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Evolutionary Patterns of Diversity in Spadefoot Toad Metamorphosis (Anura: Pelobatidae)

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The larvae of spadefoot toads exhibit extreme developmental/endocrinological diversity. For example, New World spadefoot toads (*Scaphiopus* and *Spea*) have the shortest larval periods known among anurans, and the tadpoles of Old World spadefoot taxa (*Pelobates*) are among the largest known. To analyze the patterns of this diversity in an evolutionary context, we generated comparable larval growth and development data from 10 of the 11 taxa of spadefoot toads and from one taxon of parsley frog (*Pelodytes*), the nearest spadefoot toad relative. We found dramatic differences in growth and development among taxa, which indicated that taxon-specific physiology, rather than phenotypic plasticity, underlies larval period diversity. For all eight response variables (development rate, three growth rates, time to forelimb emergence, time to tail resorption, mass at tail resorption, and body length at tail resorption), taxa within genera were similar to each other and were different from taxa in other genera. Larvae of *Scaphiopus* were small with short larval periods, larvae of *Spea* were large with short larval periods, larvae of *Pelobates* were large with long larval periods, and larvae of *Pelodytes* were small with long larval periods. Even though taxa within the same genus live in different environments, larval growth and development correlated with phylogenetic groupings rather than breeding habitat. Mapping larval data onto a molecular phylogeny indicated that short larval periods, as well as rapid embryonic development and high temperature tolerance, originated within the spadefoot toad family.

THE New World spadefoot toad genera (*Scaphiopus* and *Spea*: Pelobatidae) are monophyletic and form a monophyletic sister group to the Old World spadefoot toads (*Pelobates*: Pelobatidae; Cannatella, 1985; Ford and Cannatella, 1993). The parsley frogs (*Pelodytes*: Pelodytidae) are the next closest relatives to the spadefoot toads. Despite the small number of taxa, spadefoot toads display almost the entire range of amphibian tadpole size and larval period length under natural conditions (Stebbins, 1951; Schleich et al., 1996; Kuzmin, 1999). The average larval period for the New World spadefoot toads is 26 days with a range of 8–86 days, and the average for Old World taxa is 130 days with a range of 36–180 days, not including overwintering examples (Wright and Wright, 1949; Busack and Zug, 1976; Kuzmin, 1999). In addition, the tadpoles of *Scaphiopus* are among the smallest at metamorphosis and *Pelobates* among the largest.

The above data from natural history observations may not be reliable for comparing larval traits across taxa. Spadefoot taxa have distinct breeding habitats, and their tadpoles grow in different natural conditions (Bragg, 1965; Diaz-Paniagua, 1988; Kuzmin, 1999). For example, most New World taxa live in xeric environments whereas the Old World taxa live in mediterranean climates or temperate forests (Stebbins, 1951; Conant and Collins, 1991; Arnold and Burton, 1992). Because larval development is greatly influenced by growing conditions, different conditions experienced across genera and species could explain differences in larval growth and development. For example, *Sc. couchii* breeds in desert pools where temperatures and food supply are high and the water level rapidly decreases. All these conditions favor short larval periods in tadpoles (Leips and Travis, 1994; Beck, 1997; Denver, 1997). Because of phenotypic plasticity (Wilbur and Collins, 1973; Travis, 1984; Newman, 1994), perhaps other taxa would metamorphose just as quickly if subjected to the same growing conditions as *Sc. couchii*. Thus, differences in growing conditions and taxon-specific physiological controls over the larval period are confounded in the previous studies.

Our experiments examined the diversity of larval growth and development across taxa, and we use these data to address ecological and evolutionary issues. First, we compared larval periods across spadefoot taxa to test whether New World taxa have intrinsically shorter larval periods than their Old World relatives. We reared tadpoles under laboratory conditions established previously (Buchholz and Hayes, 2000) to obtain growth and development data for all of the New World spadefoot taxa (*Scaphiopus cou-*

chii, Sc. holbrookii, Spea bombifrons, Sp. hammondii, Sp. intermontana, Sp. multiplicata), three of four Old World spadefoot taxa (*Pelobates cultripes, Pb. syriacus, Pb. varaldii*), and one of three taxa of parsley frogs (*Pelodytes ibericus*). Second, we used these data to evaluate whether larval period characteristics correlated with phylogeny or breeding habitat. Third, we tested the hypothesis that accelerated development evolved in the immediate ancestors of the New World spadefoot toads and is not ancestral for the family.

