

# Pesticide use is linked to increased body size in a large mammalian carnivore

M. LETNIC<sup>1,2\*</sup> and M. S. CROWTHER<sup>3</sup>

<sup>1</sup>Centre for Ecosystem Science, University of New South Wales, Sydney, NSW 2052, Australia

<sup>2</sup>Evolution and Ecology Research Centre, University of New South Wales, Sydney, NSW 2052, Australia

<sup>3</sup>School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia

Received 29 March 2020; revised 7 May 2020; accepted for publication 11 May 2020

Pollution and pesticide use have been linked to evolution of chemical resistance and phenotypic shifts in invertebrates, but less so in vertebrates. Here we provide evidence that poisoning directed towards a mammalian carnivore, the dingo (*Canis dingo*), is linked to an increase in dingo body mass. We compared the skull length of dingoes, a proxy for size, from three regions where dingo populations were controlled by distributing poisoned meat baits and an unbaited region, before and after the introduction of the toxin sodium fluoroacetate (Compound 1080). Following 1080 introduction, dingo skull length increased in baited regions but not in the unbaited region. We estimate that after 1080 introduction, the skull length of female and male dingoes in baited regions increased by 4.49 and 3.6 mm, respectively. This equates to a 1.02- and 0.86-kg increase in mean body masses of female and male dingoes, respectively. We hypothesize that dingo body size has increased in baited regions due to 1080 selecting for animals with larger body size or because a reduction in dingo abundance in baited areas may have removed constraints on growth imposed by intraspecific competition and prey availability. Our study provides evidence that pesticide use can prompt phenotypic change in comparatively large and long-lived large vertebrates.

ADDITIONAL KEYWORDS: 1080 – dingo – large carnivore – pesticide – phenotypic change.

## INTRODUCTION

Human activities can impose strong selection pressure on species and in doing so drive rapid phenotypic change (Darimont *et al.*, 2009). Such changes can represent both genetic change and phenotypic plasticity, and may be abrupt because novel environmental changes can impose strong directional selection. Documented drivers of rapid phenotypic change in vertebrates include selective harvesting of populations (Conover & Munch, 2002; Coltman *et al.*, 2003; Conover *et al.*, 2009), introductions of invasive species (Phillips & Shine, 2004), environmental changes resulting from climate shifts (Millien *et al.*, 2006; Ozgul *et al.*, 2010) and shifts in the availability of food resources due to human activities (Yom-Tov, 2003).

Pesticides and pollutants are particularly well-documented environmental drivers of phenotypic change (Hoffmann & Willi, 2008). A consequence of selective mortality resulting from pollution and

pesticide use has been the rapid evolution of chemical resistance and changes in morphology (McKenzie & Batterham, 1994; Cook *et al.*, 2012). That pesticides and pollutants can drive phenotypic changes has been well documented among invertebrates but there are comparatively few instances of vertebrates displaying phenotypic changes following exposure of their populations to pesticides and pollutants (Greaves & Ayres, 1969; Twigg *et al.*, 2002).

Australia's largest terrestrial predator, the dingo (*Canis dingo*) is a pest to livestock producers because they attack livestock, particularly sheep (Letnic *et al.*, 2012). Many methods including shooting, trapping, exclusion fencing and poisoning have been used to control dingo populations (Letnic *et al.*, 2012). Since the 1960s–1970s, the distribution of meat baits impregnated with the tasteless and odourless poison sodium monofluoroacetate (hereafter referred to as 1080) has become a widespread method to control dingo populations (Allen & Sparkes, 2001). Dingoes readily consume meat baits and the periodic (typically once or twice per year) distribution of 1080 meat baits

\*Corresponding author. E-mail: [m.letnic@unsw.edu.au](mailto:m.letnic@unsw.edu.au)

from aircraft or at places where dingoes frequent has proven to be an effective way to suppress dingo populations. This is because baits impregnated with 1080 are more palatable and thus more effective at controlling dingo populations than baits containing the bitter-tasting poison strychnine (Best *et al.*, 1974; Allen & Sparkes, 2001). In addition, baiting is relatively inexpensive compared to labour-intensive approaches such as trapping and shooting.

Following initial introduction of the 1080 toxin, dingo populations were reported to have declined dramatically and to such an extent that the use of other methods of control rapidly waned (Allen & Sparkes, 2001). Further evidence of the effectiveness of 1080 baiting is provided by studies reporting short-term reductions of dingo populations by 50–100% following baiting campaigns (McIlroy *et al.*, 1986; Thomson, 1986, 1992) and concomitant increases in the abundance of dingoes' preferred prey, kangaroos, in baited regions due to relaxation of dingoes' predatory effects on their populations (Letnic & Crowther, 2013).

