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## EVOLUTION AND DEVELOPMENT OF THE EXTRAEMBRYONIC MEMBRANES IN LIZARDS: HETEROCHRONIES AND PLACENTOTROPHY

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**Abstract.**—A conserved pattern of development and arrangement of the extraembryonic membranes is well supported within Squamata; however, the interaction of the extraembryonic membranes and its external environment varies with reproductive mode (oviparous/viviparous) and with the pattern of embryonic nutrition (lecithotrophy/placentotrophy). Because those variations can be related to structural and functional changes in the developmental timing of the extraembryonic membranes, we analyzed heterochrony on the ontogenetic sequences of the morphogenesis of the extraembryonic membranes within a phylogenetic framework in Scincidae. We employed event-pairing analytical techniques that allow the estimation of the directionality of heterochronic changes, whether precocious or delayed, and also the identification of shared (synapomorphy) or parallel/convergent (homoplasy) changes on the phylogenetic tree. We used published data of extraembryonic membranes development in Squamata, including nine species of lizards with different reproductive modes and patterns of embryonic nutrition, six lineages of Scincidae, and three species as outgroups. We identified 18 homologous events and mapped them in a phylogenetic tree. Whereas the oviparous species and several lecithotrophic/viviparous placentotrophic species have no substantial changes in their ontogenetic sequences, significant heterochronic changes were determined in two highly placentotrophic lineages with the most complex placentas known within Squamata (*Pseudemoia entrecasteauxii* and *Mabuya* clade). Critical changes are related to the morphogenesis of the yolk sac; it shows an evident ontogenetic dissociation relative to other extraembryonic membranes and probably influences ontogenetic changes on them. Those detected heterochronies seem to be instrumental in the origin of evolutionary novelties, such as the emergence of a complex placenta and placentotrophy in these highly placentotrophic lizards.

**KEY WORDS.**—amnion, chorioallantois, embryo development, event-pairing analytical techniques, placentotrophic lizards, reproductive mode, yolk sac.

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### INTRODUCTION

The evolution of the extraembryonic membranes in amniotes has made possible the development of terrestrial eggs and has facilitated the great morphological variation underlying different reproductive patterns observed in amniotes (Mess et al. 2003). Information on the development and topological relationships of the extraembryonic membranes is limited in Reptilia; there are few studies on the early morphogenesis of the extraembryonic membranes in important groups of Reptilia (Archosauria, Rynchocephalia, and Testudines) and current knowledge is based on studies done in species of Squamata, especially in viviparous species (Stewart 1997). Although in Squamata there is an apparent conserved pattern of development and arrangement of the extraembryonic membranes (Stewart 1997), there is a considerable variation in the interaction of the extraembryonic membranes and their external environment. Thus, there is probably an important variation in the functional role of specific structures during embryonic development (Stewart 1997). Within Squamata there is a considerable variation of reproductive modes and ways in which nutrients are provided to the embryo. Such variation is displayed in

a continuum from oviparous, oviparous with egg-retention, lecithotrophic viviparous, and placentotrophic viviparous species (Stewart and Blackburn 1988; Blackburn 1994). This continuum of reproductive modes and of the sources of nutrients for embryonic development could be related to functional and structural variations during the ontogeny of the extraembryonic membranes and therefore they underlie the interaction of the extraembryonic membranes with the environment.

Furthermore, there is evidence of changes in developmental timing of the extraembryonic membranes when comparing oviparous with viviparous lecithotrophic lizards. Stewart and Thompson (2003) observed that the time of development of the extraembryonic membranes changes between oviparous and viviparous species of Lygosominae lizards compared in a phylogenetic framework. This suggests that the ontogenetic sequence of the extraembryonic membranes could change among lizards in relation to their reproductive pattern, and that such variation can be interpreted as heterochronic changes in which particular morphological specializations could be related to the accelerated or delayed developmental timing of

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important ontogenetic events during embryonic development.

Comparative studies of the morphogenesis of extraembryonic membranes within Squamata can be related to the origin of evolutionary novelties such as the evolution of the placental complexity. In some genera of the family Scincidae, placentotrophic viviparity has evolved more than once (Stewart and Thompson 2000), involving the deployment of extraembryonic membranes for nutrient transfer to the embryo. Those morpho-physiological specializations may be correlated with changes in the sequences of critical developmental events of the extraembryonic membranes. Therefore, scincids are an excellent model for the study of the evolution of the placentotrophic viviparity and its relationship to changes in developmental timing during embryonic development. Within this group viviparity has evolved repeatedly, moreover there is a continuum of reproductive modes and embryonic nutrition patterns; thus, scincids can help to elucidate the mechanisms that underlie the patterns of morphological and physiological evolution of the extraembryonic membranes. A study of the ontogeny of the extraembryonic membranes in a phylogenetic framework will allow identification of which events during development of the extraembryonic membranes are early shifted or delayed, and therefore the extent and direction of heterochronic changes can be detected. Heterochronic changes would favor the interaction of some structures earlier or later during ontogeny allowing those structures to contact with other tissues in different moments and places. Therefore, the origin of some of specialized placental structures described in this group can be correlated to the new interaction of tissues (i.e. extraembryonic membranes and uterus) during development.