Materials and Methods

Collection, care, and breeding of adults.—Breeding adults of *Sc. couchii* and *Sp. multiplicata* were collected near Buenos Aires National Wildlife Refuge in Pima County, Arizona, on 3–5 July 1996. Breeding adults of *Sp. hammondii* were collected from Corral Hollow Road, San Joaquin County, California, in January 1997. Breeding adults of *Sp. intermontana* were collected from the campus of Deep Springs College, Inyo County, California, on 18 May 1997. Breeding adults of *Sp. bombifrons* were collected near mile marker 410 on Highway 80, Cochise County, Arizona, on 26 July 1997. Breeding adults of *Sc. holbrookii holbrookii* were purchased from Charles D. Sullivan, Inc. (Nashville, TN) in October 1996 (no locality data available). Breeding adults of *Pb. syriacus* were collected from Osman Gazi, Bursa, Turkey in April 1997. Two clutches of embryos of *Pb. varaldii* were collected from La Mamora Forest, Kenitra Province, Morocco on 2 December 1997. Breeding adults of *Pb. cultripes* were collected along Highway 612 just north of the entrance to the Reserva Biologica de Doñana, Andalucia Province, Spain on 25 November 1997. Breeding adults of *Pd. ibericus* were collected near the Palacio in the Reserva Biologica de Doñana, Andalucia Province, Spain on 24 and 26 November 1997.

Adult spadefoot toads were maintained in screen-covered plastic boxes (55 × 35 × 22 cm) in 7 cm of 50% utility sand/50% potting soil, and adult *Pelodytes* were maintained in screen-covered plastic boxes (30 × 20 × 25 cm) with a dish of water and hiding places. All frogs were fed appropriately sized $CaCO_3$-dusted crickets two to five times per week. To induce breeding, adults were injected intraperitoneally once with 20–100 μL of 1 μg/100 μL GnRH agonist {des-Gly^{10},[D-His(Bzl)^6]-luteinizing hormone releasing hormone ethylamide, Sigma}. Spadefoot toads placed in 50-liter tanks (50 × 30 × 40 cm) at 24 or 28 C with 20 liters of filtered tap water or 10% Holtfreter's solution (Holtfreter, 1931) overnight laid eggs on several plastic strips that angled into the water from stiff plastic mesh at the water's surface. Breeding tanks for *Pelodytes* had water 4 cm deep with vertical plastic strips around which clumps of eggs were laid. The morning after fertilization, embryos and water were transferred to 20-liter tanks and aerated. *Pelodytes varaldii* embryos were transported from La Mamora Forest to the University of California at Berkeley before feeding began (Gosner stage 25; Gosner, 1960). When feeding began, tadpoles were assigned to experimental treatments. Ten individuals were reared per taxon per temperature.

Sample size.—We chose our sample sizes based on our previous work on spadefoot toad larval periods (Buchholz and Hayes, 2000). These studies showed that for all six clutches of *Sc. couchii* and *Sp. multiplicata* the variation between sibships was significantly less than variation between species such that a single clutch was representative of its population (Buchholz and Hayes, 2000:fig. 5). Based on these results, one clutch per taxon was used, or two clutches with five tadpoles per clutch per temperature for *Sp. hammondii* and *Pb. varaldii.* The two clutches for these taxa were not significantly different within temperature for any response variable and were grouped together for all analyses. Because populations may have evolved in local environmental conditions, the populations from which we collected breeding adults may or may not represent the entire species. Nevertheless, we are comparing these taxonomic units in the current study because the evolutionary relationships among our populations are the same as the relationships of the species. It would be an improbable event if we chose a population that developed exceptionally slowly (Old World taxa) or quickly (New World taxa) compared to other populations in the species. This real possibility is mitigated because we would have to have chosen an exceptional population for each of the species within a genus. Under these circumstances, our conclusion would not be in error, but we could not extrapolate beyond the populations used in this study.

Experimental rearing conditions.—The experiments to compare larval period duration across taxa consisted of rearing tadpoles under identical laboratory conditions across three temperatures. Tadpoles were reared in isolation in 5-liter tanks (28 × 18 × 12 cm). Ten percent Holtfreter's solution was changed every other day. Tadpole tanks within each temperature room were randomized by taxon and kept on the same shelf to avoid the effects of tempera-

ture stratification. The photoperiod for all treatments was 12:12 h L:D (lights on at 0700 h). Because rabbit chow resulted in the largest tadpoles and the shortest larval period in previous studies (Buchholz and Hayes, 2000), tadpoles were fed ground rabbit chow ad libitum.