Historically, the dose of 1080 contained within meat baits has varied through time and between administrative regions. Today, baits typically contain 6 mg of 1080. The dose of 1080 required to kill a dingo is dependent on body mass, with larger animals needing to ingest more poison to receive a lethal dose (McIlroy, 1986). The relationship between the dose of 1080 required to kill a dingo and body mass can be described by the relationship  $LD_{50} \text{ (mg kg}^{-1}\text{)} = -0.478 \cdot \ln \text{ body weight (kg)} + 2.16$  (McIlroy, 1986). The recommended dose of 6 mg

of 1080 in meat baits is considered to be lethal to all dingoes that entirely ingest freshly laid baits (Fleming *et al.*, 2001). However, the amount of poison contained within baits may decline after they are laid due to leaching and degradation by micro-organisms (Fleming *et al.*, 2001). Thus, in environments where 1080 is used, larger animals may have a selective advantage over smaller animals because baits need to contain more poison to kill larger individuals than smaller ones.

Here, we test the hypothesis that the distribution of meat baits impregnated with 1080 has driven an increase in body size in dingoes. We did this by comparing a proxy measurement of body size, skull length, before and after the introduction of 1080 baiting in three regions of arid Australia where dingo populations were subject to poisoning and a region where poisoning did not occur. If 1080 baiting has driven an increase in the body size of dingoes, we predicted that the size of dingoes should increase following the introduction of 1080 in baited regions but not the unbaited region; also, the increase in body size should be greater in females than males because male dingoes tend to be larger than female dingoes (Corbett, 1995) and hence should be more resistant to 1080. We also tested alternative hypotheses to explain differences in the size of dingo skulls in arid Australia through time (Table 1), including that skull size was a function of food resource availability during ontogeny (Yom-Tov & Geffen, 2011), and that skull size was best predicted by the year of collection, a proxy for climate change (Millien *et al.*, 2006; Sheridan & Bickford, 2011).

**Table 1.** Hypotheses tested and AIC values for linear mixed effects models investigating trends in the length of dingo skulls from three regions of Australia between 1930 and the present. The predictor variables were sex (male, female), average annual temperature (temp), rainfall in the 3 years preceding collection (rainfall), baiting (pre-1080, post-1080) and year (the year of collection).

Hypothesis tested	Terms	AIC	$\Delta$ AIC	Rank
1080 baiting has resulted in an increase in the size of dingoes more so for females than males	Sex × baiting	2295.196	0	1
Male dingoes are larger than female dingoes and baiting has resulted in an increase in the size of both male and female dingoes	Sex + baiting	2298.205	3.005	2
Baiting has resulted in an increase in the skull length of dingoes	Baiting	2413.025	117.825	3
Null model	Intercept	2431.657	136.457	4
Skull size positively correlates with rainfall during ontogeny because rainfall is correlated positively with ecosystem productivity	Rainfall	2439.371	144.171	5
Increases in temperatures associated with global warming should result in a decrease in the body size of dingoes	Year	2414.243	119.043	6

## MATERIAL AND METHODS

## STUDY REGIONS AND INFORMATION ON 1080 USAGE

We measured the condylobasal length (CBL) of dingo skulls from four regions in arid Australia (Fig. 1: receiving <350 mm mean annual rainfall). We restricted our analysis to regions where a reasonable sample size ( $N > 20$ ) of dingo skulls was available both before (1930 to the year that baiting commenced) and after the commencement of 1080 baiting (Table 2). We restricted our analyses to skulls from arid regions because the incidence of hybridization between dingoes and domestic dogs has been very low in these regions (Newsome *et al.*, 2013; Stephens *et al.*, 2015). Although for some regions we were able to obtain measurements for skulls that pre-dated 1930 and in some case pre-dated European settlement, we chose the year 1930 as the commencement data of the pre-1080 period. We did this because livestock grazing had commenced in each of the pastoral regions by this time and we were able to obtain measurements of skulls and estimates of annual rainfall from this time-period for all regions.

The bounds of the three baited regions (Kalgoorlie, Pilbara and South Australian pastoral) were determined by administrative boundaries for government agencies that provide support, including administration of the use of 1080 poison, for rangeland livestock producers (Fig. 1; Supplementary Information). The distribution of meat

