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Supersize me: heavy eastern grey kangaroo mothers have more sons

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Abstract The Trivers-Willard hypothesis predicts that, in po-
lygynous and sexually dimorphic mammals, mothers able to
provide a large amount of care should produce more sons.
Tests of this prediction, however, have generated equivocal
results, possibly because multiple factors, including environ-
mental conditions, simultaneously influence progeny sex ra-
tio. We tested the influence of maternal mass, condition, size,
previous reproduction and age class on offspring sex ratio in
two populations of eastern grey kangaroos (*Macropus
giganteus*). In one population, heavier and taller mothers pro-
duced more sons than did lighter mothers, but maternal con-
dition had no effect on progeny sex ratio. Primiparous fe-
males, however, produced an even offspring sex ratio despite
being smaller than multiparous females. A strong year effect
on progeny sex ratio suggested a likely effect of environmen-
tal conditions. In the second population, none of the variables
tested influenced progeny sex ratio. Different environmental
conditions between the two populations could partly explain
these results. Because maternal size and mass likely correlate
with reproductive potential, we suggest that our results sup-
port the Trivers-Willard hypothesis, but other variables likely
also influence progeny sex ratio.

Fisher ([1958](#)) suggested that because reproductive values of
males and females are equivalent in randomly mating popula-
tions, natural selection should favour unity sex ratio at the end
of parental care. If one sex becomes rare, production of that
sex will provide greater fitness returns; therefore, production
of young of the rare sex should increase until population sex
ratio reaches unity, and production of either sex is equally
advantageous (Fisher [1958](#); Hamilton [1967](#)). Following
Fisher's principle, work on adaptive progeny sex ratio prolif-
erated, but often led to equivocal results, especially for mam-
mals (Clutton-Brock and Iason [1986](#); Hewison and Gaillard
[1999](#); Cockburn et al. [2002](#)). Several hypotheses were devel-
oped arguing that some parents may obtain greater fitness
returns from producing offspring of a particular sex
(Clutton-Brock [1991](#); Cockburn et al. [2002](#); West [2009](#)).

The Trivers-Willard hypothesis (TWH) has likely received
the most attention of all hypotheses on adaptive offspring sex
ratio manipulation, especially from studies of polygynous,
sexually dimorphic mammals (Trivers and Willard [1973](#)). It
predicts that mothers in good condition would reap higher
fitness returns by producing more offspring of the sex with
greater reproductive variance (males in most mammals), while
the reverse is predicted for mothers in poor condition. This
hypothesis makes three assumptions: maternal condition is
related to offspring condition at the end of parental care, juve-
nile condition correlates with adult condition and male repro-
ductive success is more variable than female reproductive
success. If these assumptions hold, then a mother able to pro-
vide a large amount of maternal care should produce a son,
because the extra maternal care should assist in competition
with other males and a very successful son would have many

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more offspring than a successful daughter (Trivers and Willard 1973).

Although the hypothesis appears straightforward and is intuitively appealing, testing it has been difficult. First, measuring individual body condition on live animals is challenging, so that most tests of the TWH used proxies of condition, including body mass (Blanchard et al. 2005), dominance rank (Kruuk et al. 1999), maternal state defined by body condition, current environment, past history and age (Martin and Festa-Bianchet 2011), visual condition scores (Cameron et al. 1999), or other variables. Reviews of studies testing the TWH have underlined the problems of heterogeneous condition measurements, as different proxies may not capture the same information (Cameron 2004; Sheldon and West 2004). Second, the timing of condition measurement has a major impact on whether or not results support the TWH. When all studies are considered, irrespective of when condition was measured, few support the TWH (Cameron 2004; Sheldon and West 2004). If only studies that measured condition near the time of conception are considered, however, a strong majority supports the TWH (Sheldon and West 2004). Third, several environmental factors can affect sex ratio bias, partly explaining the inconsistent empirical results. Several studies have suggested that population density and climate affect sex ratio (Hewison and Gaillard 1999; Kruuk et al. 1999; Mysterud et al. 2000; Weladji and Holand 2003; Martin and Festa-Bianchet 2011). Fourth, few studies have explicitly tested the model's assumptions, due notably to the difficulty in gathering the required data (Hewison and Gaillard 1999). Fifth, there is likely a publication bias, as studies supporting the hypothesis appear more likely to be published, especially in journals with high visibility (Festa-Bianchet 1996).

