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M. B. Thompson · B. K. Speake · J. R. Stewart
K. J. Russell · R. J. McCartney

Placental nutrition in the Tasmanian skink, *Niveoscincus ocellatus*

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Abstract *Niveoscincus ocellatus* is an important species in historical analyses of the evolution of viviparity because it is the species upon which the type II chorioallantoic placenta was based. Here we describe the net nutrient uptake across the placenta of *N. ocellatus* for comparison with other species of skinks with complex placentae. *N. ocellatus* is highly placentotrophic, with neonates being 1.68-times larger in dry matter than the fresh eggs. There is an increase of nitrogen from 6.3 ± 0.2 mg to 9.2 ± 0.6 mg, and ash from 3.8 ± 0.3 mg to 6.7 ± 0.6 mg. The increase in ash is made up by a more than two-fold increase in the amounts of calcium, potassium and sodium. There is no significant difference in lipids in the neonates compared to fresh eggs, so considerable lipid must have crossed the placenta to provide energy for embryonic development. *N. ocellatus* is significantly more placentotrophic than *Niveoscincus metallicus*, which also has a complex chorioallantoic placenta. Discovery of substantial placentotrophy in this genus confirms that two lineages of Australian lygosomine skinks (represented by the genera *Pseudemoia* and *Niveoscincus*) have evolved this pattern of embryonic nutrition and supports the hypothesis that the evolution of reptilian placentotrophy involves specialisations in addition to structural modifications of the chorioallantoic placenta.

Key words Viviparity · Lizard · Lipid · Embryo · Yolk

Abbreviations *16:0* palmitic acid · *18:1n-9* oleic acid · *18:2n-6* linoleic acid · *18:3n-3* α-linolenic acid · *20:4n-6* arachidonic acid · *20:5n-3* eicosapentaenoic acid · *20:6n-3* docosahexaenoic acid · *PC* phosphatidylcholine · *PE* phosphatidylethanolamine · *PS* phosphatidylserine · *SVL* snout-vent length

Introduction

Knowledge of transport of nutrients across placentae of intermediate complexity allows interpretation of the stages involved in the evolution of complex placentae in lizards in particular, and amniotes in general. The first synthesis of placental types in reptiles identified three morphologically distinct chorioallantoic placental types, called in order of increasing complexity, types I, II and III (Weekes 1935). Definition of type II placentae, at the time considered intermediate (Weekes 1935), was based on *Niveoscincus ocellatus*, with *Niveoscincus metallicus* and *Niveoscincus pretiosus* said to have the same placental type (Weekes 1930). Although recent analyses (Stewart and Thompson 1994) have modified that view, the chorioallantoic placenta of *N. ocellatus* is complex compared to most viviparous species, but is simple compared to species of *Mabuya* (Blackburn et al. 1984; Blackburn 1993) and *Eumecia anchietae* (Flemming and Branch 2001). Thus, *N. ocellatus* provides an important model for our study of nutrient transport in species with placentae of intermediate complexity.

Here, we compare lecithotrophic (from the yolk) and placental nutrition in *N. ocellatus*, and then compare placental nutrition in *N. ocellatus* to data for *N. metallicus* (Thompson et al. 1999b). Although both species share some structural characteristics of their placentae, many aspects of their reproductive biology differ (Jones

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M. B. Thompson (✉) · K. J. Russell
School of Biological Sciences and Wildlife Research Institute,
Zoology Building (A08), University of Sydney,
NSW 2006, Australia
E-mail: thommo@bio.usyd.edu.au
Tel.: +61-2-93513989; Fax: +61-2-93514119