The purpose of the present study is to compare the ontogenetic sequences of the extraembryonic membranes of the family Scincidae within a phylogenetic framework. We aim to identify the existence of heterochronic processes that underlie morphological changes and evolutionary innovations of the extraembryonic membranes development as fundamental ontogenetic and evolutionary mechanisms to achieve the highly derived placental structures found in Scincidae.

### MATERIALS AND METHODS

We compared the ontogenetic sequences of morphogenesis of the extraembryonic membranes in nine species of squamate lizards within a phylogenetic framework, identifying possible instances of heterochronic changes using the methodology proposed by Jeffery et al. (2002). All developmental sequences were derived from the literature (Stewart 1985; Stewart and Thompson 1996; Blackburn and Callard 1997; Stewart and Florian 2000; Jerez and

Ramírez-Pinilla 2001, 2003; Stewart and Thompson 2003, 2004; Stewart et al. 2004; Villagrán et al. 2005), choosing the highest number of equivalent events present in all the species we used in the study. We derived ontogenetic sequences from the morphogenesis of the extraembryonic membranes of six species of the family Scincidae (*Pseudemoia entrecasteauxii*, *Niveoscincus ocellatus*, *Bassiana duperreyi*, *Eumeces fasciatus*, *Mabuya* spp., and *Chalcides chalcides*), three species of lizards were used as an external group (*Elgaria multicarinata*, *Sceloporus mucronatus*, and *Lacerta vivipara*). Ontogenetic sequences are composed by general events of the ontogenetic sequences, taking only "homologous" events shared by the analyzed species. Some of the ontogenetic events are probably a generalized version of the ontogenetic processes of the species used herein and perhaps does not reflect all the variability observed in the morphogenesis of the extraembryonic membranes in Squamata, such as the allantois development (see Stewart, 1993). However, the ontogenetic events used in this work try to capture the major ontogenetic events of the extraembryonic membrane development, which could be codified for the majority of the taxa included in the analysis. Furthermore, more detailed events will be applicable for a reduced number of taxa, which accounting for the limited number of ontogenies in the literature would lead to an increased fraction of inapplicable events for most of the taxa. Hence, those characters would be unique to a particular species and for that reason uninformative for the analysis.

This method compares the relative times of event pairs; the ontogenetic sequences of each species were codified in the form of event-pairs, assigning to each event-pair a "state of character" according to the position of an event with respect to the other. In this way each sequence is "re-written" as event-pairs, and coded into a matrix of event-pairs shared by all nine species. For example, for coding the ontogenetic sequence of a particular species, such as *L. vivipara*, we create an event-pair matrix for the *L. vivipara* ontogenetic sequence resulting in number event-pairs. The relative timing of one event in relation to the other is coded, for instance for two events A and B forming the event-pair AB (A column event and B row event), if A occurs before B (0), if they are simultaneous (1), if A occurs after B (2) and if one or both events are not in the ontogenetic sequence of some of the species studied (?). In this way a matrix of event-pairs was derived for the number of event-pairs for the nine species.

The event-pairs were taken as characters and they were mapped in a phylogenetic tree (Mesquite v 1.1, Maddison and Maddison 2008). Because there is not a phylogeny that includes all the terminals studied here, a phylogeny was built from the phylogenies published for the different groups. The South American *Mabuya* lineage is a monophyletic clade (Mausfeld et al. 2002), which share similar topology and specializations of the

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**TABLE 1.** Homologous events of the sequences of development. \* Stages of embryonic development following the table of development of *Lacerta vivipara* (Dufaure and Hubert 1961).

Number of total events	Events	Description of the events
1	1	Embryo between stages 5 and 10*
2	2	Embryo between stages 15 and 27*
3	3	Embryo between stages 29 and 30 *
4	4	Embryo between stage 31 and 34*
5	5	Embryo between stage 35 and 37*
6	6	Embryo between stage 38 and 40 DH*
7	a	Amnion completely surrounds the embryo.
8	b	Emergence of the allantoic bud.
9	c	Allantois expansion reaching the equatorial region of the egg or embryonic chamber
10	d	Formation and extension of choriovitelline membrane over the yolk surface.
11	e	The choriovitelline membrane disappears by mesoderm delamination.
12	f	Beginning of intravitelline invasion of the yolk sac by mesoderm migration.
13	g	The isolated yolk mass is formed by the intravitelline mesoderm within the yolk sac.
14	h	Beginning of yolk sac vascularization.
15	i	Resorption of the isolated yolk mass.
16	j	Yolk sac completely vascularized.
17	k	Expansion of the allantoic vesicle toward abembryonic hemisphere.
18	l	The allantois completely encircles the yolk sac and the embryonic chamber.

extraembryonic membranes (Leal and Ramírez-Pinilla 2008). The position of *Mabuya* spp. in relation to the group *Eugongylus* (Austin and Arnold 2006), the relationships inside the group *Eugongylus* (Austin and Arnold 2006), the relationships of *C. chalcides* and *E. fasciatus* regarding to the group Lygosominae (*Mabuya* spp. and *Eugongylus*; Brandley et al. 2005). Three species representing three different families were used as outgroups: *L. vivipara*, *E. muticarinata*, *S. mucronatus* (Estes et al. 1988; Evans and Barbadillo, 1997, 1998).