Marsupials have long been considered interesting models for the study of progeny sex ratio (Cockburn 1990; Robert and Schwanz 2011), because of their very short gestation and long period of maternal care. Cockburn (1990) noted that biased sex ratios appear more frequent among marsupials than among eutherians. Recent studies on marsupials suggest that multiple factors affect offspring sex ratio. Several studies on tammar wallabies (*Macropus eugenii*) showed patterns consistent with the TWH (Green et al. 1988; Sunnuck and Taylor 1997; Robert et al. 2010; Robert and Brown 2012), but Schwanz and Robert (2014) found support for both TWH and the local resource competition (LRC) hypothesis. For polygynous and sexually dimorphic mammals, the LRC makes the opposite prediction to the TWH, reasoning that when competition for local resources is strong and one sex is philopatric, production of the dispersing sex (males in most mammals) should be favoured (Clark 1978; Silk 1983). One study of the common brushtail possum (*Trichosurus vulpecula*) supported the LRC, because competition for den sites was positively related with the production of sons (Johnson and Richie 2002). A second study on the same species, however, provided evidence supporting both LRC and TWH (Isaac et al. 2005). Studies on marsupials, therefore, suggest that hypotheses predicting opposite biases in sex ratio may not be mutually exclusive but reflect adaptations to different ecological circumstances.

Macropodid marsupials (kangaroos and wallabies) appear to be a very suitable taxon for tests of adaptive sex ratio theory (Stuart-Dick and Higginbottom 1989). First, as relatively large, sexually dimorphic, polygynous herbivores, they share numerous characteristics with ungulates, a taxon in which the TWH has been frequently tested (Sheldon and West 2004). Second, recent studies on macropodids have suggested potentially important effect of maternal characteristics on offspring sex ratio (Robert et al. 2010; Robert and Brown 2012; Schwanz and Robert 2014).

We examined progeny sex ratio variation in the eastern grey kangaroo (*Macropus giganteus*), a large polygynous herbivore with strong sexual dimorphism (Jarman 1983, 1991; Coulson et al. 2006). Like other macropods, it has a very short gestation and a long lactation period (Robert and Schwanz 2011). Eastern grey kangaroos likely meet the assumptions of the TWH and should be a suitable candidate to test its predictions. Offspring are weaned after about 18 months of maternal care (Poole 1975); so, we expected a mother's condition to be correlated with that of her young, and that this relation would persist through adulthood. Also, the strong sexual dimorphism suggests that variability in reproductive success is greater for males than that for females; so, dominant males should be able to monopolize many paternities whereas males in poor condition may not mate at all (Miller et al. 2010). Previous studies of juvenile sex ratio in eastern grey kangaroos reported a male bias (Caughley and Kean 1964; Stuart-Dick and Higginbottom 1989) in some populations and even sex ratios in others (Poole 1975). Juvenile sex ratio was also linked with environmental conditions in this species, as populations in drier parts of its range may show a male bias (Johnson and Jarman 1983).

Studies of progeny sex ratio studies continue to face the challenge of disentangling the different factors affecting it (Cockburn et al. 2002). Long-term studies can help overcome this challenge by monitoring individuals over a range of environmental conditions. We analyzed unusually detailed individual-level data from two populations of kangaroos monitored for 6 and 7 years to explore how maternal characteristics and environmental variables affected progeny sex ratio. We tested whether eastern grey kangaroos met the first two assumptions of the TWH: a correlation between maternal and offspring condition near the end of parental care exists and persists through adulthood. We then tested the TWH predictions, expecting that mothers in better condition should produce more sons and mothers in poor condition should produce more daughters.

Material and methods

Study areas and populations

Wilsons Promontory-Wilsons Promontory National Park (38′ 57′ S, 146′ 17° E) is on the southern tip of Victoria, Australia, and includes about 1 km² of grassland around an emergency landing strip, with a density of about 4–6 kangaroos/ha (Glass 2013). Introduced red foxes *Vulpes vulpes* are the main predator of juveniles. Vegetation consists of a mosaic of grasses, herbs, sedges and ferns (Davis et al. 2010).

Kangaroos were captured by Zoletil injection, mostly using a telescopic pole syringe (King et al. 2011). About 10 % were captured with a veterinary crossbow. Animals were marked with unique collar and ear-tag combinations and sexed at first capture. In total, 659 individuals were marked between August 2008 and November 2013. During captures, we measured foot, leg and arm length, weighed each kangaroo and assigned an incisor wear score from 0 (teeth completely worn out) to 3 (almost no wear). Females were assigned to one of three age groups partly based on incisor wear: 'young' females were known-age individuals first caught as pouch young or subadults and aged 3–5 years while reproductively active during this study; 'prime-age' females had an incisor wear score of 1 to 2.5 and 'old' females a score of 0 to 0.5 (Gélin et al. 2013). For young females, we knew the year of primiparity, but some females assigned to the prime-age category may have been primiparous.

