

# Phenotype and gene flow in a marsupial (*Antechinus flavipes*) in contrasting habitats

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Ecological factors are important drivers of phenotypic divergence, which may lead to incipient speciation. A variety of habitats should be preserved to maintain evolutionary potential. We used the marsupial, the yellow-footed antechinus (*Antechinus flavipes*) as a model species for investigating phenotypic differentiation between animals inhabiting two habitat types in south-eastern Australia: flood-plain river red gum and box-ironbark forests. All tested phenotypic characteristics varied between years at the same sites and therefore were not useful for investigating morphological specialization that may lead to speciation. Males generally were significantly heavier when antechinus densities were lower, but exceptions were found, possibly related to food availability. Teat-number variation recently has been shown to be associated with habitat specialization and incipient speciation within *Antechinus agilis*. We investigated genetic differentiation associated with this trait in *A. flavipes*. Population genetic analyses of microsatellite genotypes and mitochondrial DNA haplotypes revealed that sympatric 12-, 13- and 14-teat females in Chiltern forest were part of one freely interbreeding population. Our parentage analyses found two cases where 13-teat mothers produced 12-teat daughters. This suggests either plasticity or paternal genetic influence on the offspring's teat-number phenotype. Laboratory matings may be required to resolve the extent to which teat number is heritable in *A. flavipes*. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 303–314.

**ADDITIONAL KEYWORDS:** body mass – body size – box-ironbark forest – life-history trait – microsatellite – mitochondrial DNA – phenotypic divergence – realized habitat quality – river red gum forest – teat number.

## INTRODUCTION

Anthropogenic actions, which include degradation or destruction of habitats as well as direct persecution, have led to the decline and extinction of many species (Sala *et al.*, 2000). Attempts to conserve some species under the threat of extinction usually focus on a short timescale (e.g. Goossens *et al.*, 2006). However, little attention is paid to the future evolutionary requirements of species (see Ashley *et al.*, 2003), which will play a substantial role in longer-term persistence given climate change and other ongoing or future threats. There are significant gaps in our knowledge about the importance of preserving a variety of habitats to maintain the evolutionary potential of species.

Studies have demonstrated the importance of ecological factors in instigating phenotypic divergence in species despite gene flow and, in some cases, incipient speciation (e.g. Smith *et al.*, 1997; Schneider *et al.*, 1999; Beheregaray & Sunnucks, 2001). These studies show the importance of preserving a variety of habitats occupied by a species to allow for potential speciation in the future.

In Australia, recent taxonomic analyses of the genus *Antechinus*, which are small, carnivorous marsupials with synchronized, obligatory male die-off after the annual mating season (life-history strategy I, Lee, Woolley & Braithwaite, 1982), resulted in the recognition of a previously cryptic species, *Antechinus agilis*, from the *Antechinus stuartii* complex (Dickman *et al.*, 1998). It is possible that speciation may have been promoted by habitat specialization (and/or different responses to changes in

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the rates of photoperiod, Crowther & Blacket, 2003; McAllan, Dickman & Crowther, 2006) and thus, given its widespread distribution, further revisions within the genus may be necessary. For example, the yellow-footed antechinus (*Antechinus flavipes*) exhibits subspecific divergence (Menkhorst & Knight, 2001; Crowther *et al.*, 2002) and may consist of multiple or incipient species (Crowther *et al.*, 2002; How *et al.*, 2002). *A. flavipes* is the most widely distributed antechinus, found from northern Queensland to the southern part of South Australia, as well as in southern Western Australia (Menkhorst & Knight, 2001).

The genus *Antechinus* offers a good opportunity to test life-history theory (Lee & Cockburn, 1985), which attempts to understand how characteristics of organisms are shaped by natural selection (Stearns, 2000). In *Antechinus*, major life-history traits either are easily measured (e.g. size at maturity of both genders, reproductive lifespan of females, maternity and survival of young) or have very small ranges in value (e.g. age at maturity of both genders, reproductive lifespan and ageing of males) (Lee & Cockburn, 1985), so that there is no need to measure them. Reproductive output is maximized for males by short-term intensive mating effort followed by stress-related die-off and for females by annual production of single litters coinciding with the highest food availability (Lee & Cockburn, 1985).

Teat number in females of all *Antechinus* spp. dictates the maximum number of offspring that can be weaned in a year (Cockburn, Lee & Martin, 1983) and is equivalent to a maximum possible fecundity for those females that do not survive to a second year. *A. flavipes* exhibits the greatest variation in teat number of any Australian species of antechinus (8–14), with all females in most locations having the same teat number (Cockburn *et al.*, 1983; Coates, 1995; How *et al.*, 2002; Beckman *et al.*, 2007). Chiltern forest, in north-eastern Victoria is the only known location of 14-teat *A. flavipes* females, which may be expected to have the highest reproductive output, yet are rare and occur in sympatry with the more abundant 12- and 13-teat females (Coates, 1995). Beckman *et al.* (2007) reported apparent reproductive isolation between 6- and 10-teat populations of *A. agilis* in the Otway region of Victoria and suggested that intermediate-teat-number females were a result of hybridization between these populations. Also, Cockburn *et al.* (1983) stated that 'intrapopulation variance in nipple number is very low or zero outside transition zones and we thus suspect nipple number is determined genetically'. It is not known whether the different teat-number females of *A. flavipes* in Chiltern are one population or whether 13-teat females result from hybridization between 12-teat and 14-teat 'populations'. This may be

resolved through population genetic analysis of 12-, 13- and 14-teat females. Lada *et al.* (in review) reported that, when animals from across northern and central Victoria are analysed together, individuals from Chiltern form a separate, uniform, genetic cluster. However, it is unknown whether Chiltern females also form a uniform cluster, if analysed in isolation from males and from populations in other forests.

We equate habitat quality with the probability that a population can persist in a given location. Therefore, 'source' habitats are of 'high' quality because there are sufficient resources to produce a surplus of individuals, while 'sink' habitats are the reverse (Fretwell, 1972). Lada *et al.* (2007) showed that sites in river red gum forest with higher timber loads, more large trees and that are near to flooded areas supported more individuals of *A. flavipes*. Such areas can be regarded as more optimal habitat for *A. flavipes* than sites where very few animals were found. All individuals of *A. flavipes* trapped in a given year, except for second-year females, are the realized reproductive success of the previous-year population. We consider trapping rates of females and of second-year females with suckled teats to be excellent measures of realized habitat quality for *A. flavipes*. This is because females limit young produced and, given that females are relatively philopatric (Coates, 1995; Marchesan & Carthew, 2004; Lada, Mac Nally & Taylor, 2007) they are less likely than males to move between 'source' and 'sink' habitats. Second-year females with suckled teats have survived to breed again and probably have successfully weaned offspring. We sought to determine whether a relationship exists between trapping rate and body mass, which is equivalent to investigating whether physical condition of animals reflects habitat suitability, by addressing the questions: (1) do animals in seemingly more optimal habitat (higher realized habitat quality) weigh more than those in less optimal habitat?; (2) Is body mass lower when population density is relatively higher?

