IS MORPHOMETRIC VARIATION ASSOCIATED WITH TEAT-NUMBER DIFFERENCES IN *ANTECHINUS AGILIS* AND *A. SWAINSONII* ? OBSERVATIONS FROM THE OTWAY RANGES, VICTORIA.

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That there is intraspecific variation in teat-number in *Antechinus agilis* (agile antechinus) and *A. swainsonii* (dusky antechinus) has been known for a long time. Our aim was to determine whether other key morphometric traits differed among individuals with different numbers of teats. External body dimensions and pelage characteristics were measured on live individuals and compared. Within-species variation in some external body dimensions (tail, pes, and snout-vent length) coincided with teat-number differentiation in both antechinus species in the Otway Ranges, Victoria. Disparities in pelage colour and markings were apparent between *A. agilis* 6- and 10-teat phenotypes, but were not obvious among *A. swainsonii* teat phenotypes. Although small sample sizes obviated statistical analysis, we tentatively concluded that female *A. agilis* with 7, 8, or 9 teats in the Otway Ranges probably displayed morphometric and pelage characteristics intermediate between those of 6- and 10-teat individuals. A comparison of morphometric traits among 6-teat *A. agilis* from different geographic areas (Otways, Portland and Wilsons Promontory) also revealed some variation. This result was consistent with an expectation of clinal variation in external morphology among antechinus. Overall, this study indicates that localized disparities that are associated with teat-number, as well as clinal differences occurring over large geographic distances, contribute to intraspecific variation in external morphology in antechinus.

Keywords: *Antechinus agilis*, *Antechinus swainsonii,* morphology, phenotype, teat-number, trait variation.

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Intraspecific variation in morphological traits can be common, particularly in species with large geographical ranges (Avise 2004; Endler 1977). One remarkable example of within-species trait diversity evident in some litter-bearing marsupials is teat-number variation. Intraspecific differences in number of teats have been recorded in the Virginia opossum *(Didelphis virginiana)* and among a few Australian dasyurids, including five species of antechinus and their close relative *Phascogale tapoatafa* (Cockburn *et al.* 1983; Dickman *et al.* 1998; Rhind *et al.* 2001; Tyndale-Biscoe and Renfree 1987 and references therein). Investigations to resolve long standing taxonomic uncertainties in the genus *Antechinus* have revealed a large degree of intraspecific differentiation and interspecific overlap in morphometric traits, often making the animals difficult to identify in the field (Crowther 2002a,b; Dickman *et al.* 1998; Van Dyck 1982; Van Dyck and Crowther 2000). Some reports have suggested that morphological traits other than teat number, may also vary among teat-number phenotypes in two antechinus species, the mardo (*Antechinus flavipes*) (How *et al.* 2002) and the agile antechinus (*A. agilis*) (Crowther 2002a,b). Our trapping of antechinus has given us an opportunity to test this prediction for *A. agilis* and *A. swainsonii.*

Across their species' geographic range, female *A. agilis* can have 6, 8, or 10 teats, but in the Otway Ranges in Victoria generally only 6- or 10-teat females are found. The currently recognized *A. agilis* (Crowther 2002a; Dickman *et al.* 1998) was recently found to be a monophyletic taxon according to mitochondrial cytochrome *b* sequence analyses (Beckman *et al.* 2007). However, high levels of divergence in nucleic microsatellites indicate that one group of *A. agilis* is substantially genetically differentiated from the rest

(Beckman *et al.* 2007). This distinct group comprises the 6-teat phenotypes in the Otway Ranges and the Portland area in southwest Victoria and means that as well as being divergent in teat-number, the 6- and 10-teat phenotypes currently present in the Otways are also genetically different from each other. Microsatellite DNA provides us with neutral markers with regard to trait inheritance (Sunnucks 2000), and thus does not provide a direct signal that teat number is genetically controlled. To date, investigations into a genetic basis of teat-number inheritance in mammals have been limited, although polygenic control is suspected in domestic pigs (*Sus scrofa*) (King *et al.* 2003; Kim *et al.* 2004). It is of intrinsic interest therefore to determine whether other morphological traits differ with teat-number, because associated variation in a number of traits may be a useful indirect indicator of a genetic link between them, particularly where characteristics are controlled by quantitative trait loci (Frankham *et al.* 2002).

The incidence of individuals with teat-numbers intermediate between those of the surrounding populations has given rise to the suggestion that these phenotypes are the result of hybridization between different teat number phenotypes (Braithwaite *et al.* 1978; Lee and Cockburn 1985; Cockburn 1992; Shimmin *et al.* 2000). During the course of this study, females with an intermediate number of teats (7, 8 and 9), and 10-teat animals were trapped within an ~300m^2 trapping grid in two areas in the Otway Ranges. Genetic analysis of all individuals caught at these two sites indicated that genetic intermixing indicative of interbreeding between teat-number phenotypes was evident (Beckman *et al.* 2007). Determining characteristic differences in traits other than teat-number among animals with an intermediate number of teats may show some evidence of a genetic link. Quantifying any differences may also be useful for field recognition of the probable origin of males and for juvenile females whose teat-number cannot readily be determined.

A. agilis inhabits a large geographic range of forested habitats throughout SE Australia, though the 6-teat phenotype has been found in just two areas at Wilsons Promontory and throughout the Portland-Otways region in south-west Victoria (Cockburn *et al.* 1983). Microsatellite analysis indicated that, in addition to being genetically discernible from ten teat animals, the south-west *A. agilis* 6-teat animals are also genetically distinguishable from the 6-teat Wilsons Promontory population. This finding has raised questions as to the evolutionary origin of these two geographically and genetically distinct groups of *A. agilis*, that both have a 6-teat phenotype (Beckman *et al.* 2007). Any differences in morphological traits that may exist between genetically similar populations from different geographic regions and genetically dissimilar, same teat-number phenotypes could therefore be of interest in the context of the possible role of environmental interactions.

Comparatively less information is available about variation in morphology in *A. swainsonii* however, its teat-number distribution tends to reflect that of other antechinus species in south-eastern Australia (Cockburn *et al.* 1983). Females with fewer teats are generally found in coastal areas, the number of teats increasing with distance inland (Cockburn *et al.* 1983). Similarly to *A. agilis, A. swainsonii* can have 6, 8, or 10 teats throughout their range, but in the Otway Ranges have either 6 or 8 teats, with the occasional 7-teat female being found.