Mothers were captured mostly between August and December. Young were classified following Jaremovic and Croft (1991) as small (SPY), medium (MPY) or large pouch young (LPY). We attempted to capture all reproducing females when the young had reached the LPY stage at about 7–8 months of age. At that stage, a fully furred head was at times visible protruding from the pouch. Captured offspring were sexed, measured (mass, head, foot and leg length) and marked using a unique colour combination of small plastic ear tags. We recaptured most surviving young when aged about 2 years, generally in March.

We refer to females captured in August to December as 'preconception' with respect to the young produced the following year. Although births can occur all year, at the Wilsons Promontory over half occur in December and January, after a 36-day gestation (Poole 1975). Mean capture date for females included in this study was October 7, whereas mean birth date of their next young was January 11; thus, on average, females were captured 96 days before parturition and 60 days before conception (SD=49 days). Parturition date was estimated using sex-specific growth curves for pouch young (Poole et al. 1982). We used the average estimated birthdate based on head, foot and leg lengths at capture. We defined August 1st as day 1 for the reproductive season; thus, birth dates were estimated as the number of days after August 1st. Of all juveniles with estimated birthdates at the Wilsons Promontory, 96 % were born in November–April and would have been available to tag as LPYs in August–December.

Anglesea-Anglesea Golf Club is in the town of Anglesea (38′ 24° S, 144′ 10° E), Victoria, Australia. Vegetation is regularly watered and fertilized and is mostly grass (Coulson et al. 2014). Predators include both domestic dogs and red foxes. Capture techniques were the same as at the Wilsons Promontory. Capture effort was distributed throughout the year; thus, juveniles were caught at all stages of development. As the specific objectives of the Anglesea research changed over time, so did the timing of female captures. Since 2007, 467 individuals were marked. Of these, 29 adult females received contraceptive implants (Wilson et al. 2013). Females at Anglesea were not systematically captured every year. To provide results comparable among the two populations, only females captured when they had a large pouch young were included in most analyses.

Indices of maternal condition

An individual's body condition should measure its energetic or nutritional state, usually accounting for its structural size (Peig and Green 2009). Several different indices of body condition exist, mostly attempting to scale mass to a morphometric measurement (Green 2001; Schulte-Hostedde et al. 2005; Peig and Green 2010). No consensus, however, has emerged as to which index is more effective. We used two indices. The relative condition is the ratio of the observed mass of an individual (M_i) to its predicted mass ($M_p = a\,L_i^{b}$). The values of the coefficients a and b for predicted mass correspond to the intercept and the slope of an ordinary least square regression (OLS) of mass against leg length, both log-transformed (Le Cren 1951; Peig and Green 2010). We also considered the scaled mass index (Peig and Green 2009), which quantifies the relationship between an individual's mass and skeletal size after accounting for its skeletal size in relation to the range measured in the study population. Although ideally condition should not be correlated with structural size (Schulte-Hostedde et al. 2005), both condition indices were correlated with leg length; we assumed that a stronger correlation coefficient indicated a less reliable index. Thus, because the correlation of leg length with relative condition was weaker ($r=0.18$, $P=0.013$) than its correlation with the scaled mass index ($r=-0.35$, $P<0.0001$), we present only results with the relative condition index. Results using the scaled mass index were very similar.

Statistical analyses

All analyses were performed with R 3.0.2 (R Development Core Team 2013) and Rstudio user interface v. 0.98.501 (RStudio Integrated Development Environment, Boston).

Since individual females were measured up to 6 times, we fitted a generalized mixed model with female ID as random effect to avoid pseudo-replication. Log-likelihood ratio tests were used to assess significance of random effect (Pinheiro and Bates 2000; Zuur et al. 2009). Sex ratio analyses included 166 female-pouch young pairs at Wilsons Promontory National Park, of which 14 mothers were known primiparae. We used the 'glmer' function in the 'lme4' package to run mixed logistic regression of sex of pouch young coded as zero for females and one for males (Bates et al. 2014). The probability of having a son was modeled according to maternal age class, parturition date, survival of the young born the previous year ($t-1$), maternal mass preconception, date of capture, identity of the person taking measurements, year, leg length preconception and an interaction between maternal mass preconception and year. We selected final models through backward stepwise selection, removing variables until we obtained the simplest model that could not be reduced further without significantly decreasing its explanatory power. Analyses presented in supplementary material were performed with a similar complete model replacing maternal mass with both body condition and leg length and an interaction between leg length and year. For Anglesea, analyses were performed on 21 female pouch young pairs. The complete model included the probability of having a son as function of parturition date, date of capture, year and maternal mass preconception. The number of variables differed between the two populations due to the difference in sampling effort over time at Anglesea. The Trivers and Willard assumptions were tested using the same approach, first assessing the significance of the random factor. To test the correlation between mass of the young at the LPY stage or at age 2 and maternal mass, the function lmer in the lme4 package was used to run a mixed linear regression of the mass of the young according to its sex, mother mass, capture date and year (Bates et al. 2014). To assess whether mass of the young at different ages (LPY, 2 and 3 years) was affected by maternal mass during lactation, we used the function 'lm' in the 'stats' package. We performed a linear regression of the mass of the young according to its sex, maternal mass, capture date and year (R Development Core Team 2013).