*Antechinus flavipes* can be used as a model species for investigating phenotypic divergence in habitats. Teat-number differences have been reported widely among antechinus populations (Cockburn *et al.*, 1983; Coates, 1995). There also have been reports that other phenotypic characters, such as body size and coat colour, which are some of characteristics that distinguish *A. agilis* from *A. stuartii* (Dickman *et al.*, 1998), also vary among Victorian populations of *A. flavipes*. Lada *et al.* (in review) showed that there is gene flow among almost adjacent upland box-ironbark forests and flood-plain river red gum (*Eucalyptus camaldulensis*) forests in two locations in Victoria. However, it has not been reported whether

phenotypic differences occur in *A. flavipes* between these markedly different habitat types. In the absence of river regulation, river red gum forests flood in spring when rainfall is high and snow melts feed major rivers. Elevated emergence of macroarthropods following recession of water (Ballinger, Mac Nally & Lake, 2005) provides much food for the carnivorous *A. flavipes* and may be responsible for increases in antechinus population sizes by prolonging lifetimes of individuals. Flood-plains also may serve as refuges during droughts. Ground cover is provided by fallen timber and grey–reddish fallen bark. Box–ironbark forests differ in many ways that might be expected to affect *A. flavipes*. These have mostly grey and black bark, are drier than flood-plains, are not subjected to inundations and contain many species of ground-cover plants and shrubs (Muir, Edwards & Dickins, 1995; Environment Conservation Council, 1997).

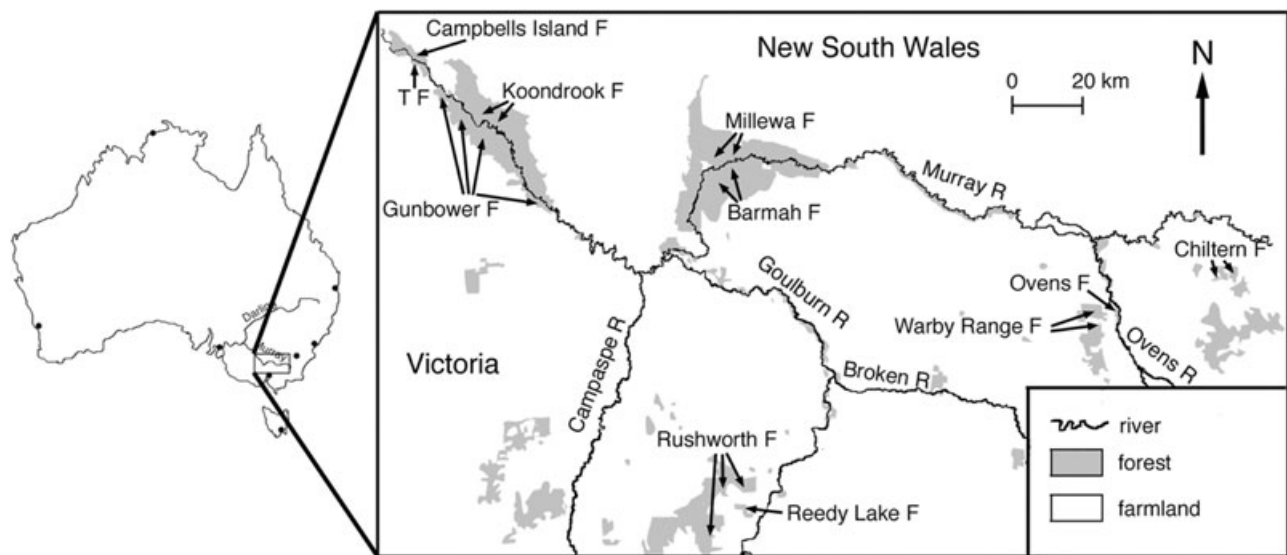
We compared body length, mass and pelage colour among males and females of *A. flavipes* inhabiting river red gum and box–ironbark forests to determine whether there is phenotypic differentiation of antechinus between these two forest types. We also assessed whether an association exists between realized habitat quality and adult mass and whether animals were heavier at lower population densities. Twelve microsatellite loci and the control region of mtDNA were used to discern if sympatric 12-teat, 13-teat and 14-teat females in one forest are part of one population.

## MATERIAL AND METHODS

### STUDY SITES, ANIMAL CAPTURES AND MEASUREMENTS

Individuals of *A. flavipes* were trapped in the austral summer, autumn and winter in 2004 and 2005 at randomly selected, 0.25 ha sites in eight river red gum (*Eucalyptus camaldulensis*) forests and in three box–ironbark forests (dominated by grey box *Eucalyptus microcarpa* and red ironbark *Eucalyptus sideroxylon* and *Eucalyptus tricarpa* in northern and central Victoria, respectively) (Fig. 1). The river red gum forests (RRG) included Gunbower<sup>RRG</sup>, Guttrum<sup>RRG</sup>, Koondrook<sup>RRG</sup>, Campbells Island<sup>RRG</sup>, Barmah<sup>RRG</sup>, Millewa<sup>RRG</sup>, Ovens<sup>RRG</sup> and Reedy Lake<sup>RRG</sup> forests. The box–ironbark forests (BI) were Chiltern<sup>BI</sup> forest (part of Chiltern–Mt Pilot National Park), Rushworth<sup>BI</sup> forest and Warby Range<sup>BI</sup> forest (Killawarra section of Warby Range State Park). Gunbower<sup>RRG</sup>, Guttrum<sup>RRG</sup>, Rushworth<sup>BI</sup> and Reedy Lake<sup>RRG</sup> were also visited in winter 2003. There were between 13 and 158 sites in the 11 forests.

All antechinuses were measured by the one person (HL) and uniquely marked with ear-biopsy holes (2 mm), tissue from which was collected and placed into 96% ethanol for subsequent genetic analysis. Snout-vent length was measured with calipers, tail length with a ruler, and mass using a 100-g Pesola balance. Ventral and dorsal pelage colours were measured; methods available from the corresponding



**Figure 1.** Study areas. Arrows indicate locations of study sites within each forest, F denotes forest, T is Guttrum. Forests centred on: 35°46'S, 144°16'E (Gunbower); 35°35'S, 144°04'E (Guttrum); 35°44'S, 144°17'E (Koondrook); 35°34'S, 144°04'E (Campbells Island); 35°52'S, 145°01'E (Barmah); 35°50'S, 145°00'E (Millewa); 36°13'S, 146°15'E (Ovens); 36°42'S, 145°05'E (Reedy Lake); 36°08'S, 146°36'E (Chiltern), 36°39'S, 145°01'E (Rushworth) and 36°13'S, 146°11'E (Warby Range).

author. Teat number and teat position in females were recorded, including the number of suckled (long, white) and unsuckled (small, red) teats in second-year females.

#### STATISTICAL ANALYSES OF PHENOTYPIC DIFFERENCES

Phenotypic variables [mass, snout-vent length, ratio of tail to snout-vent length (RTS), and ventral pelage] may covary so it is appropriate to undertake a preliminary multivariate analysis of variance (MANOVA) to assess covariation among variables with respect to region and year. However, given that individuals were not trapped in some locations in some years, we were unable to undertake MANOVAs that were directly appropriate for the treatments of interest. Instead, we compared adult antechinus males from five river red gum forests (Gunbower<sup>RRG</sup>, Barmah<sup>RRG</sup>-Millewa<sup>RRG</sup>, Ovens<sup>RRG</sup> and Reedy<sup>RRG</sup>) in 2004 and 2005 to test whether there was differential variation among regions and among years for the four phenotypic variables. The four phenotypic variables varied differently at regional scales and among years (results not shown), so ANOVA (PopTools 2.7; Hood, 2005) was conducted for each year and each variable separately, for all adult males, first-year females and second-year females. Dorsal pelage was invariant so is not considered further. Comparisons for males and first-year females in the same year were made among sampling units derived in two ways: (1) different regional river red gum forests, to explore if there were phenotypic differences among populations inhabiting the same habitat types; and (2) two different nearby forest types, one of box-ironbark and one of river red gum, to infer, from phenotypic differences, whether there was any habitat specialization within a relatively small area. A third type of comparison (3) was made for the one forest over time to: (a) infer variability in phenotypes that may relate to different environmental conditions among years; and (b) investigate the extent to which phenotypes change during a year. Because of small sample sizes (from one to seven individuals) in six of eight forests, second-year females from all box-ironbark forests were combined and compared with data combined for the river red gum forests. ANOVA also was used to assess differences in the number of suckled teats among second-year females in Chiltern<sup>BI</sup> and river red gum forests.

