# Adaptive biases in offspring sex ratios established before birth in a marsupial, the common brushtail possum *Trichosurus vulpecula*

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Offspring sex ratios in the common brushtail possum are male biased in many populations, and there is evidence that inter-population differences in sex ratios represent adaptive responses to local conditions. However, how these biases are produced is not known. Using comparisons between populations with and without biased offspring sex ratios, we show that biases in this species are not produced by sex-differential mortality between birth and weaning or sex-selective termination of pregnancy. Rather, adjustment in the sex ratio of offspring are evidently due to shifts in the probability of conceiving male and female offspring. *Key words:* brushtail possums, local resource competition, Marsupialia, primary sex ratio, sex ratio mechanisms, *Trichosurus vulpecula.* *[Behav Ecol 13:653–656 (2002)]*

A number of theories identify circumstances in which adaptive variation in the sex ratio of offspring should occur, and there is often an excellent match between predicted and observed variation in sex ratios among invertebrates (Godfray and Werren, 1996). For vertebrates, however, the evidence on adaptive variation in the sex ratio is less clear, and it has been argued that vertebrates may have limited capacity for adaptive control of primary sex ratios (Packer et al., 2000; Palmer, 2000; Williams, 1979). Sex ratios of offspring have been reported for many species of marsupials, and significant departures from parity are common (Cockburn, 1990). Not only are biased sex ratios found at the level of species, but in some species there is evidence of significant variation among populations (Cockburn et al., 1985; Dickman, 1988; Johnson et al., 2001; Johnson and Jarman, 1983). In *Antechinus* and the common brushtail possum *Trichosurus vulpecula,* such inter-population variation is consistent with the hypothesis of local resource competition (Clark, 1978). Male-biased sex ratios occur where resource competition between mothers and daughters is likely to be high (Cockburn et al., 1985; Johnson et al., 2001); in *T. vulpecula* the limiting resource has been identified as den sites in tree hollows.

These observations suggest that females in these species have the capacity to adjust the sex ratio of their offspring in response to local ecological conditions. Biases in the sex ratio observed for dependent young in marsupials could originate in a number of ways: (1) sex-biased mortality between birth and weaning; (2) a longer duration of dependence in one sex; (3) discrimination by mothers against one sex of offspring at birth; (4) sex-selective loss between conception and birth; or (5) a biased probability of conception of males and females. Here we discriminate among these mechanisms using comparisons of populations of *T. vulpecula* with and without biased offspring sex ratios. Before doing this, we review data

on sex ratios of offspring in *T. vulpecula* to determine the prevalence of biased sex ratios in populations of this species. Because marsupial young are born at an early stage of development and then spend a long period attached to a teat (often in a pouch), data on offspring sex ratios are easy to collect. This accounts for the fact that many estimates of the sex ratio of offspring in marsupials have been reported, but there is a correspondingly high risk of selective reporting in favor of data sets that show significant departures from parity or that support one of the theories of adaptive sex ratio adjustment. Selective reporting can be revealed by plotting estimates of sex ratio against sample size, as described by Palmer (2000). We used this approach to test whether our understanding of sex ratio variation in *T. vulpecula* has been distorted by selective reporting and to determine whether the populations analyzed in this study are representative of the range of variation in sex ratios for the species.

## METHODS

In *T. vulpecula,* a single young is born after a gestation of approximately 18 days, attaches to a teat, and remains in the pouch for about 5 months, after which it rides on its mother's back for another 1–2 months. Females are capable of breeding at any time of the year and can conceive again to replace a young that dies before being weaned, but in many populations there is a concentration of births between April and July, and most females produce only one offspring per year (Fletcher and Selwood, 2000).

