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Development of red-eyed treefrog eggs affects efficiency and choices of egg-foraging wasps

KAREN M. WARKENTIN*†, CHRISTINE R. BUCKLEY* & KELLY A. METCALF*

*Department of Biology, Boston University
†Smithsonian Tropical Research Institute, Panama

The arboreal eggs of red-eyed treefrogs, *Agalychnis callidryas*, are preyed on by social wasps, *Polybia rejecta*. Embryos hatch rapidly up to 30% before their typical undisturbed hatching age when attacked by wasps, so many embryos escape. We hypothesized that the escape ability of older embryos would reduce wasp foraging success or efficiency, and that wasps would therefore prefer to prey on younger eggs. To characterize wasp foraging abilities and preferences, we videotaped the behaviour of individual foragers at an outdoor feeding station. We presented wasps with pairs of clutches differing in age by 1 day, from newly laid versus 1 day through 4 versus 5 days. Eggs become competent to hatch at 4 days. Wasps foraged more efficiently and successfully on older eggs than on younger ones. Among not-yet-hatchable clutches, wasps preferred older eggs, but there was no evidence for discrimination between late unhatchable eggs and hatchable eggs (3–5 days), and wasps readily attacked embryos likely to escape. The thick egg jelly of younger clutches makes it difficult for wasps to break into eggs. The poorly developed embryos also disintegrate easily, making them harder to carry. Thus, at least when wasps have experience with a range of egg stages, they kill few young eggs. Older eggs frequently escaped by hatching, so the heaviest mortality fell on intermediate developmental stages, both at the feeding station and at a natural breeding site. Structural variation in egg clutches and developmental changes in embryos may affect interactions between other small, mobile predators and amphibian eggs.

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Foraging theory predicts that animals should allocate foraging effort based on the value of resources as well as the costs and risks related to their acquisition. For instance, predators may choose more profitable prey, prey that are easier to handle or prey that are less likely to escape (Stephens & Krebs 1986; Sih & Christensen 2001; Lima et al. 2003). Eggs are typically considered profitable, easy to handle, low-risk prey; they are richly supplied with nutrients for the developing embryo, generally incapable of fleeing or fighting and rarely toxic (Orians & Janzen 1974). Thus, eggs often suffer high levels of predation (Tejedo 1991; Bosque & Bosque 1995; Drewes & Altig 1996; Chalcraft & Andrews 1999; Vonesh 2000). Some taxa have evolved as specialized egg predators (Scanlon &

Shine 1988; Kuris 1993), and more generalized predators often include eggs as part of their diet.

Well-known egg defences include parental care, cryptic oviposition sites, tough egg coverings and distasteful chemicals (Orians & Janzen 1974; McDiarmid 1978; Bruce 1998; Green 1999). Like the resource value of eggs, these defences may change little over the course of embryonic development, providing little reason for egg predators to forage selectively. However, it has become clear that some amphibians, fish and invertebrates also have a developmentally variable egg defence: during later stages of embryonic development, they are capable of hatching prematurely to escape from egg-stage risks (Warkentin 1995; Chivers et al. 2001; Li 2002; Wedekind 2002). In such species, less developed embryos that are not yet capable of hatching may be more secure prey for egg predators. Thus, egg predators may evolve or learn preferences for particular developmental stages of embryos.

The red-eyed treefrog, *Agalychnis callidryas*, is the best-studied case of inducible early hatching as an egg defence. These frogs attach their eggs to vegetation overhanging ponds and swamps, and the tadpoles fall into the water

Correspondence and present address: K. M. Warkentin, Department of Biology, Boston University, 5 Cummington St., Boston, MA 02215, U.S.A. (email: kwarken@bu.edu). C. R. Buckley is now at the Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, U.S.A. K. M. Metcalf is now at Purdue University School of Veterinary Medicine, West Lafayette, IN 47907, U.S.A.

upon hatching. Thus, hatching involves a habitat shift, which creates a trade-off of risks between egg and larval stages. Eggs are safe from aquatic predators of tadpoles, and hatching allows escape from arboreal and aerial egg predators. Embryos hatch as much as 30% earlier than the typical undisturbed hatching time in response to a variety of egg-stage risks, including egg-eating snakes and wasps and pathogenic fungus (Warkentin 1995, 2000b; Warkentin et al. 2001). The predator-induced hatching is a rapid behavioural response of embryos to attack, and allows most embryos in clutches that are attacked late in development to escape. This clearly reduces the food value of these egg clutches for their predators.

The most important predators of red-eyed treefrog eggs are snakes and wasps. Both prey on *A. callidryas* eggs at all of our field sites. Snakes attack over half the egg clutches laid at a pond in Corcovado National Park, Costa Rica, and wasps attack about half the clutches that are laid at Ocelot Pond, near Gamboa, Panama (Warkentin 1995, 2000b). For egg-eating snakes crawling through the vegetation, the search time involved in locating clutches is likely to be the highest cost of foraging. The time and effort required to eat a clutch once it is found is comparatively small; thus, there seems little reason not to attack any clutch encountered, even if many embryos would escape. However, the relative costs and benefits of selective foraging may be different for wasps.