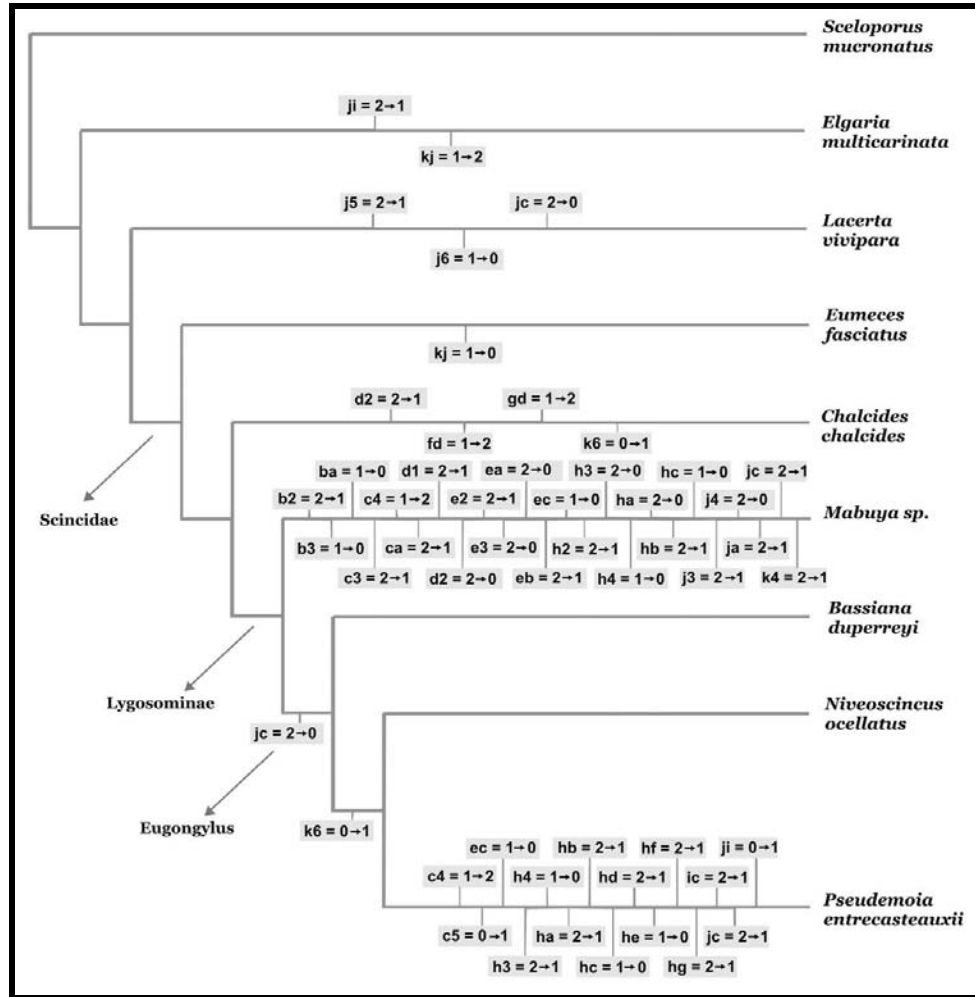
Following Jeffery et al. (2002), once the characters were optimized, we identified the transformations for each one of the branches, representing these transformations, as potential heterochronic changes. Each branch was separately analyzed, identifying the constituent events of the event-pairs that suffered transformation in the same branch. To determine the heterochronic changes, the direction of changes for each event, which we included in an event-pair, were analyzed taking into account whether the event was a row or a column event. Then, we looked if decreases in the character state were from 2 to 0 or 1, or from 1 to 0, reflecting earlier shifts of the row-event relative to the column-event (change is defined as negative), and if increases in state were from 0 to 1 or 2, or from 1 to 2

(change is defined as positive), reflecting later shifts. We determined the total relative change for each event (see Jeffery et al. 2002) according to the event-pairs for which they were involved in a particular branch. Therefore, the final result is very conservative and recovers just the movements showing a high magnitude of change in the ontogenetic sequences for a particular branch. Thus the “actively moving events” were identified and correspond to the events that have a coherent relative change and usually a “great magnitude.” Therefore, they are consistent whether they are row or column events.

Finally, those events that were equal to or above the average of the total relative changes of all events were selected. These were the actively moving events, leaving out the apparently moving events, in contrast to hitchhikers that show apparent movements derived by its changes in the ontogenetic sequence relative to the actively moving events. Therefore, the final result is very conservative and recovers just the movements showing a consistent and high magnitude change in the ontogenetic sequence for a particular branch. With the purpose of conserving only those actively moving events, the changes that were not included in the events that were conserved in the previous step were retained. In this way the events conserved are the events that

**TABLE 2.** Ontogenetic sequences of extraembryonic membrane development in nine lizard species. Events within parentheses indicate that they are simultaneous events.

