a tree in which the rates of diversification (speciation – extinction and a parameter for the ratio of speciation to extinction) are potentially dependent on the states of a binary character. We contrasted a model in which gains and losses of crests were estimated separately to one in which gains of crests were possible but losses were very unlikely (rate = 1E-15; given that setting this rate to zero gives an undefined likelihood). We then compared the likelihoods of these models using the AIC.

The phylogeny shows many species with crests interdigitating among species without crests, which raises the question of how species without crests should be coded for characters associated with variation in the crests. Furthermore, maximum-likelihood analyses (in Mesquite) did not allow the inclusion of species with unknown states. It was therefore necessary to code the noncrested species in some way for each crest-related character.

Many species lacking dorsal crests have a tail crest. These tail crests may provide some insight into the condition of the crest in species lacking a dorsal crest (given that these crests are typically continuous in species with dorsal crests). In species having the tail crest but lacking the dorsal crest, the tail crest has a smooth dorsal margin, a posterior anterior extent and coloration that is indistinct from the adjacent tail (e.g. some *Lissotriton*, *Notophthalmus*; references for morphology in Appendix S2). Therefore, we tentatively coded species without dorsal crests as having state 0 for characters 2, 3, 4 and 5. An alternative coding strategy is to treat those species lacking the crest as having a third character state for those characters associated with variation in the crests (although the use of three states required the use of a single-rate model). This analysis gave similar results to those obtained from coding crestless species with state 0 (results not shown).

Lissotriton vulgaris has intraspecific variation in some crest-related characters (i.e. characters 2 and 3 vary among populations; Nöllert *et al.*, 2010). We coded this species as having these apparently derived modifications of the crest, and most methods for coding within-species variation for ancestral-state reconstruction emphasize the presence of the derived state in a species (such that alternative coding methods should give the same reconstruction; Wiens, 1999b). Furthermore, genetic structure in *L. vulgaris* is inconsistent with major geographic variation in morphology, making it difficult to justify treating this taxon as two or more separate species (Babik *et al.*, 2005).

Correlations between crest characters

We tested the hypothesis that different modifications of the dorsal crest (characters 2–5) are correlated with each other, using Pagel's (1994) maximum-likelihood method (in Mesquite v. 2.73). We compared the relative likelihoods of a 4-parameter, uncorrelated model (rate of gains and losses for each character independent) with an 8-parameter, correlated model (rates of gains and losses of each character dependent on the other character). Statistical significance of differences in likelihoods was evaluated by comparing AIC scores, as described earlier. Although Mesquite calculates *P*-values for these comparisons using simulations, these *P*-values did not appear to be conservative. Specifically, simulation replicates in which characters were constant (invariant) decreased the *P*-value, and these replicates often occurred frequently (e.g. > 50%). The initial choice of which character to consider dependent vs. independent was not clear and was made arbitrarily (the character with the higher number was considered dependent), and we confirmed that choice of a variable as independent vs. dependent led to only minor changes in likelihood differences between models. Only the first set of results for each pair of characters is presented.

Pagel's test can only be implemented with binary characters in which all taxa are assigned to one of two states. Therefore, we coded species lacking crests to one of the two alternate states (state 0; see above). This practice might potentially bias analyses of correlation between crest-related traits, as these traits can only evolve in those taxa having crests, not in any species in the tree (but see comments above regarding tail crests). However, among those species having crests, crest-related traits could still evolve independently of each other, with no overlap in the set of branches on which they evolve. Restricting the analyses to only those 13 species with crests showed no correlation between any crest characters (results not shown).

Correlations between morphology and behaviour

We also tested for potential correlations between the evolution of crests and the evolution of behavioural traits related to male courtship displays and reproduction. We assembled a data set (Appendix S3) of 31 behavioural characters from the literature for 40 salamandrid species that have relatively well-documented mating behaviour. These 40 species included all genera with dorsal crests and closely related genera from Europe (e.g. *Calotriton* and *Euproctus*), Asia (*Cynops*, *Laotriton*, *Pachytriton* and *Paramesotriton*) and North America (*Notophthalmus* and *Taricha*). We used Pagel's (1994) likelihood method to test for a possible correlation between each behavioural character (independent variable) and the presence of crests (dependent variable). Preliminary analyses suggested that treating behavioural characters as dependent variables had little impact on the results. Some behavioural characters lacked data for a few species, and these species were simply pruned from analyses including that character. Characters describing behaviours present only in one species, present only in females (one character), or that are present only in crestless outgroups (i.e. *Euproctus*, *Notophthalmus* and *Taricha*) were not included. Nevertheless, all characters are listed and described (along with literature sources) in Appendix S3.