We used temperatures of 24, 28, and 32 C. These temperatures are generally within the natural range experienced for the New World taxa. *Scaphiopus couchii*, *Sp. multiplicata*, and *Sp. bombifrons* experience breeding pool temperatures of 20–38 C (Pomeroy, 1981; Newman, 1989), *Sp. intermontana* experiences 13–31 C (D. Reznick, pers. com.), *Sp. hammondii* typically experiences 11–32 C (D. Reznick, pers. com.), and *Sc. holbrookii* experiences 4–33 C (Gosner and Black, 1955). Equivalent data for Old World taxa are not available. However, the temperature tolerance range for parsley frogs is from 7–30 C (Balcells, 1955). Also, a laboratory study showed 35% mortality at 32 C and no mortality at 25 C in *Pb. cultripes* (Cei and Crespo, 1971).

Measurements.—As measures of comparison for growth and development across taxa, we recorded stage, mass, body length (snout–vent), and total length (snout–vent plus tail) every other day for *Pelobates* and *Pelodytes* and daily for New World spadefoot toads. Stages 25–31 and 41–46 were assigned according to Gosner (1960). Stages 32–38 were assigned according to Gosner but made explicit for spadefoot toads (Busack and Zug, 1976). Stages 39 and 40 were assigned by the partial and complete degeneration of the cloacal tail piece, respectively. Forelimb emergence (stage 42) was monitored every 12 h, and tail resorption (stage 46) was monitored daily. Tadpoles and metamorphs were blotted and weighed to the nearest 0.01 g. Body and total length were measured to the nearest 1 mm with a millimeter ruler.

Calculations and analyses.—We statistically analyzed development and growth rates across taxa as well as time to forelimb emergence, time to tail resorption, and size at tail resorption. Development rate was calculated as the least squares slope of the linear portion (stages 27–46) of the stage-versus-time curves. Growth rates were calculated as the slope of the linear portion (stages 26–38) of the mass, body length, and total length-versus-time curves. Although mass increased exponentially during the larval period, the growth rate was calculated as a linear function because r^2-values were rarely below 0.90 and never below 0.75. Multivariate ANOVA was performed across all eight response variables (development rate, growth rates for mass, body length and total length, time to forelimb emergence and tail resorption, and mass and body length at tail resorption) to test for significant effects of genus and temperature because many response variables were highly correlated. Because genus and temperature were significant factors in the MANOVA, a univariate ANOVA examined further the response variables of significant effects. For all analyses of variance, data were checked for equality of variances by the Equality of Variances *F*-test in the SuperANOVA statistical software (Abacus Concepts, Berkeley, CA). If *F*-tests showed significant heteroscedasticity, a ln-transformation of the data solved the problem. Because there were significant effects of genus and temperature, posthoc comparisons were performed for each response variable with the Tukey-Compromise test with significance accepted at $\acute{a} = 0.05$. *Z*-tests on correlation coefficients between growth and developmental variables tested for significant relationships between pairs of response variables.

Results

Growth curves.—With the exception of overall tadpole size, spadefoot tadpole growth curves with respect to stage were similar among taxa (Fig. 1). At the beginning of the larval period, spadefoot taxa were indistinguishable in mass, body length, and total length ($P > 0.05$). Body length and total length increased linearly with stage until the beginning of metamorphic climax (forelimb emergence, stage 42), whereas mass increased exponentially in all taxa. Maximum mass occurred between stages 38–41, maximum body length occurred between stages 37–40, and maximum total length occurred between stages 39–41. Similar results were obtained at 28 and 32 C, except that higher temperatures increased the slopes of the curves (data not shown).

Effects of genus and temperature.—The statistical tests revealed significant differences across genera and temperature. In the full factorial MANOVA with genus and temperature as factors, genus (Wilks' Lambda $F_{24,33} = 18.4$, $P = 0.0001$) and temperature (Wilks' Lambda $F_{24,33} = 2.81$, $P = 0.02$) were significant factors. Univariate ANOVAs showed that genus was a significant factor for all eight response variables, and temperature significantly affected all development variables (development rate and times to forelimb emergence and tail resorption) and one of the five growth variables (growth rate for body length; Table 1). There was no genus by temperature interaction. For the ANOVAs, we as-