Species	Reproductive mode- Nutritional pattern	Ontogenetic sequences
<i>Sceloporus mucronatus</i>	Viviparous - lecithotrophic	(1)(2)(3,a,b,f,g,d)(4,c,e,h)(i,5)(6,k,j)
<i>Elgaria multicarinata</i>	Oviparous - lecithotrophic	(1)(2)(3,a,b,f,d,g)(4,c,h,e)(k,5)(6,i,j,l)
<i>Lacerta vivipara</i>	Oviparous - lecithotrophic	(1)(2)(3,a,b,f,d,g)(4,c,e,h)(5,k,j)(6,i,l)
<i>Eumeces fasciatus</i>	Oviparous - lecithotrophic	(1)(2)(3,a,b,f,d,g)(4,c,h,e)(5,k,i)(6,j,l)
<i>Bassiana duperryi</i>	Oviparous - lecithotrophic	(1)(2)(3,a,b,f,d,g)(4,c,h,e)(5,j,k)(6,i,l)
<i>Niveoscincus ocellatus</i>	Viviparous - placentotrophic	(1)(2)(3,a,b,f,d,g)(4,e,h,c)(5,j)(6,k,i)
<i>Chalcides chalcides</i>	Viviparous - placentotrophic	(1)(2,d)(3,a,b,f,g)(4,c,e,h)(5,i)(6,k,j)
<i>Pseudemoia entrecasteauxii</i>	Viviparous - placentotrophic	(1)(2)(3,a,b,f,d,g,h)(4,e)(5,c,j,i)(6,k)
<i>Mabuya</i> spp.	Viviparous - placentotrophic	(1,d)(2,e,h,b)(3,a,c,j)(4,k)(5)(6,l)



**FIGURE 1.** Mapping of the event-pairs changes of ontogenetic sequences of the extraembryonic membrane development in the nine species analyzed, illustrating non ambiguous transformation. Grey boxes contain event-pairs and denoted their transformations for each branch. The number followed by an arrow illustrates the change of an ancestral state character to derived one. Note the great number of changes in the ontogenetic sequence of the extraembryonic membranes in the branch of *Mabuya* spp. and fewer in the branch of *Pseudemoia entrecasteauxii*, which indicate heterochronic changes. Those changes also reflect the most derived ontogenetic sequence of extraembryonic membranes of the *Mabuya* clade.

have actively changed in the position of the ontogenetic sequence and the heterochronic changes were calculated according to if they are positive or negative the total relative change, thus a range of -1 to 1, negative values indicating early movement (advances) and positive values indicating later movement (delays; see Jeffery et al. 2002).

### RESULTS

We found 18 homologous events from the literature of the morphogenesis of the extramembryonic membranes of the nine species studied herein (Tables 1 and 2). These homologous events were coded as 153 “characters” or “homologous event-pairs” (Appendix). The branches

of *Pseudemoia entrecasteauxii* and *Mabuya* spp. were the only branches that presented actively moving events that were not involved in events that apparently move (Fig. 1). Those actively moving events represent heterochronic changes in the ontogenetic sequences (Table 3, Fig. 2). In the branch of *P. entrecasteauxii*, the events that showed coherent and high magnitude heterochronic changes were as delayed event, the expansion of the allantois reaching the equatorial region of the egg or embryonic chamber, and as precocious or early events (the beginning of yolk sac vascularization and the regression of the isolated yolk mass). In the branch of *Mabuya* spp. the events that showed coherent heterochronic changes with high magnitude were as delayed events, (1) the stage of

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**TABLE 3.** Accepted-final events active in the branch of *Mabuya* spp. and *Pseudomoia entrecasteauxii*. All the events that do not show active movements were eliminated; therefore, those events that presented incoherent movement were deleted.

Branches	Events	Constituent events (accepted final events)	Relative change		
			Event as row	Event as column	Total relative change
<i>Mabuya</i> spp.	b-a = 1 to 0	a	0	-2	2
	c-a = 2 to 1				
	e-b = 2 to 1	e	-2	0	-2
	e-c = 1 to 0				
	h-b = 2 to 1	h	-2	0	-2
	h-c = 1 to 0				
	j-c = 2 to 1	j	-1	0	-1
	b-2 = 2 to 1	2	0	-2	2
	d-2 = 2 to 0				
	b-3 = 1 to 0	3	0	-2	2
	c-3 = 2 to 1				
	c-4 = 1 to 0	4	0	-2	2
	h-4 = 1 to 0				
	<i>Pseudomoia entrecasteauxii</i>	c-4 = 1 to 2	c	2	-2
c-5 = 0 to 1					
e-c = 1 to 0					
j-c = 2 to 1					
h-4 = 1 to 0		h	-8	0	-8
h-a = 2 to 1					
h-b = 2 to 1					
h-d = 2 to 1					
h-e = 1 to 0					
h-f = 2 to 1					
h-g = 2 to 1					
h-3 = 2 to 1					
j-i = 0 to 1		i	0	1	-1

embryonic development within 15 and 34 (the stages of embryonic development from neurulation to the differentiation of the digits in the hindlimb), and (2) the complete expansion of the amnion around the embryo. As early shifted events we found: (1) the choriovitelline membrane is replaced by the chorioallantoic membrane; (2) the beginning of yolk sac vascularization; and (3) the completion of its vascularization.