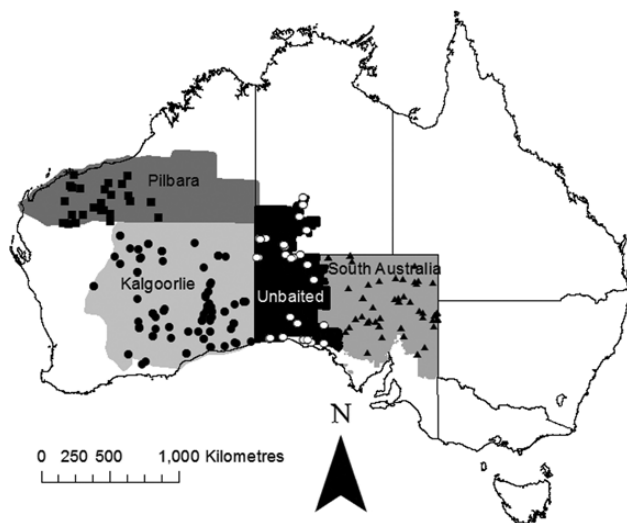
baits containing 1080 was introduced to the Kalgoorlie and Pilbara regions of Western Australia in 1964, and to the pastoral region of South Australia in 1972 (Supplementary Information). In the Kalgoorlie and Pilbara regions, the distribution of 1080-impregnated meat baits was conducted using both aircraft and the distribution of baits from the ground. In South Australia, poison baiting campaigns were conducted primarily by distributing 1080-impregnated baits from the ground in areas frequented by dingoes such as livestock watering points and along vehicular tracks.

The unbaited region comprised the western portions of the Australian jurisdictions of South Australia and Northern Territory (Fig. 1). This region consists of indigenous-owned lands and conservation reserves that have not been used for commercial livestock grazing. A dingo-proof fence exists along the southern boundary of this region and much of the eastern boundary (Letnic *et al.*, 2012) and thus limits the movement of dingoes between the unbaited region and adjoining South Australian pastoral region. We could find no documented evidence of systematic 1080 baiting being conducted in this region. However, in the past dingo populations in this region were harvested by hunters to obtain bounty payments.

## MEASUREMENTS OF DINGO SKULLS AND BODY MASS

To investigate changes in skull size through time in three baited regions and the unbaited region, we measured CBL of 559 dingo skull specimens held in museums for which data on the date of collection and provenance were available (Supporting Information, Fig. S1). This sample represented all of the skulls with provenance data that we could locate from each region that were held in the collections of the Australian Museum, Sydney, New South Wales; The Australian National Wildlife Collection, CSIRO, Canberra, ACT; National Museum of Victoria, Melbourne, Victoria; Queensland Museum, Brisbane, Queensland; South Australian Museum, Adelaide, South Australia; Western Australian Museum, Perth, Western Australia; Western Australia Vertebrate Pest Unit; and Centre for Ecosystem Science, University of New South Wales. Of these specimens, 345 from baited regions also had information on their sex. Only adult specimens were measured. Specimens were classified as adults if all the 'adult' teeth were fully erupted and the bone sutures of the skull were fused (Jones, 1990). All measurements were made to the nearest 0.01 mm using Mitsuoyo digital callipers.

For many mammals, CBL can be used as a proxy for body mass because the two measures are correlated (Ralls & Harvey, 1985). To validate the use of CBL as a proxy for body mass in dingoes we performed linear regression of CBL vs. body mass for 534 skull specimens from arid regions (<350 mm annual rainfall)



**Figure 1.** Collection locations of 559 dingo skulls from three baited regions (Kalgoorlie, shaded circles; Pilbara, squares; South Australian pastoral, triangles) and an unbaited region (open circles) used in our analyses comparing condylobasal length of dingo skulls before and after the introduction of 1080 baiting. The areas of the regions are indicated by shading (Kalgoorlie = light grey, South Australian pastoral = medium grey, Pilbara = dark grey, Unbaited region = black).

**Table 2.** The number of skulls from the pre- and post-1080 periods for which condylobasal length (CBL) was measured in three baited regions (Kalgoorlie, Pilbara and South Australian) and an unbaited region, and the difference between the mean, 25<sup>th</sup> percentile and 75<sup>th</sup> percentile of CBL for skulls collected during the pre-1080 and post-1080 periods. For the unbaited region the date of 1080 introduction has been specified first as the year 1964 when 1080 was introduced in the adjacent Kalgoorlie and Pilbara regions and second as 1972 when 1080 was introduced in the adjacent South Australian pastoral region. *P* represents the statistical significance of permutation tests comparing the difference in values of statistics for the pre- and post-1080 periods. Significant ( $P < 0.05$ ) values are in bold type.