Polybia rejecta (Vespidae, Polistinae, Epiponini), the main wasp that preys on *A. callidryas* eggs, is a swarm-founding social wasp that is common through Central America and into South America, broadly overlapping the Middle American range of *A. callidryas* (Duellman 1970; Carpenter 1991; Jeanne 1991). Foraging *Polybia* locate *A. callidryas* clutches and other prey types, primarily soft-bodied arthropods, through an aerial search, interspersed with short periods of walking on the vegetation (Raveret Richter & Jeanne 1991). Egg clutches are often abundant in the vegetation around breeding ponds, and wasps may visit several clutches before attacking any eggs (K.W., personal observation). Attacks involve biting and pulling at eggs to extract an individual embryo that the wasp then carries away, presumably to its nest. Individual *Polybia* foragers return repeatedly to a site while prey remain, taking what they can carry each time (Raveret Richter & Jeanne 1985, 1991; Raveret Richter 2000). From *A. callidryas* clutches, the wasps remove one embryo at a time, sometimes returning until the entire clutch has been killed (Warkentin 2000b). Thus, foraging wasps have the opportunity to be selective about the particular clutches upon which they prey and might benefit from such selectivity.

To examine the foraging preferences of *P. rejecta* with respect to the developmental stage of *A. callidryas* eggs, we offered wasps choices between pairs of egg clutches of different ages at a feeding station. We videotaped and analysed wasp behaviour to evaluate the foraging efficiency of wasps preying on clutches of different ages, as well as their foraging choices. We hypothesized that wasps would prey more efficiently on younger embryos that could not escape from them by hatching, and that they would prefer such prey if given a choice.

METHODS

Egg Collection and Maintenance

Young *Agalychnis callidryas* egg clutches, and the leaves to which they were attached, were collected from Ocelot Pond, 2 km south of Gamboa, Panama. Mean ± SD clutch size was 39 ± 11.8 eggs (range 16–75). Eggs were aged based on developmental stage (Warkentin 1999, 2002); development is very consistent within and between clutches at a site. Clutches, on their leaves, were mounted on plastic cards for support and placed over water in individual cups in a laboratory with ambient temperature (25–30°C) and humidity (high). Eggs were misted several times daily with rainwater to maintain hydration and were covered with screening to exclude insects until they were used in experiments. Hatchlings were returned to their native pond after experiments. This research was conducted under permits from the Panamanian National Authority for the Environment, and the research and animal use were approved by the Smithsonian Tropical Research Institute in April 2000.

Wasp Foraging Behaviour and Choice Tests

We recruited foragers of *Polybia rejecta* to come to an outdoor feeding station in Gamboa, where we offered them *A. callidryas* eggs. The feeding station was established to record vibrations that occur in egg clutches during wasp attacks (e.g. Warkentin 2005, unpublished data). The experiments reported here were conducted in intervals between recordings.

The feeding station was a square concrete post, to which the cards supporting egg clutches were taped. Wasps were recruited by one of two methods. (1) Egg clutches were left continuously exposed at the feeding station, and wasps sometimes found them, especially when other wasps were regularly coming and going from the station. (2) *Polybia rejecta* observed near the feeding station, but away from other food sources, were offered a hand-held *A. callidryas* clutch, on its card. Wasps that landed on the clutch were carried to the feeding station while they investigated and attacked the eggs. Once a wasp had obtained prey from a clutch at the feeding station, she was marked on the thorax with a coloured dot from a paint pen for individual identification. Most of these wasps returned to the feeding station frequently over the next several days (maximum 13 days). Attempts to recruit *P. rejecta* foragers using meat baits (e.g. tuna or ground beef; see Jeanne et al 1995) were unsuccessful, as were attempts to recruit wasps foraging on fruit or nectar using hand-held frog eggs. We were also unable to recruit foragers directly from the nest, using either hand-held eggs or clutches left exposed within a few metres of the nest.

Foraging choice tests were conducted between 0600 and 1800 hours, in July–August 2000. For each test, a pair of egg clutches differing in age by 1 day were hung side by side, about 5 cm apart, at the feeding station. The relative positions of older and younger clutches (right/left) were reversed between trials, and the height of test clutches on the post was varied haphazardly. We exposed five pairs

of 0-day-old (newly laid) versus 1-day-old clutches, and 10 pairs each of ages 1 versus 2 days old through 4 versus 5 days old. Clutches were exposed for about an hour each ($\overline{X} \pm \text{SE} = 59 \pm 3.2$ min). Exposure times were adjusted based on the frequency of wasp visits: clutch pairs that experienced lower wasp activity were exposed longer in an attempt to record sufficient wasp–clutch interactions for analysis. Clutches that hatched rapidly under attack were, necessarily, exposed for shorter times. Most egg clutches were used only once. A few clutches that were completely undamaged when presented as the younger in a pair of 0- versus 1-day or 1- versus 2-day clutches were re-used several days later as the older member of a different pair. For tests that included hatchable eggs, a container of aged tap water was placed below the clutches to catch hatchlings, and pairs of clutches were chosen so that their hatchlings could be easily distinguished by yolk sac colour. Yolk colour is consistent within clutches, readily visible through the body wall of hatchlings, and ranges from gold to cream to lime green to turquoise between clutches. Seventeen *P. rejecta* were used in the choice tests, with a mean $\pm$ SE of 2.7 ± 0.2 wasps visiting each clutch pair (range 1–7). There was no difference in the number of wasps visiting pairs of different ages (Kruskal–Wallis test: $H_4 = 3.73$, $P = 0.44$). Based on flight directions of wasps departing with prey, at least three colonies contributed foragers to the experiment. We videotaped the clutches and their wasp visitors and recorded additional observations, including embryos taken by wasps as well as periodic counts of intact eggs, broken eggs and hatched tadpoles, on the audio track of the tape.