We compiled all available data on sex ratios of offspring between birth and weaning in *T. vulpecula.* We restricted the survey to data sets from single geographic localities, representing local populations, and with sample sizes equal to or above the minimum required to demonstrate a significant departure from parity by a binomial test (i.e., $n = 6$ offspring). Twenty-eight estimates of the sex ratio met these criteria; 23 represented populations from throughout the species' natural range in Australia, and a further 5 were from populations in New Zealand, where *T. vulpecula* has been introduced. We conducted a more detailed analysis of data from six North Queensland populations of *T. vulpecula* (described in Johnson et al., 2001); in this series of populations, male biases in
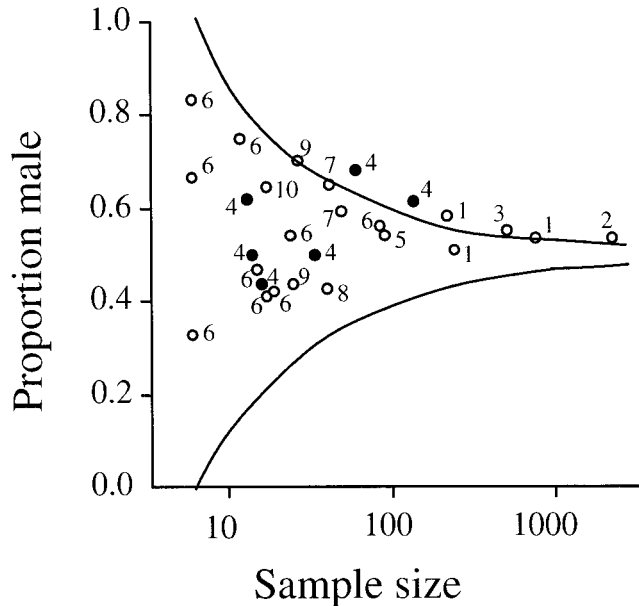
**Figure 1**
Reported sex ratios of offspring of *T. vulpecula,* plotted against sample size (number of offspring). Sex determinations were for young between birth and weaning age, when they are either resident in the pouch or are carried on the mother's back. The two lines show the limits at or beyond which departures of parity are significant by a binomial test (values taken from Table Q in Rohlf and Sokal, 1995). Filled circles represent the populations analyzed in this paper. Data sources: 1, Coleman and Green (1984); 2, Efford (1998); Caughley and Kean (1964); 3, Efford M, unpublished data; 4, Johnson et al. (2001); 5, Kerle and Howe (1992); 6, Hope (1972); 7, Clinchy (1999); 8, Winter (1976); 9, Pieters (1985); 10, Dunnett (1956).



**Figure 2**
Sex ratios of young in different age classes from birth to weaning, in North Queensland populations of *T. vulpecula* with male-biased offspring sex ratios (unshaded) and without bias (shaded). Samples sizes (number of offspring) are shown.

the sex ratio were significantly associated with relative shortages of den sites. All populations occupied open eucalypt woodland with a grassy understory and were distributed over an area of about 40-km radius. Populations were studied by live-trapping on 40-ha grids for periods up to 4 years, and sex was determined for as many pouch-young and young-on-back as possible. In all, 311 offspring from 142 mothers were sexed. Head length was measured for all offspring, and pouch-young were aged from head length by a standard curve established for these populations (Johnson, unpublished data). Because growth of the head was linear during pouch life, a standard measure of growth rate could be calculated for any animal measured twice by dividing the change in head length by the time interval between measurements. We calculated growth rate only when the two measurements were separated by more than 30 days because estimates over shorter intervals were subject to a relatively large measurement error. When more than two measurements were available for a pouch young, we used the earliest and latest to calculate a single estimate of growth rate. Reproductive rates of adult females were measured as the number of offspring produced per year for females monitored for 2 years or more.

