

Reproductive mode plasticity: Aquatic and terrestrial oviposition in a treefrog

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Diversification of reproductive mode is a major theme in animal evolution. Vertebrate reproduction began in water, and terrestrial eggs evolved multiple times in fishes and amphibians and in the amniote ancestor. Because oxygen uptake from water conflicts with water retention in air, egg adaptations to one environment typically preclude development in the other. Few animals have variable reproductive modes, and no vertebrates are known to lay eggs both in water and on land. We report phenotypic plasticity of reproduction with aquatic and terrestrial egg deposition by a frog. The treefrog *Dendropsophus ebraccatus*, known to lay eggs terrestrially, also lays eggs in water, both at the surface and fully submerged, and chooses its reproductive mode based on the shade above a pond. Under unshaded conditions, in a disturbed habitat and in experimental mesocosms, these frogs lay most of their egg masses aquatically. The same pairs also can lay eggs terrestrially, on vegetation over water, even during a single night. Eggs can survive in both aquatic and terrestrial environments, and variable mortality risks in each may make oviposition plasticity adaptive. Phylogenetically, *D. ebraccatus* branches from the basal node in a clade of terrestrially breeding species, nested within a larger lineage of aquatic-breeding frogs. Reproductive plasticity in *D. ebraccatus* may represent a retained ancestral state intermediate in the evolution of terrestrial reproduction.

aquatic egg-laying | evolution of reproductive mode | *Hyla ebraccata* | phenotypic plasticity | climate change

The evolution of terrestrially developing eggs from ancestral aquatic eggs is a repeated trend in both invertebrates and vertebrates (1–10). In both groups, aquatic predators and constraints on oxygen uptake are hypothesized to select for terrestrial eggs (2, 7–11). Terrestrial eggs can improve the embryonic respiratory environment, allow oviposition over fast-moving streams where aquatic eggs might be swept away, and allow animals to colonize habitats without permanent water bodies (2, 4, 7–10). However, terrestrial eggs experience new risks from desiccation and terrestrial predators (2, 4, 7–10). Because aquatic and terrestrial environments select for different traits, eggs are usually well adapted to only one environment (2, 4). Adaptations for terrestrial oviposition have evolved independently in several groups [e.g., gastropods (8, 12), insects (9, 13), and fishes and amphibians (1, 2, 4, 10)]. In all of these organisms, the divergence in reproductive mode [oviposition site and type of egg development (1, 2, 10)] occurred long ago, and it is thus difficult to directly assess selective pressures that may have influenced such evolution. Closely related species or populations that vary in their reproductive modes [e.g., between viviparity and oviparity (14–18)] offer the best opportunity to study the selective pressures leading to reproductive mode diversification. Although some foam-nesting frogs are reported to place nests in diverse locations (10, 19, 20), there are no vertebrate species known to deposit eggs both into water and on land.

We studied the reproductive mode of the Neotropical treefrog *Dendropsophus ebraccatus* [formerly *Hyla ebraccata* (21)] in mesocosms and at three ponds near Gamboa, Panama. *D. ebraccatus* is a common, well studied Neotropical treefrog known to have semiterrestrial reproduction; its eggs are laid on vege-

tation above water and develop for 3–4 days, then aquatic tadpoles fall into the water upon hatching (Fig. 1A) (1, 2, 10, 22, 23). Using mesocosm experiments and observations of natural clutches, we report the discovery of reproductive mode plasticity in *D. ebraccatus*, and we argue that this plasticity is most likely adaptive. Adult *D. ebraccatus* are capable of laying eggs either aquatically, both at the surface of the water and fully submerged, or terrestrially, and they choose their reproductive mode based on factors that affect risk of terrestrial egg desiccation.