B. K. Speake · R. J. McCartney
Department of Biochemical Sciences,
Scottish Agricultural College, Ayr, KA6 5HW, UK

J. R. Stewart
Department of Biological Sciences, Box 70703,
East Tennessee State University,
Johnson City, TN 37614, USA

and Swain 1996; Jones et al. 1997). Whereas the snout-vent lengths (SVLs) of adult females of both species overlap (45–64 mm for *N. metallicus*, Jones and Swain 1996; 51–71 mm for *N. ocellatus*, Jones et al. 1997), *N. ocellatus* has smaller litters (2.7 ± 0.1, range 1–4, Jones et al. 1997; compared to 3.4 ± 0.1, range 1–7 for *N. metallicus*, Jones and Swain 1996) and larger eggs than *N. metallicus*. Thus, part of our interest stems from the possible differences in placentotrophy that might result from relative differences in egg size. Here, we describe the mass, ash, ion, nitrogen, lipid and energy content and fatty acid profiles of the eggs and neonates of *N. ocellatus* and make inferences from these data about transport of nutrition across the placenta during embryonic development. We then compare these data to those for other species with the aim of making more general conclusions about the evolution of complex placentae in squamates.

Materials and methods

Gravid female *N. ocellatus* ($n = 27$) were collected at The Thumbs, about 6 km south of Orford on the east coast of Tasmania (42°36′S, 147°54′E) at an elevation of 200–300 m at about the time of expected ovulation in the austral spring (9–10 October) 1996. The females were returned to the University of Sydney and maintained until parturition. They were fed mealworms (*Tenebrio molitor*) and crickets (*Acheta domestica*) dusted with calcium gluconate three times per week and water was provided ad libitum. A 25-W incandescent light bulb provided a thermal gradient for 9-h per day, followed by cooling to 19 °C each night. The light regime followed that of the local environment.

Females were assigned to one of three groups: experimental ($n = 10$), sham-operated controls ($n = 9$) and unoperated controls ($n = 8$). The females in the experimental and sham-operated groups were anaesthetised using 5% halothane in oxygen and a small incision was made in the right flank. One oviduct containing freshly ovulated eggs was removed from the experimental females, but not from sham-operated controls. Incisions were closed using monofilament polypropylene 5/0 sutures and the female allowed to recover. Only four females in each treatment group survived to successful parturition. On the day of parturition, young were killed by rapid freezing and females and neonates were measured (SVL and tail length) to 1 mm using a ruler and weighed to 10 mg on a top-pan balance (females) or 0.1 mg using an analytical balance (neonates). None of the neonates retained any residual yolk. Two eggs with little embryonic development were dissected from each of a further five females freshly captured nearby at Orford, close to sea level in October 1998, for some of the chemical (nitrogen) and other (calorimetry) analyses.

The reduced sample sizes, due to the failure of some females to give birth, precluded our original aim of assessing the effect of reduction in clutch size on neonatal growth. As there was no significant difference in size of female or neonates between groups (see below), we pooled data across treatments. Where possible, comparison of the chemical composition was based on eggs and neonates from different females.

Total lipid was extracted from yolks ($n = 5$) and neonates ($n = 12$, four from each treatment group) using standard chloroform-methanol methods (Christie 1984). Samples were homogenised in a suitable excess of chloroform-methanol (2:1, vol/vol) and the organic phase was washed with 0.88% (wt/vol) KCl. Total lipid was determined gravimetrically on a portion of the chloroform extract after evaporation of the solvent. Thin-layer chromatography on silica gel G using a solvent system of hexane-diethyl ether-formic acid (80:20:1, by volume) was used to isolate the major lipid classes (triacylglycerol, phospholipid, cholesterol, cholesteryl ester, free fatty acid) from the extracts. Bands were visualised and lipid classes were eluted from the silica as described previously (Noble et al. 1993). A sub-sample of the isolated phospholipid fraction from two pooled samples from each of two eggs and six pooled samples from each of two neonates was pooled for further fractionation. Thin-layer chromatography on silica gel D using a solvent system of chloroform-methanol-acetic acid-water (25:15:4:2, by volume) separated the major phospholipid classes (phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine), which were visualised and eluted from the silica (Noble et al. 1993). The isolated acyl-containing lipid classes were transmethylated to form fatty acid methyl esters as described by Noble et al. (1993). Fatty acid methyl esters were analysed by gas-liquid chromatography using a capillary column (Carbowax, 30 m × 0.25 mm, film thickness 0.25 μm; Alltech, Carnforth, UK) in a CP9001 Instrument (Chrompack, Middleburg, The Netherlands) connected to an EZ Chrom Data System (Scientific Software, San Ramon, Calif., USA). The Data System enabled the expression of the fatty acid compositions in terms of percentage weight and also enabled the calculation of the amount of each acyl-lipid and phospholipid class from which the methyl esters were derived. Peaks were identified by comparison with the retention times of standard fatty acid methyl ester mixtures (Sigma Chemicals, Poole, UK). Free cholesterol was determined using an enzymatic-colorimetric assay kit (Boehringer, Lewes, UK).

