



Fused Eggs and Twinning in the Australian Jacky Dragon (*Amphibolurus muricatus*)

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The production of two offspring from a single egg (i.e., twinning) has been observed in all major reptilian groups (squamates: Marion 1980; Hartdegen and Bayless 1999; turtles: Tucker and Janzen 1997; crocodylians: Platt et al. 2011). While twinning in oviparous species is often considered a rare event, this phenomenon is well known, and anecdotal observations have been reported frequently in the literature (see reviews by Piovano et al. 2011; Di Marzio et al. 2023). Although less common, many cases of twinning involve bicephalic individuals or those that are conjoined in a variety of different ways (e.g., Cooper 2009; van Schingen and Ziegler 2014; Di Marzio et al. 2023). Most observations of twinning occur during research or captive husbandry programs that typically involve artificial incubation of large numbers of eggs (Hartdegen and Bayless 1999; Di Marzio et al. 2023), which increases the likelihood of observing these rare events. Nevertheless, the rate of twinning relative to the production of singleton offspring (i.e., a single hatchling from an egg) is rarely reported, and difficult, if not impossible, to quantify in nature. Herein we report observations of fused eggs and conjoined hatchlings from wild-caught Australian Jacky Dragons

(*Amphibolurus muricatus*) during four years of egg incubation studies on this species.

Our observations were made during a series of research projects in 2003–2007 that focused on maternal effects and developmental plasticity (e.g., Warner and Shine 2005, 2011; Warner et al. 2008). Our most notable observations come from offspring produced by a single wild-caught female from Lane Cove National Park in Sydney, Australia (-33.7912, 151.1512). This female was collected at 1100 h on 13 September 2005, and housed in an outdoor field enclosure at Macquarie University located ~1 km from the collection site. This female was kept in captivity (housed with one male and four other females) and monitored for egg production until the end of the reproductive season in 2007 (see Warner et al. 2008 for details on captive husbandry). This female produced two clutches during the 2005–2006 Austral summer, which resulted in normal viable offspring. In the following summer (2006–2007), this female was housed with three males and two other females, and she produced three more clutches that resulted in several instances of fused eggs and one case of conjoined offspring (Table 1; Fig. 1). This female’s first



Figure 1. Two severely fused Jacky Dragon (*Amphibolurus muricatus*) eggs that were internally connected and produced two viable singleton offspring (listed as clutch #2, eggs 3 and 4 in Table 1) (left). Conjoined twins that hatched from an egg of normal appearance but from the same clutch as the fused eggs (listed as clutch #2, eggs 5-A and 5-B in Table 1) (right). Photographs by Daniel Warner.

Table 1. Summary of three egg clutches produced by a single female Jacky Dragon (*Amphibolurus muricatus*) in the 2006–2007 Austral summer. All hatchlings were genotyped at eight microsatellite loci (allele a/allele b). Dashes indicate cases where the allele could not be determined with confidence. Individuals that did not hatch were not genotyped and are indicated by blank cells.

		AM01 (a/b)	AM16 (a/b)	AM25 (a/b)	AM52 (a/b)	AM53 (a/b)	CP7 (a/b)	CP10 (a/b)	CP11 (a/b)
Clutch #1	Oviposition on 4 October 2006								
Egg 1	Unfused egg that did not hatch								
Egg 2	Slightly fused to egg 3, did not hatch								
Egg 3	Slightly fused to egg 2, hatched	156/184	145/181	281/305	134/186	376/376	122/122	158/160	130/134
Egg 4	Normal egg that hatched	156/184	147/147	281/291	116/186	376/376	122/122	138/158	130/130
Egg 5	Normal egg that hatched	148/148	145/181	305/364	134/186	376/376	122/—	138/140	130/130
Egg 6	Normal egg that hatched	148/148	147/147	291/364	116/186	376/376	122/—	138/140	130/130
Clutch #2	Oviposition on 11 December 2006								
Egg 1	Unfused egg that did not hatch								
Egg 2	Normal egg that hatched	156/184	149/181	305/364	116/186	—/—	124/124	158/160	130/130
Egg 3	Severely fused with egg 4, hatched	240/240	145/169	364/364	160/186	376/380	122/122	140/152	130/134
Egg 4	Severely fused with egg 3, hatched	184/240	145/169	281/291	186/186	376/380	124/124	140/160	130/134
Egg 5-A	Conjoined twin with hatchling 5-B	240/240	145/157	281/281	160/186	376/378	122/122	140/152	130/134
Egg 5-B	Conjoined twin with hatchling 5-A	240/—	145/157	281/281	160/186	—/—	122/122	140/152	130/134
Egg 6	Unfused egg that did not hatch								
Eggs 7, 8, 9	Slightly fused eggs that did not hatch								
Clutch #3	Oviposition on 27 December 2006								
Egg 1	Unfused egg that did not hatch								
Egg 2	Normal egg that hatched	240/240	145/157	281/291	186/186	376/380	122/122	140/160	130/134
Egg 3	Normal egg that hatched	128/184	145/169	364/—	160/186	376/378	124/124	140/160	130/134
Egg 4	Fused with egg 5, hatched	128/184	149/157	281/281	186/—	376/377	124/124	140/152	134/134
Egg 5	Fused with egg 4, hatched	128/184	149/169	281/291	184/186	376/380	122/122	160/—	130/134

noteworthy clutch was produced on 4 October 2006 (clutch size = 6; mean egg mass = 1.01 g \pm 0.079 SD) and contained two eggs that were fused but were obviously distinct from each other; these eggs were only slightly connected by a small amount of calcification. The eggs were carefully separated by cutting away parts of the eggshell with surgical scissors (egg masses = 1.055 g and 1.101 g), and they were then incubated in separate jars using our standard protocol (see Warner and Shine 2011). One egg produced a viable hatchling, but the smaller of the two eggs did not hatch.