Region	Sex	$N_{pre}$	$N_{post}$	Statistic	Difference (mm)	<i>P</i>
Kalgoorlie	All	106	79	Mean	3.52	<b>&lt;0.001</b>
				25th Percentile	3.14	<b>0.035</b>
				75th Percentile	4.04	<b>0.003</b>
Kalgoorlie	Female	18	25	Mean	3.99	<b>0.049</b>
				25th Percentile	3.06	0.105
				75th Percentile	2.91	0.188
Kalgoorlie	Male	22	29	Mean	3.86	<b>0.018</b>
				25th Percentile	4.73	<b>0.039</b>
				75th Percentile	1.99	0.101
Pilbara	All	26	162	Mean	4.29	<b>0.01</b>
				25th Percentile	6.33	<b>0.044</b>
				75th Percentile	4.26	0.050
Pilbara	Female	9	60	Mean	3.8	0.056
				25th Percentile	3.78	<b>0.047</b>
				75th Percentile	2.47	0.247
Pilbara	Male	11	93	Mean	3.32	0.070
				25th Percentile	5.70	<b>0.046</b>
				75th Percentile	2.71	0.141
South Australia	All	83	55	Mean	5.07	<b>&lt;0.001</b>
				25th Percentile	7.89	<b>&lt;0.001</b>
				75th Percentile	3.86	0.077
South Australia	Female	31	8	Mean	9.78	<b>&lt;0.001</b>
				25th Percentile	10.78	<b>&lt;0.001</b>
				75th Percentile	8.42	<b>0.008</b>
South Australia	Male	28	11	Mean	0.99	0.307
				25th Percentile	0.86	0.425
				75th Percentile	-0.84	0.594
Unbaited (1964)	All	15	33	Mean	0.35	0.431
				25th Percentile	1.23	0.283
				75th Percentile	0.62	0.459
Unbaited (1972)	All	23	25	Mean	-0.51	0.431
				25th Percentile	-0.43	0.276
				75th Percentile	0.60	0.452

of Australia held by the Australian National Wildlife Collection for which we were able to measure CBL (as described above) and obtain data on the mass at the time of the individuals' death (Fig. 2).

#### ENVIRONMENTAL VARIABLES

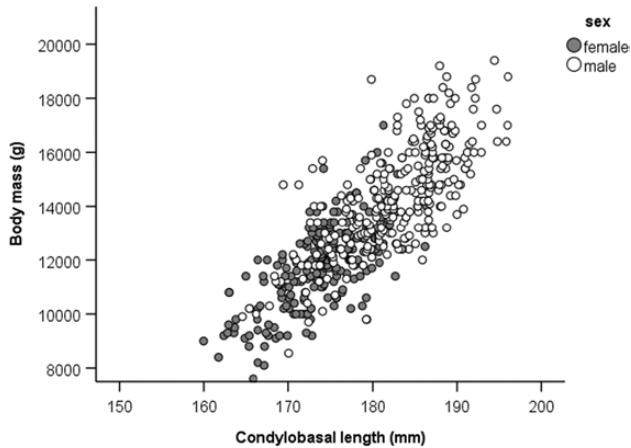
The mean annual rainfall (mm) received at the location of each dingo specimen in the 3 years before collection was derived from a digital model of monthly rainfall for Australia from the Bureau of Meteorology (<http://www.bom.gov.au/>). The resolution of the rainfall grid was 25 km. We chose a period of 3 years before

collection as a proxy for primary productivity because wild dingoes in regions where their populations are controlled in arid Australia rarely live for more than 3 years (Thomson, 1992), and because rainfall is a strong driver of arid zone primary productivity and in turn the growth and population dynamics of vertebrates, including dingoes, in arid Australia.

#### STATISTICAL ANALYSIS

Although we are unaware of biases, it is possible that the skulls in museum collections that we measured may not represent random samples of dingo





**Figure 2.** Relationship between condylobasal length (mm) and body mass (g) for 530 live-caught dingoes inhabiting areas of Australia that receive less than 350 mm average annual rainfall. Linear regression revealed a positive relationship between CBL (mm) and mass of dingoes (g) for the entire dataset [linear regression CBL (mm) vs. body mass (g):  $R^2 = 0.665$ ;  $F_{1,528} = 1047.681$ ;  $P < 0.001$ , eqn 1  $y = 254.283x - 32222.732$ ], males [linear regression CBL (mm) vs. body mass (g):  $R^2 = 0.521$ ;  $F_{1,285} = 308.531$ ;  $P < 0.001$ , eqn 2  $y = 236.755x - 28875.718$ ] and females [linear regression CBL (mm) vs. body mass (g):  $R^2 = 0.520$ ;  $F_{1,242} = 260.592$ ;  $P < 0.001$ , eqn 3  $y = 227.841x - 27781.069$ ].

populations at the time of collection. This is because we do not know if collectors targeted particular dingo phenotypes or if dingoes with certain phenotypes were more susceptible to the methods used by collectors. Hence, we performed a two-sample randomization test (Manly, 2006) to compare the differences between the means and the 25<sup>th</sup> and 75<sup>th</sup> percentiles for the distribution of CBL for all skulls, including skulls for which information on their sex was not available, before and after the commencement of baiting from the three baited regions and the unbaited region. We performed our permutation tests twice for the unbaited region to determine if there was a difference in CBL following the introduction of 1080 in the neighbouring pastoral regions of South Australia and Western Australia, respectively. In the first analysis, we specified the date of 1080 introduction in South Australia (1972) as the commencement of the post-1080 period. In the second analysis, we specified the date of 1080 introduction in the Kalgoorlie and Pilbara regions of Western Australia (1964) as the commencement of the post-1080 period. We also conducted separate permutation tests on male and female dingoes before and after the commencement of baiting in the three baited regions (Kalgoorlie, Pilbara and South Australian pastoral), but not the unbaited region because there were too few specimens with information on their sex from this

region. For all permutation tests, we conducted 10 000 randomizations (sampling with replacement) in a Monte Carlo bootstrapped simulation of the dataset to calculate a probability distribution and associated  $P$ -value (Manly, 2006) in PopTools (Hood, 2010).