Analysis of Videotaped Wasp Behaviour

We analysed 262 visits from the 17 wasps to the 45 clutch pairs. Visits began with a focal wasp's appearance on screen, and included interactions with at least one egg clutch. Visits ended when a wasp either left the visible area and did not return within 30 s or departed from the feeding station. We analysed visits to two to nine clutch pairs per wasp, with a maximum of three pairs per age combination. We analysed up to the first three visits of each focal wasp to each clutch pair. After either clutch was reduced to fewer than 10 eggs, no additional visits to that pair were analysed. We analysed visits from as many different wasps as possible for each clutch age combination ($N = 9$, 10, 14, 13 and 13 wasps for 0- versus 1-day through 4- versus 5-day clutch pairs, respectively).

Visits were analysed using a computerized event recorder (JWatcher, Animal Behaviour Laboratory, Macquarie University). We defined a set of spatial parameters, wasp behaviours and embryo behaviours (Table 1), and assigned each a keystroke. Then we watched the videotape in real time, using keystrokes to record the occurrence, time and duration of behaviours. To improve accuracy, visits to be analysed were previewed at least once before recording behaviours. For long or complex visits, data were recorded from the videotape twice to check accuracy. If necessary, data were recorded additional times until they were consistent.

Table 1. Definitions of behaviours used in analysis of videotaped visits of *P. rejecta* foragers to pairs of *A. callidryas* egg clutches

Behaviour	Definition
Wasp	
Abandons*	Drops an embryo that is not struggling
Bites	Opens and closes mandibles, with mouthparts pressed into clutch
Breaks egg*	Ruptures egg membrane while biting it, evidenced by release of perivitelline fluid, sudden loss of turgor, and/or comment recorded on audio track
Departs*	Flies away from feeding station
Eats	Ingests embryo or part thereof
Extracts*	Pulls embryo out of egg capsule, capturing it
Handles	Manipulates extracted or scavenged embryo with mouthparts and forelimbs, or drags it over the substrate
Hovers	Maintains aerial position in front of feeding station
Investigates	Is oriented towards egg clutch, touching it repeatedly with mouthparts, forelegs and/or antennae
Off screen	Is not in view on video, but still at feeding station, near clutches
Pulls	Moves backwards while mandibles are closed on jelly, egg capsule or embryo
Scavenges*	Picks up a previously hatched or extracted embryo that is stuck to the clutch or leaf
Walks	Moves over substrate or clutch without antennation or mouthpart contact with prey
Embryo	
Escapes*	Breaks free or is dropped by wasp while struggling
Hatches*	Exits the egg capsule by its own movements
Struggles	Makes vigorous movements while gripped by wasp

Note: wasps also stood, groomed, and interacted with other wasps while at the feeding station; these behaviours are not addressed here. Embryo behaviours were recorded only for the embryo with which a focal wasp was interacting.
*Behaviours that were treated as events. Other behaviours had quantifiable durations.

To characterize wasp locations, we divided the visible area into a right and left zone, each associated with a clutch and extending to a point midway between the clutches. Wasps equidistant from the clutches and interacting with neither were considered to be in neither spatial zone. We quantified behaviours in four categories: wasp interactions with clutches, wasp interactions with other wasps, noninteractive wasp behaviours (e.g. walking, grooming) and embryo behaviours (Table 1).

Statistical Analysis of Data from Videotapes

To test the effects of egg development on wasp behaviour, we pooled data across clutches or clutch pairs of the same age for each wasp, and used the average behaviour of each wasp as a data point (i.e. $N =$ number of wasps). To examine how clutch characteristics affected predation risk, we pooled data across wasps for each clutch, or clutch pair, and used the average values for clutches as data points ($N =$ number of clutches). To assess absolute,

age-specific clutch traits, or wasp experience of clutches, we included data from all equal-aged clutches, regardless of the age of clutch with which they were paired. To assess relative aspects of interactions, based on the alternatives available, we used the paired structure of the choice tests.

We examined in detail the sequences of wasp behaviours on 5-day clutches, paired with 4-day clutches, and 1-day clutches, paired with 2-day clutches. We calculated behaviour durations and probabilities of transitions between behaviours for each wasp using a matrix macro in Microsoft Excel 2001. Only transitions starting in the focal clutch spatial zone were included; movements from the alternative to the focal clutch were not included, but behaviour following a period of less than 30 s off screen, after a wasp left the focal clutch, was included.

To test for effects of relative age within pairs, and its interaction with pair type, on wasp foraging preferences, we used a MANOVA followed by univariate *F* tests of three variables: (1) spatial association (time in each clutch spatial zone, as a proportion of onscreen time), (2) investigating (time investigating each clutch, as a proportion of time spatially associated with either clutch) and (3) attacking (time biting and pulling at each clutch, as a proportion of time spatially associated with either clutch). We used Kolmogrov–Smirnov tests of normality and examined residual plots for heteroscedasticity. Investigating and attacking were rank-transformed to meet parametric assumptions, and spatial association required no transformation. For other comparisons of wasp behaviour, foraging efficiency and egg fates, we used Mann–Whitney *U* tests and Kruskal–Wallis tests. We used sequential Bonferroni criteria for multiple nonparametric tests on related variables; all significant *P* values reported met these criteria.