This field-based study was primarily conducted in the Otway Ranges in south-west Victoria and involved measuring morphological traits in *A. agilis* and *A. swainsonii* of different teat-number phenotypes. Our aims were to: 1) quantify whether patterns of variation in morphometrics and pelage characteristics are associated with conspecific teat-number variation in two species of antechinus, 2) assess whether animals of intermediate teat-number are distinguishable from "pure" teat populations on the basis of traits other than teat-number, and 3) compare 6-teat *A. agilis* from the Otways to (a) geographically distant, but genetically similar Portland individuals and (b) geographically distant, but genetically dissimilar Wilsons Promontory individuals.

METHODS

All measurements were made on live animals after capture in Elliott traps baited with oats, peanut butter and honey. To ensure that each sample was independent, only first-capture measurements taken from each individual were analysed. There is an increased probability of measurement error when taking measurements from live animals under field conditions (Blackwell *et al.* 2006). In order to minimise the potential for type II errors, where first-capture individuals were trapped more than once during any three-night trapping session, they were measured repeatedly. The recorded measurements for each individual were subsequently averaged before data analysis. Measurements of snout-vent, tail (± 1.0 mm) and head lengths (± 0.1 mm) were taken using Vernier callipers according to the methods of Wakefield and Warneke (1963). Head length was measured while the animal was held so that its nose was at a 90º angle to its throat. Right pes and 1st toe lengths were obtained by first measuring the pes (± 0.1 mm) as outlined in Wakefield and Warneke (1963) and then measuring the length of the toe and subtracting that value from the full pes length. Body mass (± 0.5 g) was measured with Pesola© scales. Body mass measurements from females that were pregnant, lactating or carrying pouch-young

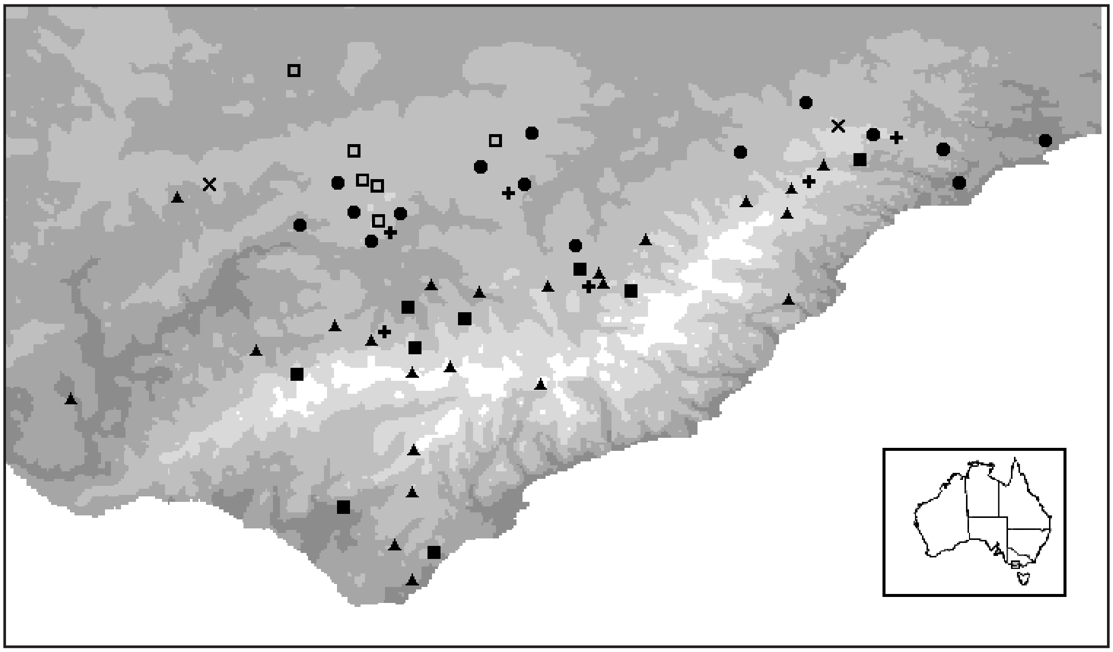


Fig. 1. Elevation map of the Otway Ranges, in south-western Victoria showing our trap sites (+), including two where *A. agilis* females had more than one teat number phenotype (×),and the distribution of known teat number locations (Cockburn *et al.* 1983 & Museum Victoria) of 6- (▲), and 10-teat (●) *A. agilis* and 6- (■), and 8-teat (□) *A. swainsonii*. Elevation intervals: 0-50m, 50-150m, 150-300m, 300-450m, and 450-675m.

were not included in analyses. Detailed descriptions of pelage colour and markings were recorded for each captured animal.

Two hundred and ninety four (215 females; 79 males) *A. agilis* were measured in the period 2000-2003, with re-capture within trapping episodes (and therefore duplicate measurements being obtained for newly-trapped individuals) ranging from 19% (*A. agilis* females) to 30% (*A. agilis* males). Trapping was conducted at 8 sites in the Otway State Forest (Fig. 1), namely three 10-teat (38°27'S 143°38'E; 38°24'S 144°05'E; 38°29'S 143°31'E) and three 6-teat locations (38°36'S 143°31'E; 38°33'S 143°44'E; 38°27'S 143°56'E), as well as two 'mixed teat-number' sites, where females had $7_{(n=5)}$, $8_{(n=4)}$ or $9_{(n=11)}$ teats (38°25'S 143°23'E; 38°25'S 143°58'E). Additional, but smaller samples of 6-teat female *A. agilis* were obtained through trapping at Wilsons $\text{Promontory}_{(n=13)}$ (39°01'S, 146°20'E) and near $\text{Portland}_{(n=8)}$ (38°12'S, 141°28'E), enabling comparisons among 6-teat females from different geographic locations to be made. Candidate regions for trapping were initially selected using previous records of teat-number distributions for *A. agilis* (Cockburn *et al.* 1983 and records kindly provided by Museum Victoria), with sites of similar elevation, aspect and abundance of animals being chosen after extensive on-ground survey trapping. Whilst pure (as opposed to mixed) teat-number populations were defined as those in which only one teat-number phenotype was found, it should be noted that 9-teat females were occasionally found in an otherwise predominantly 10-teat *A. agilis* sample site. In contrast, at pure 6-teat sites, no females with other teat-numbers were ever found . Male antechinus do not have teats, but because there was such a clear disparity in teat-number between females found at any one 'pure' site, males were confidently assigned a phenotype on the basis of the females' teat-number at the same capture site (i.e. '6', '10' or 'mixed').