Results

Wilsons promontory

From 2009 to 2013, juvenile sex ratio differed from parity only in 2009 when the proportion of males was 0.7 (40 males and 17 females, exact binomial test $P=0.003$, CI 0.18–0.43). Juvenile sex ratio over the 5 years did not differ from parity (176 males; 148 females, proportion males 0.54; exact binomial test $P=0.13$, CI 0.49–0.60).

The offspring sex ratio of 14 known primiparous females did not differ from parity (6 males; 7 females, CI 0.25–0.81). Primiparae were on average smaller (leg length: $t=-2.19$, $P=0.03$) and tended to be lighter ($t=-1.95$, $P=0.052$) than other females but showed substantial variation in leg length and mass preconception (Table 1).

For 35 pouch young captured before 102 days of age, sex ratio did not differ from unity (exact binomial test, CI 0.39–0.65, $P=0.79$). For these young, survival to LPY did not differ according to sex (males 10/15; females 16/20, $\chi^2=0.07$, $P=0.93$).

Offspring mass at LPY stage or at age 2 were both related to maternal mass. Heavier mothers had heavier offspring (Table 2). Males were heavier than females. Capture date affected the mass of LPY but not that of 2-year olds. Study year also affected offspring mass. Mass at age 3 was also related to mass at age 2 and sex; however, its correlation with mass at LPY stage was not significant (Table 3).

Offspring sex was affected by maternal mass: Heavier females were more likely to have sons (Table 4, Fig. 1). Effect size was stronger when primiparous female were excluded, but was significant in models with and without primiparae. Males were born on average 1 day before females, but when primiparae were excluded, the difference was not significant (Table 4). Capture date was kept in the final model even though it did not affect offspring sex because its removal significantly lowered the fit of models, both for all females (log-likelihood ratio test; $\chi^2=7.27$ $P=0.007$) and excluding primiparae ($\chi^2=7.48$ $P=0.006$). Year as a five-level fixed factor also affected offspring sex, as in both 2009 and 2010 sex ratio was male-biased compared to 2011. We selected 2011 as reference because the sex ratio was 0.54 and not different from parity (exact binomial test, CI 0.32–0.59, $P=0.60$). Maternal identity was significant as a random factor in both models (all females, identity variance= 2.44 $\chi^2=6.97$, $P<0.0001$; without primiparae, identity variance=2.84, $\chi^2=6.22$, $P<0.0001$). Maternal leg length preconception was not conserved in the final model, and its removal did not lower the fit of models (all females, $\chi^2=0.035$, $P=0.85$; without primiparae, $\chi^2=0.42$, $P=0.52$). When analyses were performed without maternal mass preconception, however, leg length affected offspring sex: larger mothers were more likely to have sons (Fig. S1, Supplementary Material). Leg length and mass were strongly correlated ($r=0.74$, $P<0.0001$), thus partly explained the same variance. Maternal relative condition did not

Table 1 Leg length and preconception mass of primiparous and multiparous (excluding known primiparae) female eastern grey kangaroos at Wilsons Promontory, Victoria, Australia, 2009–2013

		Minimum	Mean	Maximum
Primiparae (n=14)	Leg length (mm)	432.0	468.8	510.0
	Mass (kg)	17.5	20.2	25.8
Multiparae (n=152)	Leg length (mm)	475.0	520.5	558.0
	Mass (kg)	21.5	27.5	33.5

Table 2 Effect of maternal mass on mass of large pouch young (LPY, about 7–8 months of age) and of 2-year olds for eastern grey kangaroos at Wilsons Promontory, Victoria, Australia, 2008 to 2013

Full model
young mass~offspring sex+mother mass+capture date+year+(1|motherid)