We also tested for a general relationship between the number of behavioural courtship-related traits present in a species and the number of modifications of the crest (including crest presence). We tallied the number of crest components (the five characters listed previously; Appendix S2) and the number of male behavioural traits (the 31 characters in Appendix S3, minus the one female-only trait) in each species and tested for a relationship between them using phylogenetic generalized least squares (PGLS; Martins & Hansen, 1997). PGLS was implemented using the chronogram and the packages CAIC (Purvis & Rambaut, 1995) and Ape (Paradis *et al.*, 2004) in R, version 2.9.2. Given that the state of *Ommatotriton vittatus* is unknown for seven behavioural characters known for *O. ophryticus*, we assumed that *O. vittatus* also had these same states for these characters (rather than giving this latter species an inappropriately reduced number of traits and given that these two species are genetically very similar). The traits counted as 'present' within a species appear to be novel additions to the behavioural repertoire that have evolved within the group, based on their limited distributions among salamandrid species (note: if we simply counted the number of character states in each species, every species would have the same number). More specifically, these traits appear to be derived, given their absence in the outgroups to the European newt clade (*Euproctus*, *Notophthalmus* and *Taricha*) or their presence in only one of these three genera.

For these latter analyses, we again assumed that crests (dependent variable) evolve as a function of behaviour (independent variable). Reversing this assumption (behaviour as a function of crests) shows a weaker but still significant relationship between morphology and behaviour ($F_{1,38} = 8.9803$; $r^2 = 0.1912$; $P = 0.0048$).

In theory, we could have also addressed how crests (and behaviours) are related to sexual size dimorphism (SSD). However, previous studies of SSD in newts suggest that variation in crests and SSD are decoupled (e.g. species of *Triturus* with elaborate crests may have either male-biased or female-biased SSD; Ivanovic *et al.*, 2008).

Results

Phylogeny and divergence times

Our phylogenetic analyses yield the most comprehensive phylogeny of salamandrids to date (Fig. 1), in terms of both characters and taxa, by combining data from whole mitochondrial genomes (Zhang *et al.*, 2008) with data from extensive taxon sampling for the ND1–ND2 genes (Weisrock *et al.*, 2006; with additions, see Appendix S1). The relationships among genera estimated are identical to those postulated by Zhang *et al.* (2008), but with nearly twice as many species included. The support for intergeneric relationships and monophyly of genera is generally very strong (bootstrap support, bs = 99–100%), with the only exceptions being the clade that unites all salamandrids excluding *Salamandrina* (bs = 87%), the clade uniting *Laotriton* and *Pachytriton* (66%) and the monophyly of *Cynops* (64%). These three clades have similar support using whole mitochondrial genomes (Zhang *et al.*, 2008). There are some conflicts between generic-level relationships estimated by Weisrock *et al*. (2006 largest number of taxa) and by Zhang *et al*. (2008 largest number of characters), and in our study, these are resolved in favour of Zhang *et al.* (2008). These include the placement of *Salamandrina* and placement of the Asian newt clade (*Cynops* and relatives) among the European newts (*Calotriton*, *Ichthyosaura*, *Lissotriton*, *Neurergus*, *Ommatotriton* and *Triturus*). Hereafter, we refer to this latter group of genera as the 'European newt clade' but acknowledge that some species extend their ranges into Central Asia and the Middle East and that some European salamandrids are not in this clade. Despite the overall strong support, some relationships within genera remain weakly supported, such as within *Salamandra* and *Lyciasalamandra* and the *Triturus cristatus* group (many of these relationships are also weakly supported by Weisrock *et al.*, 2006). Overall, our results show that this sampling design can be successful, despite the fact that ~50% of the taxa have > 90% missing data (see also Wiens, 2003; Philippe *et al.*, 2004).

The divergence times estimated here (Fig. 2) are similar to those from previous studies. For example, Zhang *et al.* (2008) estimated the age of the earliest split within salamandrids to be between 97 and 69 Myr, whereas we estimate this clade to be ~91.2 Myr. Our estimated age for this clade is similar to that from nuclear and mitochondrial data (Roelants *et al.*, 2007) and nuclear data (Wiens, 2007; collectively 83.4–108.7 Myr). Similarly, Zhang *et al.* (2008) estimated the age of the European newt clade to be between 25 and 50 Myr, whereas we estimate it to be ~48.4 Myr. Our dates are also generally similar to those from Steinfartz *et al.* (2007).

Character evolution

Our results show extensive evolution of dorsal crests in the European newt clade (*Calotriton*, *Ichthyosaura*, *Lissotriton*, *Neurergus*, *Ommatotriton* and *Triturus*), but the pattern of changes inferred differs radically depending on the likelihood model used (Fig. 3). Under a one-rate model or a two-rate, no-loss model, the dorsal crest appears to have originated independently five times (i.e. in *Ichthyosaura*, *Ommatotriton* and *Triturus* and twice in *Lissotriton*) and is never unambiguously lost. Under the standard two-rate model, there has been a single origin of the dorsal crest in the ancestor of the European newt clade, followed by five losses. The support for the estimated ancestral state is very strong for most of the nodes in which different models yield different estimated states. For example, under the one-rate model and no-loss, two-rate model, there is strong support for crests being absent in the ancestor of the European newt clade (proportional likelihoods of 0.99 and 1.00), whereas under the standard two-rate model, there is strong support for crests being present at this node (proportional likelihood of 1.00). Because only one state was presumably present at a given node, this result suggests that at least one of the methods is yielding ancestral reconstructions that are incorrect and yet have strong statistical support.