Eggs ($n = 5$) and neonates ($n = 6$) were lyophilised and then homogenised using a mortar and pestle. Sub-samples were ashed in a muffle furnace at 500 °C and the ash was prepared for analysis of ions by digestion in 3 ml concentrated sulphuric acid at 140 °C overnight. Excess sulphuric acid was evaporated and 5 ml 1:1 HCl:water added. Volume was made up to 100 ml in a volumetric flask with water to give 0.25 M HCl and the samples were stored in sodium-free plastic bottles. All glass and plastic ware had been washed in 1% nitric acid. One millilitre of sample was added to 1.5 ml caesium chloride in acid-washed atomic absorption spectrophotometer tubes and the concentrations of Ca, Na and K were determined with an atomic absorption spectrophotometer (GBC 906AA). Known concentrations of Ringers solution containing NaCl, KCl and $CaCl_2$ were added to 5.9 mmol l^{-1} $CsCl_2$ to make up 2.5 ml to generate a standard curve for each ion for comparison with the unknown samples.

Sub-samples of the dried contents of seven eggs and six neonates were pressed into pellets of approximately 10 mg and burned in a Phillipson Microbomb Calorimeter. The calorimeter was calibrated using benzoic acid periodically throughout these analyses. Total nitrogen content was determined in further dried sub-samples ($n = 7$ eggs and $n = 6$ neonates) using an automated Kjeldahl procedure (Tecator System Digestion Unit 1009 and Kjeltec System 1026 Distilling Unit). A Kjeldahl catalyst tablet (High Selenium, 1.0 g sodium sulphate anhydrous and 0.05 g selenium, Labchem A, Catalogue no. 2206–1000) and 5 ml concentrated (98%) sulphuric acid was added to each sample and heated to 400 °C for 90 min. The solution was left to cool slowly before being introduced to the distillation procedure where NaOH solution (40% wt/vol in water) was added automatically prior to distillation. The distillate, collected in a flask containing 25 ml 4%(wt/vol) boric acid, was automatically titrated with 1 M HCl and the nitrogen content of the original sample was calculated using the method of Clare and Stevenson (1964). Protein content was calculated from the nitrogen values using a conversion factor of 6.25 (Thompson 1981).

Relevant comparisons were tested by one-way analysis of variance using STATISTIX. Significance was assumed if $P < 0.05$. Equality of variances was compared using Bartlett's test. If variances were not equal, data were log transformed prior to analysis. Data are presented as means ± 1SE.

Results

There was no significant difference in post-partum mass of female lizards from among the treatments

($F_{(2,8)} = 2.84$, $P = 0.117$), so data for females was pooled. Post-partum mass of females was 5.280 ± 0.296 g (range 3.313–6.846 g, $n = 11$, one was not measured) and SVL was 62 ± 0.8 (range 58–65 mm, $n = 11$). Neonatal SVL ($F_{(2,9)} = 0.31$) and wet mass ($F_{(2,9)} = 1.06$) were not significantly different for each treatment group, so data were combined. Neonates had a mean SVL of 28 ± 0.3 mm and a wet mass of 521.6 ± 17.8 mg (Table 1). Mean clutch size (live births for sham and control females and eggs + neonates of experimental females) was 2.0 ± 0.2 ($n = 12$, range = 1–3). There was no significant relationship between post-partum mass of females and mean wet mass of neonates for each female ($F_{(1,8)} = 1.23$, $P = 0.30$, $r^2 = 0.13$), so neonatal data for each treatment were pooled.