#### STATISTICAL ANALYSES OF TRAPPING RATES

We investigated whether differences in male body mass were associated with population densities (and presumably with competition for food) and/or with significant differences in realized habitat quality for *A. flavipes*. A binomial variable was used for trapping

rates of all individuals. Success was a capture of a unique individual in a given forest in a given year in  $n$  trials, with  $n$  being equivalent to the number of trap nights minus the number of all recaptures. This approach has the effect of weighting more highly sites with greater trapping effort. We tested for differences in the trapping rates among the same sets of forests as described above for phenotypes, using the GLMStat 6.0.0 software (KJ Beath). The procedure was repeated for two indicators of realized habitat quality: (1) trapping rates of females and (2) trapping rates of second-year females with suckled teats.

#### TESTING GENETIC DIFFERENCES AMONG FEMALES WITH 12, 13 AND 14 TEATS IN THE SAME FOREST

DNA extraction, genotyping of 12 microsatellite loci and of the control region of mitochondrial DNA (mtDNA) are described in Lada *et al.*, (2007), as are the tests for Hardy-Weinberg equilibrium estimates and for linkage equilibrium. Lada *et al.*, (2007) have described the parameters and criteria used for maternity assignments, which subsequently were used by Lada *et al.* (in review) to conduct maternity analysis for the Chiltern population. Lada *et al.* (in review 1) conducted maternity analysis in CERVUS 2.0 (Marshall *et al.*, 1998) by testing all offspring against all females trapped the preceding year and all second-year females trapped the same year. Eleven loci were used for simulation power analysis with: 0.2 as the proportion of candidate mothers sampled, 10 000 cycles and genotyping error rate of 0.01. The twelfth locus (shown to be involved in linkage disequilibrium and thus not suitable for inclusion in log of the odds (LOD) calculations; Marshall *et al.*, 1998) and mtDNA were used to make further exclusions after CERVUS analysis. In CERVUS, the natural LOD score and the delta score (equivalent to the gap between the highest and second-highest LOD scores) based on simulated data sets are used to obtain confidence values for applying to the real data. Maternity was accepted when candidate mothers and offspring shared at least one allele at each locus, had the same haplotype, high LOD ( $\geq 3$ ) and high delta score ( $\geq$  the 80% confidence level threshold value). We used these successful maternity assignments to determine whether sisters and mother-daughter pairs always shared the teat number. Maternity results also were used to exclude from genetic analysis one female from each 12-teat or 14-teat mother-daughter pair to avoid family related biases towards low genetic distances in the samples.

To determine whether coexisting, 12-teat and 14-teat females in Chiltern are part of the same population, total pairwise-squared genotypic distances (11 microsatellite loci) were calculated between same-teat females and between different-teat females

sampled over 2 years using GENALEX 6 (Peakall & Smouse, 2006). We used Monte-Carlo analysis in PopTools 2.7.1 (Hood, 2005) in which the values were randomized 10 000 times among the groups and the means for each randomized group were recalculated and compared with actual means.

We tested whether females formed separate clusters according to teat number (12 or 14) once males (the predominantly dispersing gender, which lack teats) were excluded, using the STRUCTURE 2.1 software (Pritchard, Stephens & Donnelly, 2000).

We carried out  $\chi^2$ -tests in PopTools (PopTools, Hood, 2005) to test whether the frequency of 14-teat females was the same between years in Chiltern forest overall and in the area where 14-teat females were most abundant.

## RESULTS

### REALIZED HABITAT QUALITY FOR *A. FLAVIPES* IN BOX-IRONBARK AND RIVER RED GUM FORESTS

Trapping rates of females and of second-year females with suckled teats, both of which we considered to be indicators of realized habitat quality for *A. flavipes*, were significantly different in most inter- and intra-forest comparisons (Table 1). Therefore, realized habitat quality was significantly higher in: Reedy Lake<sup>RRG</sup>, Rushworth<sup>BI</sup> and Chiltern<sup>BI</sup> in 2005 than in 2004; Gunbower<sup>RRG</sup> than Guttrum<sup>RRG</sup> (2004 and 2005); Reedy Lake<sup>RRG</sup> than Rushworth<sup>BI</sup> (2003, 2004 and 2005); and Ovens<sup>RRG</sup> than Warby<sup>BI</sup> (2005) (Table 1).

### TRAPPING RATES OF ALL INDIVIDUALS OF *A. FLAVIPES* IN BOX-IRONBARK AND RIVER RED GUM FORESTS

Trapping rates of all animals were significantly higher in: Reedy Lake<sup>RRG</sup> than Rushworth<sup>BI</sup> (2003, 2005), Ovens<sup>RRG</sup> than Warby<sup>BI</sup> (2005), Gunbower<sup>RRG</sup> than Guttrum<sup>RRG</sup> (2004), Guttrum<sup>RRG</sup> in 2005 than in 2004, Gunbower<sup>RRG</sup>, Chiltern<sup>BI</sup> and Reedy Lake<sup>RRG</sup> in 2005 than in 2004 (Table 1).

### IDENTIFICATION OF PLASTIC TRAITS

Phenotypic characteristics that did not vary significantly among sampling units, from the same stage of the life cycle in different years, were deemed to be least plastic and so most useful for comparisons between habitat types to investigate habitat specialization. No characteristic fitted this criterion, but mass was least useful, varying not only among forests and among years in the same forest (Tables 2 and 3), but occasionally also in an individual (of either gender) within days. In males, out of the three other characteristics, RTS appeared least plastic because it

was stable across years in all but one forest, Gunbower<sup>RRG</sup>, where it differed significantly between 2004 and 2005 (Table 2), even though environmental conditions were similar in both years (i.e. post-flood). In first-year females, snout-vent length and ventral pelage were significantly different between 2004 and 2005 in Ovens<sup>RRG</sup> and Gunbower<sup>RRG</sup>, respectively, and in both forests for RTS (Table 3). Even if temporal variation in phenotypic characteristics examined here has an adaptive component because of its fluctuating nature, it is unlikely to lead to morphological specialization and subsequently to incipient speciation.

### PHENOTYPIC DIFFERENCES AMONG MALES

In 2004 and 2005, males were significantly heavier in Rushworth<sup>BI</sup> than in the nearby Reedy Lake<sup>RRG</sup>, but the reverse was the case in 2003 (sample sizes provided in Table 2), when Rushworth<sup>BI</sup> forest seemed to be particularly affected by the drought. Each year, Reedy Lake<sup>RRG</sup> had significantly higher realized habitat quality than Rushworth<sup>BI</sup>, and higher trapping rates of animals in both 2003 and 2005 (Table 1). In four comparisons, males were heavier when trapping rates of all animals were lower (Tables 1 and 2). However, the reverse was the case in comparisons between Rushworth<sup>BI</sup> and Reedy Lake<sup>RRG</sup> in 2003, and between 2004 and 2005 in Gunbower<sup>RRG</sup> (Tables 1 and 2). These results indicate that higher mass of *A. flavipes* is not always associated with lower densities of animals and/or with lower realized habitat quality.