We used linear mixed effects models with a Gaussian distribution to further explore the relative strength of hypotheses concerning the effects that the introduction of 1080 baiting (pre-baiting/post-baiting), primary productivity (average rainfall in the 3 years before specimen collection) and year of specimen collection had on the length of dingo skulls for which information on their sex was known for the three baited regions.

Spatial autocorrelation occurs when the value of a variable at any one location in space can be predicted by the values of nearby locations. We included Administrative Region as a random term in our linear mixed effects models to account for spatial autocorrelation in the models because we knew a priori that the body size and CBL of dingoes varied between administrative regions (Corbett, 1995; Radford *et al.*, 2012).

To determine if there was unexplained spatial autocorrelation in the fitted model, we tested for spatial autocorrelation in the Pearson residuals of the top-ranking model using Moran's  $I$  in the 'APE' package for R (Paradis *et al.*, 2004). Calculations of Moran's  $I$  of the residuals revealed no spatial autocorrelation in the model's residuals (Moran's  $I = -0.006$ ,  $Z = -0.486$ ,  $P = 0.62$ ).

Because larger dingoes are more resistant to 1080 and because male dingoes are larger than female dingoes, we hypothesized that 1080 baiting has selected for larger body size in females more so than in males. If this was the case, then we would expect that the shift in the skull length of female specimens following the introduction of 1080 would be greater in females than in males. To test this hypothesis, we ran a model with a term specifying the interaction between the introduction of 1080 and sex. We also tested alternative models to explain variation in the skull length of sexed dingo specimens (Table 1). We used the package SPSS v.24.

The candidate models for both datasets were ranked using Akaike's information criterion (AIC) (Burnham & Anderson, 2002). We selected the best models by comparing AIC differences ( $\Delta_i$ ). Models with an AIC difference ( $\Delta_i < 2$  units relative to the model with the lowest AIC ( $AIC_{min}$ )) have substantial support, whilst those with  $\Delta_i > 10$  are not supported (Burnham & Anderson, 2002).

## RESULTS

The mean CBL of all dingo skulls, irrespective of their sex, increased following the introduction of

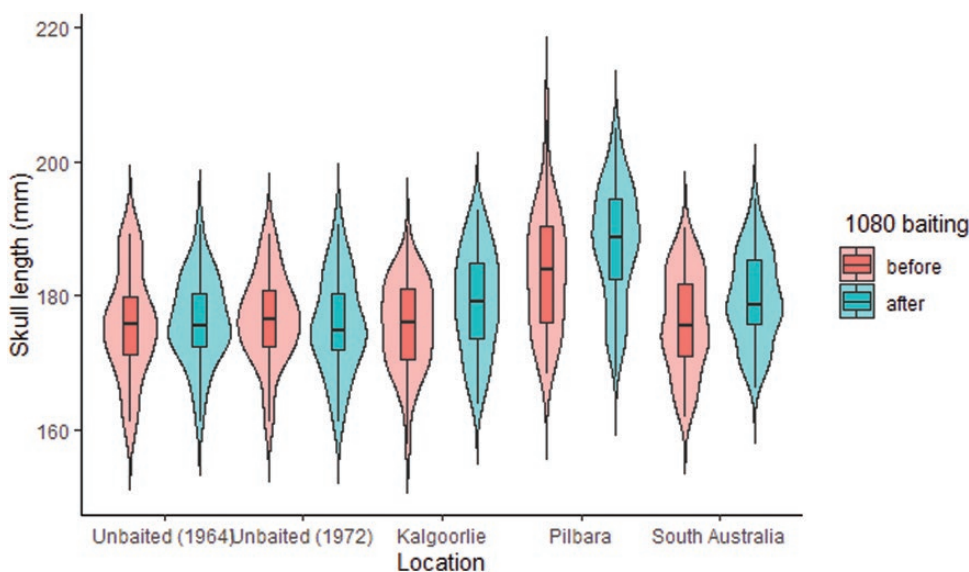
1080 in the three baited regions, but did not increase in the unbaited region following the commencement of 1080 baiting in adjacent pastoral regions (Fig. 3; Table 2). The 25<sup>th</sup> percentile of CBL increased in each of the baited regions following the introduction of 1080 (Fig. 3; Table 2). For the baited Kalgoorlie region there was an increase in the 75<sup>th</sup> percentile of the distribution of CBL after 1080 was introduced (Fig. 3; Table 2). For the baited Pilbara and South Australian pastoral regions, the increase in the 75<sup>th</sup> percentile of the distribution of CBL approached significance at  $P < 0.05$  (Table 2). In the unbaited region, there was no difference in the 25<sup>th</sup> and 75<sup>th</sup> percentiles of the distribution of dingo skulls between the pre- and post-1080 periods irrespective of whether the date for 1080 introduction was specified as that in the neighbouring pastoral regions of South Australia or Western Australia (Fig. 3; Table 2).