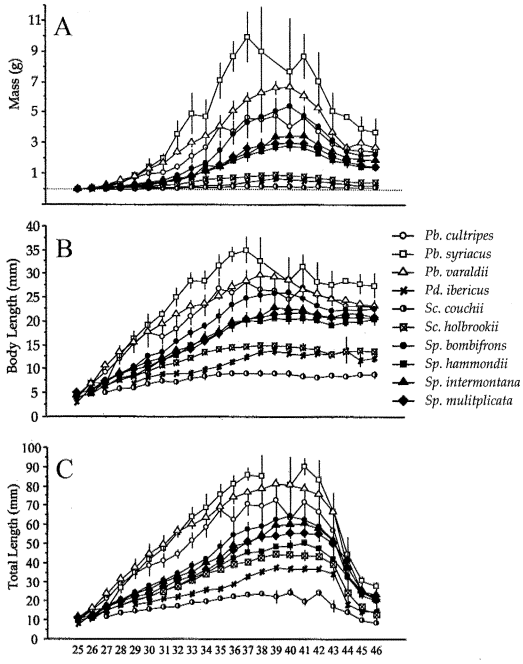

Fig. 1. Comparison of spadefoot toad and parsley frog larval growth from beginning of the larval period to the end of metamorphosis at 24 C. Mass (A), body length (B), and total length (C) are compared across Gosner stage for each taxon. Each data point shows the average and standard deviation for 10 individuals.

signed the value of the average of the 10 replicates within each temperature to each taxon. Consequently, each taxon was a replicate within its genus, and the sample size for each genus was the number of taxa in that genus.

Development rate.—New World spadefoot toads, *Scaphiopus* and *Spea*, developed faster than Old World taxa, *Pelobates* and *Pelodytes*, at each temperature ($P < 0.05$, Tukey Compromise, Fig. 2A). Also, within the New World spadefoot toads, *Scaphiopus* had significantly faster development rates than *Spea* at 24 and 28 C, but not at 32 C (Fig. 2A). Within taxa, temperature significantly increased the rate of development within *Spea* ($P < 0.05$, Tukey Compromise) but not within the other taxa.

Growth rates.—Despite the large differences in juvenile size (see below), there was only one case of significant differences in larval growth rates among taxa. *Scaphiopus* increased in mass significantly slower than *Pelobates* and *Spea* at 28 C ($P < 0.05$, Tukey Compromise, Fig. 2B). The significant effect of genus and temperature in growth rates for body length and total length (Table 1) did not reflect any single significant pairwise comparison between genera when separate analyses were carried out for each temperature ($P > 0.05$, Tukey Compromise) or genus ($P > 0.05$, Tukey Compromise) (Fig. 2C–D).

Time to forelimb emergence and tail resorption.—Consistent with development rate, New World spadefoot toads achieved forelimb emergence significantly earlier than Old World spadefoot toads and parsley frogs at all temperatures ($P < 0.05$, Tukey Compromise, Fig. 3A). There was little overlap in the ranges of the responses, such that all individuals of *Scaphiopus* reached forelimb emergence (range of 16–19 days) before any *Spea* (20–28 days), and *Spea* reached forelimb emergence before any *Pelodytes* (28–36 days) or *Pelobates* (31–49 days). In addition, New World spadefoot toads reached tail resorption before Old World spadefoot toads ($P < 0.05$, Tukey Compromise, Fig. 3B). Even though time to tail resorption in parsley frogs was not significantly different from *Pelobates* and *Spea*, there was no overlap of individuals among genera. Temperature had no significant effect on time to forelimb emergence or time to tail resorption in *Pelobates* ($P > 0.05$, $F_{2,7} = 1.4, 1.2$). In *Spea*, both time to forelimb emergence and tail resorption were not significantly different between 28 and 32 C, and both were significantly lower than values at 24 C ($P < 0.05$, Tukey Com-

TABLE 1. SUMMARY OF UNIVARIATE ANOVA *F*-VALUES FOR DEVELOPMENT RATE (DRATE), GROWTH RATES FOR MASS (MRATE), BODY LENGTH (BLRATE), AND TOTAL LENGTH (TLRATE), TIME TO FORELIMB EMERGENCE (TTM), TIME TO TAIL RESORPTION (TTTR), MASS AT TAIL RESORPTION (M), AND BODY LENGTH AT TAIL RESORPTION (BL). The average value of 10 individuals was used for each taxon and temperature. * indicates $P < 0.05$ and ** indicates $P < 0.001$.