Results

Congruent with previous reports (2, 10, 22, 23), over 5 years at two forest ponds (Bridge and Ocelot Ponds) near Gamboa, Panama, we observed only terrestrial oviposition (350 closely monitored clutches from 2003–2005, plus many additional observations during >90 nights of field work between 2003 and 2007). In 2006, at a pond in an old gravel quarry (Quarry Pond) that lacks forest canopy over oviposition sites, we observed *D. ebraccatus* laying eggs in the water, supported by aquatic vegetation. Some clutches were laid at the water surface or across the air–water interface so that some eggs were submerged, others were in contact with both air and water, and some were in air (Fig. 1B). Other clutches were submerged (Fig. 1C) at depths ranging from just under the water to ≈ 4 cm below the surface. Of the 148 clutches that we found at Quarry Pond during 2006–2007, 28% were submerged (all eggs completely under water), 48% were laid at the water surface, and 24% were laid terrestrially (no eggs in contact with water). The terrestrially laid eggs at Quarry Pond had significantly higher mortality from desiccation ($20 \pm 6\%$, mean \pm SEM) than terrestrial eggs at Bridge ($8 \pm 2\%$) or Ocelot Ponds ($0.4 \pm 0.2\%$) presumably because of differences in shading (Kruskal–Wallis test: $n_{\text{Quarry}} = 27$, $n_{\text{Bridge}} = 151$, $n_{\text{Ocelot}} = 73$, $\chi^2 = 37.97$, $P = 5.69 \times 10^{-9}$; pairwise Wilcoxon rank-sum tests, Quarry–Bridge, $P = 0.00023$, Quarry–Ocelot, $P = 5.0 \times 10^{-6}$, Bridge–Ocelot, $P = 0.0013$) (Fig. 2). Mortality of eggs laid in the water at Quarry Pond (including submerged and surface clutches) was, however, significantly lower ($45 \pm 3\%$, $n = 112$) than that of flooded terrestrial eggs previously observed at Bridge and Ocelot Ponds ($61 \pm 6\%$, $n = 52$; Wilcoxon rank-sum test, $P = 0.028$).

Our observation of 76% aquatic oviposition by *D. ebraccatus* at Quarry Pond and only terrestrial oviposition by frogs at nearby forest ponds (Ocelot and Bridge) could reflect either local genetic differentiation, with some polymorphism among individuals at Quarry Pond, or individual plastic responses to differences in their environments. To test for plasticity, we manipulated exposure to forest canopy shade, a variable that affects egg desiccation rate and clearly differs between our study ponds (see *Materials and Methods* for details). We quantified oviposition choices of frogs from all three ponds in shaded and

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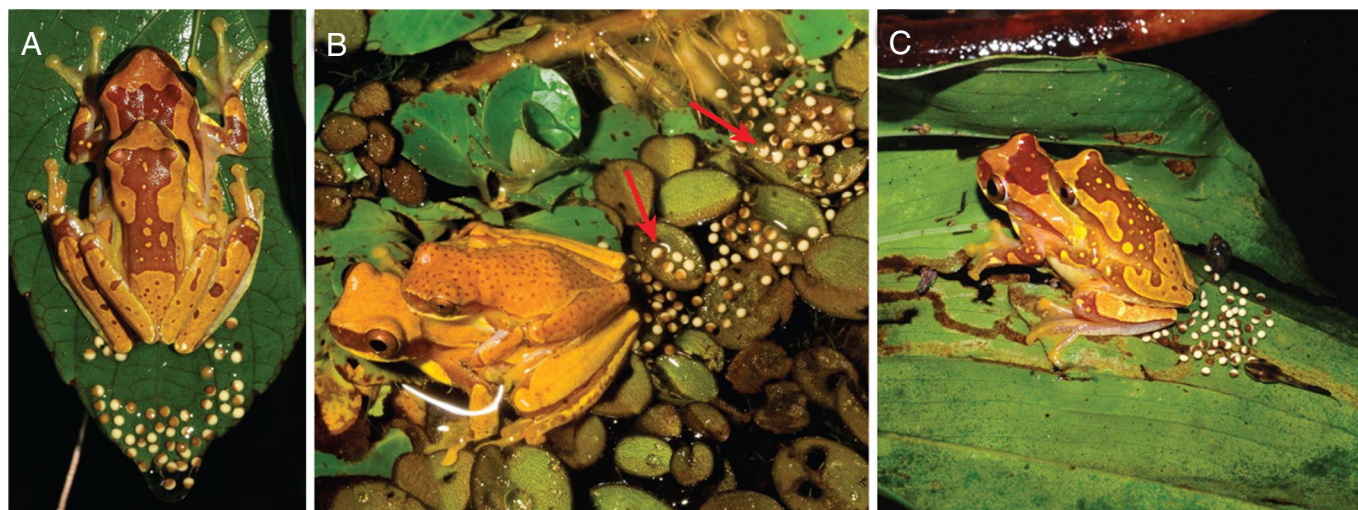


