The mating system and reproduction in the honey possum, *Tarsipes rostratus*: a life-history and genetical perspective

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

The honey possum *Tarsipes rostratus*, a marsupial endemic to south-western Australia, feeds exclusively upon nectar and pollen. It is one of the smallest marsupials, with adult females (8-12g) significantly larger than adult males (6-9g). Honey possum males have the longest sperm (356µm) recorded for any mammal and the testes represent 4.2% body weight, amongst the largest recorded for mammal species. These features suggest that sperm competition is an important part of the mating system. This study used a combination of field based studies, DNA analysis and histological examination of the female reproductive tract to investigate the life history, multiple paternity and reproduction of the honey possum in natural populations in the Fitzgerald River National Park (FRNP), on the south coast of Western Australia.

This study drew upon earlier work on the honey possum in the FRNP in order to describe its life-history. The honey possum is short-lived (1-2 years), and attains sexual maturity whilst still growing. All four teats are occupied after birth, but the litter is reduced to 2 or 3 young during pouch life. The young have a relatively slow rate of growth. Breeding occurs continuously throughout the year, but is affected by the flowering phenologies of its foodplants. The greatest proportion of females with pouch-young occurs in winter; there are fewest pouch-young in autumn, a time of year when there is a dearth of flowers. Honey possums are essentially solitary animals, with no structured social unit, and male and female home ranges overlap. In captivity they are largely tolerant of one another, but larger females are behaviorally dominant to smaller females and to males.

The densities and structure of the honey possum populations in the FRNP were analyzed from trapping data collected over 19 years. Population densities fluctuated significantly from season to season throughout the year, with changes in the flowering food resources available. There were also year-to-year differences in the intensity of those fluctuations, and these were significantly associated with rainfall in the previous year, and probably mediated through a lag effect in the flowering of the honey possum's foodplants. The greatest densities of animals occurred over winter. In years following high rainfall, mean winter densities reached 88 individuals per

hectare. The lowest densities occurred in spring, and in years following low rainfall mean spring densities fell to 8 individuals per hectare. Even at these lowest densities, there is still the potential for interaction between males and females. A succession from high to low, then back to high densities was seen during the three years of the present study (2000-2002) and this shadowed a similar succession of changes in rainfall.

The proportion of females with pouch-young was significantly affected by the season, and by rainfall in the previous year. Years following low rainfall had a lower proportion of females in a condition to breed. The autumn dip in breeding that occurred in all years was exacerbated following dry years. Of those females that did breed in 2001, a time of low resources, there was no difference in the size of the litter compared to 2000 and 2002, times of higher resource availability. The sex-ratio of pouch young was at parity, but there was a slight bias towards males among both juveniles (56%) and adults (58%). This was probably due to the greater movements shown by males. Sex ratios were not affected by changes in rainfall and density. Male-biased dispersal was detected using genetic data and the movement patterns of males showed that they moved greater distances than females during their normal activity.

Analysis of four microsatellite loci revealed extremely high levels of variation, with 28 to 50 alleles per locus and a mean expected heterozygosity of 0.95. These are amongst the highest seen in any microsatellite study of vertebrates. There was multiple paternity in 86% litters, using a minimum number of sires per litter method, and in 95% litters, using an estimated number of sires method based upon the relatedness of litter males. This indicates that multiple mating is frequent in female honey possums and is evidence for sperm competition. The estimated number of sires in a litter was often three or four. In 41% of cases, the number of sires was less than the number of young in the litter, indicating that some males were more successful at siring offspring than others. Nevertheless, no more than two offspring in a litter were known to have been sired by the same male. Despite marked fluctuations in density from high in 2000, to low in 2001, then high again in 2002, the level of multiple paternity remained equally high in all years.

Embryonic diapause and female reproduction was investigated in the honey possum. All adult females examined, both with and without pouch-young, were either close to oestrus, had ovulated or were carrying conceptuses. The honey possum has a post-partum oestrus and it was evident that this occurs approximately 2-4 days after birth. Cleavage and formation of the unilaminar blastocyst appears to occur rapidly over approximately 5 days. Embryonic diapause proceeded in a two phase manner similar to other small possum species. The unilaminar blastocyst expanded rapidly at first; and then, from about 18-20 days after birth, the diameter of the blastocyst remained constant at approximately 1.2-1.8mm. No growth or development beyond the unilaminar stage was observed during pouch-life. The first signs of reactivation occurred during lactation, after pouch exit, and expansion of the blastocyst only occurred in one post-lactational female. The development of the corpus luteum appeared different to patterns described for other marsupials, but its formation coincided with the formation of the unilaminar blastocyst. The diameter of the corpus luteum remained constant throughout diapause. The histology of the reproductive tract was generally similar to other marsupials. There were no sperm storage crypts in the female reproductive tract.

The length of pouch-life in the honey possum was 55-65 days, and the interval between litters of the same size varied between 65 and 100 days. Embryonic diapause may reduce the time between production of successive litters in the honey possum, but lifetime reproductive potential is reasonably low. Females had up to four litters over the period that they were captured. Thus, each litter represents a substantial proportion (25%) of a female's lifetime reproductive output. Reproductive amortization occurred, with 61% loss overall, due to overproduction of ova, loss of conceptuses and reduction of the litter during lactation.

The behavioural dominance of females suggests that multiple mating is an active strategy, and this presumably allows the genetic quality of their offspring to be maximized. Males that succeed in sperm competition may be of better intrinsic quality. Overproduction of conceptuses by females presents the opportunity for them to select those fertilized by intrinsically viable males or genetically compatible males. Sexually active males are present all year round. Females were not synchronous in their sexual receptivity, and this would lead to a skewed operational sex ratio, with more reproductive males than oestrous females. Since adult males are significantly

smaller than adult females and possess no ornaments or armaments, it is unlikely that males overtly fight for access to females. Rather, males appear to monitor the reproductive status of females through smell, and probably compete in their ability to locate oestrous females. The risk and intensity of sperm competition is high, sexual selection for a large investment in spermatogenesis is evident and competition after copulation is probably an important factor in the mating system. It is likely that males, as well as females mate multiply, and the mating system is promiscuous.

Table of Contents

Chapter 5: Microsatellite Analysis of Multiple Paternity

Chapter 6: Reproduction in the Female Honey Possum

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