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## Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis

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**Abstract** We tested whether temperature-induced variation in the growth rate of *Rana cascadae* tadpoles caused any variation in head width or leg length at metamorphosis, independent of the effects of temperature on body size. Body-size-adjusted head width appears to be insensitive to even large variations in tadpole growth rate. This result mirrors previous observations on the effects of variation in food level and temperature on metric shape in frogs and other ectothermic vertebrates. Leg length, on the other hand, showed a small but statistically significant response to the temperature treatment. Fast-growing tadpoles attained slightly longer legs than slowly growing tadpoles at a common metamorphic body size. This example is the first to show that variation in growth rate per se can influence metric shape (i.e., the rate at which individuals reach a common body size determines their shape at that size). Nevertheless, the induced effects were small, and our results taken together with those of previous studies suggest that environmentally induced variation in growth rate is not a major source of variation in metric shape of skeletal characters in ectothermic vertebrates.

**Key words** *Rana cascadae* · Heterochrony · Shape · Morphometrics

### Introduction

Plasticity is a pervasive feature of phenotypic variation in nature, and some traits are more plastic than others (Roff 1997; Schlichting and Pigliucci 1998). For example, skeletal characters in vertebrates are relatively canalized, and the extent to which geographic variation in morphometric shape of skeletal traits in this taxon is likely to be environmental is still not clear (James 1983;

Thorpe 1991; Jockusch 1997; Malhotra and Thorpe 1997). This question is important because size and shape of skeletal traits are still the main characters used to define vertebrate species, and because studying the role of selection in geographic variation requires understanding the genetic basis of that variation.

Here we are concerned with metric shape (functions of morphometric measurements such as the relative sizes of two body parts; e.g., Mosimann 1970; Sprent 1972; Rohlf and Bookstein 1987). The environment may control metric shape in vertebrates in several ways. First, training of particular body parts obviously controls bone growth. These effects are often observed in trophic structures whose size and shape can be modified by the size or hardness of food items (Moore 1965; Lanyon and Rubin 1985; Meyer 1987; Day et al. 1994; Robinson and Wilson 1995; Myers et al. 1996; Queral-Regil and King 1998). Second, particular morphs can be induced by social/behavioral cues such as the presence or density of prey, conspecifics, or predators (Collins and Cheek 1983; Pfennig 1992; Pettersson and Bronmark 1999). Third, environment might induce shape variation simply by controlling the overall rates of growth and differentiation. Variation in growth rate per se might affect shape in two ways (Blouin and Loeb 1991). In the trivial case, environment produces size variation simply because shape scales non-isometrically with body size and individuals are compared at different body sizes (Gould 1977). In the more interesting case, the rate at which two animals reach a common body size determines their shape at that size (i.e., changes in overall growth rate do not affect the growth rate of all body parts proportionately; Sprent 1972). Because many factors influence growth, this latter source of shape variation could be very important, particularly for ectotherms. Nevertheless, few studies have tested whether variation in growth rate per se really can induce size-independent shape variation in vertebrates.

Although environmental variation is well known to affect meristic traits in ectothermic vertebrates (counts, such as number of vertebrae or fin rays; Fox et al. 1961; Lindsey 1966; Fowler 1970; Peabody and Brodie 1975;

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Osgood 1978; Jockusch 1997), we are aware of only one example in which environmental manipulation of development rate per se might have affected size-independent metric shape for a skeletal trait. Juvenile tree frogs raised at different food levels did not differ in leg length at a common larger size, but did differ in head width (Blouin and Loeb 1991). However, this latter result might be just another example of the training effect on trophic structures. Neither temperature nor food level influenced size-adjusted head shape in snakes (Arnold and Peterson 1989; Forsman 1996). Similarly, tadpoles grown at different food levels did not differ in relative (size-adjusted) leg length or head width at metamorphosis (Blouin and Loeb 1991). So whether environmentally induced growth rate variation per se is a likely source of metric shape variation for head and limb traits in vertebrates remains an open question. Here we test whether temperature-induced variation in tadpole growth rate can cause variation in relative head width or leg length at metamorphosis.