Fig. 1. Aquatic and terrestrial oviposition by a treefrog. (A–C) *D. ebraccatus* laying eggs terrestrially as is typical in shaded habitats (A), at the water surface (B), and under water (C). Aquatic oviposition occurs in unshaded sites where terrestrial eggs desiccate rapidly. (B) Vegetation ranges from a few centimeters below the water surface (top right, rotting leaves with mass of eggs suspended in water column above them) to emergent (rosette above frogs). Arrows indicate some eggs that are partially air-exposed on leaves at the water surface. The ovipositing female is sitting in water (note reflection from flash on the water surface). (C) Entire clutch is under water on a partially submerged leaf. Note the *D. ebraccatus* tadpole, an aquatic egg predator. Photographs were taken by the authors.

unshaded experimental mesocosms. Terrestrial clutches were laid completely out of the water on emergent vegetation (e.g., Fig. 1A). Aquatic clutches were laid both at the water surface and under water (e.g., Fig. 1B and C).

Frogs in unshaded mesocosms laid more water-surface and submerged egg masses than did frogs in shaded mesocosms, where the majority of egg masses were laid terrestrially ($n = 67$ pairs tested, 293 egg masses laid; multinomial logistic regression, $\chi^2_{2,290} = 111.81$, $P < 2.0 \times 10^{-16}$) (Fig. 3). On average, water-surface egg masses were $35 \pm 4\%$ in contact with or under water and $65 \pm 5\%$ above the water. Frogs from all three ponds laid eggs aquatically in unshaded mesocosms, and 48% of pairs laid both aquatic and terrestrial egg masses in a single night. There was neither a main effect of pond nor a significant interaction of pond with shade treatment, indicating that oviposition site is plastic at all ponds tested (see *Materials and Methods* for details). There was no difference in the total number of eggs laid per pair in shaded or unshaded mesocosms (linear

model, $F_{1,65} = 2.3$, $P = 0.13$), and water-surface, submerged, and terrestrial egg mass sizes did not differ between environments ($F_{2,287} = 0.74$, $P = 0.48$). However, pairs in unshaded mesocosms partitioned their eggs into significantly smaller masses (49 ± 3 eggs) than pairs in shaded mesocosms (62 ± 2 eggs; linear model, $F_{1,287} = 12.94$, $P = 0.0004$). Pairs in unshaded mesocosms also laid their terrestrial clutches closer to the water (5.8 ± 1.3 cm high) than did pairs in shaded mesocosms (13.8 ± 1.2 cm high; linear model, $F_{1,151} = 12.43$, $P = 0.0006$).

Discussion

To the best of our knowledge, no vertebrates have been described to plastically lay eggs both in water and on land. In this study, we report such plasticity for *D. ebraccatus*. Natural variation in oviposition site is not due to local genetic differentiation between ponds or polymorphism within Quarry Pond, but instead reflects the plastic behavioral responses of frogs to their different environments. Most previously documented examples of natural variation in reproductive mode within vertebrate species occur only among genetically differentiated populations, such as lizards (14–16) and

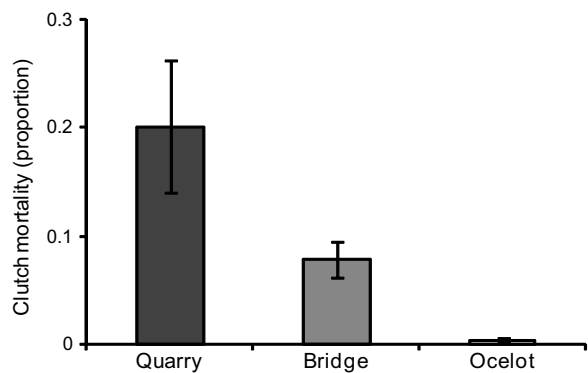


Fig. 2. Desiccation of terrestrial egg clutches of treefrogs, *D. ebraccatus*, at three ponds near Gamboa, Panama. Quarry Pond ($n = 27$ clutches) where desiccation is highest is least shaded, Bridge Pond ($n = 151$) is intermediate, and Ocelot Pond ($n = 73$) has the most shade over terrestrial oviposition sites. The ponds are located within 2 km of each other and were monitored concurrently, so clutches at all ponds experienced the same weather. Most eggs at Quarry Pond are laid aquatically, thus few terrestrial clutches were available to monitor. Data are mean \pm SEM.