Among the three models, the best-fitting model is the standard two-rate model (Table 1), with an AIC score that is lower than the other models by 3.61 (one-rate) or 3.35 (no-loss, two-rate model). We take this to be the best-supported hypothesis hereafter, but we acknowledge that these AIC differences are below the value (4) considered strong support. Use of equilibrium root frequencies showed much stronger support for the standard two-rate model (with a single gain and multiple

Fig. 1 Maximum-likelihood estimate of salamandrid phylogeny. Numbers adjacent to branches indicate bootstrap values > 50%.

losses; log likelihood = −18.1818; AIC = 40.3636), relative to the two-rate model with no losses (log likelihood = −48.0670; AIC = 98.1340). Fixing the root state to zero gave more equivocal results (standard two rate: log likelihood = −18.2111; AIC = 40.4222; two-rate no-loss: log likelihood = −19.3587; AIC = 40.7174).

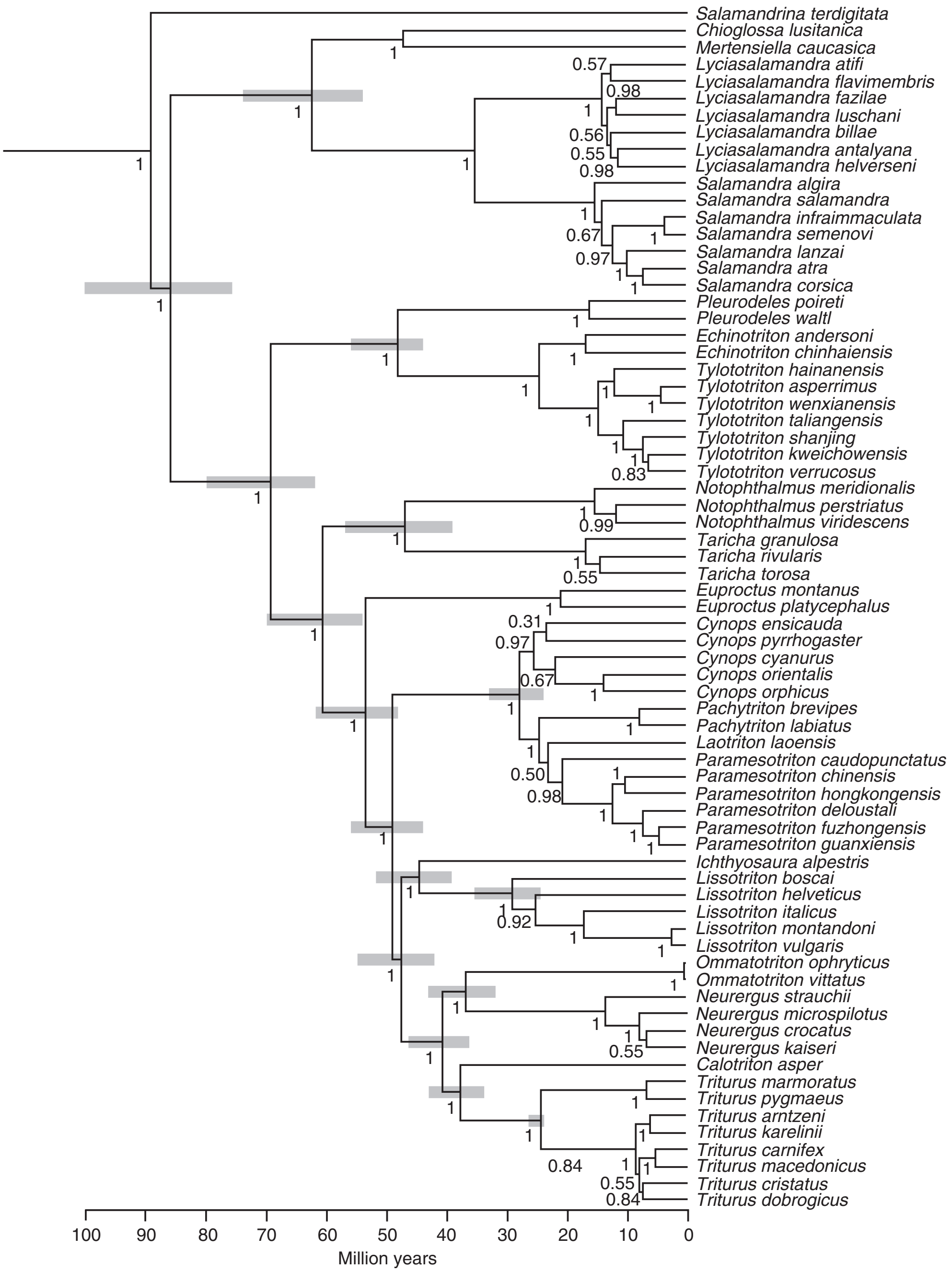

Fig. 2 Bayesian estimate of time-calibrated phylogeny for salamandrids. Numbers adjacent to nodes are posterior probabilities. The topology is the maximum clade credibility tree, and branch lengths are based on the mean values for post-burnin topologies. Grey bars indicate 95% highest posterior density intervals for ages for selected clades. Outgroup taxa are excluded.