Developmental Pattern of Wasp Predation in Nature

At the feeding station, wasps were offered clutches of different ages in close physical proximity, and individual foragers gained experience with a broad range of clutch ages. The foraging preferences and egg fates that we measured in this context may not be expressed in nature, for example if wasps do not sample sufficient clutches. To determine the developmental pattern of egg mortality caused by wasp predation in nature, we reanalysed data from a field study conducted at Ocelot Pond in 1998 (Warkentin 2000b). For 52 clutches attacked by wasps, all mortality could be assigned to circa 24-h developmental periods, between egg checks. We calculated the proportion of eggs in each clutch that were killed by wasps at each age. Checking all of the egg clutches took several hours during the day; thus, each developmental period includes parts of two sequential days.

RESULTS

The behaviour of *P. rejecta* foragers during visits to *A. callidryas* egg clutches included a series of common elements, but the pattern was strikingly different on clutches of different ages, and some behaviours occurred only on subsets of clutch ages. Figure 1 illustrates the average sequences of behaviour, with durations and transition probabilities, for wasps foraging on 5-day-old and 1-day-old egg clutches. On arrival at the feeding station, a wasp often hovered briefly before landing on or near a clutch. She typically investigated the clutch, antennating and prodding it with her mouthparts and forelimbs before biting at egg
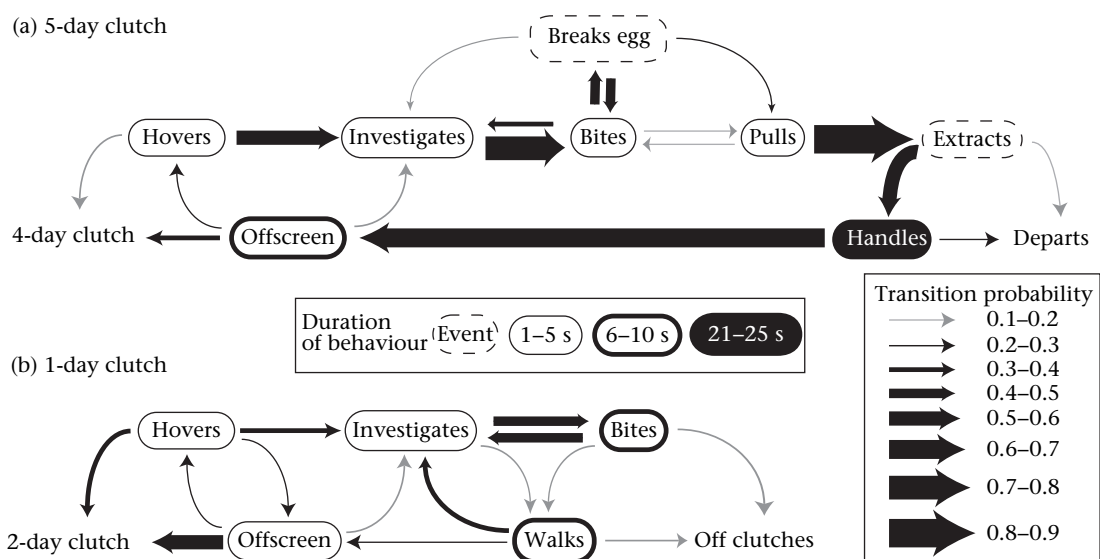

Figure 1. Contrasting sequences of behaviour for *P. rejecta* foraging on (a) 5-day-old and (b) 1-day-old *A. callidryas* egg clutches, presented in pairs with 4-day and 2-day-old clutches, respectively. Data are average durations of behaviours, and transition probabilities between behaviours, across wasps. Grooming and wasp–wasp interactions are omitted for clarity, as are transitions with a probability of less than 0.1 and the rare behaviours that followed them.

capsules. On older clutches (Fig. 1a), biting often ruptured an egg; then the wasp bit and pulled at the embryo to extract it from the egg. Older embryos are relatively robust and were usually extracted whole and alive. The wasp often handled the extracted embryo to subdue and compact it before leaving the feeding station. On young clutches (Fig. 1b), biting was rarely successful in rupturing eggs. Wasps frequently resumed investigation after unsuccessful biting, and spent substantial time walking around and over the clutch. Wasps were less likely to progress from hovering to investigating, and from investigating to biting on younger clutches (Mann–Whitney U tests: $N_{1\ day} = 10$, $N_{5\ days} = 13$, $U = 32$, $P = 0.04$; $N_{1\ day} = 11$, $N_{5\ days} = 13$, $U = 32$, $P = 0.02$, respectively).

Distribution of Wasp Foraging Effort

During visits to clutch pairs, in general, wasps spent relatively more time spatially associated with the older clutches and directed a higher proportion of their investigating and attacking behaviour towards them (Table 2, Fig. 2). The effect of relative age on spatial association and attacking varied significantly across pair types and was strongest on 2- versus 3-day and 1- versus 2-day pairs, respectively. There was no evidence that wasps discriminated between either 3- versus 4-day or 4- versus 5-day clutches (MANOVAs on association, investigation and attacking: Wilk's lambda = 0.95, $F_{3,22} = 0.4$, $P = 0.7$ for both pair types, considered separately).