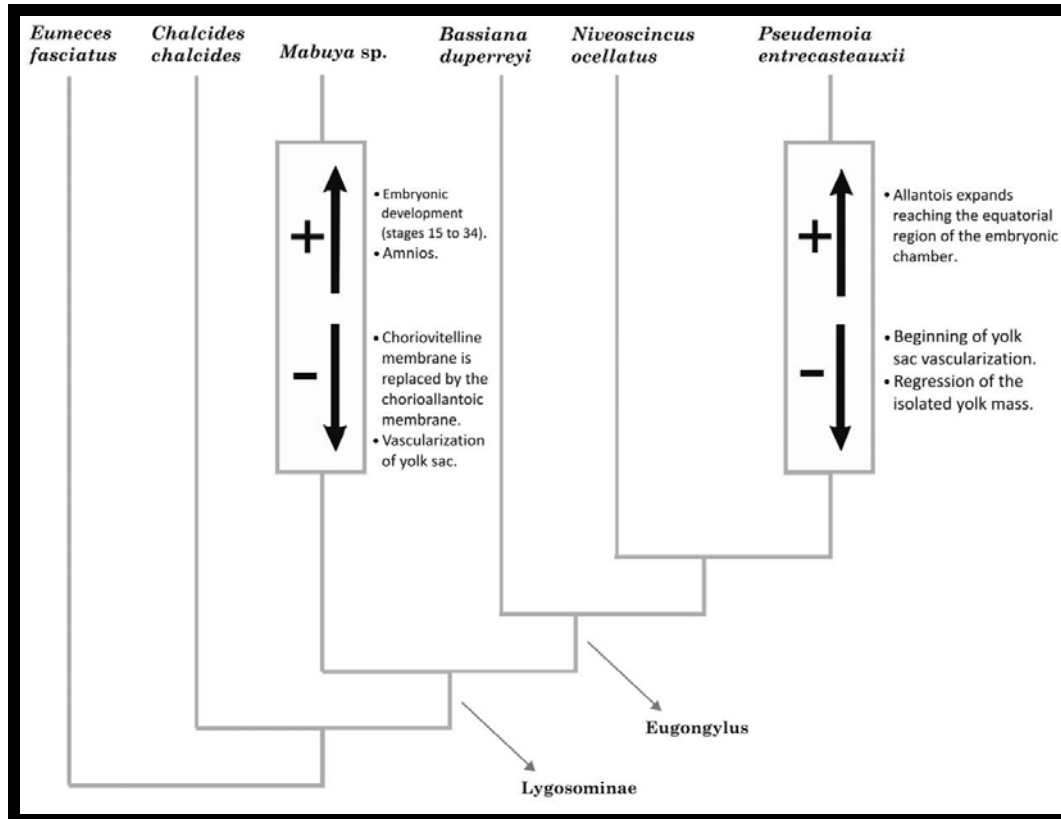
### DISCUSSION

The development of the extraembryonic membranes in Scincid lizards is conserved; however, we found that several events in the ontogenetic sequences are subject to significant heterochronic changes. The oviparous species, several lecithotrophic, and viviparous placental species did not present changes in their ontogenetic sequences in our analysis. However, the highly placental viviparous species with more complex placentas, such as *Pseudomoia entrecasteauxii* and the *Mabuya* lineage, show more dramatic heterochronic changes in their ontogenetic sequences.

In *Pseudomoia entrecasteauxii* there are three events that significantly vary in the developmental timing of the extraembryonic membranes: the early

regression of the isolated yolk mass, the precocious vascularization of the yolk sac, and a delay in the expansion of the allantois to reach the equatorial region of the egg or embryonic chamber. The early vascularization of the yolk sac and the regression of the isolated yolk mass would allow the early formation of the structurally specialized omphaloplacenta of this species that function in the histotrophic exchange of nutrients for embryonic development and that is maintained until the end of the gestation (Stewart and Thompson 1996, 2003). These precocious shifts restrict the expansion of the allantois into the abembryonic pole; the allantois is maintained in the embryonic hemisphere where it vascularizes the chorion forming the placentalome with histotrophic function in the embryonic pole and the paraplacentalome that constitutes a specialized gas exchange organ until parturition. Thus, there is a clear and direct consequence of two accelerated and permanent events with respect to the ontogeny of another structure and its placement in the ontogenetic sequence.