These results were generally robust to variation in topology and branch lengths and incorporation of potential effects of the crest on diversification. Across the 5400 trees subsampled from the BEAST analysis, the mean log likelihood for the standard two-rate model is −18.0405 (AIC = 40.0810; range = 17.1–18.9) and is lower for the single-rate (mean = −20.8194; AIC = 43.6388; range = 20.0–21.8) and no-loss models (mean = 20.7596; AIC = 43.5192; range = 19.8–21.8). Incorporating potential effects of the crest on diversification rates shows weak evidence against the model with no losses (standard two-rate model log likelihood = −307.7766; six parameters; AIC = 627.5532; two-rate, no-loss model = −309.5763; five parameters; AIC = 629.1526), with an AIC difference

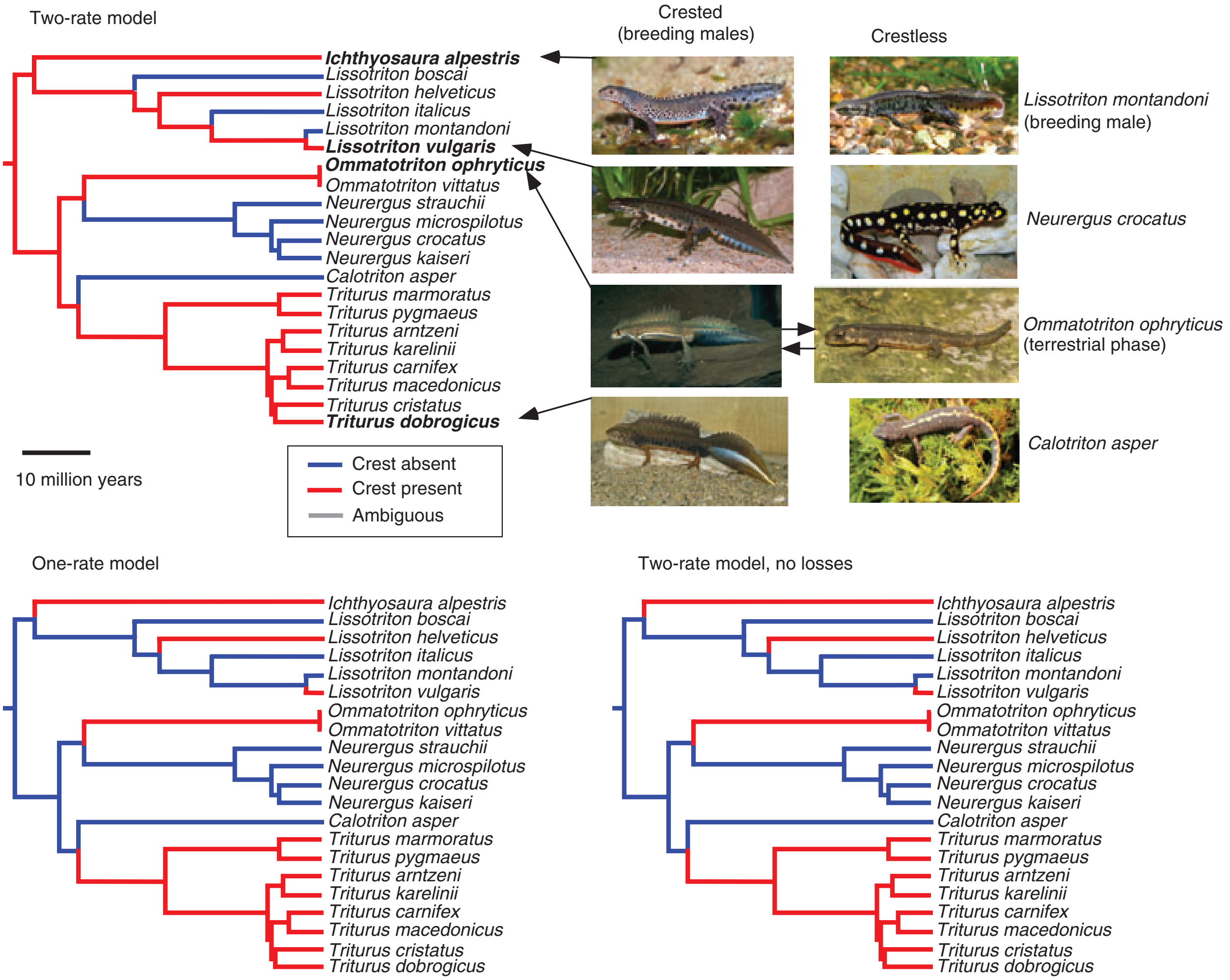

Fig. 3 Maximum-likelihood reconstructions of the evolution of dorsal crests in salamandrids under three different models, using the time-calibrated phylogeny (Fig. 2). Only the European newt clade is shown; dorsal crests are absent outside of this group (but note that analyses included all taxa). The standard two-rate model has the highest support (Table 1). Only branches that are significantly supported as having a given state are shown as being unambiguous (but note that all branches are unambiguous). Representative images of crested and crestless individuals are shown. For crested species, males in the breeding season are shown. These species also have a terrestrial, nonbreeding phase (illustrated for *Ommatotriton ophryticus*) in which crests are absent. Lineages lacking dorsal crests are also illustrated. Photo credits are Mark Aartse-Tuyn (*Calotriton asper*), Horia Bogdan (*Triturus dobrogicus*), Serge Bogaerts (*Neurergus crocatus*), Max Sparreboom (*Ommatotriton ophryticus*, breeding), Boris Timofeev (*Lissotriton montandoni*) and Henk Wallays (*Ichthyosaura alpestris, Lissotriton vulgaris, Ommatotriton ophryticus*, nonbreeding).

of 1.5994. However, setting diversification rates to be equal for both states suggests that crests actually have no influence on diversification (likelihood = −307.7677).