An increase in the mean CBL of dingo skulls following the introduction of 1080 was evident for both male and female dingoes from the Kalgoorlie region and female dingoes from the South Australian pastoral region (Table 2; Fig. 4). In the Pilbara pastoral region there was a tendency for both male and female dingo skulls to be longer in the post-1080 period, but for both male and female dingoes the permutation test was not significant at  $P = 0.05$  (Table 2; Fig. 4). Further

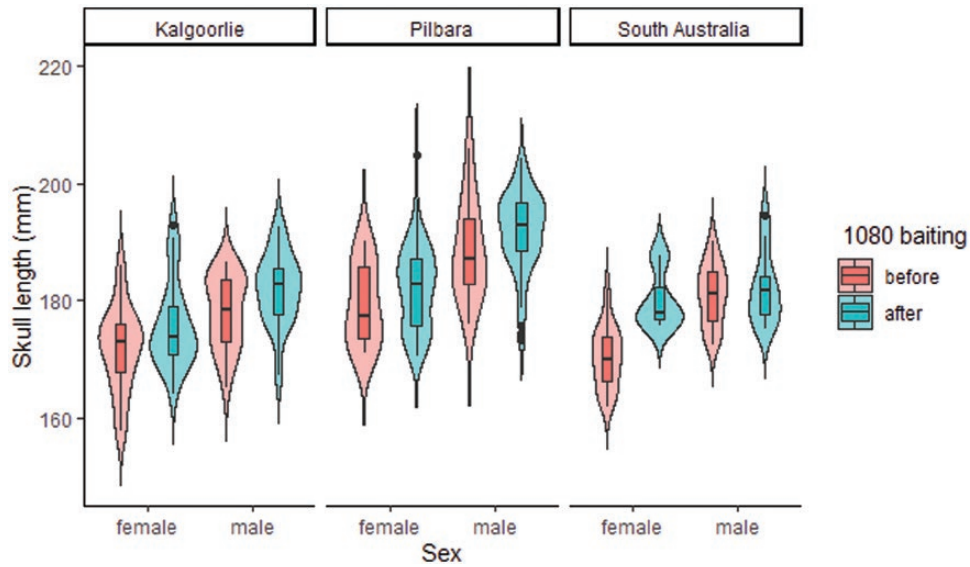
evidence for shifts in the size distribution of skulls was an increase in the 25<sup>th</sup> percentile of the distribution of CBL for female dingoes from the Pilbara and South Australian pastoral regions and for males from the Kalgoorlie and Pilbara pastoral regions (Table 2; Fig. 4). The 75<sup>th</sup> percentile of CBL for female dingoes from the South Australian pastoral region increased in the post-1080 period (Table 2; Fig. 4).

Our linear mixed effects models predicted that the increase in the length of dingo skulls following the introduction of 1080 was greater for female than for male dingoes. The top-ranked model (Tables 1, 3) was that containing the interaction term between commencement of baiting ( $F_{3,341} = 52.670$ ,  $P < 0.001$ ) and sex, followed by the additive model, baiting + sex ( $\Delta_i$  AIC = 3.009). None of the other models was well supported ( $\Delta_i$  AIC > 10; Table 1). The parameter estimates for the top-ranked model all excluded zero (Table 3).

Fitted values of the top-ranked model estimated that the mean CBL of females increased by 4.49 mm in the post-baiting period [contrast,  $t = 3.664$ ,  $P < 0.0001$ ; before, mean ( $\pm$ SE) CBL =  $173.44 \pm 2.81$  mm; after, mean CBL =  $177.93 \pm 2.76$  mm; Fig. S2], and that mean CBL of males increased by 3.6 mm (contrast,  $t = 3.187$ ,  $P < 0.002$ ; before, mean CBL =  $182.46 \pm 2.80$  mm; after, mean CBL =  $186.10 \pm 2.74$  mm; Fig. S2).