Visit duration varied across clutch pairs of different ages (Kruskal–Wallis test: $H_4 = 13.54$, $P = 0.009$), with wasps spending longer on younger clutch pairs. Wasps also investigated and bit clutches aged 0–2 days (hereafter, 'younger') more than clutches aged 3–5 days (hereafter,
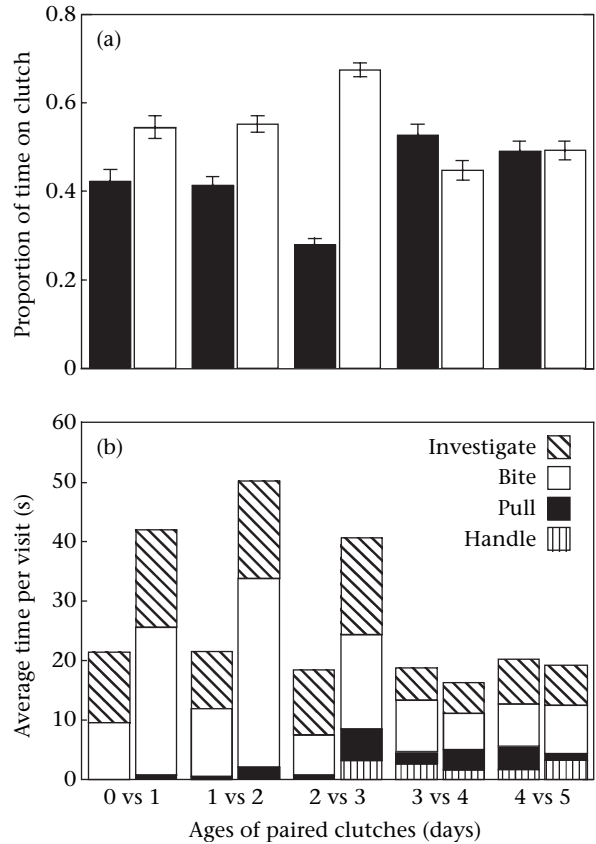

Figure 2. Distribution of effort by *P. rejecta* foragers on pairs of *A. callidryas* egg clutches of different ages. (a) Mean ± SE proportion of onscreen time that wasps spent spatially associated with each clutch. Wasps could be onscreen but associated with neither clutch. (b) Average amount of time per visit that wasps engaged in each of four different foraging behaviours, on each clutch. The bars on the left of each pair represent the younger clutches.

Table 2. Distribution of effort of *P. rejecta* foraging on pairs of *A. callidryas* egg clutches differing in age by 1 day

Multivariate analysis of variance				
Effect	**df**	**Wilk's lambda**	**F**	**P**
Relative age	3, 112	0.9205	3.22	0.025
Pair type * relative age	12, 296	0.8017	4.58	0.014
Univariate F tests				
Variable	**Effect**	**df**	**F**	**P**
Spatial association	Relative age within pair	1, 114	8.12	0.005
	Pair type * relative age	4, 114	4.64	0.002
Investigating	Relative age within pair	1, 114	4.47	0.037
	Pair type * relative age	4, 114	0.43	0.784
Biting+pulling	Relative age within pair	1, 114	6.70	0.011
	Pair type * relative age	4, 114	3.82	0.006

Spatial association is measured as a proportion of onscreen time. Investigating and attacking (biting + pulling) are measured as a proportion of time spatially associated with clutches, and rank transformed.

'older') but pulled at older eggs much more than younger eggs (Fig. 2b). Handling of captured embryos was observed only on older clutches.

Foraging Efficiency

Polybia rejecta foraged more efficiently on older egg clutches than on younger clutches (Fig. 3). As clutch age increased, wasps broke more eggs per unit time biting and extracted more embryos per unit time attacking (Kruskal–Wallis test: $H_5 = 53.4$ and 56.6, respectively, both $P < 0.0001$; Fig. 3a). Wasps were less likely to abandon extracted or scavenged prey from older clutches (Kruskal–Wallis test: $H_4 = 19.2$, $P = 0.0007$; Fig. 3b). Wasps were also more likely to leave the feeding station carrying a captured embryo from visits to older clutches (Kruskal–Wallis test: $H_5 = 52.5$, $P < 0.0001$; Fig. 3c).