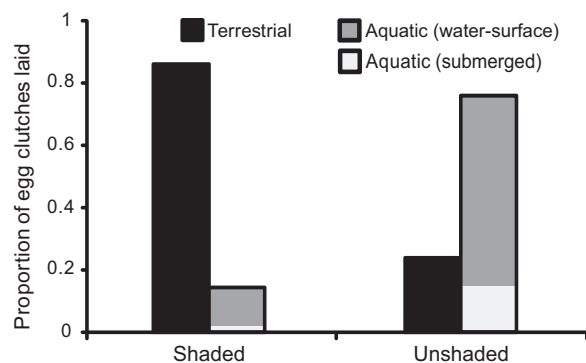


Fig. 3. Terrestrial and aquatic oviposition by treefrogs, *D. ebraccatus*, in shaded and unshaded experimental mesocosms. Frogs laid most of their egg masses in the water (in surface positions and submerged to ≈ 4 -cm depth) (Fig. 1B and C) in unshaded mesocosms and most terrestrially (Fig. 1A) in shaded mesocosms. Data are proportion of egg masses in each category ($n = 293$ egg masses laid by 67 pairs from three ponds).

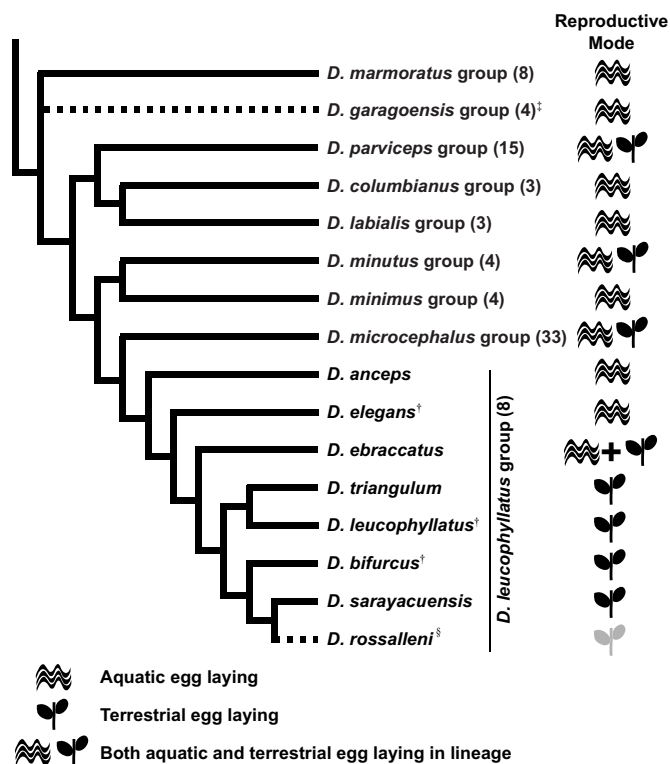


Fig. 4. Phylogeny of the *D. leucophyllatus* group, showing the distribution of known reproductive modes and relationships with other *Dendropsophus* species groups. Aquatic reproduction is ancestral in the genus and the predominant reproductive mode, but terrestrial eggs have evolved at least four times. Symbols indicate species groups where only aquatic or terrestrial egg laying is known and where both reproductive modes are known for different species within a group. *D. ebraccatus* is the only species known to exhibit both reproductive modes. The reproductive mode is unknown for ca. half the species of *Dendropsophus*. Some of these species also may have reproductive plasticity, and species with described reproductive modes may have undescribed plasticity. Numbers in parentheses indicate number of species in each group. The phylogeny is based on ref. 21, except as follows: †, based on ref. 63, which agrees with ref. 21 on placement of the members of the *D. leucophyllatus* group they have in common; ‡, tentative placement (indicated by dashed lines) based on morphological data from ref. 64; and §, tentative placement (indicated by dashed lines) based on morphological data from ref. 65. Reproductive modes are from refs. 1, 35, 53, 54, 64, and 66–69. The reproductive mode of *D. rossalleni* is speculated to be terrestrial (54), but is currently unknown.