The smaller samples obtained of the larger *A. swainsonii* (n=43: 20 females; 23 males) were a result of lower population densities in this species. *A. swainsonii* live sympatrically with *A. agilis* in the Otways and so trapping was conducted as described for *A. agilis*. At each trapping site female *A. swainsonii* had either 6 or 8 teats (Fig. 1) and males were designated as being of either 6- or 8-teat origin on the basis of the corresponding female teat-number at the capture site. Only one female with 7 teats was captured during the trapping program. There were no immediately obvious differences in pelage markings among *A. swainsonii* and so pelage characteristics were not recorded for this species.

Both species are sexually dimorphic and so males and females were analysed separately. Indeterminate growth in *Antechinus* (Cockburn and Johnson 1988) can complicate direct comparison of body dimensions. To alleviate potential biases, we used an age cohort for each species, such that we could ensure approximately equal sample sizes in which data were gathered evenly across age-bands for each teat number within each test group. The larger number of samples available for *A. agilis* females meant that they could be divided into two age cohorts, 7-10 and ≥12 months old, whereas the samples for female *A. swainsonii* formed one age cohort of 7-14 months. Male antechinus have discrete generations (Lee *et al.* 1982) and were present in the population from the age of 3 (weaning/dispersal) to 11 months (time of annual male 'die off'). Only measurements taken from animals ≥ 4 months old (sub-adults; Crowther 2002a) were included in statistical analyses. Male *A. agilis* ranged from 4-10 months old. Male *A. swainsonii* were only measured at 4 months old.

Morphometric data were graphically checked for normality prior to analysis and, where appropriate, transformed to their natural logarithms. The data for *A. agilis* at the Otway sample sites showed that the distributions satisfied the assumption of homogeneity of variances. Due to smaller sample sizes, all data for *A. swainsonii* and for *A. agilis* used in comparisons among the Wilsons Promontory, Portland and Otway populations were log natural transformed prior to analysis. Principal components analyses (PCA) is a statistical technique used to reduce the number of measured variables into a smaller number of components, while maintaining most of the variation in the data (Jolliffe 2002). Each resultant component includes those variables that show the greatest correlation with one another in order of their proportional contribution to variation (Jolliffe 2002). PCA was applied to five sets of data, with animals being grouped by species, sex and, in the case of female *A. agilis*, age. Multivariate analyses of variance (MANOVA) were used to identify any differences among teat-number phenotypes for the variables that formed PC1 in the PCA. T-tests were used to test for univariate differences between teat-number phenotypes. Analyses of variance (ANOVA) followed by planned Tukey's tests were used for comparisons among the geographically disparate populations of the Otways, Portland and Wilsons Promontory. To keep sample sizes even for this test, a random subset (n=17) of females from identical age classes were included from the Otway samples All statistical analyses were performed using Systat$^{©}$ v.10 and differences were deemed significant only after subsequent Bonferroni probability adjustment for multiple tests (Rice 1989).

RESULTS

Body mass and dimensions are expected to increase with age in antechinus, but a diagnostic check for bivariate correlations showed few obvious trends between mass and any of the other morphometric variables. One exception occurred in 10-teat male *A. agilis,* where there was an age effect on mass. As expected, younger males were shorter in body length than 10 month old males, which were also heaviest. A severe drought occurred in south-east Australia during 2003 (National Climate Centre 2004); however, within-teat group/between-year comparisons made with ANOVA revealed that measurements taken in any given year were not significantly different from those obtained in other years.

Within-species morphometric trait variation in *A. agilis and A. swainsonii*

The means of the morphometric variables measured in each species and sex are listed in Table 1. The PCA revealed some consistency in the variables contributing to PC1 among the five tests, in that tail and pes lengths were the main contributors (Table 2). This trend was apparent in each sex, but most obvious in the females of both species. Clear disparity in the distribution of the component variables was apparent between 6- and 10-teat *A. agilis*, with the most obvious difference being that between females (Fig. 2). Bivariate plots of the main variables contributing to PC1 show that for most there was only a small overlap in measurements between teat-number phenotypes in each species and sex (Figs. 3 and 4). MANOVA confirmed that tail, pes and toe lengths were the main contributors to differences between both *A. agilis* females ($F_{(6,\ 60)} = 25.1$, $p<0.001$) and males ($F_{(6,\ 53)} = 39.3$, $p<0.001$). Male *A. agilis* from 6-teat sites had longer tails ($t_{(64)} = 13.4$, $p<0.001$), and slightly longer pes ($t_{(64)} = 5.6$, $p<0.001$) and toes ($t_{(64)} = 3.7$, $p<0.05$) than those from 10-teat sites. Six-teat female *A. agilis* were generally shorter in snout-vent length ($t_{(65)} = -2.7$, $p = 0.049$), but had a longer tail ($t_{(179)} = 18.6$, $p<0.001$), pes ($t_{(178)} = 5.7$, $p<0.001$) and toes ($t_{(144)} = 4.4$, $p<0.001$) than females with 10 teats. These morphometric differences were not reflected in body mass disparities between 6- and 10-teat *A. agilis* populations in either sex. Mean body mass, head, snout-vent and pes of the morphologically larger *A. swainsonii* males ($F_{\text{males }(6,\ 15)} = 5.9$, $p<0.05$), and tail, pes and toe lengths of 6-teat *A. swainsonii* females ($F_{\text{females }(6,\ 12)} = 6.7$, $p<0.05$) were greater than those of their 8-teat conspecifics. Although the finding was not as definitive as in *A. agilis* due to smaller sample sizes, 6-teat *A. swainsonii* females appeared to have a longer tail ($t_{(18)} = 3.1$, $p = 0.04$) and pes ($t_{(18)}$

