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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 vegetation 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 ± 6%, mean ± SEM) than terrestrial eggs at Bridge (8 ± 2%) or Ocelot Ponds (0.4 ± 0.2%) presumably because of differences in shading (Kruskal–Wallis test: nQuarry = 27, nBridge = 151, nOcelot = 73, χ2 = 37.97, P = 5.69 × 10–5; pairwise Wilcoxon rank-sum tests, Quarry–Bridge, P = 0.00023, Quarry–Ocelot, P = 5.0 × 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 ± 3%, n = 112) than that of flooded terrestrial eggs previously observed at Bridge and Ocelot Ponds (61 ± 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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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., Figs. 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, $\chi^2_{1,290} = 111.81, P < 2.0 \times 10^{-16}$) (Fig. 3). On average, water-surface egg masses were 35 ± 4% in contact with or under water and 65 ± 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 plasticly 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...
a salamander (17) that vary in oviparity/viviparity and a frog that produces foamy and nonfoamy egg masses (24). A normally sexual lizard and a normally sexual shark also have both shown a presumably plastic capacity for parthenogenesis by females isolated from males in captivity (25, 26).

Many Old World treefrogs of the family Rhacophoridae lay their eggs arboreally within foam nests (2, 10), and nests attributed to single species have been found in diverse locations, including on tree trunks, on the ground near stream banks, and even floating on top of water (10, 19, 20, 27–32). Although embryos in foam nests are protected from desiccation and supplied with oxygen from the foam, rhacophorid foam nest placement might vary with environmental conditions such as humidity, temperature, or forest cover; to the best of our knowledge, this has not yet been tested. Nest placement could, however, simply reflect the availability of oviposition sites (20, 29). Several Old and New World frogs lay eggs adjacent to water, on stream banks or moss, in excavated nests, or on low-lying vegetation. These eggs can become inundated with water after heavy rains (10, 11), as can terrestrially laid D. ebraccatus clutches, so that the embryos then experience an aquatic environment. Eggs laid near the air–water interface may therefore experience more environmental variation over development than is reflected in their initial oviposition sites.

Reproductive mode plasticity is poorly documented in any taxon, and, to our knowledge, only two other species, a mosquito (33) and a dragonfly (34), are known to lay both terrestrial and aquatic eggs. Like D. ebraccatus, they choose oviposition sites in response to factors affecting egg desiccation risk. These insects are likely not the only invertebrates capable of such plasticity. Similarly, D. ebraccatus may not be the only amphibian capable of both aquatic and terrestrial reproduction. Researchers studying amphibian reproduction, ourselves included, had not previously documented plasticity in aquatic/terrestrial oviposition (1, 2, 10, 23, 35–37) perhaps because reproductive mode was considered fixed. However, the range of oviposition sites used by D. ebraccatus and the environmentally induced variation in how eggs are distributed across these sites suggests that terrestrial and aquatic oviposition are not fully dichotomous. Rather, they may represent ends of a continuum, and oviposition site plasticity may be an intermediate stage in the evolution of obligate terrestrial eggs.

D. ebraccatus eggs, although apparently not very well adapted to development either in air or under water, can develop in both environments and appear physiologically more flexible than obligate aquatic or terrestrial eggs. Aquatic eggs exposed to air typically dry out rapidly and die (e.g., 38), although some salamander egg masses can survive prolonged terrestrial stranding (39). Desiccation mortality of terrestrial amphibian eggs is, however, generally low because clutches are laid in humid locations and are surrounded by a protective, water-rich jelly (2, 4, 10). During this study, desiccation killed an average of 20% of D. ebraccatus eggs (Fig. 2). During drier periods within the rainy season, we have observed >50% desiccation mortality of terrestrial clutches at the same ponds (J.C.T. and K.M.W., unpublished data). This rate is far greater than the desiccation mortality reported for many other terrestrial breeding frogs (40–43). In contrast, D. ebraccatus embryos are more capable of aquatic development than other terrestrial amphibian eggs, which die if submerged before hatching competence (40, 42, 44–48). D. ebraccatus eggs can develop normally under water, although developmental retardation and death occur if eggs are deep in the water column, where oxygen is lower. Other more strictly aquatic eggs also can suffocate if they fall to the pond bottom (e.g., Hypsiboas rosenbergi (49)), and many aquatic-breeding amphibians attach their eggs to vegetation or other structures in the water to hold them near the water surface (2, 10, 23). Both the rapid desiccation of terrestrial D. ebraccatus eggs and their ability to survive under water are likely due to their small size (1.2–1.4 mm in diameter (23)). D. ebraccatus eggs are smaller than most terrestrial anuran eggs (10), which both reduces their oxygen demand and increases their surface area-to-volume ratio, thereby enhancing oxygen diffusion under water (50). Highlighting this point, Wells (10) referred to D. ebraccatus clutches as “little more than aquatic egg masses transferred to terrestrial oviposition sites.”