Neonatal wet mass was more than 2.5-times greater than wet mass of freshly ovulated eggs, and only some of that increase was due to uptake of water (Table 1). The neonates also took up dry matter, with neonates having 1.68-times as much dry mass as eggs. There was a net uptake of both inorganic and organic matter during development. Although the ash content (inorganic matter) almost doubled during development, the absolute amount (2.9 mg) is small compared to the size of the neonate (Table 1). Consequently, most net uptake of dry matter was organic in origin, and this is reflected in the one third increase in energy content of neonates compared to eggs (Table 1).

Table 1 Mean (±1SE) of dry mass, protein, lipid, ash, energy, calcium, potassium and sodium content of eggs and neonates of *Niveoscincus ocellatus*. Lipid (% of dry) was calculated on the basis of mean dry mass

	Eggs	*n*	Neonates	*n*
Wet mass (mg)	199.2 ± 6.6	10	521.6 ± 17.8	12
Dry mass (mg)	59.4 ± 1.4	7	100.0 ± 7.5	6
Nitrogen (mg)	6.3 ± 0.2	7	9.2 ± 0.6	6
Protein (mg)	39.3 ± 1.1	7	57.8 ± 4.0	6
Lipid (mg)	19.6 ± 2.7	5	17.2 ± 1.2	12
Total ash (mg)	3.8 ± 0.3	7	6.7 ± 0.6	6
Total energy (J)	1532 ± 46	7	2182 ± 182	6
Protein (% of dry)	67.4 ± 1.7	7	58.0 ± 1.1	6
Lipid (% of dry)	33.0 ± 4.5	7	17.2 ± 1.2	6
Ash (% of dry)	6.2 ± 0.4	7	6.7 ± 0.5	6
Energy density (kJ g^{-1} ash-free)	27.5 ± 0.6	7	23.3 ± 0.4	6
Calcium (mg)	2.19 ± 0.09	5	4.76 ± 0.41	6
Potassium (mg)	0.19 ± 0.01	5	0.36 ± 0.03	6
Sodium (mg)	0.12 ± 0.01	5	0.27 ± 0.03	6

There was no significant difference in the amounts of lipid, or of the separate lipid fractions, taken up by neonates from each treatment, so data for neonates were pooled. The total amount (milligram per egg or per neonate) of lipid recovered from the neonates did not differ significantly from the amount originally present in the eggs (Table 1). Triacylglycerol was the major lipid class of both the egg and the neonate with substantial proportions of phospholipid also present (Table 2). Free fatty acids formed a greater proportion of the total lipid of the neonate than of the egg. The proportion of cholesteryl ester in the neonate was double that in the egg whereas the proportions of free cholesterol was about the same in the egg and neonate. The absolute amounts (milligram per egg or per neonate) of triacylglycerol, phospholipid and free cholesterol recovered from the neonates were not significantly different from the amounts initially present in the eggs. However, the amount of cholesteryl ester in the neonate was significantly (two-fold) greater than the amount in the egg.

Fatty acid profiles of all of the lipid fractions for neonates from the three treatment groups were indistinguishable, so data were combined for analysis (Table 3). The major fatty acid components of the egg triacylglycerol were palmitic (16:0), oleic (18:1n-9) and linoleic (18:2n-6) acids; α-linolenic (18:3n-3) and arachidonic (20:4n-6) acids were also present. The composition of the neonate triacylglycerol was very similar to that of the egg. The egg phospholipid was also rich in 16:0, 18:1n-9 and 18:2n-6. The main C_{20-22} polyunsaturates were 20:4n-6 and eicosapentaenoic acid (20:5n-3), but the amount of docosahexaenoic acid (22:6n-3) in the egg phospholipid was very small. Neonatal phospholipid differed in several respects from that of the egg; the proportions of 18:2n-6, 18:3n-3 and 20:5n-3 were lower whereas the proportions of 18:0, 20:4n-6 and particular 22:6n-3 were higher in the phospholipid of the neonate than in the egg. The major fatty acids of the egg cholesteryl ester were 16:0, 18:1n-9 and 18:2n-6 with significant amounts of 20:4n-6 and 20:5n-3 also present. The cholesteryl ester of the neonate differed from that of the egg in that 18:1n-9 was lower and 20:4n-6 was higher. The free fatty acids of the neonate were characterised by a greater proportion of 20:4n-6 and a lower proportion of 18:3n-3 than in the egg.

Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) respectively formed

Table 2 Proportions (as a percentage) and amounts (mg) of major components that make up total lipids in eggs and neonates of *N. ocellatus*; $n = 5$ for eggs and $n = 12$ for neonates. *NS*, no significant difference between eggs and neonates

Lipid Units	Egg %	Neonate %	Egg mg	Neonate mg	Significance
Triacylglyceride	73.8 ± 2.4	62.1 ± 2.6	14.46 ± 0.46	10.74 ± 1.11	NS
Total phospholipid	15.8 ± 2.5	16.6 ± 0.9	3.10 ± 0.49	2.72 ± 0.08	NS
Cholesteryl ester	2.0 ± 0.2	4.5 ± 0.6	0.39 ± 0.03	0.76 ± 0.10	$P < 0.05$
Free cholesterol	4.7 ± 0.3	5.4 ± 0.5	0.91 ± 0.05	0.87 ± 0.05	NS
Free fatty acid	3.7 ± 0.4	11.5 ± 1.2	0.71 ± 0.08	1.93 ± 0.15	$P < 0.05$

Table 3 Mean percentage (±1SE, %w/w) of fatty acids in each of the major lipid components of newly ovulated eggs and new born neonates of *N. ocellatus*; $n=5$ for eggs and $n=12$ for neonates

Fatty acid	Triacylglycerol		Phospholipid		Free fatty acid		Cholesteryl ester	
	Egg	Neonate	Egg	Neonate	Egg	Neonate	Egg	Neonate
14:0	1.3±0.1	1.1± 0.1	0.2±0.0	0.6±0.1	1.4±0.2	0.5±0.1	2.0±0.2	1.3±0.2
16:0	14.4±0.5	13.5±0.4	23.4±0.4	22.7±0.7	12.0±0.5	11.6±0.3	33.1±2.9	41.8±1.9
16:1n-7	4.3±0.9	5.1±0.6	2.1±0.5	1.8±0.2	4.7±0.6	2.6±0.4	2.8±0.3	2.9±0.5
18:0	3.9±0.2	5.2±0.2	4.1±0.1	12.0±0.2	3.5±0.5	8.2±0.2	2.8±0.3	5.4±0.4
18:1n-9	48.8±0.5	52.1±1.0	27.0±0.4	23.4±0.6	32.6± 1.8	35.0±1.3	32.0±1.8	18.0±1.1
18:2n-6	16.6±1.1	15.4±0.5	27.2±0.9	11.5±0.5	19.7±2.2	14.9±1.5	12.7±0.6	10.7±0.7
18:3n-3	3.4±0.8	2.4±0.4	1.7±0.4	0.4±0.1	6.6±1.8	0.9±0.1	1.6±0.4	0.7±0.7
20:4n-6	2.3±0.2	1.3±0.1	8.2±0.4	15.1±0.5	4.5±0.4	18.3±1.2	5.3±0.7	14.3±0.5
20:5n-3	0.5±0.1	–	1.5±0.3	0.2±0.0	2.3±0.6	0.2±0.0	1.0±0.4	1.4±0.2
22:5n-3	0.4±0.1	–	0.5±0.1	1.0±0.1	1.0±0.2	1.0±0.1	0.1±0.1	–
22:6n-3	0.4±0.0	0.4±0.0	0.8±0.1	5.9±0.2	0.8±0.1	2.3±0.1	0.6±0.2	–