	LPY				Age 2			
	n=152				n=42			
Fixed effect	Estimate	SE	t	P	Estimate	SE	t	P
Intercept	**−1.04**	**0.78**	**−1.34**	**0.018**	**11.27**	**3.27**	**3.45**	**<0.0001**
Offspring sex	**0.24**	**0.12**	**2.03**	**0.042**	**1.93**	**0.61**	**3.16**	**0.0016**
Maternal mass	**0.079**	**0.025**	**3.17**	**0.0015**	**0.25**	**0.12**	**2.11**	**0.035**
Capture date	**0.0027**	**0.0011**	**2.31**	**0.02**	–	–	–	–
Year 2008	−0.35	0.28	−1.22	0.22				
Year 2009	**0.44**	**0.19**	**2.23**	**0.025**	–	–	–	–
Year 2010	0.36	0.18	1.93	0.054	**5.88**	**1.52**	**3.85**	**<0.0001**
Year 2012	**−0.52**	**0.18**	**−2.88**	**0.0039**	**−2.59**	**0.56**	**−4.58**	**<0.0001**
Year 2013	**−0.42**	**0.18**	**−2.29**	**0.021**	**−2.74**	**1.30**	**−2.10**	**<0.0001**
Random effect	Variance				Variance			
ID	0.15				2.83			

Estimates are from a generalized linear mixed model (GLMM) with maternal identity as random factor. The year 2011 is the reference for estimates of year effects. "Previouspysurvival" is the survival of the previous pouch young to different developmental states (0, no reproduction the previous year; 1, young did not survive to LPY stage; 2, young survived to LPY stage). Juveniles were not recaptured as 2-year olds in the first 2 years of the study
Significant effects in bold

affect offspring sex, regardless of whether or not primiparae were included (Table S1, Supplementary Material).

Anglesea

From 2007 to 2013, juvenile sex ratio did not differ from parity, globally or in any year (112 males and 109 females, exact binomial test, CI, 0.44–0.57, P=0.89). Capture timing at Anglesea varied over time. In 2007–2009, 99 of 122 (81 %) mothers were captured when they had SPYs while in 2010–2013, 71 of 99 had LPYs (χ^2=5.82, P<0.016). Juvenile sex ratio also differed between these periods, being 34 % male in the earlier period and 51 % male in the latter (χ^2=60.34, P<0.0001). Therefore, although overall, the probability that a

Table 3 Effect of mass at about 7–8 months of age (LPY) and near the end of parental care (aged 2) on mass near adulthood (age 3), for eastern grey kangaroo at Wilsons Promontory, Victoria, Australia, 2011 to 2013

Full model
offspring mass (age 3)~offspring sex+offspring mass+capture date+year

	LPY				Age 2			
	n=32				n=33			
Fixed effect	Estimate	SE	t	P	Estimate	SE	t	P
Intercept	**22.29**	**1.82**	**12.26**	**<0.0001**	**8.67**	**1.58**	**5.46**	**<0.0001**
Offspring sex	**3.88**	**0.82**	**4.73**	**<0.0001**	**2.32**	**0.52**	**4.46**	**<0.0001**
Offspring mass	0.75	0.46	1.62	0.12	**0.77**	**0.092**	**8.28**	**<0.0001**
Year 2008	2.76	2.93	0.94	0.35	–	–	–	–
Year 2010	1.79	2.61	0.69	0.50	–	–	–	–
Year 2012	−1.60	2.63	−0.61	0.55	–	–	–	–

Estimates were generated using a linear model (LM). Offspring sex was coded as 0, female; 1, males. The table presents the full model followed by estimates and statistics for variables that remained in the simplest model chosen by backward stepwise selection
Significant effects in bold

Table 4 Effect of preconception mass on the probability of producing a son for eastern grey kangaroo females at Wilsons Promontory, Victoria, Australia, 2008 to 2013

Full model

Sex~birth date+age+mass preconception+previouspysurvival+observer+capture date+year+leg preconception+mass preconception *year+(1|id)