	A. agilis						*A. swainsonii*			
	♀: 12-21 months		**♀: 7-10 months**		♂		♀		♂	
No. of teats	**6**	**10**	**6**	**10**	**6**	**10**	**6**	**8**	**6**	**8**
Parameters measured:										
Body mass (g)	22.7 (18.5-29.5)	24.1 (21.0-29.0)	18.5 (14.5-27.0)	19.3 (16.0-23.0)	29.7 (21.0-38.5)	30.3 (18.5-40.0)	51.9 (35.5-58.5)	44.8 (33.5-55.0)	45.5 (40.0-53.0)	38.5 (31-55.5)
Head length (mm)	30.8 (28.5-32.3)	30.5 (29.0-32.5)	30.4 (28.6-32.0)	30.4 (28.9-32.1)	32.4 (30.9-33.6)	32.4 (30.9-34.6)	37.2 (33-39.4)	35.9 (33.8-37.9)	37.1 (35.2-39.1)	35.7 (31.8-38.0)
Right Pes length (mm)	11.7 (11.1-12.6)	11.4 (10.6-12.3)	11.7 (11.0-12.6)	11.5 (10.6-12.5)	12.8 (11.9-13.6)	12.3 (11.3-13.0)	15.0 (14.4-15.4)	13.8 (12.8-14.7)	15.6 (14.9-16.4)	14.6 (13.6-15.7)
1st toe length (mm)	4.6 (3.0-5.6)	4.2 (3.1-5.3)	4.8 (3.4-5.9)	4.2 (3.3-5.2)	5.2 (4.1-6.8)	4.7 (3.9-5.8)	4.9 (3.1-6.1)	4.3 (3.7-5.3)	5.6 (3.8-7.2)	5.0 (4.3-6.2)
Snout-vent length (mm)	92.3 (81-104)	98.7 (92-110)	83.8 (73-92)	89.6 (81-103)	98.3 (80.5-113)	98.9 (88-109)	121.7 (116-135)	110.6 (94-129)	113.5 (106-136)	102.6 (95-119)
Tail length (mm)	92.5 (82-103)	77.8 (68-94)	93.5 (85-102)	78.5 (74-86)	98.9 (86-113)	86.3 (76-101)	99.2 (85-108)	89.9 (75-101)	98.2 (86-109)	92.7 (88-100)

Table 1. Mean values (range in parentheses) for each parameter measured for different teat numbered *A. agilis* and *A. swainsonii* from the Otway Ranges, Vic

	A. agilis						*A. swainsonii*			
	♀: 12-21 months		**♀: 7-10 months**		♂		♀		♂	
Component loading	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Body mass				0.738	0.680	0.562	0.848		0.848	
Head length		0.749		0.818	0.591		0.859		0.523	
Right Pes length	0.521	-0.806	0.714		0.845		0.880		0.782	
1st toe length	0.946	0.052	0.631			-0.564	0.739		0.547	-0.591
Snout-vent length	-0.713	-0.541	-0.726			0.632		0.976	0.552	0.628
Tail length	0.920		0.830		0.758	-0.521	0.805		0.773	
Percentage variance	**47.61**	**30.10**	**38.93**	**24.15**	**42.91**	**23.08**	**60.21**	**18.86**	**46.74**	**19.54**

*Table 2***.** Resultant component loadings from Principal Components Analyses (PCA) for morphometric parameters among *A. agilis* and *A. swainsonii.*

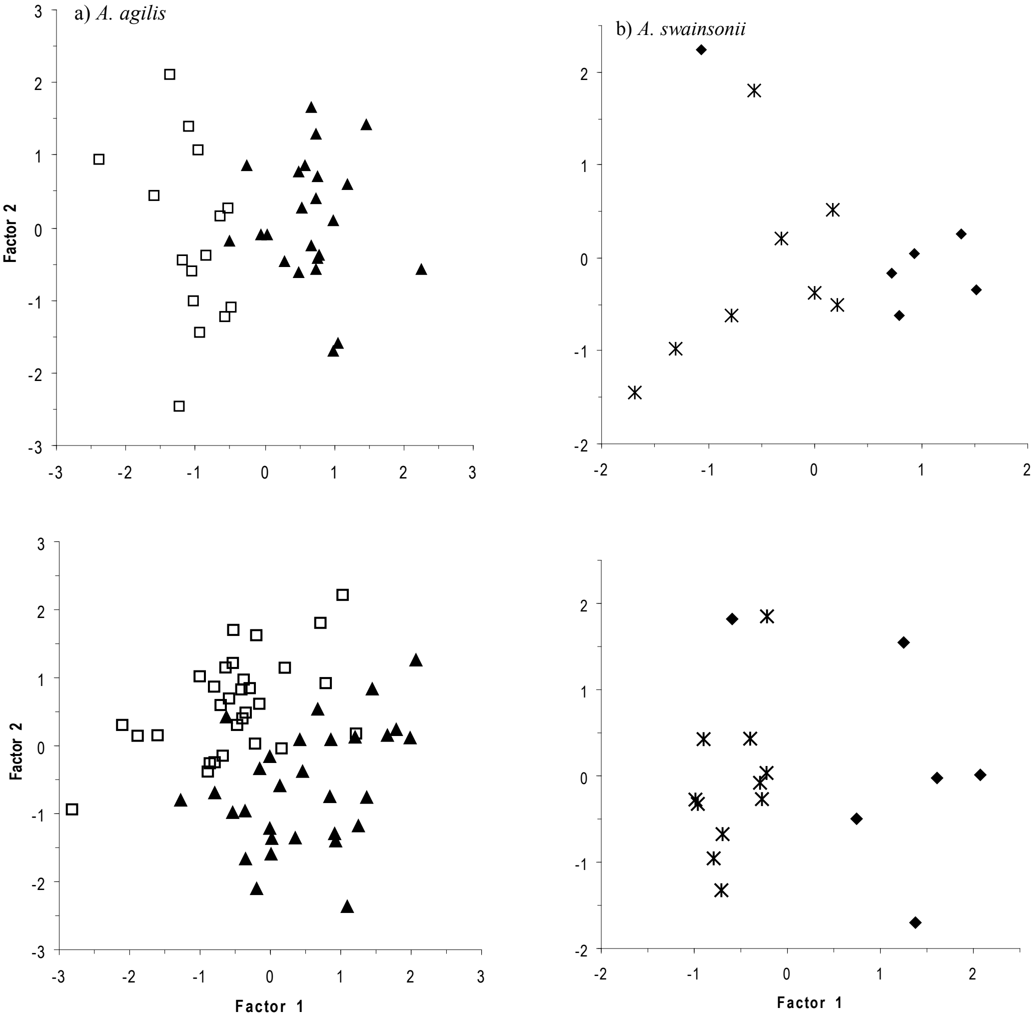
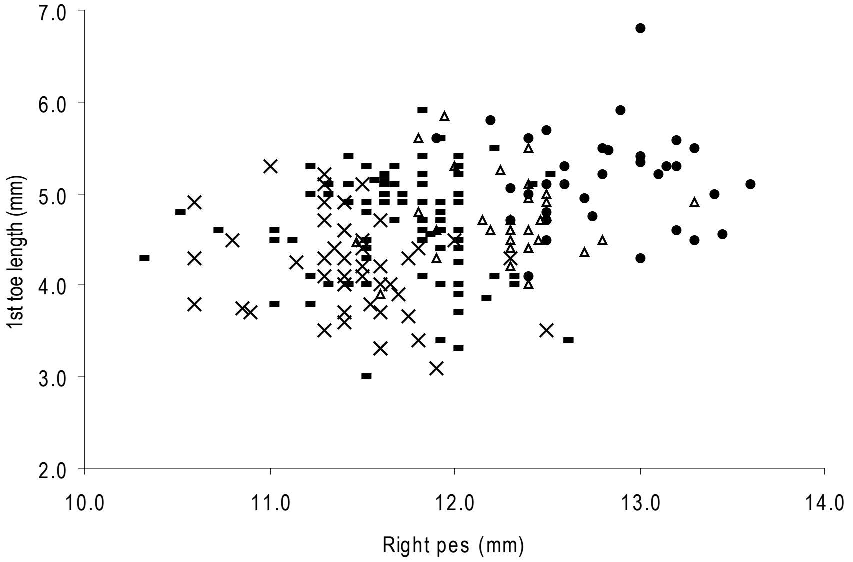