Although without using event-pairs optimization, the development of extraembryonic membranes has been analyzed in a phylogenetic framework in Lygosomine lizards. Previous studies have optimized ontogenetic characters in a phylogenetic tree (Stewart and Thompson 2003), illustrating that the most derived



**FIGURE 2.** Heterochronic changes in the ontogenetic sequences of the extraembryonic membranes of *Mabuya* spp. and *Pseudemoia entrecasteauxii* (the positive sign means delayed events and the negative sign means precocious events). In the *Mabuya* clade the development of the embryo and the amnion are delayed but the yolk sac and the replacement of the choriovitelline membrane by the chorioallantoic membrane are precocious. In *Pseudemoia entrecasteauxii* the allantois is delayed and the vascularization of yolk sac is precocious.

ontogeny inside the Lygosomine group is observed in the viviparous and placentotrophic genus *Pseudemoia* (the authors did not include the data of the *Mabuya* clade). Stewart and Thompson (2003) proposed that the ontogenetic and morphological evolutionary transformations of the extraembryonic membranes in *Pseudemoia* are achieved by a combination of heterochronic changes and structural innovations. Those changes include an acceleration of the growth of the allantois and of the omphalomesenteric vessels of the yolk sac splanchnopleure and early loss of the isolated yolk mass. Therefore those changes allow an early vascularization in the extraembryonic membranes which participate in the placentation (Stewart and Thompson 2003). Similarly, in this study we found an early vascularization and acceleration in the vascularization of yolk sac and early loss of the isolated yolk mass. However, we did not detect an acceleration of the growth of the allantois with respect to embryonic development; on the contrary, our event-pair analysis identifies it as a delay in the expansion of the allantois to the equatorial region of the embryonic chamber. Therefore, the same heterochronic change was identified relative to previous studies, but the

direction of the change is inverted. It is probable that in agreement with Stewart and Thompson (2003), the change can be interpretable as an early shift of the allantois growth rather than a delay. The interpretation of the direction of heterochronic changes could change if more ontogenetic events are added, such as in the morphogenesis of allantois and the decoupling of embryonic developmental stages in several independent events. In that way, the points of reference relative to the active movements will increase, improving the accuracy in the establishment of the direction of those heterochronic changes. It is possible that the placental structural specializations in the genus *Pseudemoia* have arisen by evolutionary mechanisms such as heterochronies. Thus, these heterochronic changes on the extraembryonic membranes are correlated with morphological innovations reflected in placental morphology that probably facilitate the earlier and efficient interaction of the embryo with the uterus in this highly placentotrophic clade.

In *Mabuya* spp. the stages of embryonic development from the formation of the neural tube until the differentiation of the digits in the hindlimb,

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and the formation of the amnion, appear as delayed events in the ontogenetic sequence; contrary to this, events such as the disappearance of the choriovitelline membrane (replaced by the yolk sac) and the beginning of yolk sac vascularization are events that are precocious. In the *Mabuya* lineage, as in the other species studied here, we observed that the complete formation of the amnion is associated with the stages 29–30 of embryonic development, suggesting that these two events show some degree of integration during embryonic development, hence the amnion can be dissociated of other extraembryonic membranes but hardly from the embryo or the embryonic development. The derived ontogeny of the extraembryonic membranes in *Mabuya* spp. can be related to the early development of the yolk sac in conjunction with the delay of embryonic development. However, it is worth mentioning that it is a preliminary study, with important limitations, such as the information related to embryonic development, which herein is considered as integrated developmental stages and not decoupled as several events. Decoupling the embryonic development in several events will increase the chance for the identification of heterochronic changes of the extraembryonic membranes and its direction (precocious/delayed), because in that case it will increase the number points in the ontogenetic sequence relative to the active movements. Therefore, it is very difficult to establish if the embryonic development is delayed relative to the development of the extraembryonic membranes or contrarily if the embryonic membranes early shifted in ontogeny relative to the embryonic development.

Regardless of the direction of those changes, it is probable that the derived ontogeny of the extraembryonic membranes in the *Mabuya* lineage could be related and facilitate the evolution of the great morpho-physiological specialization of its placenta. The specializations and morphological complexity of the allantoplacenta has occurred through morphological innovations as the placentome, chorionic areolas, different types of absorptive plaques, respiratory segments, and highly folded regions, as these morphological innovations increase the surface for the gas exchange and passage of nutrients (Jerez and Ramírez-Pinilla 2001, 2003; Leal and Ramírez-Pinilla 2008). The developmental mechanisms that give rise to those evolutionary novelties, such as the appearance of a complex allantoplacenta are still unknown; however, we have contemplated several hypotheses. One mechanism that probably underlies to the appearance of repeated serial homologous structures (placentome and the different types of absorptive plaques) is the replication of a developmental program controlling a discrete morphological structure (a whole absorptive plaque) and subsequent differentiation (Leal and Ramírez-Pinilla 2008). The regionalization and differentiation of those replicated and homologous structures could be

mediated by regionalized inductive signals, affecting the absorptive plaques by epithelial-mesenchymal interactions during its morphogenesis influenced by its position in the embryonic chamber (Leal and Ramírez-Pinilla 2008). According to this, and supported by previous studies, the early appearance of the dorsal absorptive plaque and of several smaller absorptive plaques is possibly induced by the early extension of the extraembryonic mesoderm (Jerez and Ramírez-Pinilla 2003). In fact, the morphogenesis of absorptive plaques in the embryonic chamber depends on the extension of the extraembryonic mesoderm; therefore the last absorptive plaques to appear are the ones that contact the mesoderm later in development, particularly in the abembryonic pole.