Fixed effect	Excluding primiparae				All females			
	$n=152$				$n=166$			
	Estimate	SE	z	P	Estimate	SE	z	P
Intercept	**−8.084**	**3.081**	**−2.62**	**0.0094**	**−7.12**	**2.77**	**−2.61**	**0.0091**
Mass preconception	**0.24**	**0.093**	**2.57**	**0.001**	**0.20**	**0.078**	**2.58**	**0.0098**
Birth date	0.0069	0.0050	1.41	0.16	**0.010**	**0.0051**	**2.17**	**0.030**
Capture date	−0.0020	0.0047	−0.43	0.67	−0.005	0.0050	−0.95	0.34
Year 2009	**3.13**	**0.98**	**3.20**	**0.0014**	**3.53**	**1.00**	**3.55**	**0.0004**
Year 2010	**1.46**	**0.67**	**2.19**	**0.029**	**1.53**	**0.69**	**2.22**	**0.026**
Year 2012	1.18	0.66	1.80	0.072	1.28	0.68	1.88	0.061
Year 2013	0.45	0.67	0.66	0.51	0.57	0.68	0.83	0.41
Random effect	Variance				Variance			
ID	1.814				2.28			

Estimates are from a generalized linear mixed model (GLMM) with maternal identity as random factor. The year 2011 is the reference for estimates of year effects. "Previouspysurvival" is the survival of the previous pouch young to different developmental states (0, no reproduction the previous year; 1, pouch young did not survived to LPY stage; 2, pouch young survived to LPY stage). The table presents the full model followed by estimates and statistics for variables that remained in the simplest model chosen by backward stepwise selection

Significant effects in bold

captured young was a male had a nearly significant relationship with its developmental stage (Table 5), we cannot distinguish between sex-biased mortality and year effects on offspring sex ratio.

At Anglesea, maternal mass preconception had no effect on offspring sex (Table 6). Analyses included only offspring captured as LPY to be consistent with analyses at Wilsons Promontory. Maternal leg length preconception did not affect juvenile sex ratio as this variable was not selected in the model simplification.

Discussion

Offspring sex ratio at Wilsons Promontory National Park varied with preconception maternal characteristics: heavier mothers

Fig. 1 Probability to have a son (0, daughter; 1, son) for female eastern grey kangaroos according to mass pre-conception. *Circles* are observed data excluding known primiparous females, the logistic regression curve is from a generalized linear mixed model also excluding primiparae. *Filled points* are proportions of offspring (±SE) at intervals of 2.4 kg excluding primiparae. *The triangle* is the offspring sex ratio for primiparae and corresponds to the average mass of primiparous females
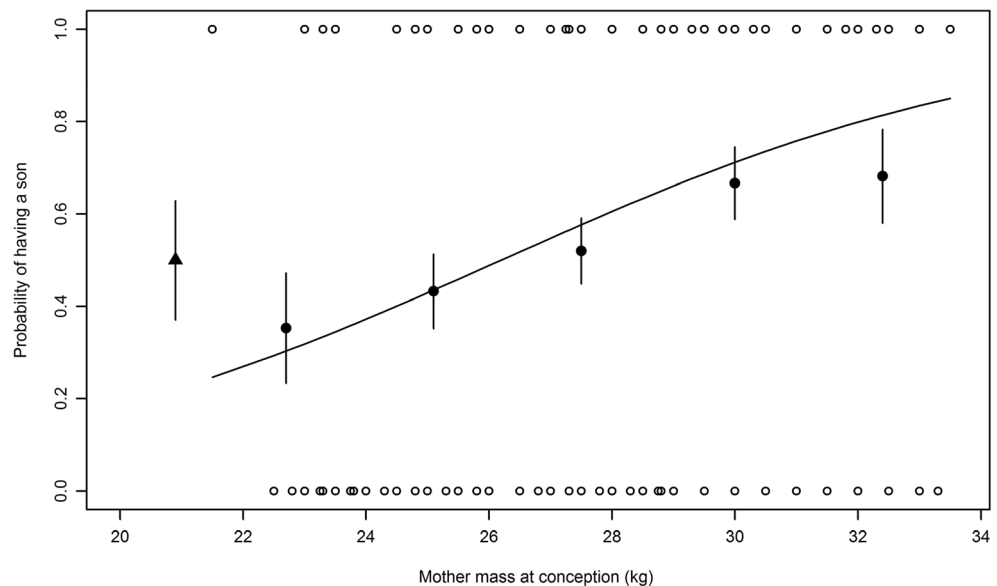
Table 5 Probability of an offspring being a male according to its developmental stage for eastern grey kangaroos at Anglesea, Victoria, Australia, 2007–2013

Full model sex~pydevelopment+year+pydevelopment*year+(1|id)

	Estimate	s.e.	z value	p value
Intercept	−0.19	0.19	−0.998	0.32
PY development	0.51	0.28	1.80	0.072
	Variance			
Id	0.35			

Estimates from a generalized linear mixed model (GLMM) based on 221 observations with female identity as random variable. PY development was coded as 0, unfurred small pouch young (SPY); 1, furred large pouch young (LPY). The table presents the full model followed by estimates and statistics for variables that remained in the simplest model chosen by backward stepwise selection

were more likely to have sons. Excluding known primiparous females, an increase in maternal mass of 2 kg from the mean (27.5 kg) led to an 11 % increase in the probability of having a son, from 0.58 to 0.69. A study on Tammar wallabies reported similar results (Sunnuck and Taylor 1997). Maternal mass was not, however, related to offspring sex ratio for kangaroos at Anglesea, where we had a small sample size. The absence of effect of maternal mass on juvenile sex ratio in one of two study populations corresponds with the inconsistency often reported by studies of progeny sex ratio (Hewison and Gaillard 1999; Cockburn et al. 2002; Sheldon and West 2004).