*Fig. 2***.** Plots of the first two factor scores from PCA analyses of body dimension measures for a) *A. agilis* six- (▲) and ten-teat (□) and b) *A. swainsonii* six- (◆) and eight-teat (∗) females (top) and males (below). Results for female *A. agilis* aged 7-10 months are shown.



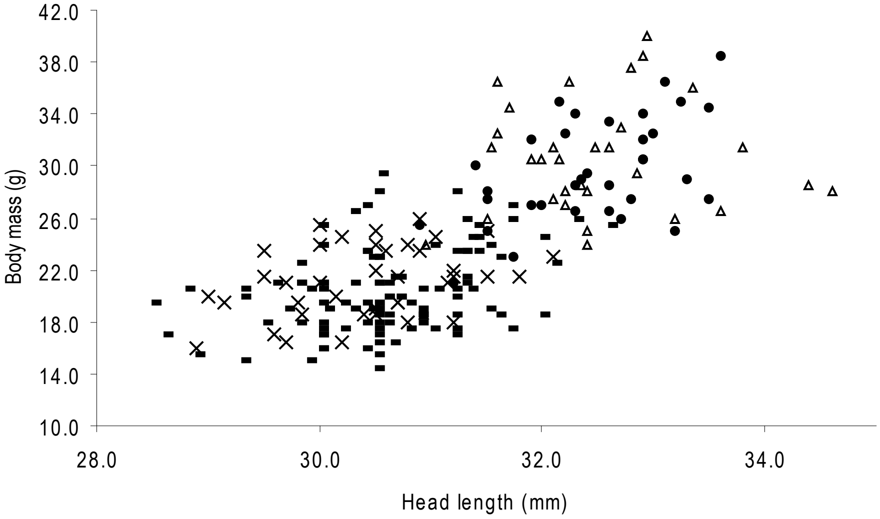


Fig. 3. Bivariate comparisons of morphometric data collected from individual *A. agilis* females with 6- (▪) and 10-teats (×), and males from 6- (●) and 10-teat (Δ) populations. (Continued on next page.)

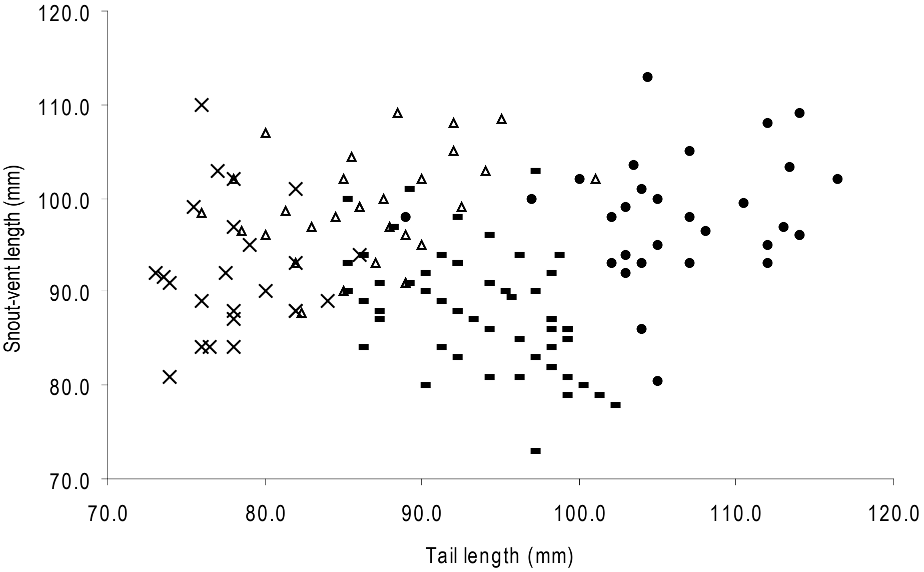


Fig. 3. Bivariate comparisons of morphometric data collected from individual *A. agilis* females with 6- (▬) and 10-teats (×), and males from 6- (●) and 10-teat (Δ) populations. (Continued from previous page.)