70.2 ± 1.0, 21.7 ± 0.9 and 6.8 ± 1.5% of the total phospholipid of the eggs. In the hatchlings, PC, PE and PS respectively formed 51.7 ± 2.0%, 28.4 ± 1.0% and 14.0 ± 1.7% of the total phospholipid; sphingomyelin (6.0 ± 0.7%) was also present in the phospholipid of the hatchlings. The PC of the egg was characterised by high proportions of 16:0 and 18:1n-9 and particularly of 18:2n-6 (Table 4). The proportions of the $C_{20–22}$ polyunsaturates were, however, relatively low in the PC fraction. The PE and PS fractions of the egg were characterised in particular by very high proportions of 20:4n-6 and a relatively low proportion of 16:0. In comparison with the egg, all three phospholipid classes in the neonate displayed markedly reduced proportions of 18:2n-6 and 18:3n-3 and much greater proportions of 22:6n-3.

There was a significant uptake of calcium ($F_{(1,9)}=57.56$, $P<0.001$), sodium ($F_{(1,9)}=47.78$, $P<0.001$) and potassium ($F_{(1,9)}=30.28$, $P<0.001$) during development. The amount of each ion approximately doubled during development (Table 1).

Discussion

Discovery of substantial placentotrophy in *N. ocellatus* adds a fifth lineage of squamate reptiles in which substantial placentotrophy has evolved; the other lineages (*Mabuya*, *Chalcides*, *Eumecia*, *Pseudemoia*) are also scincid lizards (Thompson et al. 2000). *N. ocellatus* is of particular interest because it occurs in a lineage in which there are different patterns of embryonic nutrition among species. As in all highly placentotrophic species, the contribution of placental transfer to neonatal inorganic content exceeds that of the yolk contribution in *N. ocellatus*, and the neonate:egg dry mass ratio exceeds unity.

The amount of total lipid recovered in the neonate was not significantly less than the amount initially present in the egg. Since a considerable proportion of the original egg lipid must be oxidised by the embryo for energy during development, these results provide strong circumstantial evidence for the transfer of lipid across the placenta. The amount of total cholesterol (free plus esterified) was higher in the neonate than in the egg. If there is no synthesis of cholesterol by the embryo, our result provides further evidence for the net transfer of at least some classes of lipid by the placenta during gestation. Like other placentotrophic lizards (Thompson et al. 1999a, 1999b, 1999c), the egg lipid of *N. ocellatus* contains a lower proportion of triacylglycerol and commensurately higher proportions of phospholipid and free cholesterol than have been reported for eggs of oviparous lizards (Speake and Thompson 1999, 2000).

Table 4 Mean percentage (±1SE) of fatty acids in each of the major phospholipid classes of newly ovulated eggs and newborn neonates of *N. ocellatus*; $n=2$ pooled samples from two eggs, and $n=6$ pooled samples from two neonates

Fatty acid	Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylserine	
	Egg	Neonate	Egg	Neonate	Egg	Neonate
16:0	26.7±0.3	33.7±0.4	4.9±0.3	7.8±0.3	6.0±0.4	8.1±1.2
16:1n-7	1.4±0.1	2.2±0.2	0.7±0.1	1.3±0.2	0.8±0.1	0.6±0.1
18:0	3.6±0.4	7.0±0.2	8.1±0.4	20.6±0.5	18.5±4.0	20.9±2.0
18:1n-9	27.1±1.4	27.3±0.6	35.6±0.9	21.3±0.7	25.1±1.0	17.1±0.8
18:2n-6	30.8±0.4	13.6±0.5	20.4±1.4	8.3±0.5	14.0±1.2	7.5±0.8
18:3n-3	2.0±0.8	0.4±0.1	1.6±0.4	0.4±0.1	1.0±0.1	0.3±0.0
20:4n-6	6.8±0.8	8.4±0.4	18.6±0.9	23.3±0.8	28.1±2.3	27.8±1.0
20:5n-3	0.9±0.1	0.3±0.1	2.2±0.4	1.4±0.2	2.0±0.3	1.7±0.2
22:5n-3	0.4±0.1	0.4±0.1	0.9±0.1	1.8±0.1	1.1±0.1	1.9±0.1
22:6n-3	0.5±0.1	2.2±0.1	1.3±0.0	6.4±1.3	1.6±0.5	8.3±0.3