All models agree that there have been repeated origins of some crest components (Fig. 4), including denticulated margins (char. 2) and separation of dorsal and tail crests (char. 4). For the extension of the crest onto the head (char. 3) and the distinct coloration on the crests (char. 5), the reconstructions differ somewhat depending on the model, but the model with multiple origins has the lowest AIC score, even if the differences are slight (results not shown). These different aspects of the crest generally appear to evolve in a correlated fashion (Table 2), with significant correlations among four of six pairs of characters. However, none of these four characters have an identical distribution of states among taxa (Appendix S2).

Many behavioural characters show nonrandom associations with the evolution of crests (Table 3), including fan, whip, lean-in, cat-buckle, wiggle-tail bent and push back (described in Appendix S3). However, no characters have distributions fully concordant with that of the crest,

Table 1 Relative support for different models of evolution for the dorsal crest of newts.

Models	One rate	Standard two rate	No-loss, two rate
−ln Likelihood	20.1836	17.3772	20.0519
Estimated rates	0 to 1: 0.0033	0 to 1: 1.5798E−8	0 to 1: 0.0033
	1 to 0: 0.0033	1 to 0: 0.0249	1 to 0: 0
Parameters	1	2	1
AIC	42.3672	38.7544	42.1038

and some show only limited concordance (i.e. fan and wiggle-tail bent). The behavioural characters with distributions that most closely match the crest are whip, lean-in and cat-buckle. The functional linkage between the whip behaviour and the dorsal crest is not clear. However, the lean-in and cat-buckle behaviours may have both evolved to accentuate the crest during courtship displays, by raising the body (lean-in) and arching the back (cat-buckle). Using the standard two-rate model, it is not clear whether these three characters evolved in the ancestor of the European newts or evolved independently in the crested lineages (results not shown).

The number of crest modifications that are present in a species is significantly related to the number of behavioural traits present ($F_{1,38} = 14.8601$; $r^2 = 0.2811$; $P = 0.0004$), using phylogenetic methods (PGLS). This relationship is not perfect. For example, species of *Lissotriton* that lack crests have many behavioural display traits (although the absence of crests in these *Lissotriton* may reflect recent loss). Nevertheless, species with the most highly modified crests also have large numbers of novel behavioural traits (e.g. *Ommatotriton*), and species