Source	df	DRate	MRate	BLRate	TLRate	TTM	TTTR	M	BL
Genus	3	26.7**	9.73**	6.51**	3.84*	72.0**	71.3**	60.1**	63.9**
Temp	2	10.7**	0.82	3.86*	2.85	8.93*	11.5**	0.74	0.01
G×T	4	2.93	0.32	1.17	1.35	2.54	1.60	0.29	0.20

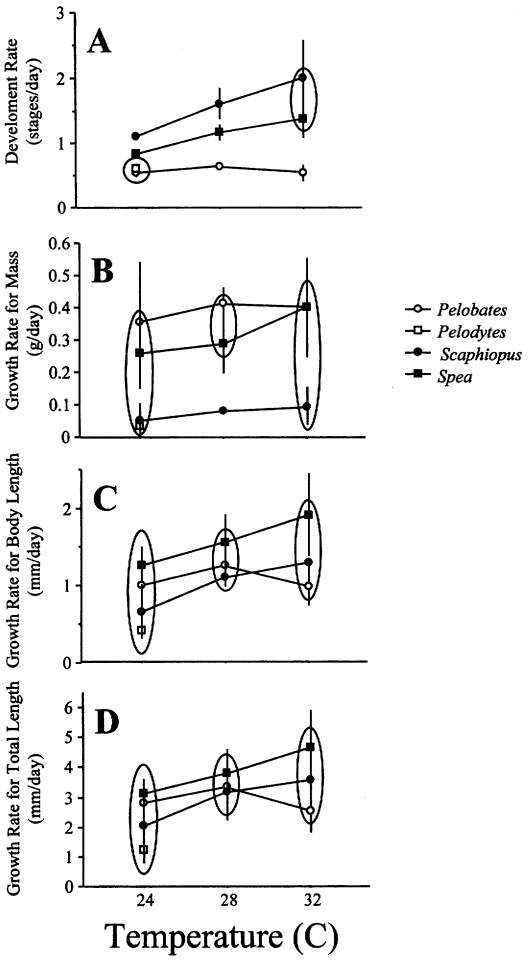

Fig. 2. Comparison of spadefoot and parsley tadpole development and growth rates. (A) Development rates calculated from Gosner stage 27–46 are compared for tadpoles reared at 24, 28, and 32 C. Tadpole growth rates for mass (B), body length (C), and total length (D) were calculated from the size versus time curves between Gosner stages 27 and 38 for tadpoles reared at 24, 28, and 32 C. Only data for 24 C is available for tadpoles of *Pelodytes*. For clarity, each data point shows the average and standard deviation for each genus instead of each species. Uncircled data points and circled groups of data points represent significance groups based on Tukey-Compromise posthoc tests.

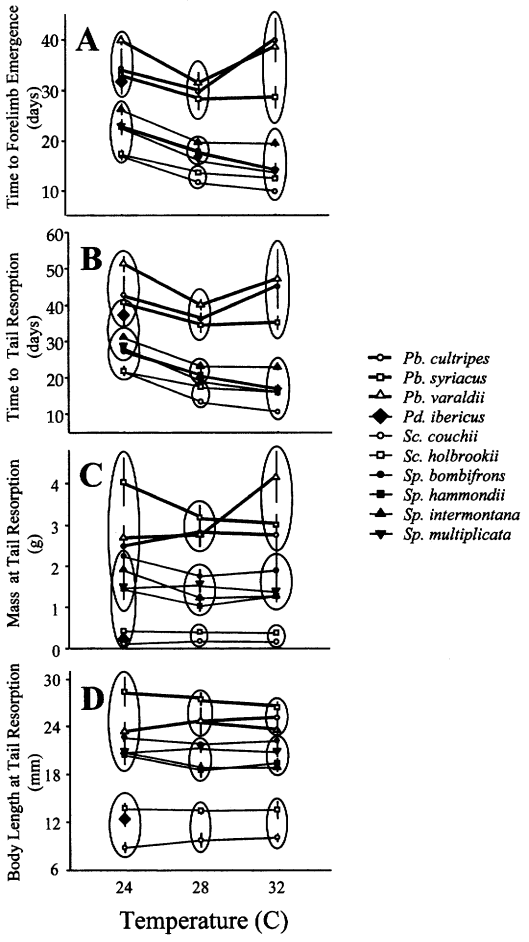

Fig. 3. Comparison of spadefoot and parsley frog larval period length and size at metamorphosis. Time to forelimb emergence (A), time to tail resorption (B), mass (C), and body length (D) at tail resorption are compared for tadpoles reared at 24, 28, and 32 C. Each data point shows the average and standard deviation from 10 individuals. Uncircled data points and circled groups of data points represent significance groups based on Tukey-Compromise posthoc tests.

promise). In *Scaphiopus*, time to forelimb emergence at 28 and 32 C was significantly shorter than at 24 C ($P < 0.05$, Tukey Compromise).