Most wasps simply carried away entire embryos or large parts of them. Seven of the 17 wasps occasionally also ate parts of eggs, particularly when embryos fell apart. There was a nonsignificant tendency for an effect of clutch age
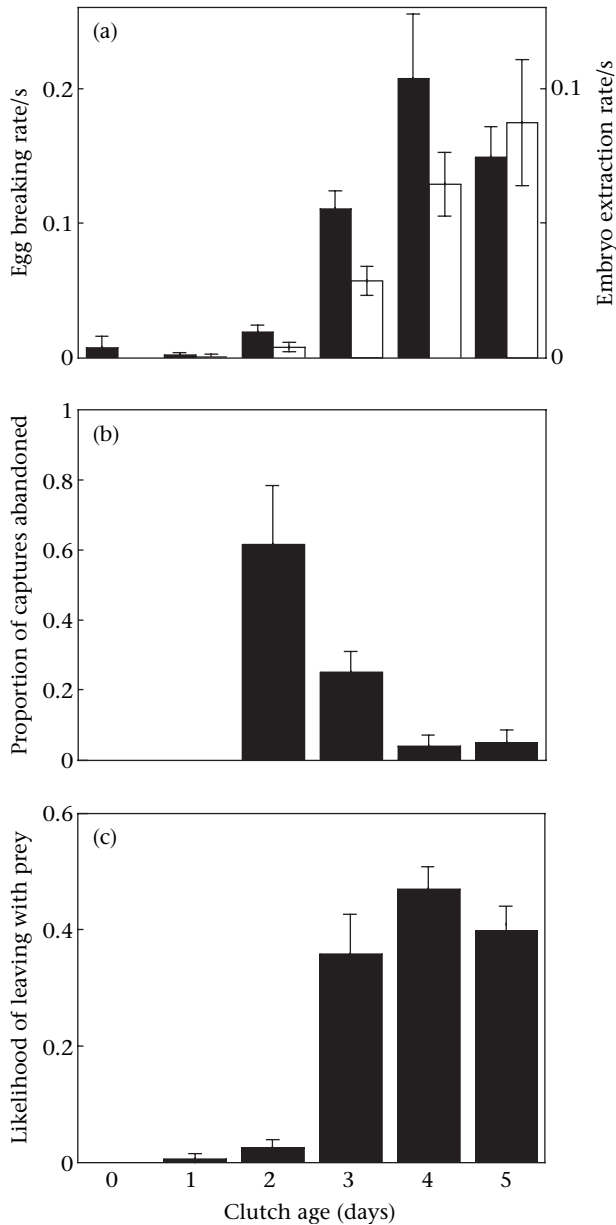
Figure 3. *Polybia rejecta* foraging efficiency on *A. callidryas* clutches of different ages. (a) Average rate at which wasps broke egg capsules, per second biting (■), and rate at which wasps extracted embryos, per second biting and pulling (□, right axis). (b) Proportion of captured embryos that wasps abandoned, including both extracted and scavenged embryos and only prey that wasps dropped, not embryos that escaped by struggling. No 0-day embryos and only one 1-day-old embryo was captured. (c) Likelihood of a wasp leaving with prey from a clutch at the end of a visit. Clutches were presented in pairs; thus, if wasps did not discriminate and were always successful, these values would be 0.5. Some wasps spent more than 30 s off screen, ending the visit, but later returned to a clutch, and some left the feeding station without prey. Error bars are SE.

on egg-eating time (Kruskal–Wallis test: $H_5 = 10.1$, $P = 0.07$). No wasp ate 0- or 5-day eggs, and the most time was spent eating 2- and 3-day eggs ($\overline{X} \pm SE = 1.1 \pm 0.8$ and 2.3 ± 1.3 s per visit, respectively).
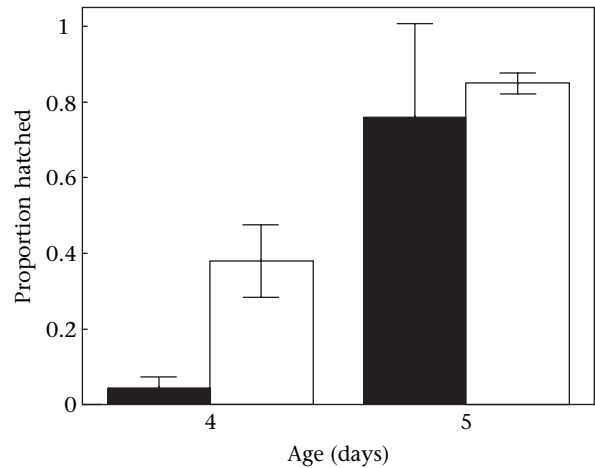

Figure 4. Mean $\pm$ SE escape hatching rate of *A. callidryas* embryos of different ages. ■: Average proportion of focal embryos that hatched while under direct attack by a wasp. Not all unhatched embryos were killed, because wasps sometimes ceased attacking or switched to another egg. □: Proportion of embryos that hatched and escaped into the water from clutches left exposed to wasps until all eggs had hatched or been killed.

Escape Hatching and Egg Fates

Embryos became capable of hatching at 4 days, and the likelihood of hatching when attacked increased developmentally after that (Fig. 4). A few older embryos also escaped from wasps by struggling after capture. The total proportion of embryos escaping from the wasps, calculated from a subset of clutches that were left exposed at the feeding station until all embryos had escaped or been taken, was substantially higher at 5 days than at 4 days (Mann–Whitney U test: $N_{4\ days} = 7$, $N_{5\ days} = 13$, $U = 7.5$, $P = 0.003$; Fig. 4).