phylogenetic reconstructions of ancestral traits functionally related to terrestrial and aquatic egg development (e.g., egg size and clutch structure) should improve our understanding of both the evolution of terrestrial reproduction and the role of plasticity in evolution. An interesting candidate for comparison to *Dendropsophus* is the diverse African treefrog genus *Hyperolius*, which contains >100 species, some of which breed aquatically, others terrestrially, at least one that lays water-surface eggs in between the leaves of floating plants, as well as many species for whom the reproductive mode is not yet known (55–58).

The risk of terrestrial egg desiccation will likely increase in the future (59), both because of habitat disturbance and because rainfall patterns are changing in Panama (60, 61). By 2080–2099, the air temperature during June, July, and August, peak breeding months for *D. ebraccatus*, is predicted to have increased at least 2.0–2.5°C, whereas precipitation is predicted to have decreased 10–15% and become more sporadic (61). Less predictable rainfall and more disturbed habitats will

increase the mortality of terrestrially laid eggs (45), but reproductive mode variation may help *D. ebraccatus* to persist despite these changes. Frogs from all three ponds laid a small subset of their eggs aquatically even in shaded mesocosms, suggesting that aquatic oviposition also may occur at a low frequency in forest ponds, perhaps as a bet-hedging strategy (Fig. 3). This would provide a buffer against unpredictable environmental variation, protecting a subset of eggs from the possibility of desiccation. Additionally, frogs from ponds where only terrestrial oviposition had been observed laid most of their egg masses aquatically in unshaded mesocosms, suggesting that such plasticity may be widespread in this species (Fig. 3). To the extent that the cues for aquatic oviposition accurately predict egg desiccation risk, this plasticity should improve *D. ebraccatus*' ability to survive in disturbed habitats or under altered rainfall patterns. Frogs in unshaded mesocosms laid smaller egg masses, which would allow them to spread eggs among more different microsites, but probably also increases desiccation by increasing the ratio of edge to central eggs in masses. Terrestrial egg masses were, however, laid closer to the water in unshaded mesocosms, which would increase their chances of transitory flooding in nature. This behavior may be similar to that of amphibians that lay eggs adjacent to water in the anticipation of flooding (11).

The proximate cues that stimulate *D. ebraccatus* adults to reproduce terrestrially or aquatically are unknown, but candidates include light level, temperature, and humidity. Clearly, cues are ascertained shortly before oviposition because frogs moved from their native pond to experimental mesocosms responded to their new environment within a single night. If light level is the primary cue, then the frogs will likely respond appropriately to forest clearing, but not to changing rainfall patterns. However, if air temperature or humidity is the indicator of open habitats, then plasticity, as well as bet-hedging, might improve *D. ebraccatus*' chances of persistence in a changing climate.

Materials and Methods

Field Monitoring. We located aquatically and terrestrially laid egg clutches at Quarry Pond and terrestrial egg clutches at Ocelot and Bridge Ponds the morning after oviposition during 2006–2007 and checked them twice daily for the first 48 h. Afterward, aquatically laid eggs were hatching-competent, making the fates of missing eggs ambiguous. Clutches were categorized based on their contact with water and air (air only, terrestrial; water and air, surface; water only, submerged). Terrestrial clutches were found on vegetation overhanging water and emergent vegetation. At Quarry Pond, aquatic egg clutches were primarily attached to *Salvinia* vegetation; this plant did not occur at Ocelot Pond and was in very low density at Bridge Pond. Surface clutches included eggs in contact with water and exposed to air on surface or near-surface leaves. Submerged clutches were suspended to ≈4 cm deep in gaps between plants or attached to roots or other underwater vegetation. At each observation, we recorded the number of eggs that had died from desiccation (for terrestrial eggs), from hypoxia (for aquatic eggs, characterized by developmental retardation before death), or missing because of predation (for all eggs). We similarly monitored fates of terrestrially laid clutches at Ocelot and Bridge Ponds that had been flooded during a different time period when pond levels fluctuated more (2003–2005).