Just before the 2004 mating season, males were smallest in Gunbower<sup>RRG</sup> ( $96.2 \pm 5.6$  mm) and largest in Guttrum<sup>RRG</sup> ( $110.4 \pm 2.1$  mm), which was a significant difference (Table 2). Ventral pelage and RTS were not significantly different among any forests (Table 2). In cases where, nearby, different forest types were compared, significant differences in antechinus phenotypes (excluding mass) were not found (Table 2).

### PHENOTYPIC DIFFERENCES AMONG FIRST-YEAR FEMALES

There were no significant differences in mass between Ovens<sup>RRG</sup> and Warby<sup>BI</sup> first-year females in 2005, or Chiltern<sup>BI</sup> females between February and July 2004 (Table 3). The latter indicates that females already reached their full-adult mass 2 months after weaning. The biggest first-year females were in Warby Range<sup>BI</sup> in April 2005 ( $93.1 \pm 1.8$  mm), which were significantly larger than those in the nearby Ovens<sup>RRG</sup> forest in April 2005 ( $88.2 \pm 2.0$  mm), but not in April 2004 (Table 3). In 2005, Warby Range<sup>BI</sup> and Ovens<sup>RRG</sup> females also differed significantly in ventral pelage and RTS, while no differences were observed between Rushworth<sup>BI</sup> and Reedy<sup>RRG</sup> females in 2004 (Table 3).

**Table 1.** Comparisons of trapping rates of *Antechinus flavipes* suckled-teats (s-t) females and of all females (both equivalent to realized habitat quality for *A. flavipes*) and of all individuals, among forests and years

Groups	TR s-t females	TR females	TR all	<i>n</i> s-t females	<i>n</i> females	<i>n</i> all	<i>n</i> trials
Guttrum <sup>RRG</sup> June 2004	0	0.006	0.019	0	2	6	318
Guttrum <sup>RRG</sup> June 2005	0	0.019	0.094	0	5	25	266
Koondrook <sup>RRG</sup> May 2005	0.007	0.032	0.081	4	19	48	590
Gunbower <sup>RRG</sup> June 2004	0.005	0.032	0.076	5	32	76	993
Gunbower <sup>RRG</sup> June 2005	0.009	0.042	0.122	7	34	99	811
Gunbower <sup>RRG</sup> July 2005	0	0.011	0.042	0	3	11	264
Millewa <sup>RRG</sup> May 2005	0.013	0.055	0.113	4	17	35	309
Barmah <sup>RRG</sup> May 2005	0.005	0.040	0.091	1	8	18	198
Rushworth <sup>BI</sup> June 2004	0.001	0.004	0.012	1	4	14	1123
Rushworth <sup>BI</sup> June 2005	0	0	0.014	0	0	6	435
Reedy Lake <sup>RRG</sup> June 2004	0.003	0.008	0.020	2	5	13	640
Reedy Lake <sup>RRG</sup> June 2005	0.015	0.062	0.161	3	12	31	193
Warby <sup>BI</sup> April 2005	0	0.005	0.012	0	4	10	801
Ovens <sup>RRG</sup> April 2005	0.009	0.074	0.101	2	16	22	217
Chiltern <sup>BI</sup> July 2004	0.003	0.021	0.045	3	24	52	1159
Chiltern <sup>BI</sup> July 2005	0.029	0.071	0.179	12	30	75	419
Guttrum <sup>RRG</sup> June 2003	0	0.003	0.025	0	1	9	361
Gunbower <sup>RRG</sup> June 2003	0	0.015	0.030	0	18	37	1221
Rushworth <sup>BI</sup> July 2003	0	0	0.008	0	0	2	238
Reedy Lake <sup>RRG</sup> July 2003	0	0.014	0.071	0	1	5	70
Koondrook <sup>RRG</sup> May 2004	0.002	0.024	0.048	1	10	20	416
Chiltern <sup>BI</sup> February 2004	0.003	0.018	0.039	4	22	47	1197
GLM for groups							
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2004	*	NS	NS				
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2005	*	*	*				
Ovens <sup>RRG</sup> , Warby <sup>BI</sup> April 2005	*	*	*				
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2004	*	*	*				
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2005	*	*	NS				
GT <sup>RRG</sup> June 2004, June 2005	*	*	*				
G <sup>RRG</sup> June 2004, June 2005	NS	NS	*				
C <sup>BI</sup> July 2004, June 2005	*	*	*				
RL <sup>RRG</sup> June 2004, June 2005	*	*	*				
Rus <sup>BI</sup> June 2004, June 2005	*	*	NS				
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2003	*	*	*				
G <sup>RRG</sup> June 2003, June 2004, June 2005	NS	NS	NS				
GT <sup>RRG</sup> June 2003, June 2004, June 2005	NS	NS	NS				
C <sup>BI</sup> February 2004, July 2004	NS	NS	NS				

Differences among trapping rates: \* $P < 0.05$ ; NS, not significant.

Forests: Guttrum (GT), Koondrook (K), Gunbower (G), Rushworth (Rus), Reedy Lake (RL), Chiltern (C). The number (*n*) of unique individuals caught (successes) in the number of trap nights (trials) is a binomial dependent variable. Trapping rate (TR) is the (number of successes)/(number of trials).

#### PHENOTYPIC DIFFERENCES AMONG SECOND-YEAR FEMALES

Second-year females in box-ironbark forests did not significantly differ in mass ( $31.8 \pm 4.0$  g) or in ventral pelage ( $10.7 \pm 1.9$ ) from river red gum ones ( $30.3 \pm 5.4$  g,  $10.1 \pm 1.9$ , respectively). There were significant differences in snout-vent length and in RTS: box-ironbark second-year females were larger

( $99.9 \pm 2.6$  mm) and had lower RTS ( $0.85 \pm 0.02$ ) than the river red gum ones (snout-vent length  $95.8 \pm 4.4$  mm and RTS  $0.93 \pm 0.06$ ).

#### TEAT NUMBER IN FEMALES

The number of suckled teats in second-year females, indicating the maximum possible number of weaned

**Table 2.** ANOVA analyses of *Antechinus flavipes* male mass, snout-vent length (SV), ventral pelage (VP) and ratio of tail to snout-vent length (RTS) in box–ironbark (BI) and river red gum (RRG) forests