Mass and body length at tail resorption.—All genera of spadefoot toads and parsley frogs were significantly different from each other in size at tail resorption at 28 and 32 C ($P < 0.05$, Tukey Compromise, Fig. 3C–D). At 24 C, *Scaphiopus* and *Pelodytes* were significantly different from *Pelobates* in mass and body length at tail resorption ($P < 0.05$, Tukey Compromise). *Spea* was intermediate in mass between *Scaphiopus* and *Pelobates* and grouped with *Pelobates* in body length ($P < 0.05$, Tukey Compromise).

Comparison of growth and development.—We used *Z*-tests to evaluate significance of correlations within growth variables and within development variables at different taxonomic levels. Across genera, correlations between development variables (development rate, time to forelimb emergence, time to tail resorption) were signif-

TABLE 2. CORRELATION ANALYSIS OF GROWTH AND DEVELOPMENT VALUES ACROSS GENERA AT 24 AND 28 C USING *Pelobates, Scaphiopus, Spea* AND *Pelodytes* (24 C ONLY). The hypothesized correlation of zero was evaluated using the *Z*-test. TTM = time to forelimb emergence, TTTR = time to tail resorption, DevRate = development rate, M = mass at tail resorption, BL = body length at tail resorption, MRate = growth rate for mass, BLRate = growth rate for body length.

Comparison	Temperature	Correlation	*Z*-value	*P*-value
TTM *v* TTTR	24	0.994	7.58	<0.0001
	28	0.997	7.78	<0.0001
TTM *v* DevRate	24	−0.975	−5.79	<0.0001
	28	−0.959	−4.74	<0.0001
M *v* BL	24	0.960	5.14	<0.0001
	28	0.962	4.84	<0.0001
M *v* MRate	24	0.914	4.11	<0.0001
	28	0.927	4.01	<0.0001
BL *v* BLRate	24	0.720	2.40	0.0163
	28	0.338	0.86	0.3881
TTM *v* M	24	0.651	2.06	0.0398
	28	0.936	4.18	<0.0001
TTM *v* BL	24	0.573	1.73	0.0844
	28	0.858	3.15	0.0016

icant, that is, *Z*-tests showed the correlations were significantly different from zero (Table 2). Similarly, growth variables were significantly correlated with most other growth variables across taxa (Table 2). Within taxa, equivalent correlations were usually not observed for each taxon at each temperature (data not shown). As exceptions, the three development variables, time to forelimb emergence, time to tail resorption, and development rate, were not correlated with each other for *Sc. couchii* and *Sc. holbrookii* at 24 C or in *Pb. cultripes* at 28 C. However, the lack of these correlations within taxa did not occur across all temperatures. The only consistent difference within taxa compared to between taxa was that body length was correlated with mass between taxa and not within.

To compare growth with development, we examined correlations between time to forelimb emergence and mass and body length at tail resorption. Across taxa, time to forelimb emergence was correlated with mass and not significantly correlated with body length at tail resorption (Table 2). At 28 C (a temperature at which *Pelodytes* did not survive), time to forelimb emergence was highly correlated with both measures of size at tail resorption (Table 2). When *Pelodytes* was not included in the analysis at 24 C, both mass and body length were correlated with time to metamorphosis (data not shown). Within each taxon, time to forelimb emergence did not correlate with mass or body length at any temperature (data not shown).

Embryological development and mortality.—We report embryological development and mortality differences between taxa because they may relate to tadpole physiology and ecology. Differences across taxa were observed in the time to the beginning of feeding at stage 25. New World spadefoot toads had rapid embryological development such that feeding began on day 3 postfertilization. Feeding began later in the other taxa, *Pb. cultripes* (day 6), *Pb. syriacus* (day 7), and *Pd. ibericus* (day 5). In New World spadefoot toads, mortality ranged from 0–50% and averaged 20% with no pattern among temperatures. Mortality in *Pelobates* was between 0 and 10% at forelimb emergence for all temperatures and at tail resorption for 24 and 28 C. However, at 32 C, two to four tadpoles per taxon of *Pelobates* died between forelimb emergence and tail resorption (20–40%). The remaining *Pelobates* tadpoles at 32 C appeared unhealthy, lethargic, and grossly edematous. In the case of *Pb. syriacus*, we removed two runts and two giants at 24 C and one giant and one runt for both 28 and 32 C from analyses because they showed no signs of metamorphic development at the time when siblings metamorphosed. In *Pelodytes*, at 24 C, there was 10% mortality. At 28 C, all but two tadpoles died before forelimb emergence, and these two died before tail resorption. At 32 C, *Pelodytes* tadpoles did not survive longer than the first four days of larval life.