Eastern grey kangaroo appear to satisfy the two first assumptions of the Trivers and Willard hypothesis: maternal mass was correlated with offspring mass at the LPY stage and at 2 years, after weaning. Although mass at age 2 was correlated with mass at age 3, near adulthood, mass at the LPY stage was not significantly correlated with mass at age 3. Given the limited sample size of offspring monitored to age

Table 6 Probability of an offspring being a male according to maternal mass in eastern grey kangaroos at Anglesea, Victoria, Australia, 2007–2013

Full model
Sex~birth date+year+capture date+mass preconception+(1|id)

	Estimate	s.e.	z value	p value
Intercept	57.56	4721	0.012	0.99
Mass preconception	−0.028	0.022	−1.27	0.21
Age 2	−17.12	3177	−0.005	0.996
Age 3	−40.75	7255	−0.006	0.996

Estimates from a generalized linear model (GLM) based on 21 observations from 2007 to 2013 with female identity as a random variable. Age is defined as 1, young; 2, prime aged and 3, old females. Young females are the reference group. The table presents the full model followed by estimates and statistics for variables that remained in the simplest model chosen by backward stepwise selection

3, however, we could only detect strong effects, and given the positive trend, it seems reasonable to assume that weaning mass is correlated with adult mass. We could not test the assumption that male reproductive success is more variable than female reproductive success. Male reproductive success in semi-captive eastern grey kangaroo increases with body mass and dominance rank (Miller et al. 2010), similarly to species such as red deer (*Cervus elaphus*) where a more variable reproductive success for males than for females has been documented (Clutton-Brock et al. 1986). Given also the strong sexual dimorphism, it seems reasonable to expect that for eastern grey kangaroo, reproductive success is more variable for males than that for females.

At Wilsons Promontory, models excluding maternal mass showed that maternal leg length, but not body condition, affected juvenile sex ratio (Table S1). Mothers with longer legs had a higher probability to give birth to sons than to daughters. Excluding known primiparous females, an increase of 10 mm from the mean (520 mm) was associated with an 8 % greater chance of having a son. Maternal mass can vary with both structural size and body condition (Green 2001), but the concordant results for leg length, and the absence of an effect of body condition, suggest that its relationship with offspring sex ratio is mostly due to changes in structural size. Although the association of offspring sex appeared stronger with maternal mass than with structural size, the two variables are closely correlated, as leg length explained 54 % of the variability in mass: heavy mothers are also large mothers. Interestingly, primiparous females deviated from the pattern seen for older mothers for both mass and leg length, because primiparae produced an even offspring sex ratio. The reason for this age-dependent effect is unknown, and future work will address the age-specific fitness consequences of producing sons and daughters. Isaac et al. (2005), found a similar age-dependent effect for brushtail possums: multiparous females in better condition produced more sons, but primiparae did not follow this trend.

Trivers and Willard (1973) predicted that maternal body condition should affect offspring sex ratio, assuming that body condition was correlated with ability to provide care. Instead, our analyses indicate a relationship between offspring sex and maternal mass, not maternal condition. We found that large mothers, not fat mothers, produced more sons. Large females likely have a higher reproductive potential than small ones and may be able to provide greater maternal care. Studies on tammar wallabies support this interpretation, reporting a positive correlation between maternal mass, milk production and growth of the young (Green et al. 1988). Robert et al. (2010) used cross-fostering to investigate variation in maternal reproductive potential of tammar wallabies according to the sex of the offspring birthed. Females that produced sons were more likely to wean the young assigned to their pouch, regardless of its sex. Robert et al. (2010) suggested that females with higher reproductive potential are more likely to give birth to a son,

although neither mass nor condition of mothers were related to offspring sex. Robert and Braun (2012) revealed that the milk of mothers of sons contained more protein than the milk of mothers of daughters between 100 and 215 days post-partum. In that study, the smallest mothers only produced daughters, but overall maternal foot length was not a significant predictor of offspring sex. A recent study of the same species found that females in better condition were more likely to have sons in only 1 of 2 years (Schwanz and Robert 2014).