On the other hand, the early formation of the yolk sac in *Mabuya* probably has an influence in the regionalization of the incubation chamber during embryonic development, such as the induction for the early formation of a novel placental structure in the abembryonic hemisphere, the absorptive plaques. Jerez and Ramírez-Pinilla (2003) observed that in an Andean *Mabuya* there is an early disappearance of the choriovitelline membrane and its substitution by the yolk sac. As well there is an early and complete vascularization of the yolk sac. Thus, these events in relation to the yolk sac development show an early appearance relative to the embryonic development. Therefore, *Mabuya* possesses a derived ontogeny of the extraembryonic membranes regarding other lizards and one of the main divergences can be related with the lack of an isolated yolk mass and the precocious development of the yolk sac. Likewise, the dissociation of events such as the disappearance of the choriovitelline membrane and its replacement by the yolk sac, the early vascularization of the yolk sac (all of them appearing early regarding to the development of the other extraembryonic membranes) could give rise to ontogenetic changes in other extraembryonic membranes, such as the accelerate growth of the allantois and therefore early formation of the chorioallantois. The latter has a very important consequence; it allows a precocious interaction of the chorioallantois with the uterus. Therefore, it is possible that heterochronic changes could underlie the origin of evolutionary novelties, and a suit of characters that constitute the highly specialized placenta of the *Mabuya* clade.

Although we were not able to identify significant heterochronic changes in other viviparous placentotrophic lizards, such as *Chalcides chalcides*, we did show, however, changes in the ontogenetic sequence related to the morphogenesis of the yolk sac. Despite the ontogenetic changes in the branch of *C. chalcides*, the technique does not recover them at the end. However, it is interesting to note that the development of the yolk sac probably plays an important role in the placentotrophy in this lizard as well. Moreover, it will be interesting to test the role of

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the yolk sac in the morphogenesis of the extraembryonic membranes in other placental lizards.

Some heterochronies in *Mabuya* spp. and *Pseudemoia entrecasteauxii* are related to the morphogenesis of the yolk sac, which shows an evident ontogenetic dissociation relative to other extraembryonic membranes. These heterochronies probably would play an important role, influencing possible ontogenetic changes on the other extraembryonic membranes. It is possible that these heterochronies were instrumental in the origin of evolutionary novelties, as the emergence of a complex placenta in these placental lizards.

In this work the development of the allantois was analyzed using only three generalized events. Those events represent an oversimplification of the variability of the allantois development in relation to the yolk sac on late stages of the extraembryonic membrane ontogeny. Future analysis of comparative development of the extraembryonic membranes in Squamata requires a better refinement of some of the ontogenetic events, especially those related to the allantois development. However, those studies will require an increased number of taxa in order to avoid an increased fraction of unique and uninformative events. Therefore, it is important to study the ontogeny of the extraembryonic membranes in other Squamata, which better represent the ontogenetic variation. Likewise, the study of basal lecithotrophic (yolk-dependent embryo nutrition) oviparous squamates, will give us a better picture of primitive versus derived ontogenies of the extraembryonic membranes, a prerequisite for the understanding of the extraembryonic membrane evolution and development.

Finally, we have to acknowledge that future analyses will require more detailed ontogenetic sequences, including detailed descriptions of embryonic development rather than consider it as developmental stages as we did. Furthermore, new methodological tools have been developed to analyse event-pair data sets, such as the method described by Jeffery et al. (2005) called Parsimov, which together with an increasing number of events (reference points), will allow the detection of additional changing events that will influence the recovery of more heterochronic changes and will improve the accuracy inferring its direction (precocious or delayed).

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APPENDIX (Continued). Data Matrix. Includes 153 event-pairs as characters. The abbreviations follow the Table 1.

	Event-pairs																																
	c 6	c a	c b	d 1	d 2	d 3	d 4	d 5	d 6	d a	d b	e c	e 1	e 2	e 3	e 4	e 5	e 6	e a	e b	e c	e d	f 1	f 2	f 3	f 4	f 5	f 6	f a	f b	f c	f d	f e
<i>Sceloporus mucronatus</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	1	2	2	2	1	0	0	0	1	1	0	1	0
<i>Lacerta vivipara</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	2	2	2	2	1	0	0	0	1	1	0	1	0
<i>Elgaria multicarinata</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	1	2	2	2	1	0	0	0	1	1	0	1	0
<i>Chalcides chalcides</i>	0	2	2	2	1	0	0	0	0	0	0	0	2	2	2	1	0	0	2	2	1	2	2	2	1	0	0	0	2	2	0	2	0
<i>Mabuya</i> sp.	0	1	2	1	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	2	?	?	?	?	?	?	?	?	?	?
<i>Eumeces fasciatus</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	2	2	2	2	1	0	0	0	1	1	0	1	0
<i>Niveoscincus ocellatus</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	2	2	2	2	1	0	0	0	1	1	0	1	0
<i>Bassiana duperreyi</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	0	2	2	2	1	0	0	0	1	1	0	1	0
<i>Pseudemoia entrecasteauxii</i>	0	2	2	2	2	1	0	0	0	1	1	0	2	2	2	1	0	0	2	2	1	2	2	2	1	0	0	0	1	1	0	1	0

Continued on next page

Reproduction in Reptiles from Genes to Ecology  
 Leal and Ramírez-Pinilla.—Extraembryonic membranes in lizards.