DISCUSSION

Our data showed that New World spadefoot toads have shorter larval periods than their clos-

est relatives, Old World spadefoot toads and parsley frogs. We eliminated differences due to environmental factors to show that larval period differences are due to intrinsic, taxon-specific developmental/endocrinological factors. Because phenotypic plasticity greatly affects larval period traits, it was not clear that previous reports suggesting differences in spadefoot larval growth and development observed under natural conditions were not merely the result of those conditions (Wright and Wright, 1949; Busack and Zug, 1976; Kuzmin, 1999). To compare taxa under comparable conditions and to eliminate differences caused by environmental factors, we reared tadpoles under controlled laboratory conditions established previously (Buchholz and Hayes, 2000). That study examined 15 rearing conditions, varying in temperature, larval density, and food type, to compare growth and development in *Sc. couchii* and *Sp. multiplicata*. We found that 32 C, one tadpole per tank, and ground rabbit chow were the best conditions examined for both taxa. Under each of the 15 conditions, *Sc. couchii* developed faster than *Sp. multiplicata* such that there was no overlap between taxa in time to forelimb emergence or size at tail resorption across all sibships and individuals. In the current study, we used two temperatures in addition to the one best for *Sc. couchii* and *Sp. multiplicata* because 32 C is high and may be a special adaptation of the desert-dwelling spadefoot toads (Bentley, 1966). All the other potential environmental factors that affect larval growth and development were held constant. Because of the myriad of environmental factors and interactions that affect larval growth and development, including diurnal temperature fluctuations and variable diet, it may not be possible to determine an optimal growing condition. Nevertheless, just considering the environmental factor temperature in the present study, the best temperature for growth and development for *Pelobates* was 28 C, for *Pelodytes* 24 C, and for *Scaphiopus* and *Spea* 32 C. Thus, comparing at 24 and 28 C, even at these suboptimal temperatures for New World taxa that were best for Old World taxa, *Scaphiopus* and *Spea* still developed significantly faster than *Pelobates* and *Pelodytes*. In addition, the consequence of different development rates, yet similar growth rates across taxa, was a difference in size of the toadlets. Therefore, larval physiology contributes to the large diversity in spadefoot tadpole growth and development observed under natural conditions.

We found no evidence for a relationship between breeding habitat and larval period duration in New World spadefoot toads. *Scaphiopus couchii*, *Sp. multiplicata*, *Sp. bombifrons*, and *Sp. intermontanus* live in desert environments, whereas *Sc. holbrookii* lives in sandy areas in eastern forests and *Sp. hammondii* lives in the foothills of the California coastal range (Wright and Wright, 1949; Stebbins, 1951). Our null hypothesis was that taxa living in similar habitats would have similar larval growth and development (e.g., the desert-inhabiting *Sc. couchii* and *Sp. multiplicata* may have similar larval period characteristics). Alternatively, tadpole growth and development may correlate with phylogenetic relationships (e.g., *Sc. couchii* and the nondesert-inhabiting *Sc. holbrookii* may have similar larval periods). Our data confirmed the second hypothesis: New World taxa that live in nondesert habitats are similar to other members in their genus, and taxa in different genera that live in the same desert pools do not have similar tadpoles.

This lack of correlation between larval period characteristics and breeding habitat may be understood from at least two perspectives. First, similar larval periods may be related to similar breeding situations. Both *Sc. couchii* (desert-dweller) and *Sc. holbrookii* (nondesert dweller) breed in shorter-lived pools on average than *Spea* sp. (pers. obs. and Bragg, 1945; Bragg, 1961; Bragg, 1965), perhaps explaining similarity among larvae of *Scaphiopus* and differences between larvae of *Scaphiopus* and *Spea*. However, different spadefoot taxa within the same genus have very different levels of desiccation risk. Although *Scaphiopus* has high desiccation risk, *Sp. hammondi* has moderate risk and *Sp. intermontana* has little risk in study populations (Morey and Reznick, 2000). Therefore, the breeding site characteristics such as pool ephemerality may not be as important as the larval strategies for growth and development. From this second standpoint, the differences between *Scaphiopus* and *Spea* may be caused by different larval niches. Also, in line with Bragg's hypothesis that spadefoot toads evolved in desert environments with subsequent dispersal into non-desert environments (Bragg, 1945; Bragg, 1961), there may be an evolutionary inflexibility or lag in the larval physiology to match the environment.