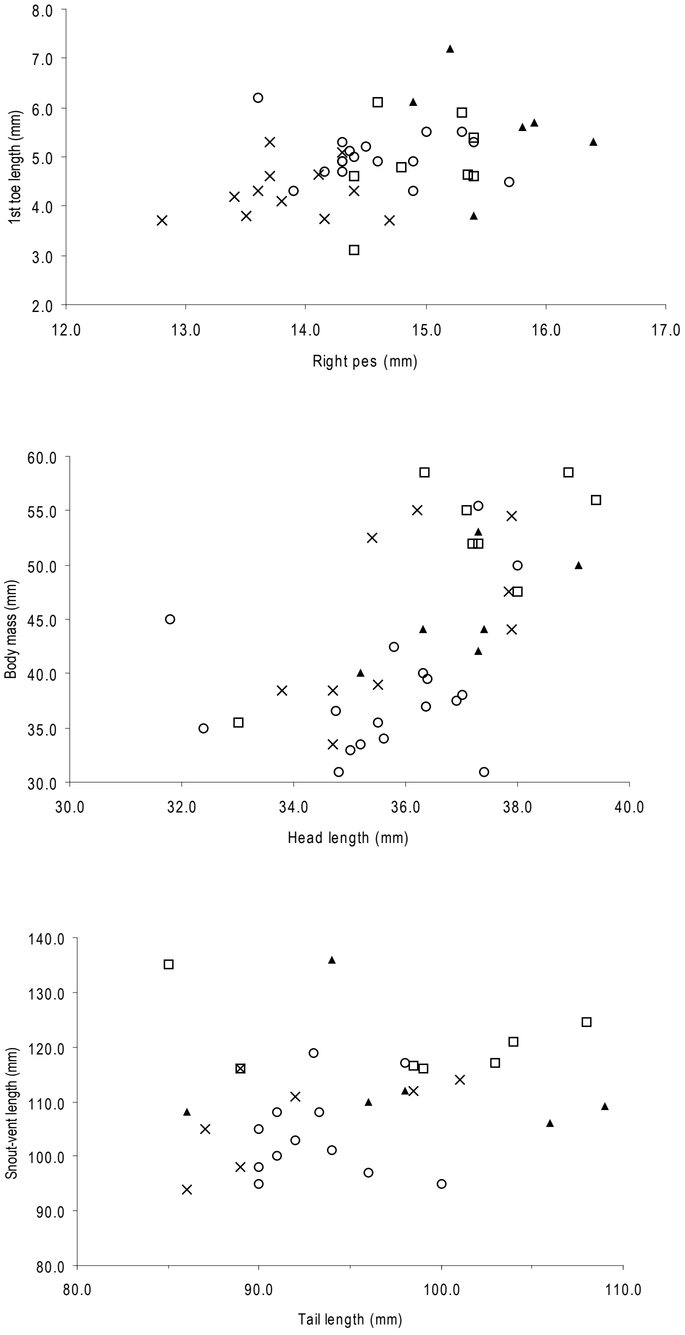


Figure 4. Bivariate comparisons of morphometric data collected from individual *A. swainsonii* females with 6- (□) and 8-teats (×), and males from 6- (▲) and 8-teat (○) populations. Note that male *A. swainsonii* are smaller in some measures than their female conspecifics because they were sampled at a younger age.

= 4.95, p<0.001) than 8-teat females. In contrast to the situation in *A. agilis*, 6-teat *A. swainsonii* males and females generally had longer snout-vent lengths than their 8-teat conspecifics, although this trend was not statistically significant in the corrected t-tests. As might be expected, there was also a strong indication that head length increased as a function of body size in both species and sexes.

Pelage variation among *A. agilis* that differ in teat-number in the Otway Ranges

Observable differences in pelage colour and markings existed between 10- and 6-teat *A. agilis* in the Otways. The most notable of these was the presence of a pale line of fur on the upper and lower eyelids, forming "eye rings", that were present in all *A. agilis* captured in 10-teat sites, but were never present in 6-teat individuals. Considerable differences in general coat colour were also evident between teat-number phenotypes. The dorsal fur of 6-teat animals was dark chocolate brown, with the distal ends a rufous/orange colour, giving the effect of 'warm' flecks on the coat. In contrast, the coat fur of 10-teat animals was a uniform grey, with paler, yellowish, post-auricular patches and tufts along the flanks. Short, yellowish fur covered both manus and pes in the 10-teat animals, whereas these areas were covered by short, brown fur in 6-teat animals. Pelage colour differences between teat-number phenotypes were most obvious on the animals' ventral surface. Ten-teat animals had very pale ventral fur, which became yellowish along the flanks, whereas the ventral fur of the 6-teat animals was a rich rufous colour. Ventro-dorsal bicolouration of the tail was obvious in the 10-teat animals (dorsal- grey, ventral - pale), but absent in the unicoloured brown tails of 6-teat animals. Throughout the study, we observed that 10-teat animals apparently had flatter snouts and broader faces than 6-teat animals, but further investigation is required to provide clarification of whether or not this was a visual illusion attributable to differences in facial fur colouration.

Males and females caught at 'mixed-teat' sites can have pelage characteristics that vary among individuals and which do not wholly conform to the 'norms' for either 6- or 10-teat animals outlined above. Sample sizes were too small to tease out consistent patterns, but many individuals at these sites had faint or incomplete "eye rings" and an overall appearance that was intermediate in colour and features between the 6- and 10-teat animals.

Tail length variation among female *A. agilis* with intermediate teat-numbers

Sample sizes were insufficient to permit significance testing to determine whether there were morphometric differences among *A. agilis* females with 7, 8, or 9 teats.