APPENDIX (Continued). Data Matrix. Includes 153 event-pairs as characters. The abbreviations follow the Table 1.

	Event-pairs																																	
	g 1	g 2	g 3	g 4	g 5	g 6	g a	g b	g c	g d	g e	g f	h 1	h 2	h 3	h 4	h 5	h 6	h a	h b	h c	h d	h e	h f	h g	i 1	i 2	i 3	i 4	i 5	i 6	i a	i b	
<i>Sceloporus mucronatus</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	1	2	1	2	2	2	2	2	2	2	1	0	2	2
<i>Lacerta vivipara</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	2	2	1	2	2	2	2	2	2	2	1	2	2	
<i>Elgaria multicarinata</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	1	2	1	2	2	2	2	2	2	2	1	2	2	
<i>Chalcides chalcides</i>	2	2	1	0	0	0	1	1	0	2	0	1	2	2	2	1	0	0	2	2	1	2	1	2	2	2	2	2	2	1	0	2	2	
<i>Mabuya</i> sp.	?	?	?	?	?	?	?	?	?	?	?	?	2	1	0	0	0	0	0	0	0	2	1	0	0	?	?	?	?	?	?	?	?	
<i>Eumeces fasciatus</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	1	2	0	2	2	2	2	2	2	1	0	2	2	
<i>Niveoscincus ocellatus</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	1	2	0	2	2	2	2	2	2	1	2	2		
<i>Bassiana duperreyi</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	2	1	0	0	2	2	0	2	1	2	2	2	2	2	2	1	2	2		
<i>Pseudemoia entrecasteauxii</i>	2	2	1	0	0	0	1	1	0	1	0	1	2	2	1	0	0	0	1	1	0	1	0	1	1	2	2	2	2	1	0	2	2	

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APPENDIX (Continued). Data Matrix. Includes 153 event-pairs as characters. The abbreviations follow the Table 1.

	Event-pairs																																	
	i c	i d	i e	i f	i g	ih	j 1	j 2	j 3	j 4	j 5	j 6	j a	j b	j c	j d	j e	j f	j g	j h	j i	k 1	k 2	k 3	k 4	k 5	k 6	k a	k b	k c	k d	k e	k f	
<i>Sceloporus mucronatus</i>	1	2	1	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2
<i>Lacerta vivipara</i>	2	2	2	2	2	2	2	2	2	2	1	0	2	2	2	2	2	2	2	2	0	2	2	2	2	2	1	0	2	2	2	2	2	2
<i>Elgaria multicarinata</i>	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1	2	2	2	2	2	1	0	2	2	2	2	2	2
<i>Chalcides chalcides</i>	1	2	1	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2
<i>Mabuya</i> sp.	?	?	?	?	?	?	2	2	1	0	0	0	0	1	0	2	2	?	?	2	?	2	2	2	1	0	0	2	2	2	2	2	2	2
<i>Eumeces fasciatus</i>	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	0	2	2	2	2	2	2
<i>Niveoscincus ocellatus</i>	2	2	2	2	2	2	2	2	2	2	1	0	2	2	2	2	0	2	2	2	0	2	2	2	2	2	2	1	2	2	2	2	2	2
<i>Bassiana duperreyi</i>	2	2	2	2	2	2	2	2	2	2	1	0	2	2	2	2	2	2	2	2	0	2	2	2	2	2	1	0	2	2	2	2	2	2
<i>Pseudemoia entrecasteauxii</i>	1	2	1	2	2	2	2	2	2	2	1	0	2	2	1	2	1	2	2	2	1	2	2	2	2	2	2	1	2	2	2	2	2	2

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Reproduction in Reptiles from Genes to Ecology  
 Leal and Ramírez-Pinilla.—Extraembryonic membranes in lizards.

APPENDIX (Continued). Data Matrix. Includes 153 event-pairs as characters. The abbreviations follow the Table 1.

	Event-pairs																					
	kg	kh	ki	kj	l1	l2	l3	l4	L5	l6	la	lb	lc	ld	le	lf	lg	lh	li	lj	lk	
<i>Sceloporus mucronatus</i>	2	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Lacerta vivipara</i>	2	2	0	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1	2	2	2
<i>Elgaria multicarinata</i>	2	2	1	1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1	1	1	1
<i>Chalcides chalcides</i>	2	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Mabuya</i> sp.	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2
<i>Eumeces fasciatus</i>	2	2	2	1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1	1	1
<i>Niveoscincus ocellatus</i>	2	2	1	2	?	?	?	?	?	?	2	2	2	2	2	2	2	2	1	2	1	1
<i>Bassiana duperreyi</i>	2	2	1	2	2	2	2	2	2	1	?	?	?	?	?	?	?	?	?	?	?	?
<i>Pseudemoia entrecasteauxii</i>	2	2	2	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?