Testing for Reproductive Mode Plasticity. We constructed twelve 1.3-m-diameter pond mesocosms containing both floating aquatic vegetation and emergent vegetation and placed half under thick forest canopy (shaded) and the rest nearby in an open field (unshaded). On eight nights in 2006 and 2007, we collected pairs of frogs that had not yet begun laying eggs from all three ponds (no more than four pairs per pond each night; $n = 21, 23,$ and 23 total pairs from Quarry, Ocelot and Bridge Ponds, respectively) and allowed them to breed in the mesocosms overnight. Only one pair was used per mesocosm per night, thereby preventing competition for oviposition sites and allowing us to identify all egg masses laid by each pair. Because variation in weather conditions might affect oviposition choices, we always tested frogs from all three ponds on each night. We recorded the

number of egg masses laid and the location of each mass (terrestrial, surface, or submerged). For a subset of surface masses ($n = 66$ masses by 28 pairs), we also counted the number of eggs in terrestrial, surface, and submerged positions within the mass.

Oviposition site was modeled by using a multinomial logistic regression (MLR) in R version 2.6.0 (62). Predictors were: (i) shade, (ii) pond of origin, (iii) mating pair, (iv) cage, (v) date, and (vi) a shade \times pond of origin interaction. Shade was the only significant factor, thus other variables were excluded from the final model (MLR: shade, $\chi^2_{2,290} = 111.81$; pond, $\chi^2_{4,286}$

$= 6.51$; pair, $\chi^2_{2,284} = 0.73$; cage, $\chi^2_{2,282} = 2.20$; date, $\chi^2_{2,280} = 0.45$; shade \times pond interaction, $\chi^2_{4,276} = 3.89$).