Groups ( <i>n</i> )	Mean ± SD Mass (g)	Mean ± SD SV (mm)	Mean ± SD VP	Mean ± SD RTS
Guttrum <sup>RRG</sup> June 2004 (4)	45.9 ± 3.4	110.4 ± 2.1	10.7 ± 1.5	0.94 ± 0.07
Guttrum <sup>RRG</sup> June 2005 (17)	38.7 ± 5.5	103.8 ± 4.0	9.7 ± 1.2	0.97 ± 0.04
Koondrook <sup>RRG</sup> May 2005 (27)	35.2 ± 5.6	100.3 ± 2.9	11.3 ± 1.5	0.98 ± 0.06
Gunbower <sup>RRG</sup> June 2004 (41)	30.4 ± 5.1	96.2 ± 5.6	11.5 ± 1.2	1.00 ± 0.07
Gunbower <sup>RRG</sup> June 2005 (21)	39.4 ± 5.7	104.5 ± 4.5	10.4 ± 1.5	0.95 ± 0.04
Gunbower <sup>RRG</sup> July 2005 (8)	56.4 ± 6.0	110.5 ± 3.2	9.5 ± 1.2	0.90 ± 0.03
Millewa <sup>RRG</sup> May 2005 (16)	39.0 ± 6.6	102.8 ± 4.0	10.3 ± 1.4	0.97 ± 0.06
Barmah <sup>RRG</sup> May 2005 (11)	47.5 ± 9.7	104.5 ± 5.6	10.0 ± 1.9	0.98 ± 0.05
Rushworth <sup>BI</sup> June 2004 (10)	49.9 ± 6.5	106.8 ± 7.6	11.7 ± 1.8	1.00 ± 0.10
Rushworth <sup>BI</sup> June 2005 (6)	45.8 ± 6.6	102.3 ± 5.5	11.0 ± 2.0	1.01 ± 0.10
Reedy Lake <sup>RRG</sup> June 2004 (7)	41.4 ± 8.3	99.7 ± 6.5	12.0 ± 1.6	1.01 ± 0.06
Reedy Lake <sup>RRG</sup> June 2005 (14)	37.9 ± 6.3	102.6 ± 4.9	10.8 ± 1.4	1.02 ± 0.06
Warby Range <sup>BI</sup> April 2005 (6)	45.4 ± 4.1	108.9 ± 4.8	10.3 ± 1.7	0.98 ± 0.11
Ovens <sup>RRG</sup> April 2005 (6)	45.7 ± 6.3	107.7 ± 3.3	11.6 ± 1.5	0.97 ± 0.04
Chiltern <sup>BI</sup> July 2004 (19)	53.5 ± 6.1	112.8 ± 4.1	11.0 ± 1.5	0.87 ± 0.05
Chiltern <sup>BI</sup> July 2005 (10)	46.7 ± 6.0	108.8 ± 4.8	9.6 ± 1.1	0.85 ± 0.06
Guttrum <sup>RRG</sup> June 2003 (7)	43.4 ± 7.9			
Gunbower <sup>RRG</sup> June 2003 (17)	33.6 ± 5.1			
Rushworth <sup>BI</sup> July 2003 (2)	32.5 ± 2.1			
Reedy Lake <sup>RRG</sup> July 2003 (3)	57.0 ± 7.0			
Koondrook <sup>RRG</sup> May 2004 (8)	32.7 ± 3.7			
Chiltern <sup>BI</sup> February 2004 (24)	35.7 ± 6.5			
ANOVA for groups				
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2004	5.66 (1, 15)*	4.10 (1, 15) NS	0.12 (1, 15) NS	0.04 (1, 15) NS
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2005	6.58 (1, 18)*	0.02 (1, 19) NS	0.07 (1, 19) NS	0.16 (1, 18) NS
Ovens <sup>RRG</sup> , Warby <sup>BI</sup> April 2005	0.01 (1, 10) NS	0.29 (1, 11) NS	1.87 (1, 11) NS	0.11 (1, 11) NS
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2004	34.97 (1, 43)*	24.69 (1, 43)*	1.19 (1, 43) NS	2.78 (1, 42) NS
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2005	0.13 (1, 36) NS	0.22 (1, 37) NS	2.24 (1, 37) NS	1.92 (1, 37) NS
Guttrum <sup>RRG</sup> June 2004, June 2005	–	10.19 (1, 19)*	2.21 (1, 19) NS	1.22 (1, 19) NS
Guttrum <sup>RRG</sup> June 2003, June 2004, June 2005	5.96 (1, 19) NS			
Gunbower <sup>RRG</sup> June 2004, June 2005	–	34.91 (1, 61)*	10.03 (1, 61)*	9.54 (1, 60)*
†Gunbower <sup>RRG</sup> June 2003, June 2004, June 2005	20.41 (2, 76)*			
Chiltern <sup>BI</sup> July 2004, July 2005	8.26 (1, 27)*	5.46 (1, 27)*	6.70 (1, 27)*	1.02 (1, 27) NS
Chiltern <sup>BI</sup> February 2004, July 2004	84.14 (1, 41)*			
RL <sup>RRG</sup> June 2004, June 2005	1.22 (1, 19) NS	1.40 (1, 20) NS	3.24 (1, 20) NS	0.15 (1, 20) NS
Rus <sup>BI</sup> June 2004, June 2005	1.48 (1, 14) NS	1.61 (1, 14) NS	0.51 (1, 14) NS	0.01 (1, 13) NS
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2003	21.08 (1, 3)*			

\* $P < 0.05$ , NS; not significant, *n* in parenthesis indicates number of males. ANOVA results reported as: *F* (numerator d.f., denominator d.f.)\*, or *F* (d.f., d.f.) NS.

†Tukey post-hoc comparison: 2004 < 2003 < 2005.

Forests: Guttrum (GT), Koondrook (K), Gunbower (G), Rushworth (Rus), Reedy Lake (RL), Chiltern (C).

offspring, was not significantly different (ANOVA,  $F_{1,30} = 2.02$ ,  $P > 0.05$ ) between the two forest types:  $9.3 \pm 4.0$  SD in river red gum forests collectively and  $11.7 \pm 1.0$  SD in Chiltern<sup>BI</sup>.

In Chiltern, teat number differed within a family in two of three successfully assigned mother–daughter

pairs with known teat number (assigned at 95% confidence level). Mothers had 13 teats and daughters 12. This indicates that teat number in *A. flavipes* probably is influenced by paternal genes and/or environment.

There was no significant difference between means of genetic distances among same-teat-number

**Table 3.** ANOVA analyses of *Antechinus flavipes* first-year female mass, snout-vent length (SV), ventral pelage (VP), and ratio of tail to snout-vent length (RTS) in box–ironbark (BI) and river red gum (RRG) forests