Given spatial and temporal variability in factors affecting terrestrial desiccation risk and aquatic oxygen availability, as well as in environment-specific predators, aquatic/terrestrial reproductive plasticity may be adaptive. We observed higher desiccation mortality of terrestrial eggs at Quarry Pond than at either Bridge or Ocelot Ponds likely because of less shade above clutches (Fig. 2). In contrast, mortality of aquatically laid eggs at Quarry Pond was lower than that of flooded eggs previously observed at Bridge and Ocelot Ponds. Aquatic egg predators, including several species of fish and tadpoles, are abundant in all three ponds. When clutches laid on terrestrial vegetation become flooded, they are exposed to these predators in the water column. However, most clutches laid in the water at Quarry Pond are on or in a dense mat of floating vegetation (mostly Salvinia) that may shield eggs from detection and attacks by predators in the more open water below. In addition, hypoxia is more likely for flooded clutches, which may be deep in the water column, than for eggs associated with floating vegetation, which remain near the better-oxygenated surface of the water even as water level fluctuates.

Variation among and even within ponds in factors such as tree canopy, aquatic vegetation, depth, and predator communities affects aquatic and terrestrial egg risks, altering selection on aquatic and terrestrial oviposition. Reproductive mode variation in D. ebraccatus occurs at the level of the individual, not across populations (14–17), so that morphologically and physiologically similar eggs naturally experience a wide range of conditions. This species therefore offers an excellent opportunity to study the ecological factors influencing the evolution of terrestrial and aquatic reproduction.

Reproductive plasticity also may occur in other species, particularly in clades within which terrestrial-breeding animals evolved from aquatic-breeding ancestors and extant species vary in reproductive mode. D. ebraccatus belongs to the D. leucophyllatus species group, a lineage of eight Central and South American species (21). D. ebraccatus is the sister species of all other terrestrially breeding frogs in the lineage, and all species branching from more basal nodes breed aquatically (Fig. 4) (1, 21, 35, 51, 52). Reproductive plasticity in D. ebraccatus could therefore represent an intermediate stage in the evolution of terrestrial reproduction, combinations a retained ancestral capacity for aquatic development with a derived ability for terrestrial oviposition and development. If reproductive plasticity was present in the common ancestor of D. ebraccatus and its relatives, it may have helped facilitate the transition to obligate terrestrial egg development. Alternatively, a plastic capacity for aquatic reproduction may have evolved secondarily from terrestrially breeding ancestors of D. ebraccatus.

The genus Dendropsophus contains ~90 species of treefrogs in nine species groups [plus some unassigned to any species group (21)], among which terrestrial reproduction has evolved independently at least four times (Fig. 4) (1, 35, 53, 54). A complete phylogeny of the genus is lacking, as is information about the reproductive mode of nearly half the species. Thus, additional origins of terrestrial reproduction and/or other reproductively plastic species may exist among these poorly studied taxa. It also is possible that other well studied species, like D. ebraccatus, harbor undocumented flexibility in egg deposition site and the capacity of embryos to develop in different environments. Further research within this genus and
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 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), hatching-limited, 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 submersed). 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 x pond of origin interaction. Shade was the only significant factor, thus other variables were excluded from the final model (MLR: shade, $x_2^{2.282} = 111.81; $pond, $x_2^{4.286} = 6.51$; pair, $x_2^{2.184} = 0.73; $cage, $x_2^{2.282} = 2.20$; date, $x_2^{2.280} = 0.45$; shade x pond interaction, $x_2^{4.276} = 3.89$).

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