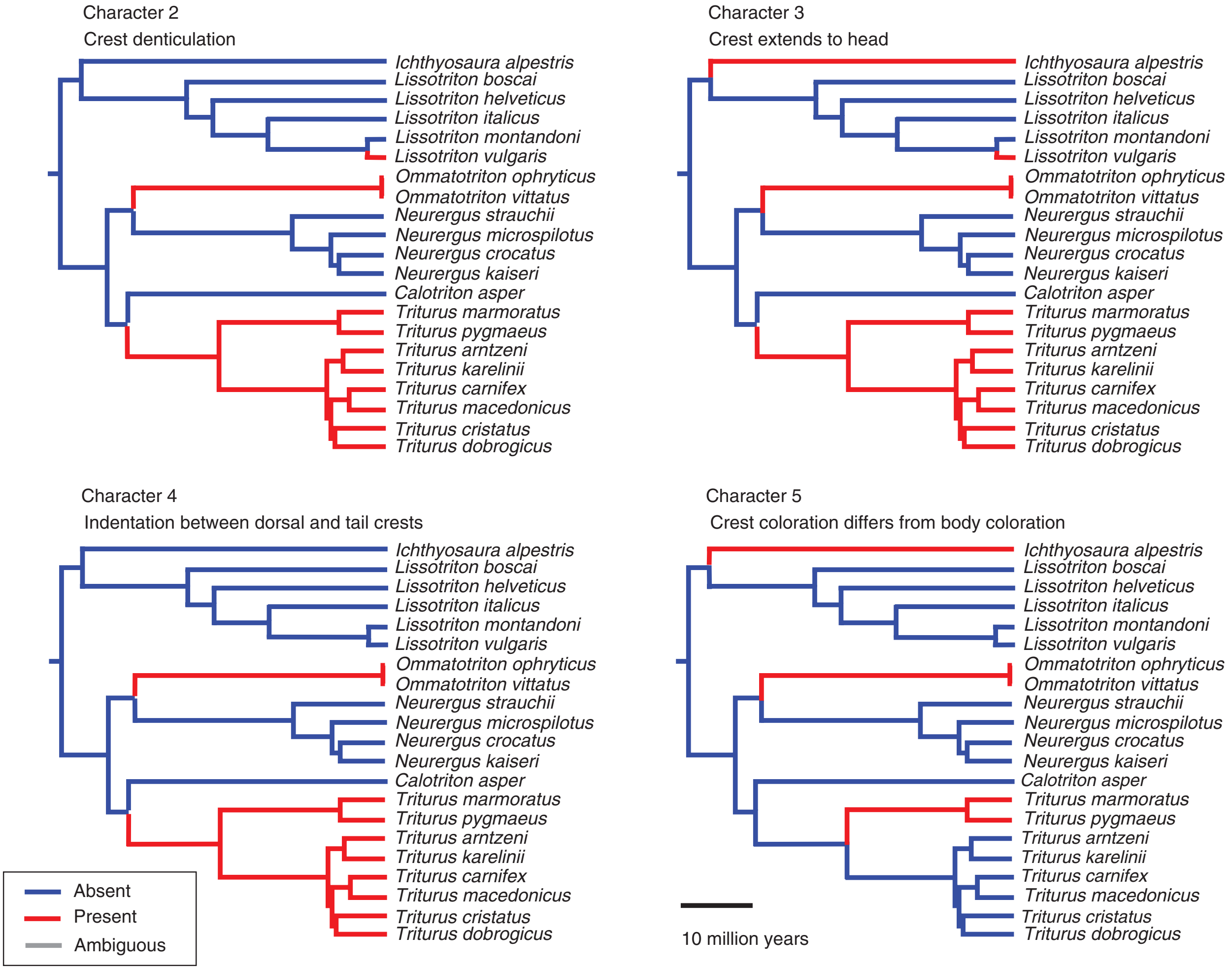

Fig. 4 Maximum-likelihood reconstructions of the evolution of four crest-related characters in salamandrids, using the time-calibrated phylogeny (Fig. 2). Reconstructions are based on the best-fitting model (lowest AIC) for each character. Only branches that are significantly supported as having a given state are shown as being unambiguous. Only the European newt clade is shown; dorsal crests are lacking outside of this group (but note that analyses included all taxa).

Table 2 Summary of comparisons of uncorrelated (four-parameter) and correlated (eight-parameter) likelihood models for pairs of characters describing variation in the crests, based on differences in the AIC score (positive values > 4 strongly favour the correlated model).

	Char. 3	Char. 4	Char. 5
Char. 2	12.0632	11.1206	−3.3240
Char. 3.	–	4.4802	5.8094
Char. 4		–	−0.7068

Table 3. Results of analyses of correlation between behavioural characters and the presence or absence of a dorsal crest. Boldface values of dAIC (delta AIC) indicate strong support for the correlated model (values > 4).

Behavioural character	Likelihood (uncorrelated)	Likelihood (correlated)	dAIC
Fan	−32.2368	−25.1138	**6.2463**
Wave	−21.0567	−15.7590	2.5953
Whip	−26.6713	−17.1232	**11.0962**
Flick	−21.5384	−17.8028	−0.5289
Lean-in	−30.4230	−16.1216	**20.6026**
Cat-buckle	−25.5552	−18.3767	**6.3568**
Shake out	−20.8835	−17.2368	−0.7065
Rocking	−19.6255	17.5319	−3.8128
Wiggle-tail bent	−31.0509	−20.1918	**13.7183**
Retreat display	−20.7013	−16.5623	0.2779
Flamenco	−27.2021	−26.0994	−5.7946
Creep	−23.7753	−21.4495	−3.3483
Brake	−25.4052	−20.5169	1.7764
Push back	−27.6776	−17.9240	**11.5073**
Caudal capture	−21.4974	−20.4798	−5.9648
Direct sperm transfer	−22.2590	−19.4294	−2.3409
Cloacal rub	−22.2590	−19.2918	−2.0656
Aggression	−32.0667	−27.3237	1.4860

with crests have a greater number of novel behavioural traits than species without crests (Appendix S3).