Groups ( <i>n</i> )	Mean ± SD mass (g)	Mean ± SD SV (mm)	Mean ± SD VP	Mean ± SD RTS
Guttrum <sup>RRG</sup> June 2004 (2)	29.5 ± 1.4	101.2 ± 2.5	9.5 ± 0.7	0.89 ± 0.02
Guttrum <sup>RRG</sup> June 2005 (2)	22.5 ± 2.8	90.1 ± 2.7	10.0 ± 0	0.98 ± 0.03
Koondrook <sup>RRG</sup> May 2005 (13)	22.8 ± 3.6	86.8 ± 4.0	9.7 ± 1.3	1.00 ± 0.08
Gunbower <sup>RRG</sup> June 2004 (27)	20.9 ± 2.9	83.6 ± 4.5	10.9 ± 1.5	1.03 ± 0.07
Gunbower <sup>RRG</sup> June 2005 (12)	22.4 ± 2.1	87.7 ± 4.1	9.7 ± 1.2	0.98 ± 0.05
Millewa <sup>RRG</sup> May 2005 (9)	23.2 ± 1.4	87.5 ± 3.2	10.0 ± 1.3	1.00 ± 0.05
Barmah <sup>RRG</sup> May 2005 (4)	24.6 ± 0.5	90.7 ± 3.9	10.2 ± 2.2	1.00 ± 0.02
Rushworth <sup>BI</sup> June 2004 (3)	29.3 ± 3.8	90.7 ± 3.7	10.0 ± 0.9	0.94 ± 0.06
Reedy Lake <sup>RRG</sup> June 2004 (3)	24.5 ± 1.8	90.4 ± 2.7	10.7 ± 1.2	0.94 ± 0.01
Reedy Lake <sup>RRG</sup> June 2005 (8)	23.2 ± 3.1	90.3 ± 3.1	10.7 ± 1.4	0.98 ± 0.05
Warby Range <sup>BI</sup> April 2005 (4)	25.2 ± 1.0	93.1 ± 1.8	8.0 ± 1.4	0.97 ± 0.09
Ovens <sup>RRG</sup> April 2004 (5)	22.2 ± 2.6	91.2 ± 2.7	12.8 ± 1.6	0.91 ± 0.05
Ovens <sup>RRG</sup> April 2005 (12)	23.3 ± 1.9	88.2 ± 2.0	11.0 ± 1.8	1.04 ± 0.05
Chiltern <sup>BI</sup> July 2004 (14)	25.4 ± 2.9	92.4 ± 3.5	10.3 ± 1.9	0.93 ± 0.05
Chiltern <sup>BI</sup> July 2005 (7)	27.9 ± 3.2	94.1 ± 3.4	9.6 ± 2.5	0.91 ± 0.30
Koondrook <sup>RRG</sup> May 2004 (8)	21.0 ± 2.9			
Gunbower <sup>RRG</sup> June 2003 (12)	23.1 ± 6.3			
Guttrum <sup>RRG</sup> March 2004 (2)	22.7 ± 2.5			
Chiltern <sup>BI</sup> February 2004 (17)	24.7 ± 3.2			
ANOVA for groups				
Ovens <sup>RRG</sup> , Warby <sup>BI</sup> April 2005	3.77 (1, 14) NS	18.63 (1,14) *	9.45 (1, 14) *	5.03 (1, 14) *
RL <sup>RRG</sup> , Rus <sup>BI</sup> July 2004	3.93 (1, 4) NS	0.01 (1, 4) NS	0.57 (1, 4) NS	0.06 (1, 4) NS
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2004	17.35 (1, 27) *	29.92 (1, 27) *	1.53 (1, 27) NS	6.50 (1, 27) *
GT <sup>RRG</sup> , G <sup>RRG</sup> June 2005	0.01 (1, 12) NS	0.58 (1, 12) NS	0.08 (1, 12) NS	0.00 (1, 12) NS
Gunbower <sup>RRG</sup> June 2004, 05	–	7.18 (1, 37) NS	5.05 (1, 37) *	4.13 (1, 37) *
Gunbower <sup>RRG</sup> June 2003, 04, 05	1.60 (2, 52) NS			
Chiltern <sup>BI</sup> July 2004, 2005	3.32 (1, 19) NS	1.11 (1, 19) NS	0.52 (1, 19) NS	0.49 (1, 18) NS
Chiltern <sup>BI</sup> February 2004, July 2004	0.35 (1, 29) NS			
Guttrum <sup>RRG</sup> June 2004, June 2005	9.80 (1, 2) NS	17.99 (1, 2) NS	1.00 (1, 2) NS	9.10 (1, 2) NS
Ovens <sup>RRG</sup> April 2004, June 2005	1.05 (1, 15) NS	6.50 (1, 15) *	3.82 (1, 15) NS	28.87 (1, 15) *
Ovens <sup>RRG</sup> April 2004, Warby <sup>BI</sup> April 2005†	4.90 (1, 7) NS	1.41 (1, 7) NS	21.33 (1, 7) *	1.82 (1, 7) NS
RL <sup>RRG</sup> June 2004, June 2005	0.47 (1, 9) NS	0.01 (1, 9) NS	0.01 (1, 9) NS	1.45 (1, 9) NS

\* $P < 0.05$ , NS, not significant; *n* in parenthesis indicates number of first-year females. ANOVA results reported as: *F* (numerator d.f., denominator d.f.)\*, or *F* (d.f., d.f.) NS.

†Comparison between Ovens females in April 2004 and Warby females in April 2005 because only one female trapped in Warby in April 2004.

Forests: Guttrum, (GT) Koondrook, (K) Gunbower, (G) Rushworth, (Rus) Reedy Lake, (RL) Chiltern, (C).

females and different-teat-number females when 12-teat and 14-teat females were compared. In the STRUCTURE analysis, the most likely number of clusters was one (posterior probability = 1) (data not shown). These results indicate that females with 12 and 14 teats are part of the same random-mating population. Three mtDNA haplotypes found in the four 14-teat females also were found in the 12-teat females.

In 2004 and 2005, respectively, four of 21 and none of 30 Chiltern females had 14 teats, a difference that bordered on statistical significance ( $\chi^2 = 3.84$ ,

$P \sim 0.05$ ). In the part of Chiltern forest where 14-teat females were most abundant, three of five females had 14 teats in 2004, but none did of three in 2005, which was not significant ( $\chi^2 = 0.89$ ,  $P > 0.05$ ). These 14-teat females could not be daughters of one 14-teat mother because they each had different mtDNA haplotype.

Twelve-teat females were found in all nine forests in which teat number was recorded and were the most common group in eight of these, while in Guttrum, one female had 12 teats and another 11 teats (Table 4).

**Table 4.** Number of teats in *Antechinus flavipes* females in box–ironbark (BI) and river red gum (RRG) forests in south-eastern Australia, *n* in parenthesis indicates number of females with known teat number

Geographical location	Teat number ( <i>n</i> )
Chiltern <sup>BI</sup>	12 (33), 13 (12), 14 (4)
Gunbower <sup>RRG</sup>	12 (19), 13 (2)
Guttrum <sup>RRG</sup>	11 (1), 12 (1)
Koondrook <sup>RRG</sup>	10 (1), 11 (1), 12 (4)
Barmah <sup>RRG</sup>	12 (1)
Millewa <sup>RRG</sup>	12 (7)
Rushworth <sup>BI</sup>	12 (1)
Reedy Lake <sup>RRG</sup>	12 (7)
Ovens <sup>RRG</sup>	12 (1)

## DISCUSSION

### PLASTICITY OF PHENOTYPIC CHARACTERISTICS

Box–ironbark and river red gum forests are markedly different habitats for *Antechinus flavipes*. River red gum forests are subjected to and dependent on flooding. We found that all phenotypic characteristics tested here (mass, snout-vent length, pelage colour and ratio of tail to snout-vent length) were plastic. Even though they might reflect trait adaptation being affected by inter-year variation in selective forces, this variation is unlikely to cause speciation. If morphological specialization exists for living on floodplains compared with in box–ironbark forests, it seems not to involve any of the phenotypic characteristics measured here. By sampling over time in different localities at the same time of the year, we were able to investigate plasticity of phenotypic characteristics and limit confounding influences of different life stages.

### BODY MASS OF MALES

In some cases, poorer realized habitat qualities for *A. flavipes* and lower trapping rates of animals were associated with heavier males; for example, in Guttrum<sup>RRG</sup> compared with Gunbower<sup>RRG</sup> in 2004, and in Rushworth<sup>BI</sup> compared with Reedy Lake<sup>RRG</sup> in 2005 (Table 2). Kraaijeveld-Smit, Ward & Temple-Smith (2003) found a similar phenomenon in the closely related *A. agilis*, in which male mass was higher in the year when animal densities were lower (but see Lunney, Cullis & Eby, 1987). Genetic composition and competition for food were proposed as reasons for the intra- and inter-year differences in body mass, respectively (Kraaijeveld-Smit *et al.*, 2003). Marchesan & Carthew (2004) reported that *A. flavipes* in habitat remnants were significantly heavier than those in a nearby extensive forest. They invoked the edge effects

explanation of Bowers *et al.* (1996) for a similar phenomenon observed in meadow voles (*Microtus pennsylvanicus*). Bowers *et al.* (1996) observed that territories were larger and more exclusive, residence times longer and breeding frequency of females higher at the edges than at the interior. However, edge effects cannot explain the differences in mass in our study because almost all sites within all forests were > 500 m from forest edges.