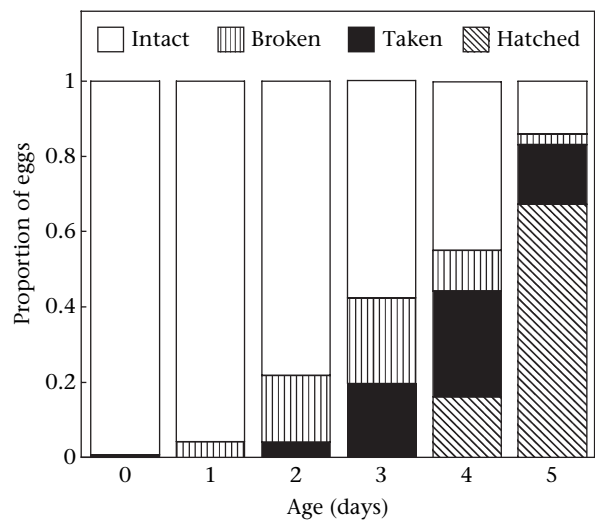Overall embryo fates after exposure to wasps varied strikingly across clutch ages (Fig. 5). In the youngest

Figure 5. Fates of *A. callidryas* embryos in clutches of different ages after exposure to *P. rejecta* foragers at a feeding station for circa 1 h. Eggs in the clutch were either intact or had their egg capsule broken. Embryos no longer in the clutch had been taken by wasps or hatched.

clutches, most eggs remained intact and unharmed, with only one egg broken out of the five 0-day clutches, but the proportion of intact eggs left decreased with development. Intermediate-aged clutches (2–3 days) were left with more broken eggs. Wasps took increasing proportions of embryos from 2- to 4-day clutches, but at 5 days most embryos hatched during the exposure period.

Developmental Pattern of Egg Mortality in Nature

Wasps killed a mean ± SE of 48 ± 5% of the eggs in *A. callidryas* clutches that they attacked at Ocelot Pond ($N = 52$ clutches). This number includes embryos taken by wasps as well as those killed and left in broken eggs or extracted but abandoned on the leaf. Most mortality occurred at intermediate ages, and wasps killed relatively few very young or hatchable embryos (Fig. 6).

DISCUSSION

Do Wasps Prefer Young, Unhatchable Eggs?

As they develop, red-eyed treefrog embryos become increasingly likely to hatch when attacked. We hypothesized that this escape ability of older embryos might lower the success or efficiency of wasps foraging on older eggs and therefore result in a preference for younger eggs that are incapable of escaping. Our results do not support these hypotheses. *Polybia rejecta* were more efficient at foraging on older eggs. Consistent with this result, wasps preferred older, more developed eggs among those eggs not yet capable of hatching.

There was, however, no evidence for a preference among 3–5-day eggs, which range from fairly well developed but not yet hatchable to highly likely to escape by hatching. Although some measures of foraging efficiency
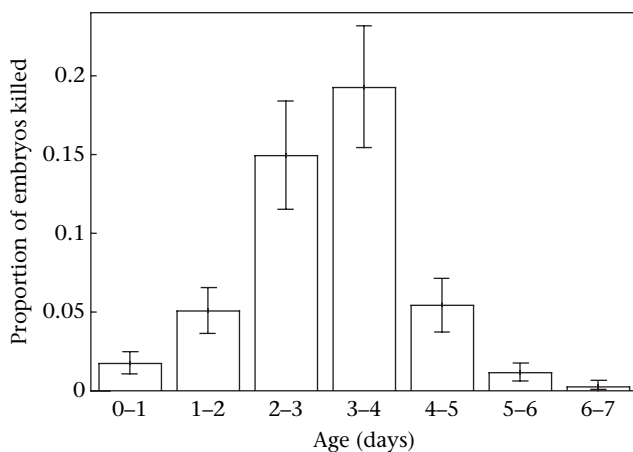

Figure 6. Developmental pattern of mortality (mean ± SE embryos killed) from wasp predation on *A. callidryas* egg clutches at Ocelot Pond. Data include both embryos taken by wasps and those killed but left in the clutch ($N = 52$ attacked clutches). Each interval includes the later part of one day, after the daily egg check, and the earlier part of the next.

(embryo extraction rate) increased across the full age range tested, others (likelihood of leaving with prey) were low for 0–2-day eggs and high for 3–5-day eggs. Increasing ease of extraction may combine with increasing escape likelihood to make 3–5-day embryos worth essentially similar effort to the wasps.

Why are Young Eggs Difficult Prey?

Both clutch structure and embryo structure change developmentally and contribute to the inefficiency of wasps foraging on younger clutches. Newly laid *A. callidryas* eggs are individually surrounded by a layer of jelly and then embedded in a substantial jelly matrix that attaches them to the substrate. As the embryos develop, water moves into the perivitelline space, enlarging it, so that the jelly layer surrounding each egg thins (Pyburn 1963; Warkentin 2000a, 2002; Warkentin et al. 2005). Simultaneously, the enlarging eggs move to the surface of the clutch to form a tightly packed layer, supported by a core of jelly. To reach embryos, wasps must break through the jelly layers and perivitelline membrane. With the youngest eggs, this act requires the wasps to press their heads into a jelly layer that is clearly thicker than their mandible length. Moreover, young eggs are less spatially constrained by each other, so they slide away from pressure against their surface. Wasps can reach young embryos, as evidenced by the field data, but doing so requires substantial time and effort. As the jelly thins and eggs become more spatially constrained, wasps require much less time to break into them.