Combining the best-fitting likelihood reconstruction with the time-calibrated phylogeny (Fig. 3) suggests that dorsal crests have been present in European newts for at least ~48.4 Myr. Dorsal crests are phenotypically plastic in all species in which they are present. Therefore, phenotypic plasticity has been maintained for at least ~48.4 Myr. However, there is some uncertainty in the reconstruction of the crest (Fig. 3). The oldest clade in which crests are unambiguously present is the ancestor of *Triturus*, which is 24.9 Myr (Fig. 3). Thus, this phenotypically plastic trait is maintained in newts for at least 24.9 Myr, but possibly for > 48 Myr.

Discussion

In this study, we use a phylogenetic approach to examine the evolution of dorsal crests in European newts, a well-known sexually selected character system first noted by Darwin (1871). We find several intriguing results, which may have important implications for many evolutionary studies, especially relating to ancestral reconstructions, sexual selection, phenotypic plasticity and the origin of novel traits.

First, our results show that likelihood can yield misleading reconstructions with strong statistical support. Although the true ancestral state is unknown, when different models show strong support for different states at one node, at least one reconstruction must be incorrect. This result shows that it is crucial to test the robustness of ancestral reconstructions to different models of character evolution (Fig. 3). Although maximum likelihood may reflect the uncertainty in ancestral reconstructions better than parsimony (e.g. Schluter *et al.*, 1997), this uncertainty may be invisible when only one model is analysed.

We speculate that heterogeneity in rates of character change across the tree may be the underlying cause of these problematic likelihood reconstructions. In a similar study, Wiens *et al.* (2007) showed strong statistical support for different states for key nodes in hemiphractid frogs based on different likelihood models. In both studies, the character of interest was largely invariant on branches close to the root but varied extensively in a more nested clade, suggesting a dramatic shift in rate. Maximum-likelihood approaches often assume that rates of trait gain and loss are each homogeneous across a phylogeny, but this does not appear to be the case in these two studies. Unfortunately, incorporating rate heterogeneity across branches using the gamma parameter had little impact in salamandrids. Accommodating rate heterogeneity in the likelihood analyses of character evolution remains an important priority for future research (see also Skinner, 2010).

The best-supported model for our data suggests that dorsal crests have evolved once and then been lost five times within newts (Fig. 3), although the support for this hypothesis is not overwhelming and is contingent on the pattern of evolution being correctly reconstructed. Other broad-scale phylogenetic studies have also found losses of sexually selected traits to be roughly 4–5 times more common than gains (e.g. Burns, 1998; Wiens, 1999a). Additional studies show that such losses occur commonly in many different clades (e.g. Wiens, 2001; Emlen *et al.*, 2005; Ord & Stuart-Fox, 2006). The repeated loss of sexually selected traits suggests that sexual selection favouring these traits has weakened, that natural selection against them has become stronger, or both (review in Wiens, 2001). Previous studies have found that loss of these traits may be associated with the loss of female preference for those traits (e.g. Wong & Rosenthal, 2006) and with shifts in habitat usage (e.g. Wiens, 1999; Ord & Stuart-Fox, 2006).

What changes might favour crest loss in salamandrids? In some cases, these losses may be related to habitat shifts. Based on a survey of breeding habitats across salamandrids (using the Global Amphibian Assessment; IUCN 2010; http://www.iucnredlist.org/initiatives/amphibians), we find that most of the species with crests occur in ponds and other slow-moving waters, whereas crests are absent in species breeding only in streams. Within the European newt clade (which may have crests ancestrally), only *Calotriton* and *Neurergus* breed exclusively in streams, and our results suggest that these genera may have lost dorsal crests (Fig. 3).

In *Lissotriton*, crests appear to have been lost three times, but species with and without dorsal crests breed in similar, slow-water habitats (IUCN, 2010). Interestingly, *Lissotriton* are smaller than other European newts, with maximum total lengths from 8.5 to 11 cm (vs. 12–19 cm for the other genera in this clade; references in Appendix S2). In *Lissotriton*, smaller size may make males more vulnerable to predation (e.g. Shine, 1979), possibly causing the costs of crests associated with natural selection to outweigh their benefits due to sexual selection. Clearly, further research will be needed to understand the ecological and behavioural changes underlying the loss of crests (if this is what occurred).

Our results also suggest extensive parallel evolution of different components of the crests in different lineages (Fig. 4). These characters do not simply evolve randomly among lineages but, instead, seem to be correlated with each other, leading to the parallel evolution of similar, highly modified crest morphologies in some nonsister lineages (with the caveat that the gain and loss of crests may influence this analysis).

Our results also show a general relationship between the evolution of display morphology (the crest), and some male display behaviours (Table 3). Although it may seem obvious that display ornaments and display behaviours should be tightly correlated in their evolution, few studies have addressed this question using statistical phylogenetic methods (but for nonstatistical, phylogenetic examples see Prum, 1990; McLennan, 1991, 1996). In fact, the only study to do so did not support a relationship between the evolution of display morphology and behaviour (Wiens, 2000). In that study, most of the changes in display morphology involved losses, but these losses seemingly had little impact on the behaviour. Our study demonstrates that behavioural displays and morphological ornaments can be significantly correlated in their evolution, although we never found the distribution of dorsal crests and behaviours to be perfectly concordant among species.