It is possible that the total mass of *A. flavipes* that can be supported in a forest is limited by prey abundance. Prey availability may be limiting the maximum number of 'heavy' males in a forest. Males may: (1) face fewer competitors for food if antechinus densities are lower; and (2) need to be heavier than those in more optimal habitat because traversing longer distances to find a breeding partner may be required when the abundance of females is low (i.e. lower realized habitat quality). Exceptions to this apparent trend included Gunbower<sup>RRG</sup> over time, and Rushworth<sup>BI</sup> and Reedy Lake<sup>RRG</sup> in 2003, which followed a year with very low rainfall. In 2003, only two animals, both males, were trapped in Rushworth<sup>BI</sup> from 390 trap nights, indicating very low abundance, yet these males weighed significantly less than those in nearby Reedy Lake<sup>RRG</sup>, where five animals were caught after 72 trap nights and where realized habitat quality was significantly higher. In 2005, both realized habitat quality and trapping rates of animals were again lower in Rushworth<sup>BI</sup>, in which males were significantly heavier than those in Reedy Lake<sup>RRG</sup>. These results may indicate that males usually are heavier when there are relatively lower abundances of antechinuses and so presumably reduced competition for food, unless conditions are extremely harsh, as in 2003, when males seemed unable to reach a heavy mass. Another exception may include particularly good conditions, which support relatively greater numbers of heavy males, as seemed to be the case in Gunbower<sup>RRG</sup> in 2005 compared with 2004. Greater abundance of relatively heavy second-year females (these with suckled teats, a vast majority, were used here as an indicator of realized habitat quality) may also have negative influence on male mass. Availability of food in a forest would need to be estimated to assess importance of inter-individual competition for food. Male body size/mass at maturity is a life-history trait shown to be associated with paternity success in *A. stuartii* and *A. agilis* (Kraaijeveld-Smit *et al.*, 2003; Holleley *et al.*, 2006). In both species, heavier males sired more offspring overall by fertilizing more females, but not by fathering a higher proportion of offspring per litter (Kraaijeveld-Smit *et al.*, 2003; Holleley *et al.*, 2006). Heavier males of *A. stuartii* were more dominant socially and survived for longer after their first

mating than subordinate males (Fisher & Cockburn, 2006). Given that *A. stuartii*, *A. agilis* and *A. flavipes* share the same life-history strategy (Lee & Cockburn, 1985), it is possible also that the heavier males of *A. flavipes* sire more offspring. This can be investigated in wild populations through sampling of males just prior to the mating season and of mothers and pouch young during lactation.

#### TEAT NUMBER IN FEMALES

Lee & Cockburn (1985) doubted the relevance of the fecundity/survival trade-off hypothesis (Charnov & Krebs, 1974) to females of *Antechinus*. The hypothesis asserts the longest survival of females in populations with low teat number. Our data for *A. flavipes* also do not support this hypothesis. Females in Chiltern<sup>BI</sup> have 12, 13 or 14 teats, the highest known number of teats for any species of *Antechinus*, with 14 being the maximum for the species and, to our knowledge, not found in any other population. Yet, in 2005, 37% of females trapped at Chiltern<sup>BI</sup> were at least second-year females, the highest proportion among all the forests sampled. This indicates high survival of lactating females to their second year despite large teat number. Suckled teats and relatively high trapping rates of first-year individuals indicated that the relatively good survival rate of second-year females was not as a result of the loss of litters. Lada, Mac Nally and Taylor (2008) found significantly higher trapping rates in Chiltern<sup>BI</sup> compared with Warby<sup>BI</sup> or Rushworth<sup>BI</sup>, in Reedy Lake<sup>RRG</sup> compared with Rushworth<sup>BI</sup>, and in Ovens<sup>RRG</sup> compared with Warby<sup>BI</sup>. Here, we reported significantly higher trapping rates of second-year females with suckled teats in Gunbower<sup>RRG</sup> than in Guttrum<sup>RRG</sup>, in Ovens<sup>RRG</sup> than in Warby<sup>BI</sup>, and in Reedy Lake<sup>RRG</sup> than in Rushworth<sup>BI</sup>, in all years tested (Table 1). The presence of 14-teat females in Chiltern<sup>BI</sup> may indicate that this forest offers the most optimal conditions for survival and rearing of offspring, at least in some years. This assertion is supported by previous observations of Chiltern<sup>BI</sup> females producing litters in a third year (Coates, 1995) (we are unaware of a third-year female *A. flavipes* being trapped anywhere else). From direct trapping during teat attachment and lactation, Coates (1995) reported 11 to be the maximum observed litter size weaned in Chiltern<sup>BI</sup> in the early 1990s. Counting suckled teats, we could infer only the maximum possible number of weaned offspring per female, which varied from 10 to 13. We did not find evidence for 14-teat females weaning 14 offspring because no second-year females with 14 suckled teats were trapped (Coates, 1995). Cockburn *et al.* (1983) suggested that Ashmole's (1963) hypothesis, which was based on that of Lack (1947), about variation in

brood size in birds, also applies to *Antechinus* spp. This implies that antechinus females, instead of reducing litter size to breed for the second time (e.g. Charnov & Krebs, 1974) or to avoid predation (e.g. Cody, 1966), produce the maximum possible number of offspring that can be successfully nourished under the given environmental conditions (Ashmole, 1963; Cockburn *et al.*, 1983). It is possible that Chiltern<sup>BI</sup> forest, systematically degraded since European colonization, rarely, or no longer, offers conditions favourable for weaning 14 offspring.

We have conclusive evidence that 12-teat and 13-teat females are part of the same population because there were two cases of daughters having different teat number from their mothers: 13-teat females produced 12-teat daughters. However, as we do not know the teat number of paternal grandmothers, we cannot determine if our results provide evidence for plasticity of teat number or for an influence of father's genotype on his daughter's teat number. A study of a captive breeding colony may be needed to resolve definitively the nature of inheritance of teat number in *A. flavipes*. There also was no evidence for genetic differentiation between 14-teat and 12-teat females because the STRUCTURE analysis suggested only one genetic cluster was present, and the means of individual, pairwise, squared genetic distances between same-teat females did not differ from those between different-teat females. When both males and females were included, there was no evidence of separate genetic clusters at Chiltern<sup>BI</sup> either (Lada *et al.*, in review). Population genetics studies of closely related *A. agilis* at mixed-teat sites, although not explicitly testing for interbreeding between different teat-number animals, also found no evidence for departure from random mating among sampled animals (Kraaijeveld-Smit, Lindenmayer & Taylor, 2002). Therefore, the phenomenon of free interbreeding in mixed teat-number populations probably is not unique to *A. flavipes* and may differ among populations. Genetic analyses of *A. flavipes* in other areas of Australia, where different-teat-numbered females coexist, are needed to determine if free interbreeding irrespective of teat number is a general phenomenon in *A. flavipes*, unlike in *A. agilis*, in which nearby populations of 6-teat and 10-teat in the Otway region of southern Victoria diverged in both microsatellite allele identity and frequencies, but not in mtDNA haplotypes (Beckman *et al.*, 2007).