**Figure 3.** Violin-plot of the skull length (condylobasal length, mm) of dingo skulls in the pre- and post-1080 periods from the baited Kalgoorlie (pre-1080  $N = 106$ , post-1080  $N = 79$ ), Pilbara (pre-1080  $N = 26$ , post-1080  $N = 162$ ) and South Australian pastoral (pre-1080  $N = 83$ , post-1080  $N = 55$ ) regions and the unbaited region. For the unbaited region the comparison of the pre- and post-1080 periods has been specified first as the year 1964 when 1080 was introduced in the adjacent Kalgoorlie and Pilbara regions (pre-1080  $N = 15$ , post-1080  $N = 33$ ) and second as 1972 when 1080 was introduced in the adjacent South Australian pastoral region (pre-1080  $N = 23$ , post-1080  $N = 25$ ). Lines within boxes indicate medians, boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and bars indicate 1.5 times the interquartile range.



**Figure 4.** Violin-plot of skull length (condylobasal length, mm) of male and female dingo skulls in the pre-1080 and post-1080 periods from the Kalgoorlie (male pre-1080  $N = 22$ , post-1080  $N = 29$ ; female pre-1080  $N = 18$ , post-1080  $N = 25$ ), Pilbara (male pre-1080  $N = 11$ , post-1080  $N = 93$ ; female pre-1080  $N = 9$ , post-1080  $N = 60$ ) and South Australian pastoral (male pre-1080  $N = 28$ , post-1080  $N = 11$ ; female pre-1080  $N = 31$ , post-1080  $N = 8$ ) regions. Lines within boxes indicate medians, boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and bars indicate 1.5 times the interquartile range. Outliers are represented by dots.

**Table 3.** Model parameters and their standard errors (SE),  $t$  statistics, 95% confidence intervals and  $P$  values from the most parsimonious linear mixed effects model (sex  $\times$  baiting onset) examining factors influencing the condylobasal length of dingo skulls in three regions of arid Australia between 1930 and the present.

Model term	Coefficient	SE	$t$	$P$	Lower 95% CI	Upper 95% CI
Intercept	182.464	2.978	65.202	<0.001	176.959	187.968
Post-1080 $\times$ female	-4.530	1.190	-3.805	<0.001	-6.871	-2.188
Post-1080 $\times$ male	3.633	1.140	3.187	0.002	1.391	5.876
Pre-1080 $\times$ female	-9.029	1.233	-7.323	<0.001	-11.454	-6.604
Pre-1080 $\times$ male	0*					

\*Set to zero.

## DISCUSSION

Our study provides support for the notion that pesticide use can engender phenotypic change in a relatively large-bodied mammalian predator. Consistent with our hypothesis that 1080 usage may lead to increased body mass in dingoes, the mean CBL of all dingo skulls, irrespective of their sex, increased following the introduction of 1080 in the three baited regions, but did not increase in the unbaited region over the same time-period. Also in agreement with our hypothesis that 1080 baiting may drive an increase in the body mass of dingoes, an increase in the mean CBL of dingo skulls following the introduction of 1080 was evident for both male and female dingoes from

the Kalgoorlie region, and for female dingoes from the South Australian pastoral region. Further evidence for shifts in the size distribution of skulls came from an increase in the 25<sup>th</sup> percentile of the distribution of CBL for female dingoes from the Pilbara and South Australian pastoral regions and for males from the Kalgoorlie and Pilbara pastoral regions.

Applying eqn 1 for the relationship between CBL and body mass for dingoes (Fig. 2), the average increase in skull length of dingoes following the introduction of 1080 equates to a body mass increase of 0.90, 0.85 and 1.09 kg for the baited Kalgoorlie, South Australian and Pilbara pastoral regions, respectively. Applying eqns 2 and 3 for the relationship between CBL and

body mass for male and female dingoes (Fig. 2), the increase in skull length predicted by the top-ranked linear mixed effects model equates to an increase in the mass of females and males of 1.02 and 0.86 kg, respectively. In the post-1080 period we estimate that the average mass of male (pre-1080 period = 14.3 kg, post-1080 period = 15.2 kg) and female (pre-1080 period = 11.7 kg; post-1080 period = 12.8 kg) dingoes increased by 6.0% and 8.7% in the pre-1080 period, respectively. Due to the mass–dose relationship of the 1080 toxin, such increases in body mass would mean that, on average, the dose of 1080 required to kill a dingo in the baited regions has increased since the introduction of the 1080 toxin.