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- Crump ML (1974) *Reproductive Strategies in a Tropical Anuran Community* (University of Kansas Museum of Natural History, Lawrence, KS).
- Duellman WE, Trueb L (1986) *Biology of Amphibians* (Johns Hopkins Univ Press, Baltimore).
- Lombardi J (1998) *Comparative Vertebrate Reproduction* (Kluwer, Boston).
- Martin KLM (1999) Ready and waiting: Delayed hatching and extended incubation of anamniotic vertebrate terrestrial eggs. *Am Zool* 39:279–288.
- Wake MH (1992) Evolutionary scenarios, homology and convergence of structural specializations for vertebrate viviparity. *Am Zool* 32:256–263.
- Wake MH (2003) Reproductive modes, ontogenies, and the evolution of body form. *Anim Biol* 53:209–223.
- Stewart JR (1997). *Amniote Origins: Completing the Transition to Land*, eds Sumida SS, Martin KLM (Academic, San Diego, CA), pp 291–326.
- Adiyodi KG, Adiyodi RG, eds (1983) *Reproductive Biology of Invertebrates: V. 1. Oogenesis, Oviposition, and Oosorption* (Wiley, New York).
- Hinton HE (1981) *Biology of Insect Eggs* (Pergamon, Oxford, UK).
- Wells KD (2007) *The Ecology and Behavior of Amphibians* (Univ of Chicago Press, Chicago).
- Brown RM, Iskandar DT (2000) Nest site selection, larval hatching, and advertisement calls of *Rana arathooni* from southwestern Sulawesi (Celebes) Island, Indonesia. *J Herpetol* 34:404–413.
- Conn DB (1991) *Atlas of Invertebrate Reproduction and Development* (Wiley-Liss, New York).
- Corbet PS (1999) *Dragonflies: Behavior and Ecology of Odonata* (Cornell Univ Press, Ithaca, NY).
- Arrayago MJ, Bea A, Heulin B (1996) Hybridization experiment between oviparous and viviparous strains of *Lacerta vivipara*: A new insight into the evolution of viviparity in reptiles. *Herpetologica* 52:333–342.
- Qualls CP, Shine R, Donnellan S, Hutchinson M (1995) The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. *J Zool* 237:13–26.
- Smith SA, Shine R (1997) Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Aust J Zool* 45:435–445.
- Garcia-Paris M, Alcobendas M, Buckley D, Wake DB (2003) Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution (Lawrence, Kans)* 57:129–143.
- Buckley D, Alcobendas M, Garcia-Paris M, Wake MH (2007) Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. *Evol Dev* 9:105–115.
- Inger RF, Stuebing RB (1997) *A Field Guide to the Frogs of Borneo* (Natural History Publications and Science and Technology Unit, Kota Kinabalu, Malaysia).
- Taylor EH (1962) The amphibian fauna of Thailand. *Univ Kan Sci Bull* 43:265–499.
- Faivovich J, et al. (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: Phylogenetic analysis and taxonomic revision. *Bull Am Mus Nat Hist* 6:228.
- Duellman WE (2001) *The Hylid Frogs of Middle America* (Society for the Study of Amphibians and Reptiles, Ithaca, NY).
- Savage JM (2002) *The Amphibians and Reptiles of Costa Rica* (Univ of Chicago Press, Chicago).
- Seymour RS, Roberts JD (1991) Embryonic respiration and oxygen distribution in foamy and nonfoamy egg masses of the frog *Limnodynastes tasmaniensis*. *Physiol Zool* 64:1322–1340.
- Watts PC, Buley KR, Boardman W, Ciofi C, Gibson R (2006) Parthenogenesis in komodo dragons. *Nature* 444:1021–1022.
- Chapman DD, et al. (2007) Virgin birth in a hammerhead shark. *Biol Lett* 3:425–427.
- Alcala AC, Brown WC (1998) *Philippine Amphibians: An Illustrated Field Guide* (Bookmark Press, Makati City, Philippines).
- Malkmus R, Manthey U, Vogel G, Hoffman P, Kosuch J (2002) *Amphibians and Reptiles of Mount Kinabalu (North Borneo)* (ARG Gantner, Ruggell, Liechtenstein).
- Schleich HH, Kästle W, eds (2002) *Amphibians and Reptiles of Nepal: Biology, Systematics, Field Guide* (ARG Gantner, Ruggell, Liechtenstein).
- Utsunomiya Y, Utsunomiya T (1983) On the eggs and larvae of frog species occurring in the Ryukyu archipelago. *J Fac Appl Biol Sci, Hiroshima Univ* 21:255–270.
- Kuramoto M (1972) The amphibians of Iriomote of the Ryukyu Islands: Ecological and zoogeographical notes. *Bull Fuk Univ Educ* 22:139–151.
- Brown WC, Alcala AC (1994) Philippine frogs of the family Rhacophoridae. *Proc Calif Acad Sci* 48:185–220.
- Madeira NG, Macharelli CA, Carvalho LR (2002) Variation of the oviposition preferences of *Aedes aegypti* in function of substratum and humidity. *Mem Inst Oswaldo Cruz* 97:415–420.
- Martens A (1991) Plasticity of mate-guarding and oviposition behaviour in *Zygonyx natalensis* (Martini) (Anisoptera: Libellulidae). *Odonatologica* 20:293–302.
- Duellman WE (1978) *The Biology of an Equatorial Herpetofauna in Amazonian Ecuador* (Univ of Kansas Museum of Natural History, Lawrence, KS).