## Materials and methods

### Justification for traits and environmental variable used

Head width and leg length in frogs are functionally important traits (Emerson 1978, 1985; Duellman and Trueb 1986) that show substantial between-population variation (Calhoun and Jameson 1970; Vogt and Jameson 1970; Mackey 1958) and are often used in traditional systematics (Duellman 1970; Jameson and Richmond 1971; Gaudin 1974; Trueb 1977). Although Blouin and Loeb (1991) found no effect of larval food level on size-adjusted shape at metamorphosis, the effects of temperature have never been examined. Temperature is one of the most important variables controlling growth in ectotherms. The effects of temperature are also worth studying because temperature controls growth and differentiation in a fundamentally different way than does food level (e.g., Smith-Gill and Berven 1979). Whereas food level variation generates a negative correlation between size at metamorphosis and length of larval period, temperature variation induces a positive correlation (i.e., differentiation rate is slowed more than growth rate by cold, whereas growth rate is slowed more than differentiation rate by low food). The hormones that control metamorphosis also control limb and skull development in frogs, and are very sensitive to temperature (Emerson 1986; Wassersug and Hoff 1982). So there are a priori reasons to suspect that relative head and limb sizes at metamorphosis will be sensitive to temperature (Emerson 1986; Emerson et al. 1988). Indeed, the types of heterochronic changes predicted are exactly the sort that explain the difference in relative limb length between two species of tree frog (timing of limb bud initiation, and limb vs body growth rates; Blouin 1991).

### Experimental procedures

Approximately 50 eggs from each of two recently laid egg masses of the Cascades frog, *Rana cascadae*, were collected from a roadside pond on route 20 near Santiam Pass, Oregon. Hatchlings were raised individually in 1.5-l bowls of deionized tap water, and fed a 3:2 mixture by weight of ground rabbit chow and flake fish food. Twelve hatchlings from each clutch were raised at 25°C, and 12 per clutch were raised at 18°C, both under a 12:12 light:dark cycle. These two temperatures were chosen in order to generate a large variance in growth rate. They are well within the range of temperatures experienced by the species in the field. Each met-

amorph was weighed on the day of first forelimb emergence, and then allowed to completely resorb its tail in a slanted bowl of water (approximately 10–12 days). We then anesthetized each animal with MS-222, and used a pair of calipers to measure, to the nearest 0.1 mm, its tibiofibula length (TFL), snout-vent length (SVL), and head width (HW) at the corners of the jaws. We used the average of two measurements on each trait. One larva died in the cold room shortly after the experiment was set up, but the remaining 23 developed normally and appeared healthy. All 24 developed normally in the warm room. We inadvertently let 7 warm-room animals dry out during tail resorption, so our final sample sizes for morphometric analyses were 17 from the warm room and 23 from the cold room.

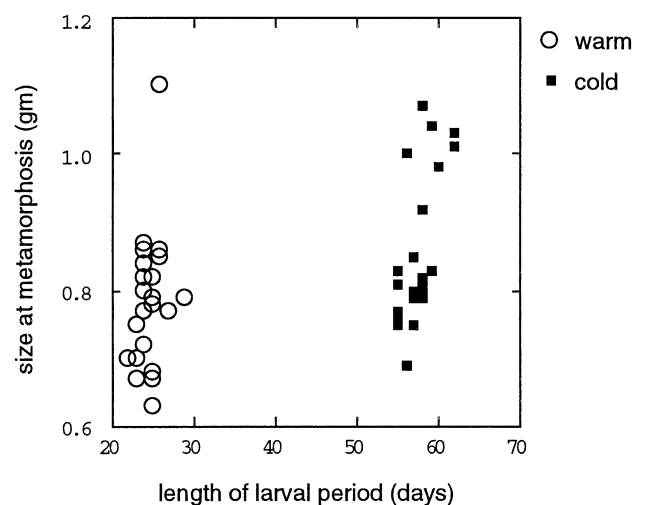
## Results and discussion

### Growth rate

As expected, the temperature treatment had a large effect on development. Cold-room tadpoles took twice as long to metamorphose as the warm-room animals (median larval period of 24 vs 58 days), and were slightly larger at metamorphosis (warm median=0.79 g, cold median=0.81 g; Fig. 1).