In general, the fatty acid profiles of the lipid and phospholipid classes of the freshly ovulated eggs of *N. ocellatus* are similar to those of *N. metallicus*, *Pseudemoia pagenstecheri* and *Pseudemoia spenceri* (Thompson et al. 1999a, b, c) and the oviparous lizard *Bassiana duperreyi* (Speake et al. 1999). In particular, the abundance of 18:2n-6 and 20:4n-6, the presence of 20:5n-3 and the relative lack of 22:6n-3 are common features of the eggs of these Australian scincid lizards and may be partly a consequence of their general insectivorous diet (Speake and Thompson 1999, 2000). $C_{20–22}$ polyunsaturated fatty acids have important roles in the embryonic development of vertebrate animals (Neuringer et al. 1988). In particular, 22:6n-3 may be essential for the functional development of the central nervous system, forming a large proportion of the fatty acyl groups of the phospholipids of the brain and retina (Neuringer et al. 1988). The egg phospholipid of *N. ocellatus* is rich in 20:4n-6 and contains a significant amount of 20:5n-3, but is relatively deficient in 22:6n-3. However, the proportions of both 20:4n-6 and 22:6n-3 are much greater in the phospholipid of the neonate than in that of the egg. The biomagnification of the proportions of 20:4n-6 and 22:6n-3 during development also occurs in *N. metallicus*, *P. pagenstecheri* and *P. spenceri* (Thompson et al. 1999a, b, c) and may reflect the requirement of the developing nervous system for these polyunsaturates (Speake and Thompson 1999, 2000). Possible explanations for this biomagnification include: (i) biosynthesis of 20:4n-6 from 18:2n-6 and of 22:6n-3 from 18:3n-3 or 20:5n-3, (ii) selective resistance of 20:4n-6 and 22:6n-3 to β-oxidation in the embryo, and (iii) the transfer of these two polyunsaturates across the placenta.

The structure of the chorioallantoic placentae of *N. ocellatus* and *N. metallicus* were said to be identical and designated as type II in complexity (Weekes 1930). The defining characteristics of type II chorioallantoic placentation include hypertrophied chorionic epithelial cells apposed to a uterine epithelium in which blood vessels course through raised ridges covered by a squamous epithelium (Weekes 1935). This morphology is illustrated in two photomicrographs of *N. ocellatus* (Weekes 1930) and was described and illustrated as including the entire chorioallantoic placenta. The chorioallantoic placenta of *N. metallicus* differs in that a stratified cuboidal chorionic epithelium apposed to vascularized uterine ridges covered by a squamous epithelium occurs only in a narrow zone about the periphery of the chorioallantoic placenta (Stewart and Thompson 1994). The remainder, and largest surface area, of the chorioallantoic placenta has the morphology described by Weekes (1935) as type I. In addition to chorioallantoic placentation, *N. metallicus* has a complex omphaloplacenta. The omphaloplacenta of *N. ocellatus* is undescribed. Neonates of *N. ocellatus* are 1.68-times larger in dry mass than the eggs, whereas neonatal *N. metallicus* are smaller in dry mass than, or similar to, freshly ovulated eggs (Stewart and Thompson 1994; Thompson et al. 1999b, 2000). If the omphaloplacentae of these two species are structurally and functionally identical, the greater degree of placentotrophy of *N. ocellatus* could result from an increase in the surface area of structural specialisation of the chorioallantoic placentae. Thus, the evolution of substantial placentotrophy would be the result of an increase in the size of an established structural-functional system.