- Haddad CFB, Prado CPA (2005) Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. *Bioscience* 55:207–217.
- Resetarits WJ (1996) Oviposition site choice and life history evolution. *Am Zool* 36:205–215.
- Forester DC, Lykens DV (1988) The ability of wood frog eggs to withstand prolonged terrestrial stranding—An empirical study. *Can J Zool* 66:1733–1735.
- Marco A, Blaustein AR (1998) Egg gelatinous matrix protects *Ambystoma gracile* embryos from prolonged exposure to air. *Herpetol J* 8:208–211.
- Lips KR (2001) Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a Neotropical treefrog. *Oecologia* 128:509–518.
- Hawley TJ (2006) Embryonic development and mortality in *Hyalinobatrachium pulveratum* (Anura: Centrolenidae) of south-western Costa Rica. *J Trop Ecol* 22:731–734.
- Warkentin KM (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Anim Behav* 60:503–510.
- Kam YC, Chuang ZS, Yen CF (1996) Reproduction, oviposition-site selection, and tadpole oophagy of an arboreal nester, *Chirixalus eiffingeri* (Rhacophoridae), from Taiwan. *J Herpetol* 30:52–59.
- Kam YC, Yen CF, Hsu CL (1998) Water balance, growth, development, and survival of arboreal frog eggs (*Chirixalus eiffingeri*, Rhacophoridae): Importance of egg distribution in bamboo stumps. *Physiol Zool* 71:534–540.
- Neckel-Oliveira S (2004) Effects of landscape change on clutches of *Phyllomedusa tarsius*, a neotropical treefrog. *Biol Conserv* 118:109–116.
- Pyburn WF (1970) Breeding behavior of the leaf-frogs *Phyllomedusa callidryas* and *Phyllomedusa dacnicolor* in Mexico. *Copeia* 1970:209–218.
- Warkentin KM (2002) Hatching timing, oxygen availability, and external gill regression in the tree frog, *Agalychnis callidryas*. *Physiol Biochem Zool* 75:155–164.
- Gomez-Mestre I, Warkentin KM (2007) To hatch and hatch not: Similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs. *Oecologia* 153:197–206.
- Kluge AG (1981) *The Life History, Social Organization, and Parental Behavior of Hyla rosenbergi Boulenger, a Nest-Building Gladiator Frog* (Museum of Zoology, Univ of Michigan, Ann Arbor, MI).
- Seymour RS (1999) Respiration of aquatic and terrestrial amphibian embryos. *Am Zool* 39:261–270.
- Bastos RP, Haddad CFB (1996) Breeding activity of the neotropical treefrog *Hyla elegans* (Anura, Hylidae). *J Herpetol* 30:355–360.
- Aichinger M (1987) Annual activity patterns of anurans in a seasonal neotropical environment. *Oecologia* 71:583–592.
- Weygoldt P, Peixoto OL (1987) *Hyla ruschii* n.sp., a new frog from the Atlantic forest domain in the state of Espirito Santo, Brazil (Amphibia: Hylidae). *Stud Neotrop Fauna E* 22:237–247.
- Rodriguez LO, Duellman WE (1994) *Guide to the Frogs of the Iquitos Region, Amazonian Peru* (Univ of Kansas Museum of Natural History, Lawrence, KS).
- Channing A (2000) *Amphibians of Central and Southern Africa* (Cornell Univ Press, Ithaca, NY).
- Channing A, Howell KM (2006) *Amphibians of East Africa* (Cornell Univ Press, Ithaca, NY).
- Rodel MO (2000) *Herpetofauna of West Africa* (Edition Chimaira, Frankfurt, Germany).
- Shiots A (1999) *Treefrogs of Africa* (Edition Chimaira, Frankfurt, Germany).
- Donnelly MA, Crump ML (1998) Potential effects of climate change on two neotropical amphibian assemblages. *Clim Change* 39:541–561.
- Windsor DM (1990) *Climate and Moisture Variability in a Tropical Forest: Long-Term Records from Barro Colorado Island, Panamá* (Smithsonian Institution Press, Washington, DC).
- Christensen JH, et al. (2007) in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds Solomon S, et al. (Cambridge Univ Press, Cambridge, UK), pp 847–940.
- R Core Development Team (2007) (R Foundation for Statistical Computing, Vienna, Austria).
- Chek AA, Lougheed SC, Bogart JP, Boag PT (2001) Perception and history: Molecular phylogeny of a diverse group of neotropical frogs, the 30-chromosome *Hyla* (Anura: Hylidae). *Mol Phylogenet Evol* 18:370–385.
- Kaplan M, Ruiz PM (1997) Two new species of *Hyla* from the Andes of central Colombia and their relationships to other small Andean *Hyla*. *J Herpetol* 31:230–244.
- De la Riva I, Duellman WE (1997) The identity and distribution of *Hyla rossalleni* Goin. *Amphibia-Reptilia* 18:433–436.
- Bastos RP, Pombal JP, Jr (1996) A new species of *Hyla* (Anura: Hylidae) from eastern Brazil. *Amphibia-Reptilia* 17:325–331.
- Lutz B (1973) *Brazilian Species of Hyla* (Univ of Texas Press, Austin, TX).
- Gutiérrez G, Lüddecke H (2002) Mating pattern and hatching success in a population of the Andean frog *Hyla labialis*. *Amphibia-Reptilia* 23:281–292.
- Fouquette MJ, Jr (1960) Isolating mechanisms in three sympatric treefrogs in the canal zone. *Evolution (Lawrence, Kans)* 14:484–497.