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## REFERENCES

- Ashley MV, Willson MF, Pergams ORW, O'Dowd DJ, Gende SM, Brown JS. 2003. Evolutionarily enlightened management. *Biological Conservation* **111**: 115–123.
- Ashmole NP. 1963. The regulation of numbers of tropical oceanic birds. *Ibis* **103b**: 458–473.
- Ballinger A, Mac Nally R, Lake PS. 2005. Immediate and longer-term effects of managed flooding on floodplain invertebrate assemblages in south-eastern Australia: generation and maintenance of a mosaic landscape. *Freshwater Biology* **50**: 1190–1205.
- Beckman J, Banks SC, Sunnucks P, Lill A, Taylor AC. 2007. Phylogeography and environmental correlates of a cap on reproduction: teat number in a small marsupial, *Antechinus agilis*. *Molecular Ecology* **16**: 1069–1083.
- Beheregaray LB, Sunnucks P. 2001. Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. *Molecular Ecology* **10**: 2849–2866.
- Bowers MA, Gregario K, Brame CJ, Matter SF, Dooley JL. 1996. Use of space and habitats by meadow voles at the home range, patch and landscape scales. *Oecologia* **105**: 107–115.
- Charnov EL, Krebs JR. 1974. On clutch-size and fitness. *Ibis* **116**: 217–219.
- Coates T. 1995. *Reproductive ecology of the yellow-footed antechinus, Antechinus flavipes (Waterhouse), in north east Victoria Ecology and Evolutionary Biology*. Melbourne: Monash University.
- Cockburn A, Lee AK, Martin RW. 1983. Macrogeographic variation in litter size in *Antechinus* (Marsupialia: Dasyuridae). *Evolution* **37**: 86–95.
- Cody ML. 1966. A general theory of clutch size. *Evolution* **20**: 174–184.
- Crowther MS, Blacket MJ. 2003. Speciation and biogeography within the Dasyuridae, or why are there so many species of dasyurids? In: Jones M, Dickman CR, Archer M, eds. *Predators with pouches: the biology of carnivorous marsupials*. Melbourne: CSIRO Publishing, 124–130.
- Crowther MS, Spencer PBS, Alpers D, Dickman CR. 2002. Taxonomic status of the mardo, *Antechinus flavipes leucogaster* (Marsupialia: Dasyuridae): a morphological, molecular, reproductive and bioclimatic approach. *Australian Journal of Zoology* **50**: 627–647.
- Dickman CR, Parnaby HE, Crowther MS, King DH. 1998. *Antechinus agilis* (Marsupialia Dasyuridae), a new species from the *A. stuartii* complex in south-eastern Australia. *Australian Journal of Zoology* **46**: 1–26.
- Environment Conservation Council. 1997. *Box-ironbark. Forest and woodlands investigation. Resources and issues report*. Melbourne: Environment Conservation Council (Victoria).
- Fisher DO, Cockburn A. 2006. The large-male advantage in brown antechinuses: female choice, male dominance, and delayed male death. *Behavioral Ecology* **17**: 164–171.
- Fretwell SD. 1972. *Population in a seasonal environment*. Princeton: Princeton University Press.
- Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau P, Bruford MW. 2006. Genetic signature of anthropogenic population collapse in orang-utans. *PLoS Biology* **4**: 285–291.
- Holleley CE, Dickman CR, Crowther MS, Oldroyd BP. 2006. Size breeds success: multiple paternity, multivariate selection and male semelparity in a small marsupial, *Antechinus stuartii*. *Molecular Ecology* **15**: 3439–3448.
- Hood GM. 2005. *PopTools*, Version 2.7.1.. Canberra: CSIRO.
- How RA, Cooper NK, Girardi L, Bow BG. 2002. The Mardo: an examination of geographic variation in morphology and reproductive potential in *Antechinus flavipes* in southwestern Australia. *Records of the Western Australian Museum* **20**: 441–447.
- Kraaijeveld-Smit FJL, Lindenmayer DB, Taylor AC. 2002. Dispersal patterns and population structure in a small marsupial, *Antechinus agilis*, from two forests analysed using microsatellite markers. *Australian Journal of Zoology* **50**: 325–338.
- Kraaijeveld-Smit FJL, Ward SJ, Temple-Smith PD. 2003. Paternity success and the direction of sexual selection in a field population of a semelparous marsupial, *Antechinus agilis*. *Molecular Ecology* **12**: 475–484.
- Lack D. 1947. The significance of clutch-size. *Ibis* **89**: 302–352.
- Lada H, Mac Nally R, Taylor AC. 2007. Genetic reconstruction of population dynamics of a carnivorous marsupial (*Antechinus flavipes*) in response to floods. *Molecular Ecology* **16**: 2934–2947.
- Lada H, Mac Nally R, Taylor AC. 2008. Responses of a carnivorous marsupial (*Antechinus flavipes*) to local habitat factors in two forest types. *Journal of Mammalogy* **89**: 398–407.
- Lada H, Thomson JR, Mac Nally R, Horrocks G, Taylor AC. 2007. Evaluating simultaneous impacts of three anthropogenic effects on a floodplain-dwelling marsupial *Antechinus flavipes*. *Biological Conservation* **134**: 527–536.
- Lee AK, Cockburn A. 1985. *Evolutionary ecology of marsupials*. Cambridge: Cambridge University Press.
- Lee AK, Woolley P, Braithwaite RW. 1982. Life history strategies of Dasyurid marsupials. In: Archer M, ed. *Carnivorous marsupials*. Sydney: Royal Zoological Society of New South Wales, 1–11.
- Lunney D, Cullis B, Eby P. 1987. Effects of logging and fire on small mammals in Mumballa State Forest, near Bega, New South Wales. *Australian Wildlife Research* **14**: 163–181.

- McAllan BM, Dickman CR, Crowther MS. 2006.** Photoperiod as a reproductive cue in the marsupial genus *Antechinus*: ecological and evolutionary consequences. *Biological Journal of the Linnean Society* **87**: 365–379.
- Marchesan D, Carthew SM. 2004.** Autecology of the yellow-footed antechinus (*Antechinus flavipes*) in a fragmented landscape in southern Australia. *Wildlife Research* **31**: 273–282.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998.** Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639–655.
- Menkhorst P, Knight F. 2001.** *A field guide to the mammals of Australia*. South Melbourne: Oxford University Press.
- Muir AM, Edwards SA, Dickins MJ. 1995.** *Description and conservation status of the vegetation of the box–ironbark ecosystem in Victoria*. Flora & Fauna Technical Report 136. Melbourne: Department of Conservation and Natural Resources, Victoria.
- Peakall R, Smouse PE. 2006.** GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–295.
- Pritchard JK, Stephens M, Donnelly P. 2000.** Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.
- Sala OE, Chapin FSIII, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH. 2000.** Global biodiversity scenarios for the year 2100. *Science* **287**: 1770–1774.
- Schneider CJ, Smith TB, Larison B, Moritz C. 1999.** A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 13869–13873.
- Smith TB, Wayne RK, Girman DJ, Bruford MW. 1997.** A role for ecotones in generating rainforest biodiversity. *Science* **276**: 1855–1857.
- Stearns SC. 2000.** Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* **87**: 476–486.