Once wasps penetrate egg capsules, they must extract the embryos. However, those embryos change substantially, converting yolk into much more cohesive tissue as they develop (Pyburn 1963; Warkentin 1999). Wasps attempting to extract young embryos from their egg capsules almost always break the yolk, which mixes with perivitelline fluid to form a thin slurry. This slurry may drip off the clutch or dry in place. Wasps can ingest yolk slurry, and they occasionally entirely consume young clutches in the field. At the feeding station in our study, however, wasps rarely ate broken yolk. As embryos develop, the yolk is less likely to break. It also comprises less of the body, so if it does break, the wasp loses less of its prey. The high incidence of wasps abandoning 2-day-old embryos may be caused by the frequent disintegration of these embryos after the wasp has removed them from the egg capsule. Younger embryos tend to disintegrate before extraction, and older embryos typically remain intact, so that the wasp can carry them away.

How Do Developmentally Changing Defences Affect Egg Fates?

The difficulty that *P. rejecta* has in breaking into the youngest eggs, and their disinclination to persist until they are successful, results in few newly laid and 1-day-old eggs being killed. Wasps readily attack the oldest, hatchable clutches and easily break into eggs. However, many of these eggs hatch and escape during direct attacks or attacks on

their clutchmates, so few are killed. Eggs of intermediate age, which wasps readily attack but that are not yet capable of hatching, suffer the highest mortality. Wasps take eggs from these clutches, but they also break and leave many eggs, in which embryos subsequently die from their initial injuries or later desiccation. Ability to survive in broken capsules improves with development, so, unless they are severely injured, many 4-day and almost all 5-day embryos left in broken capsules will later hatch.

The maternally provided jelly surrounding *A. callidryas* eggs appears to function as a defence in two ways: directly, by hindering wasp predation, and indirectly, by shifting wasp attention away from young embryos and towards older embryos that may have a chance of escaping. The indirect mechanism should be most relevant for wasps that have experience with multiple clutch ages, especially for those that have simultaneous choices. Thus, at ponds where wasp predation is heavy, there could be an advantage to *A. callidryas* of choosing oviposition sites near older, more attractive clutches.

The patterns of egg mortality at Ocelot Pond reflect those at the feeding station, suggesting that the preferences detected in our experimental context can be expressed in nature. Egg-foraging wasps either sample sufficient clutches to find the older ones among them, or they are simply less likely to attack younger clutches, even without other options. Under normal rainy season conditions, *A. callidryas* breeds frequently enough that clutches of multiple ages are usually present at Ocelot Pond, and other major breeding sites (K.W., personal observation), giving wasps a choice. However, even when we removed all other clutches from the feeding station, our trained wasps, which had experience with older clutches, did not persist sufficiently in attacking 0- or 1-day-old clutches to cause substantive mortality. In contrast to the age-selective pattern of wasp predation at Ocelot Pond, there is no evidence for age-selective predation by snakes in Corcovado Park, Costa Rica (Warkentin 1995, unpublished data).

Learning and Prey Choices

For this and a related study, 35 individual *P. rejecta*, which had probably never before encountered *A. callidryas* eggs, were readily trained to forage on egg clutches. Individuals returned to the feeding station as often as every 15 min during daylight hours, for up to 13 days. Other foragers encountered on fruit or nectar would not even investigate frog eggs. This observation is consistent with other reports of individual learning and foraging specialization in social wasps (Raveret Richter 2000). Individual learning may also be involved in the developmental-stage preferences of wasps foraging on eggs.

Polybia rejecta are capable of completely consuming even newly laid *A. callidryas* clutches, if they invest sufficient effort (Warkentin 2000b). This behaviour did not occur at the feeding station, and at Ocelot Pond wasps kill substantial portions of only a few young clutches. At smaller breeding sites where clutches are present only sporadically and few at a time, wasps encountering young clutches would be less likely to have experience with older clutches. Observations of substantial predation on young clutches in this context (K.W., personal observation) suggest that new clutches may be sufficiently valuable, compared to caterpillars and other potential resources, for wasps to invest the effort required to prey on them. Thus, the breadth of experience of foraging wasps could substantially affect egg fates.

Polybia rejecta also forage on the arboreal eggs of *Agalychnis saltator* and *Hyla ebraccata*, which share breeding sites with *A. callidryas* (K.W., personal observation). A species of *Polybia* preys on *Hyalinobatrachium colymbiphyllum* (McDiarmid 1978; Drake & Ranvestel 2005), and wasps feed on other frog eggs as well (Lacey 1979). Although it is not known whether embryos of other species can escape from wasps by hatching, the physical structure of the egg clutches varies between species and probably affects wasp foraging. For instance, the jelly surrounding *A. saltator* eggs at oviposition is much thinner than that around *A. callidryas* eggs, and it remains thin; the eggs are also tightly packed (K.W., personal observation). The jelly around *H. ebraccata* eggs is initially thin, but it swells with rainfall and thins with desiccation (J. C. Touchon & K. W. Warkentin, unpublished data). This structural variation between egg clutches of different species, as well as environmental effects on clutch structure and developmental changes in embryos, may affect the foraging behaviour of wasps and other small mobile egg predators. This variation may be particularly important at sites where several frog species breed simultaneously, as commonly occurs in tropical environments. Similarly, the presence of alternative developmental stages or species of embryos may alter risk of egg predation, and consequently alter natural selection on parents and embryos. Not all eggs are equal to foraging wasps.

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