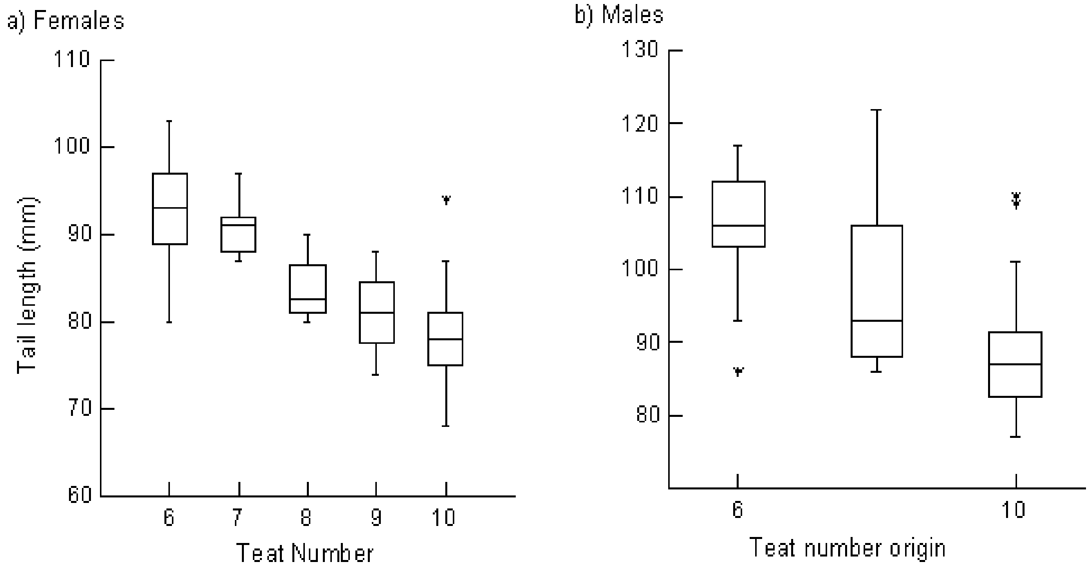


Fig 5. Box-plot comparisons of tail lengths for different teat number *A. agilis* females (a), and males (b) captured in the Otway State Forest. Boxes represent the central 50% of the data distribution (internal horizantal line shows the median). The whiskers specify the range of each upper and lower quartile and points outside indicate outliers. The central distribution in graph b) consists of measurements from males captured at mixed teat number sites, hence their teat number origin could not be determined (see text).

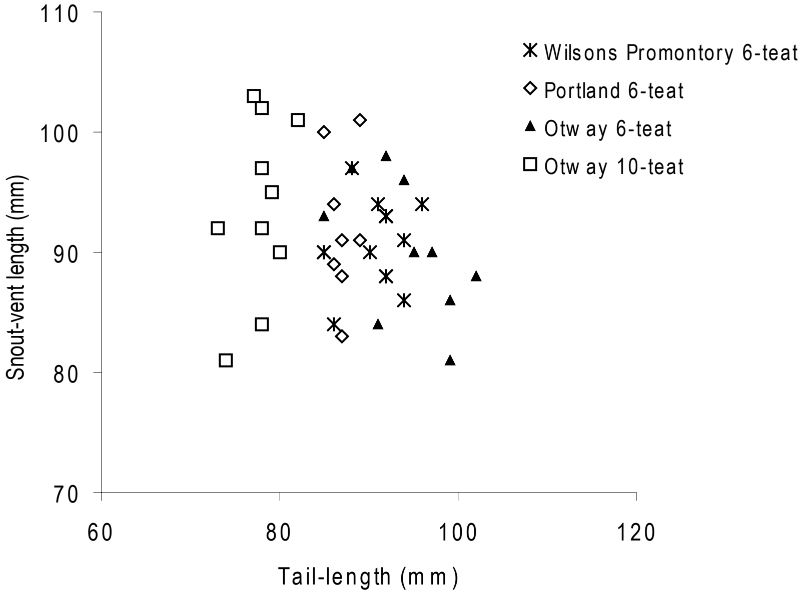


Fig. 6. Comparison depicting body length measurements of females from geographically disparate 6-teat *A. agilis* (Portland, Wilsons Promontory and Otway samples) with 10-teat *A. agilis* from the Otway region.

Graphical representation suggests that collectively these females had intermediate tail lengths and that as teat-number increased, tail length decreased (Fig. 5). A comparison of tail length of males caught at 'mixed' and 'pure' teat-number sites showed a comparatively larger overlap in the values, but essentially the pattern conformed to the trend apparent in females. The greater overlap can be attributed to our inability to make direct teat-number comparisons among males, forcing us to treat all the males from 'mixed' sites as one group of unknown teat-number phenotype. We emphasise that these proposed trends in tail length in intermediate teat-number antechinus require substantiation through larger samples and statistical testing.

Morphometric trait variation among 6-teat female *A. agilis* from different geographic locations

Some significant differences were observed in mean body dimensions among females trapped at each of the three geographically separate 6-teat locations ($F_{(12, 26)} = 8.42$, $p<0.001$). *Post-hoc* tests revealed no difference in either pes or toe length between females from Portland and the Otways. Both parameters were, however, significantly greater in the Portland and Otway females than in those from Wilsons Promontory ($F_{pes\ (2, 29)} = -0.48$, p=0.006, $F_{toe\ (2, 29)} = 0.58$, p=0.017). Interestingly, the tails of 6-teat individuals were significantly longer in the Otways population than in the Portland samples ($F_{(2, 29)} = 7.20$, p=0.001), but the mean tail length of Wilsons Promontory antechinuses was not different from that of either of these populations. However, ANOVA confirmed that 6-teat females from all three locations (Otway, Portland and Wilsons Promontory) had significantly longer mean tail lengths ($F_{(3, 35)} = 25.5$, $p<0.00$) and significantly shorter mean snout-vent lengths ($F_{(3, 35)} = 3.17$, p = 0.036) than 10-teat Otway females (Fig. 6). These results are consistent with the between teat-number phenotype comparisons outlined above.

DISCUSSION

Morphometrics vary with teat-number in *A. agilis* and *A. swainsonii*

Substantial morphological differences among teat-number phenotypes in *A. agilis* and, to a lesser extent *A. swainsonii,* were evident in southern Victoria. Intraspecific variation in morphometric parameters appeared to be associated with teat-number differences in samples of both species in the Otway region. Of the traits measured, tail and pes length showed the clearest association with teat-number in both species, whereas the trend for snout-vent length differed between the two species. Individuals with six teats had consistently significantly longer tail and pes than their counterparts with more teats, even though superficially pes lengths showed a great deal of overlap among teat numbers. Female *A. agilis* with six teats had a shorter snout-vent length than 10-teat females. In contrast, longer snout-vent lengths were found in 6-teat *A. swainsonii* populations (both sexes) than in their 8-teat conspecifics. Pelage differences were also apparent between *A. agilis* 6- and 10-teat phenotypes. Similar differences in tail length and pelage characteristics have since been reported in a follow-up study of *A. agilis* in the Otway Ranges (Bramwell 2006). Although female *A. agilis* from the Otway region with 7, 8, or 9 teats could not be included in formal statistical analyses due to small sample sizes, we suggest with appropriate caution that they probably display morphometric and pelage characteristics intermediate between those of 6- and 10-teat individuals.