The six North Queensland populations were divided into two groups: those with sex ratios above the mean for the whole sample of populations (three populations with male proportions of 0.683, $n = 72$; 0.620, $n = 159$; 0.618, $n = 13$) and those with sex ratios below the mean (three populations with male proportions of 0.500, $n = 37$; 0.500, $n = 14$; 0.438, $n = 16$). We pooled data in each set of three populations to increase the power of the analysis to detect differences that
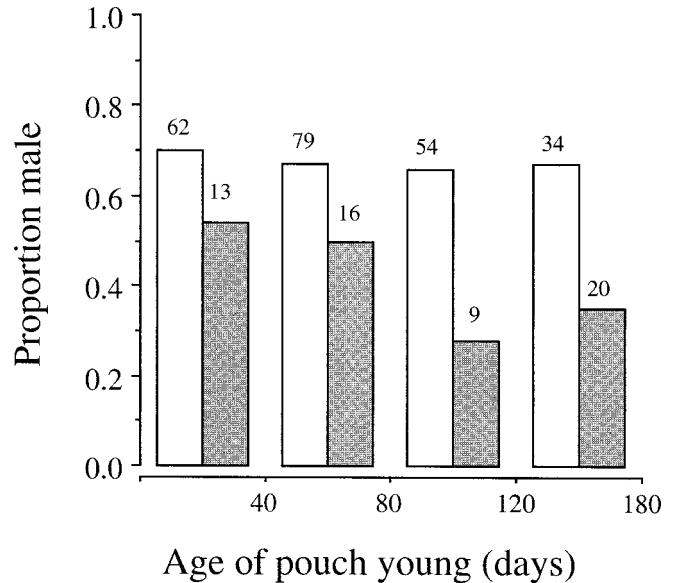
would indicate the stage of development at which sex biases were generated.

## RESULTS AND DISCUSSION

### Male-biased offspring sex ratios in *T. vulpecula*

Data on the sex ratio of unweaned offspring in 28 populations of *T. vulpecula* are shown in Figure 1. There were male biases in 19 cases and female biases in only 7 (binomial test, $p = .05$; in 2 cases sex ratios were exactly even); all 8 cases of significant departure from parity favored males (binomial test, $p = .01$).

In Figure 1, sex ratios are plotted against sample sizes to produce a funnel graph. Extreme values of the sex ratio are more likely to arise by chance when sample sizes are small, hence the increase in variance of estimates at low sample sizes, producing the funnel shape of the scatter of points. If there is a tendency to selectively report ratios that depart strongly from parity, this will be revealed by an absence of estimates close to 0.5, particularly when sample sizes are small; that is, the funnel would appear to be hollow. A tendency to selectively report values that deviate in one direction would produce a relationship between sample size and estimated sex ratio (e.g., a preference for reporting male biases would produce a negative regression of sex ratio on sample size; Palmer, 2000). The scatter of values for *T. vulpecula* falls into a solid funnel shape, and there is no relationship of sex ratio to (log10) sample size ($F_{1,26} = 0.26$, $p = .61$), suggesting that selective reporting of sex ratios has not distorted our understanding of variation in sex ratios in this species. The six populations on which the present analysis is based are typical of the variation in sex ratios reported among other populations of *T. vulpecula.*

### Origins of male biases

Generation of biased sex ratios by either sex-biased mortality between birth and weaning or a longer duration of dependence in one sex would result in a trend for sex ratios to

**Table 1**

**Comparisons of reproductive traits in populations with and without male-biased sex ratios of offspring**

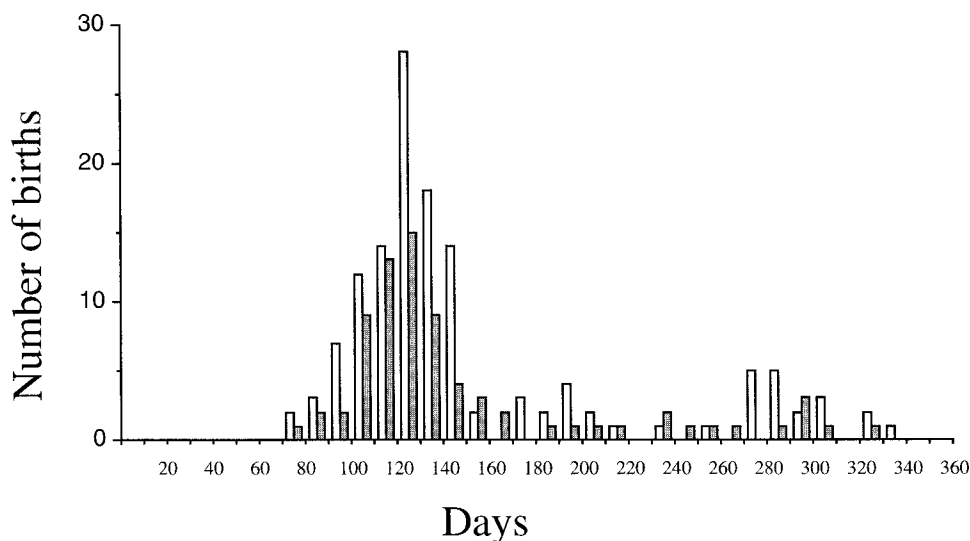| | Male-biased populations | | | Unbiased populations | | |
|---|---|---|---|---|---|---|
| | Mean | CI | $n$ | Mean | CI | $n$ |
| Growth rate, male offspring | 0.324 | 0.305–0.343 | 31 | 0.337 | 0.297–0.376 | 6 |
| Growth rate, female offspring | 0.337 | 0.310–0.364 | 17 | 0.356 | 0.328–0.383 | 7 |
| Reproductive rate (offspring/year) | 1.250 | 1.149–1.350 | 44 | 1.431 | 1.143–1.718 | 12 |