We also show that the accumulation of novel elements of the behavioural display is related to the accumulation of modifications of the crests. To our knowledge, an association between the diversity of within-species morphological and behavioural trait repertoires has not been previously tested (but for similar studies, see Prum, 1997; Ord *et al.*, 2002). This association might occur through correlated evolution of both types of traits. But this association might also arise if similar processes drive the diversification of both morphological and behavioural display traits (e.g. Prum, 1997) and may be expected under some models of sexual selection (e.g. those predicting 'perpetual evolution' of male ornaments; Mead & Arnold, 2004). The correlated addition of novel elements to both the morphological and behavioural displays might also been seen as a trend towards increasing complexity in both signal types. Further analysis of this relationship between increasing morphological and behavioural display components could be an interesting topic for future research, in both newts and other organisms.

Our results also show that phenotypically plastic traits can be maintained for relatively long macroevolutionary timescales (i.e. at least 24.9 Myr, but possibly > 48 Myr). To our knowledge, our study and that of Gomez-Mestre *et al.* (2008) are the only ones to use time-calibrated phylogenies to address the timescale over which plasticity is maintained. The latter study suggested that plasticity in treefrog hatching times was maintained for ~37–50 Myr, a similar timescale to that found here. Furthermore, if plasticity for crests was indeed present in the ancestor of the European newt clade (Fig. 3), then the loss of crests may offer another example of how canalization of ancestral plasticity can contribute to morphological divergence among species (e.g. Emlen *et al.*, 2005; Gomez-Mestre & Buchholz, 2006).

Finally, the dorsal crests of newts may also provide some insight into how morphological novelties can arise, an important topic in evolutionary biology (e.g. Futuyma, 2005; Müller & Newman, 2005). Many morphological novelties are thought to arise through duplication and individualization of existing structures (e.g. Futuyma, 2005), where a structure is repeated in the body (duplication) and the two copies eventually vary independently (individualization). In contrast, newt crests may arise through expansion of an existing structure into a new region of the body, with subsequent separation from the original structure and individualization (Fig. 5). In all newts with dorsal crests, a tail crest is also present and is continuous or nearly continuous with the dorsal crest. The tail crest is widespread in salamander larvae and in the adults of many aquatic species (Duellman & Trueb, 1986). Although many European newts lack a clear separation between the dorsal and tail crests (e.g. *Ichthyosaura*, *Lissotriton* and *Triturus marmoratus* group), the crest is greatly reduced in height in the area of the sacrum in some species (*Ommatotriton* and *Triturus cristatus* group; Fig. 3; Appendix S2). Only a slight reduction in crest height would separate these crests, and they appear fully separate in some individuals (Fig. 3). Furthermore, the tail and dorsal crests show some signs of individualization (e.g. the pattern of coloration and denticulation of the tail and dorsal crests are somewhat divergent in *Ommatotriton ophryticus*; denticulation patterns differ in some *T. cristatus* group newts; Fig. 3). This general pattern of expansion, subdivision and

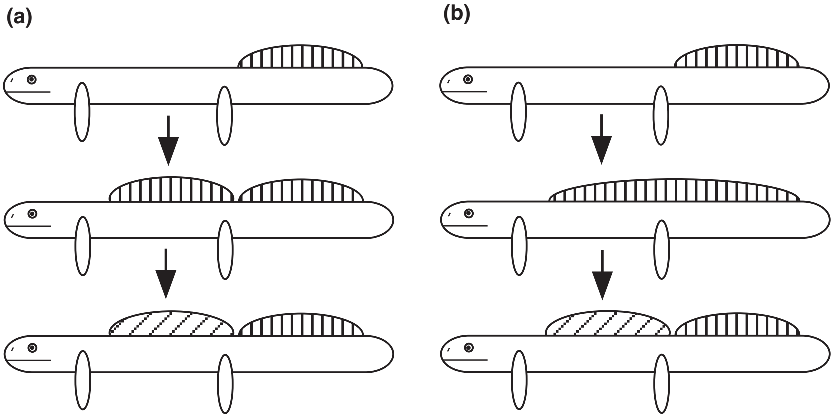

Fig. 5 Models of the origin of novel structures, illustrated with diagrammatic representations of the dorsal crest in newts. (a) Under the standard model of duplication and individualization, a second copy of the structure evolves (duplication) and then the two copies start to diverge independently (individualization). Here, the tail crest represents the original structure that is duplicated to yield the dorsal crest. (b) Under the model of expansion, separation and individualization, a structure expands into a new area of the body and then separates into two structures, which then diverge independently. We argue that dorsal crest in newts seems to follow this second model rather than the first.

individualization of existing structures may be important in the generation of other novel structures in other organisms as well.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Detailed methods for reconstructing the time-calibrated phylogeny.

Appendix S2 The distribution of the dorsal crest and crest-related characters among salamandrid species.

Appendix S3 Descriptions, references, and data matrix for behavioural characters.

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