Short larval periods evolved in the New World spadefoot toads after they diverged from their Old World relatives. By mapping the developmental data onto a spadefoot toad phylogeny (Cannatella, 1985; M. Garcia-Paris and D. R. Buchholz, unpubl. data), we identified the evolutionary origin of short larval periods in spadefoot toads based on the doublet rule for assigning the most-parsimonious character mapping (Maddison et al., 1984). Because the larval pe-

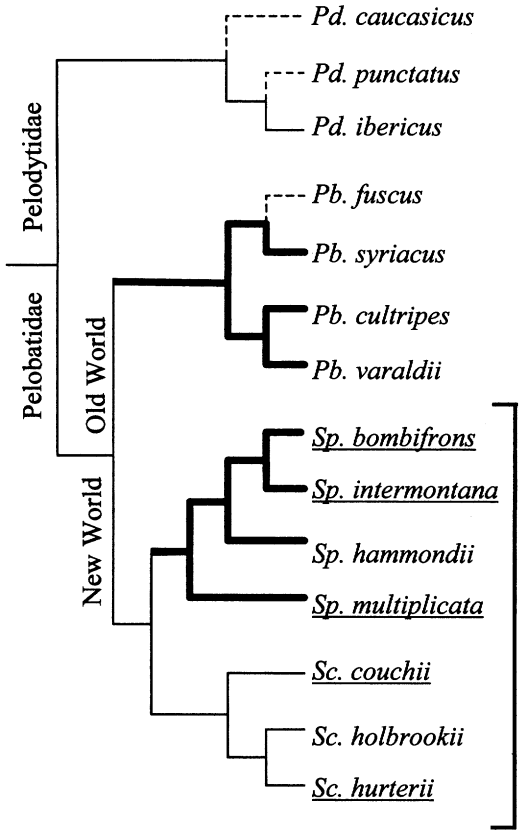

Fig. 4. Phylogenetic relationships of the spadefoot toads and parsley frogs (Cannatella, 1985; Wiens and Titus, 1991). Underlined taxa live in desert environments. Thick lines indicate taxa with large larvae (> 1 g body mass) and thin lines indicate taxa with small larvae (< 1 g body mass). The bracket indicates taxa with short larval periods (< 20 days); the other taxa have long larval periods (30–40 days). Taxa with dashed lines were not examined in this study.

riods for New World spadefoot toads were significantly shorter than the larval periods of *Pelobates* and *Pelodytes*, ancestors of the lineage leading to New World spadefoot toads did not have short larval periods (Fig. 4). Similarly, two other features have evolved within the New World spadefoot toad lineage: high temperature tolerance and rapid embryonic development.

The evolutionary origin of rapid embryonic and larval development and high temperature tolerance correlate with desert existence. New World spadefoot toads, for the most part, live in deserts (or evolved in deserts and then dispersed), whereas the Old World taxa live in mediterranean climates or forests (Kuzmin, 1999). This correlation may reflect a specific adaptation to desert environments of a New World spadefoot toad ancestor. However, evolution of desert survival occurred once within spadefoot toads. Additional studies comparing larval periods in lineages with desert and nondesert taxa, such as *Bufo* or Myobatrachidae, are required to enable phylogenetic methods to provide support that rapid development and high temperature tolerance are specific adaptations to desert existence.

An additional question is whether character traits enabling desert survival evolved before or after the divergence of *Scaphiopus* and *Spea*. No resolution has come from fossil evidence (Bragg, 1961; Kluge, 1966; Bragg, 1967) or immunological distance data (Sage et al., 1982) that suggest that *Scaphiopus* and *Spea* were diverging during the period the North American deserts were forming, about 30 mya or 21 mya, respectively. The many similarities in desert survival in *Scaphiopus* and *Spea*, in addition to the larval period traits reported here, corroborate the idea that the ancestors of the New World spadefoot toads may have evolved from a nondesert-adapted ancestor (Bragg, 1961; Bentley, 1966; Warburg, 1997). However, the large difference in size at metamorphosis between *Scaphiopus* and *Spea* may indicate that short larval periods evolved in parallel. It is possible that, after accelerated metamorphosis evolved, subsequent selection for larger growth rates in *Spea* (perhaps to allow for facultative carnivory) may have decreased its development rate as a side-effect. We are currently examining endocrinological mechanisms underlying rapid development rates to gain insight into whether the common ancestor of the New World spadefoot toads had short larval periods or whether parallel evolution occurred.

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