The foraging behaviour of eastern grey kangaroos suggests a greater energetic cost of sons, as bite rate is faster for mothers of sons than mothers of daughters (Gélin et al. 2013). Therefore, although mothers of sons are heavier and likely have a greater reproductive potential than mothers of daughters, they still must compensate for the additional cost of raising a son by increasing their feeding rate. Gélin et al. (2015) found that mothers that weaned sons were more likely to be reproductively successful the following year than mothers that weaned daughters and suggested that females with greater reproductive potential may be more likely to conceive sons.

Maternal condition had no effect on offspring sex; therefore, our results do not strictly support the Trivers and Willard hypothesis. Body condition, however, is not easily measured, and there continues to be debate about the most appropriate condition index linking the mass of an individual to its size (Green 2001; Schulte-Hostedde et al. 2005; Peig and Green 2010). Because eastern grey kangaroo are partly income breeders (Gélin et al. 2013) and have indeterminate growth, any index linking mass to skeletal size may not adequately reflect condition, partly because both mass and size can vary from 1 year to the next. Reproductive females vary substantially in size (Table 1), and it may be difficult for a small female to raise a son even when in good condition. The larger size of sons may become a problem as they grow, possibly forcing an earlier exit from the pouch of smaller mothers. Therefore, our results from Wilsons Promontory support the Trivers and Willard hypothesis in the broader sense, since the tendency for heavier females to give birth to sons appears to be adaptive. Perhaps indeterminate growth in female kangaroos is associated with a reproductive advantage of larger size. Field observation does not suggest a role of female size or dominance on access to resources, as aggressive interactions among females are extremely rare (Maguire et al. 2006). Larger mothers may be able to provide more maternal care independently of their condition, a hypothesis that will be tested by continuing to monitor this population.

Environmental effects on sex ratio are common (Kruuk et al. 1999; Weladji and Holand 2003; Baeta et al. 2012), and the strong year effect on sex ratio that we reported may be partly caused by unknown environmental effects. Both 2009 and 2010, when offspring sex ratio was male biased, appeared favourable years compared with 2011, based on the proportion of females that reproduced and on juvenile

survival (Gélin et al. 2015). Future research will investigate if favourable years are consistently associated with a higher probability of producing sons. The apparent effect of birth date on offspring sex, however, may reflect a bias in birth date estimate. Birth dates were estimated from morphometric measurements based on sex-specific growth models established from a captive population (Poole et al. 1982). Birth date estimates may be sex-biased if male and female young grew at different rates in our study population compared to captive individuals. It is also possible that in unfavourable years, early mortality may differ according to offspring sex (Clutton-Brock et al. 1985), but in macropods that relationship could be complicated by the link between reproductive potential and production of sons (Robert et al. 2010; this study). To examine these possibilities, we have begun a program of recaptures of females carrying pouch young, to measure in-pouch growth rates and assess any possible sex-differential early mortality.

At Anglesea, if year of study is ignored, early mortality in the pouch appeared biased toward daughters, making capture of sons more likely at a later developmental stage. Differential mortality according to offspring sex is a possible mechanism for biased sex ratio (Clutton-Brock 1991; Kruuk et al. 1999; Robert and Schwanz 2011). For sexually dimorphic species like kangaroos, however, one would expect male-biased early mortality (Clutton-Brock 1991). Also, sex ratios varied substantially over time in both study areas, and data on sex ratio in different years at Anglesea were mostly limited to either very small pouch young or to large young captured shortly before permanent pouch exit. Our analysis, therefore, cannot distinguish sex-differential mortality from annual differences in offspring sex ratio. It is also possible that mortality according to sex of the young varies among years. Our limited sample for the Wilsons Promontory population does not suggest sex-biased mortality of small pouch young.

In conclusion, our research suggests that maternal mass is a strong determinant of offspring sex ratio in eastern grey kangaroos. The inconsistent results for primiparous mothers, and the lack of an apparent effect of maternal mass on sex ratio at Anglesea, however, suggest that additional variables likely affect sex ratio bias in kangaroos. A prediction from our study is that sex-specific growth, survival and reproduction should also vary according to maternal size. Ongoing research is collecting the long-term individual-based data necessary to test that prediction.

Ethical standards All procedures involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted, as authorized by The University of Melbourne Animal Ethics Committee and by the Université de Sherbrooke Animal Care Committee, affiliated with the Canadian Council on Animal Care.

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