Values are means with 95% confidence intervals (CI) and samples sizes. Growth rate is measured as increase in head length in millimeters/day.

become more male biased with offspring age in populations with an overall male bias, but not in populations lacking such a bias. For neither set of populations was there a significant relationship between the age of young when sexed and their probability of being male (Figure 2, by logistic regression, $\chi^2_1$ = 2.48, $p$ = .12, $\chi^2_1$ = 1.34, $p$ = .25 in male biased and unbiased populations, respectively). There was no tendency for the rate of growth of the head to differ between the sexes ($F_{1,59}$ = 1.68, $p$ = .20) and no effect of an interaction of sex and population type (with or without a sex ratio bias) on growth rates ($F$ = 0.04, $p$ = .85; see Table 1).

Male biases could also be produced if some females who conceive female offspring prevent them from entering the pouch immediately after birth or abort them. Because *T. vulpecula* produces single young, both mechanisms would require females to terminate breeding attempts. Given that breeding is seasonal, females could either resume breeding in the same breeding season or defer reproduction to a subsequent season. A tendency for mothers to discriminate against female offspring early in the season and produce replacement young in the same season (thereby biasing the population sex ratio toward males) would be revealed by a later mean birth date of female than of male offspring. Figure 3 shows that, in populations with male-biased sex ratios, the seasonal distributions of births of male and female offspring were similar. The birth dates (in number of days after January 1) of male and female offspring did not differ significantly ($t_{175}$ = 0.65, $p$ = .51); the mean birth days for males and females (with 95% confidence limits) were 130.33 (125.29–135.36) and 127.76 (120.96–134.58), respectively (considering only the main birth period; i.e., up to the 155th day of the year). There was no relationship between birth day and offspring sex ratio

through the main birth period (by logistic regression, $\chi^2_1$ = 0.67, $p$ = .41). If females who terminated breeding attempts did not breed again until the following year, there would be no sex difference in mean birth date within a season, but fecundity would be reduced in populations with male-biased sex ratios. We measured fecundity as the mean number of pouch-young produced per year for females who were captured regularly over periods of 2 years or more; this did not differ between the two sets of populations (Table 1; $t_{154}$ = 1.55, $p$ = .13).

These analyses exclude the first four mechanisms identified above. We therefore conclude that biases were established at conception. This agrees with the only other study of a marsupial that determined the stage of development at which biased sex ratios are generated. Davison and Ward (1998) found that in a population of *Antechinus agilis* a strongly female-biased sex ratio was established at conception. Krackow (1995) has identified many physiological mechanisms that have the potential to bias the probabilities of conceiving sons and daughters in mammals, but it is not possible to say which was most likely to have been operating in *T. vulpecula*. It is clear, however, that sex ratio adjustment was achieved with little cost, as females who produced offspring in the direction of the population-wide bias were able to do so without terminating reproductive attempts. The fact that biased sex ratios are established before birth in both of the known cases of adaptive adjustment of population sex ratios in marsupials, representing two orders, together with the high incidence of biased sex ratios of offspring in marsupial species (Cockburn, 1990), suggests that the capacity to adjust sex ratios at conception might be widespread among marsupials.



**Figure 3**
Distribution of births of males (unshaded columns) and females (stippled) through the year in populations of *T. vulpecula* in North Queensland with male-biased offspring sex ratios.

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