Many factors could be interacting to influence morphometric and pelage differentiation in these mammals, making it difficult to pinpoint the reasons for differences in appearance. These factors include genetic predisposition and/or phenotypic plasticity. Trait plasticity is influenced by environmental pressures, such as habitat conditions and abundance of food, and is linked with adaptive and fitness advantages that are often not easy to decipher (Avise 2004; Endler 1977). Little is known about the heritability of teat-number in antechinus. However, genetic analysis of neutral markers indicates that interbreeding between 6- and 10- teat individuals in the Otways is uncommon relative to reproduction between same teat-number phenotypes (Beckman *et al.* 2007). Reproductive isolation of each phenotype increases the propensity for trait divergence (Avise 2004). Whether genetically controlled or due to phenotypic plasticity, there appears to be a strong association between teat-number and some morphometric parameters such as tail, pes and snout-vent length examined in these two species. Considering also that female *A. agilis* with an intermediate number of teats probably also show intermediate values in some of the measured body dimensions as well as pelage characteristics supports the contention that teat-number and some other morphological traits are linked, even if the causation is obscure.

Variation in morphology in the absence of differences in altitude and latitude

A. agilis and *A. swainsonii* both have extensive geographic ranges in south-eastern Australia (Strahan 1998). Variation with latitude and altitude over a large geographic range has been implicated as a factor contributing to intraspecific morphological variation (Crowther 2002a; Davison 1991; Dickman *et al.* 1998), including that in teat-number in antechinus (Cockburn *et al.* 1983). However, whether teat-number and other facets of morphological variation are associated with one another irrespective of altitude and latitude has not been explored. The Otway Ranges provided an ideal site in which to assess this because altitude and latitude could both effectively be held constant. Thus all of the sampling sites were within 12' of latitude of one another, had a south-easterly aspect and, with one exception at ~200m, were at a similar altitude (350 ± 20m). It is worth noting that two of our sampling sites were exceptions to the prediction of Cockburn *et al.* (1983) that teat-number in antechinus will tend to increase with altitude and distance inland. The ~200m site was inhabited by 10-teat *A. agilis*, an exception to the overall trend for larger numbers of teats to be found in individuals living at higher altitudes, and another 10-teat *A. agilis* site at ~350m was nearer to the coast (near Anglesea, 38°24'S 144°11'E) than all the 6-teat sites that we sampled. Thus, the strong association between some of the morphological differences identified here and teat-number appears to be somewhat independent of altitude and latitude.

Interestingly, *A. stuartii* are generally larger near the coast than in inland mountainous areas (Dickman *et al*. 1998) and the body size and mass of both *A. agilis* and *A. swainsonii* are generally smaller at higher than at lower altitudes in areas in and around the A.C.T. (Dickman *et al.* 1983; 1988; 1998). It is not clear whether numbers of teats also differed among the samples in some of these studies but we know that coastal antechinus generally have fewer teats than inland individuals. Perhaps some of the intraspecific clinal variation previously noted in body dimensions for these species may have also been associated with intraspecific teat-number differences (Crowther 2002a). Similarly, Wakefield and Warneke (1963) found that female *A. swainsonii* in Tasmania had a shorter mean pes length than those on the mainland. Perhaps not coincidentally, all of their Tasmanian samples were 6-teat individuals, whereas their entire mainland sample was of 8-teat individuals. An alternative explanation could be that local conditions play a significant role in 'driving' the morphological differentiation in not only teat-number, but also other associated morphological traits. For instance, differences in climate and habitat at both a large and a local (within the Otway Ranges) scale are associated with teat-number variation in *A. agilis* (Beckman *et al.* 2007).

Differences in pelage characteristics in *A. agilis*

For the practiced eye, differences in pelage markings among *A. agilis* in the Otways provide a reliable indication as to which teat-number phenotype an antechinus most likely belongs. However, using pelage colour as an indicator of affinity became markedly unreliable in areas where intermediate 7-, 8-, and 9-teat individuals were trapped in close proximity. These animals showed high variability in fur colour and clarity of pelage markings and tended to display intermediate or 'mixed' pelage features. This supports the contention that a link between pelage features and teat-number is present in Otway *A. agilis* and perhaps strengthens the argument that these individuals may be offspring produced from interbreeding between 6- and 10-teat parents.

As well as providing strong indications that there are between teat-number phenotype differences in morphological traits, this study has also demonstrated that differences occur within the phenotypes among populations from different geographic locations. Dickman *et al.* (1998) reported that no prominent ring of fur around the eye was evident in the *A. agilis* holotype, a male collected from the Brindabella Range in NSW, an area known to be inhabited by 10-teat individuals (Cockburn *et al.* 1983). In contrast, 'eye rings' were one of the defining features of 10-teat animals in our Otway samples. In addition, the present study has also demonstrated that 6-teat *A. agilis* from different areas show significant variation in some of the measured morphometric traits. That is, differences between the geographically disjunct, but genetically similar 6-teat Portland and Otways *A. agilis* populations (Beckman *et al.* 2007) provide evidence that the morphology of same teat-number animals varies geographically. This geographic variation within teat-number phenotypes supports the contention that a proportion of the variability among sites in the external morphology of antechinus is due to differences in habitats. Variation in morphological characteristics often occurs in a response to environmental gradients, and such trait plasticity can be particularly evident as differences in pelage colour and markings (Endler 1977).

Our research was limited in scope, but it demonstrates that there are intraspecific morphological differences in antechinus that are associated with teat-number variation which warrant further detailed investigation. Some of these morphological differences, such as the clear disparities in pelage markings evident between *A. agilis* teat-number phenotypes in the Otway Ranges, may yet be shown to be a highly localized phenomenon. More detailed investigations of skeletal parameters, such as cranial dimensions (particularly snout breadth in the case of *A. agilis*), and of tarsal pads, could be illuminating.

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