### Head width

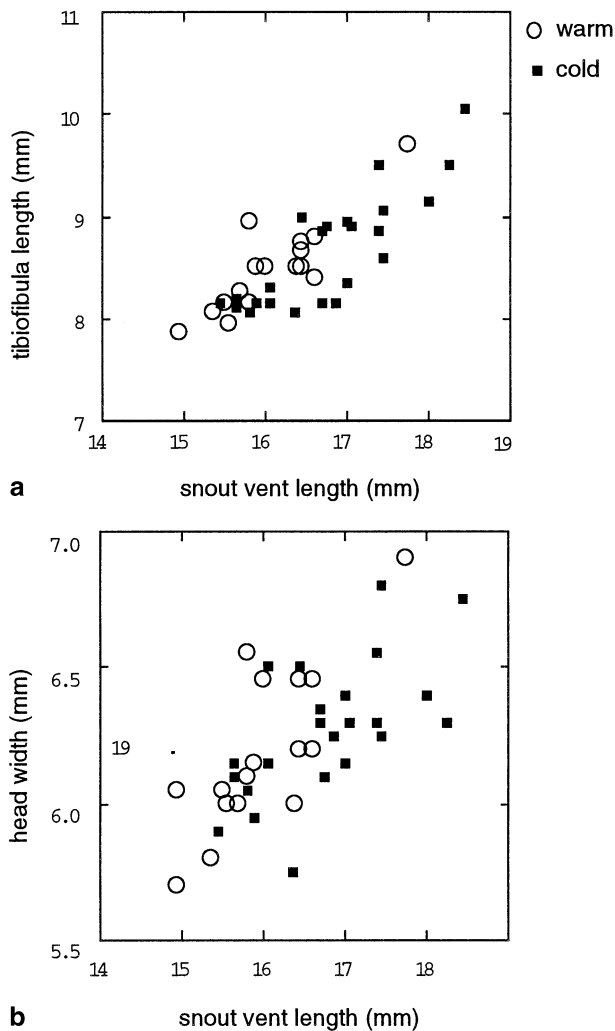
The two treatment groups had indistinguishable HWs after SVL was removed as a covariate (Table 1, Fig. 2). Note that the marginal significance of the interaction term ( $P=0.088$ ) results mostly from the one unusually large tadpole in the warm-room treatment (Fig. 1), which happened to have a wide head for its SVL, and thus high leverage in the ANCOVA (Fig. 2). Removing that individual, the P-value for the interaction becomes 0.308. Thus, the assumption of parallel lines is probably reasonable. Under the equal-slopes model, the adjusted least-



**Fig. 1** Weight at metamorphosis and length of larval period for larvae raised in warm room (circles) and cold room (squares).  $n=24$  in warm room and  $n=23$  in cold room (some points are superimposed)

**Table 1** Analysis of covariance for tibiofibula length and head width. Type I sums of squares with independent variables entered in order presented (TEMP rearing temperature, SVL snout vent length)

Source	Sum of squares	df	Mean square	F-ratio	P
<b>Tibiofibula length</b>					
SVL	7.574	1	7.574	95.31	0.000
TEMP	0.363	1	0.363	4.56	0.040
TEMP×SVL	0.004	1	0.004	0.05	0.826
Error	2.861	36	0.079		
<b>Head width</b>					
SVL	1.257	1	1.257	32.12	0.000
TEMP	0.103	1	0.103	2.64	0.113
TEMP×SVL	0.120	1	0.120	3.07	0.088
Error	1.409	36	0.039		



**Fig. 2** Tibiofibula length and head width of warm-room (circles) and cold-room (squares) metamorphs vs snout-vent length at tail resorption.  $n=17$  in the warm room and  $n=23$  in the cold room (some points are superimposed)

square mean HWs were 6.31 for warm-room and 6.20 for cold-room animals, for an observed effect size of 1.8%. Because there was no significant treatment effect, the power of the test to detect a biologically interesting effect is worth examining. If one considers a biologically interesting effect to be on the order of variation observed between conspecific anuran populations, then the data of Mackey (1958) are instructive. In his survey of 14 *Hyla regilla* populations in Oregon, he found populations that had approximately the same mean SVL but differed in mean HW by up to 8% (estimated from his Tables 9a and 11a). If we consider an effect of half that (4%) to be biologically significant, then the power to detect that effect, given our sample sizes and observed error variance, was 95% ( $\alpha=0.05$ , two-tailed test; O'Brien 1998). Thus, we had plenty of power to detect a biologically interesting effect had one existed.

### Tibiofibula length

The temperature treatment induced a small but significant difference in size-adjusted TFL (Table 1, Fig. 2). Warm-room animals had size-adjusted TFLs that were 2.6% longer than in cold-room animals (adjusted least-square mean TFLs of 8.47 and 8.69 for cold and warm, respectively). Mackey's (1958) data show mean TFL differences of up to 4% between *H. regilla* populations having similar mean SVLs (his Tables 9a and 10a). So, although the treatment effect observed in this study is small, it approaches what one might consider biologically interesting at the intraspecific level.

### Conclusions

The temperature treatment resulted in leg length variation that was independent of the effects of temperature on body size. This result is the first demonstration that environmentally induced variation in growth rate can influence shape independently of allometric effects. In other words, the rate at which individuals reach a common body size determines their shape at that size, as opposed to the shape variation resulting from body size variation and non-isometric growth of body parts. Although this mechanism for generating shape variation is interesting, it seems unlikely to be an important source of shape variation in nature. Despite a temperature treatment that induced extreme variation in growth rates (Fig. 1), there was no effect on head width, and the effect on leg length variation was relatively small. These results combined with the negative results of Forsman (1996), Arnold and Peterson (1989), and Blouin and Loeb (1991) suggest that environmentally induced growth rate variation is not a major source of size-independent metric shape variation in ectothermic vertebrates.

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