There is clearly a wide variation in the placentotrophic contribution to embryonic development within each of the two clades of Australian lygosomine skinks in which substantial placentotrophy has evolved. Within the genus *Niveoscincus*, the neonate:egg dry mass ratios vary from 0.71 (Thompson et al. 2000) or 0.91 (Thompson et al. 1999b) for *N. metallicus* (depending on the population) to 1.68 for *N. ocellatus*. Within *Pseudemoia*, the ratios vary from 1.23 in *P. spenceri* (Thompson et al. 1999c) to 2.38 in *P. pagenstecheri* (Thompson and Stewart 1994; Thompson et al. 1999a). The degree of placentotrophy among members of the genus *Pseudemoia* differs, yet the structure of the chorioallantoic placentae among species does not differ (Stewart and Thompson 1996, 1998). It is also clear that species within both lineages have evolved a substantial degree of placentotrophy. The neonate:egg dry mass ratio of 1.68 of *N. ocellatus* is the same as that of *Pseudemoia entrecasteauxii* (Stewart and Thompson 1993) and intermediate between *P. spenceri* and *P. pagenstecheri*. These three species of *Pseudemoia* all have more complex chorioallantoic placentae than *N. ocellatus* (Weekes 1930; Stewart and Thompson 1996, 1998).

N. ocellatus has a significantly smaller clutch size and larger egg size (clutch size of 1–3; egg size, 59.4 mg dry mass) than does *N. metallicus* (clutch size of 1–7, Jones and Swain 1996; egg size, 41.8 mg dry mass, Thompson et al. 1999b). *N. ocellatus* also has a significantly larger body size than *N. metallicus*. In contrast, the largest species of *Pseudemoia* for which we have data (*P. spenceri*) also has the largest eggs and smallest clutch size (Thompson et al. 1999c), but relies less heavily on placentotrophy than do the smaller congeners *P. entrecasteauxii* (Stewart and Thompson 1993) and *P. pagenstecheri* (Thompson et al. 1999a). Thus, we cannot conclude that development of placentotrophy is related to large eggs, small clutches or large maternal body size.

The basis of the categorisation of placental types by Weekes (1935) relied solely on the chorioallantoic placenta because she assumed that this structure was the only site for nutrient transfer (Weekes 1935). Weekes (1935) also argued that type II placentation was an intermediate step in the evolution of type III placentation. There are no known lineages of squamate reptiles in which both of these placental types occur, so Weekes' (1935) hypothesis of an evolutionary transition may never be able to be tested. However, she also inferred that species with type III placentation should be more highly placentotrophic than species with type II placentation. Our data reject that hypothesis. Given the wide range of placentotrophic uptake of nutrients within the

genus *Pseudemoia* where the structure of the chorioallantoic placenta is conserved, and the similarity in placentotrophy between *N. ocellatus* and *Pseudemoia* spp., which have very different chorioallantoic placental structures, perhaps the chorioallantoic placenta is not the only site of nutrient transfer as assumed by Weekes (1935). Indeed, a recent analysis suggests that a complex omphaloplacenta in species with a placentome may be quantitatively more important for placental nutrient uptake than the complex chorioallantoic placenta (Stewart and Thompson 2000). Unfortunately, the form of the omphaloplacenta of *N. ocellatus* is not known. However, on the basis of comparison of our data for nutrient uptake with data for *N. metallicus* (Thompson et al. 1999b) and species of *Pseudemoia* (Stewart and Thompson 1993; Thompson and Stewart 1994; Thompson et al. 1999a, c), we predict that *N. ocellatus* will have a complex omphaloplacenta. The totality of placental structures, including the omphaloplacenta, and the dynamic changes through embryonic ontogeny, must be considered when interpreting the evolution of viviparity. *N. ocellatus* provides a model to test the relative importance of placental complexity to the evolution of placentotrophy (Stewart and Thompson 2000). The genus *Niveoscincus* will be important in the study of the evolution of placentotrophy because of diversity in placental structure and pattern of embryonic nutrition in members of this genus.

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