This female produced a second clutch of nine eggs (mean egg mass: 1.35 g) on 11 December 2006. One egg, however, appeared to be two eggs that were severely fused (mass = 2.025 g; Fig. 1); this egg was incubated at a constant 25 °C and produced two viable offspring that emerged on 28 and 29 January 2007. Each hatchling pipped the eggshell on their respective side of the fused egg, and the individual from the smaller side

of the egg (left side in Fig. 1) was smaller (mass = 0.894 g, SVL = 28 mm) than the individual from the larger side of the egg (mass = 1.012 g, SVL = 29 mm). Upon inspection of the hatched eggshell, these fused eggs were connected internally, unlike the fused eggs from the first clutch. Another egg from this same clutch was normal in appearance and mass (1.159 g) but produced twins on 1 January 2007 that were ventrally conjoined (Fig. 1). One conjoined individual was too bent to measure accurately, but the SVL of the other individual approximated 23 mm, which is about 6.5 mm shorter (22%) than the SVL of its only sibling from a “normal” egg. Although these conjoined twins were alert and active, their locomotor coordination was severely impaired, and they were humanely euthanized according to ethics approval. Of the remaining six eggs from this clutch, three were also slightly fused (collectively weighed 2.763 g) but they never hatched; the only “normal” egg from this clutch produced a viable offspring.

On 27 December 2006, this female produced a third clutch of five eggs (mean egg mass: 1.19 g), two of which were slightly fused (total egg mass = 1.771 g); these two eggs were not separated and incubated together, and each produced a viable offspring that was similar in SVL and mass to their other siblings. Of the remaining eggs from this third clutch, two produced viable offspring and one egg did not hatch. For all three clutches, unhatched eggs were not dissected and therefore we do not know whether they were fertile, contained early embryos, or contained other twins.

Based on genetic evidence, we are confident that the conjoined individuals (Fig. 1) were identical twins, but those that hatched from the fused eggs were not. We isolated DNA from all hatchlings from these three clutches and genotyped them at eight microsatellite loci (Austin et al. 2006; Schwartz et al. 2007). Genotypes were scored by eye with the assistance of Genemapper software (more details are in Warner and Shine 2008a; Warner et al. 2010). Comparisons of genotypes across loci (Table 1) showed perfect matches for the two conjoined individuals, whereas all other individuals (including those that hatched from fused eggs) did not exhibit perfect matching. Importantly, we do not consider the two offspring from the severely fused eggs to be true twins because (1) each side of the egg resembled a distinct egg from which each hatchling emerged, and (2) their genotypic differences were not notably more or less extreme than those of their other siblings (Table 1). The mother and potential fathers were not genotyped, and therefore we could not determine if offspring were full or half siblings.

The cause of the fused eggs or conjoined twins described above is unknown, but others have attributed these and other anomalies to abnormal developmental conditions or exposure to toxins (Martínez Silvestre and Barrio-Amorós 2018). These explanations seem unlikely in the cases reported here, as mothers experienced natural conditions in their outdoor enclosures. Moreover, while eggs in our experiments experienced variation in incubation temperature, incubation conditions were within the range reported in natural nests and did not reach thermal extremes (Warner and Shine 2008b). Although some eggs experienced hormonal manipulations during our research (Warner and Shine 2005; Shine et al. 2007; Warner et al. 2009), the egg that produced the conjoined twins was not hormonally manipulated and experienced a standard incubation temperature (constant 28 °C; Warner and Shine 2011). The fact that the fused eggs and conjoined twins reported herein were produced by the same mother indicates that genetic factors might have predisposed this female to produce abnormal eggs/offspring. Importantly, these abnormal eggs and offspring were not a consequence of inbreeding because the potential sires that were housed with this female were from a different location. An additional consideration is senescence that might have led to reproductive abnormalities. The average life span of *A. muricatus* is probably around four years (Warner and

Shine 2008a). This female was collected as an adult, and her reproductive abnormalities were not observed until her second year in captivity, indicating that she was near the end of her life span (at least 3–4 years of age) during our observations. Although evidence of age-related physiological decline is rare, but variable, in wild populations of reptiles (Reinke et al. 2022), this female might have been exhibiting signs of reproductive senescence in captivity.

Over the course of our research in 2003–2007, we collected 79 females from several locations in the Sydney area (Lane Cove National Park, Botany Bay National Park, Royal National Park, Dee Why Lagoon). We observed slightly fused eggs on only three other occasions (on 1 December 2003, 13 December 2003, and 11 January 2007), each produced by a different female. None of these fused eggs were as severe as that shown in Fig. 1, and viable offspring were produced by each of these eggs. The eggs in these three cases did not appear to be connected internally and the fused eggs may have resulted from being in close proximity to each other during the in-utero egg-shelling process. Of the 1,595 eggs that we obtained during our research, 1,269 produced viable offspring (79.5% egg survival); given these numbers, the rate of fused eggs occurs once every 228 eggs produced (seven fused eggs/1,595 eggs), and the rate of identical twinning is one of 1,269 hatchlings produced (0.07%). This rate of twinning is within the range reported for six turtle species (ranging from 0.01–0.63%; reviewed in Tucker and Janzen 1997), but to our knowledge, rates of twinning have not been reported for other reptiles. Nevertheless, we consider the rate of twinning to be rare but likely underestimated due to the difficulty in making these observations in nature. Indeed, impaired locomotor capacity of the conjoined twins indicated that these individuals would have rapidly died in nature, and therefore such an observation is highly improbable in the field. Observations from captive husbandry or research programs that involve artificial incubation of eggs, such as ours, can therefore provide interesting information about rare developmental events in these animals.

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