The results of the linear mixed effects models suggest that the increase in the length of dingo skulls following the introduction of 1080 baiting was greater for female than for male dingoes. They do not, however, provide support for the hypotheses that the body size of dingoes was influenced by rainfall during ontogeny or climatic variables that may have shifted through time due to climate change. The finding that female dingoes have increased in size more than male dingoes is consistent with the hypothesis that 1080 baiting selects for larger dingoes because they are more resistant to the toxin. However, there was considerable overlap in the size distributions of dingoes from the pre- and post-1080 periods, and our ability to make inferences concerning relative differences in the sizes of sexes before and after the introduction of 1080 is limited due to the small sample sizes for individuals with known sex in some of the regions. In addition, our finding that the shift in body size of dingoes in the Pilbara pastoral region was comparable to that in the South Australian and Kalgoorlie pastoral regions goes against the hypothesis that 1080 baiting has selected for larger dingoes. This is because dingoes from the Pilbara pastoral region were on average larger than those from the two other baited regions during the pre-1080 period, but if selection for larger body size by 1080 was the driver of the shift in dingo body size, the change in body mass in the post-1080 period should have been greatest where dingoes were smallest because smaller dingoes are more vulnerable to the toxin.

Another plausible explanation for the shift in body size of dingoes following 1080 introduction is greater growth during ontogenesis due to reduced intraspecific competition for food resources and greater availability of prey. The purpose and practical effect of 1080 baiting programmes is to suppress dingo populations (Fleming *et al.*, 2001; Leo *et al.*, 2019). One hypothesized consequence of reduced density of conspecifics for the dingoes that survive baiting campaigns is that intraspecific competition for food resources will be reduced and ease constraints on

growth during ontogenesis. Support for this hypothesis comes from studies which have linked shifts in the intensity of intraspecific competition to shifts in body size (Bolnick, 2004; Segers & Taborsky, 2012). Support for the idea that dingoes in baited regions will have better access to food resources comes from studies conducted in arid Australia showing that the abundance of kangaroos and frequency of kangaroos in the diet of dingoes both increase in areas where dingo populations are suppressed (Letnic *et al.*, 2012; Letnic & Crowther, 2013; Morris & Letnic, 2017). For example, Morris & Letnic (2017) found that dingoes living at low densities in baited areas where kangaroo abundance was high consumed kangaroos more frequently than dingoes living at high densities in unbaited areas where kangaroos occurred at very low densities.

Because we have not conducted a manipulative experimental study, we cannot rule out the possibility that the shift in dingo skull length observed in baited regions could have been due to other factors that coincided with the introduction of the 1080 toxin. For example, previous studies have put forward hybridization with domestic dogs to explain shifts in body size of dingoes. This is because dingoes can interbreed with domestic dogs and many domestic dog breeds are larger than dingoes (Claridge *et al.*, 2014). Hybridization between dingoes and domestic dogs has been widespread near densely populated regions of Australia such as the forests of south-eastern Australia (Cairns *et al.*, 2020). However, the hypothesis that hybridization was a driver of the increase in skull size observed in this study is not well supported because the incidence of hybridization between dingoes and dogs has been low in the sparsely populated arid regions of Australia, where the specimens examined in this study were obtained (Stephens *et al.*, 2015).

Our study is significant because few studies have linked phenotypic changes in vertebrate populations to control of their populations using pesticides. However, that 1080 baiting may be driving shifts in the body size of dingoes is not altogether surprising because poisoning of dingo populations is effectively a form of harvest and there are numerous examples of harvesting driving shifts in the body size of vertebrates (Coltman *et al.*, 2003; Darimont *et al.*, 2009). In most documented instances, shifts in body size resulting from hunting and fishing have been towards smaller body sizes due to size-selective harvesting leading to the dominance of smaller individuals that are targeted less frequently by harvesters and or selection for smaller size at maturity (Milner *et al.*, 2007). Although we are uncertain of the mechanism that has driven the increase in body size of dingoes following the introduction of the 1080 toxin, a key implication of our study is that phenotypic change



in response to pesticide use may not be limited to invertebrates or small vertebrates but may also occur in comparatively large vertebrates. We recommend that further studies are conducted to understand why the average size of dingoes has increased since the introduction of the 1080 toxin.

### ACKNOWLEDGEMENTS

Funding was provided by the Australia Pacific Science Foundation. N. Colman assisted with measurements. We thank the many staff from museums for providing access to their collections. We thank Marco Festa-Bianchet, Fabio Andrade Machado and two anonymous reviewers for their helpful comments on the manuscript. We have no conflict of interest to declare.

### REFERENCES

- Allen LR, Sparkes EC. 2001.** The effect of dingo control on sheep and beef cattle in Queensland. *Journal of Applied Ecology* **38**: 76–87.
- Best L, Corbett L, Stephens D, Newsome A. 1974.** Baiting trials for dingoes in Central Australia, with poison '1080', encapsulated strychnine, and strychnine suspended in methyl cellulose. *CSIRO Australian Division of Wildlife Research Technical Paper* **30**: 1–7.
- Bolnick DI. 2004.** Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution* **58**: 608–618.
- Burnham KP, Anderson DR. 2002.** *Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn.* New York: Springer.
- Cairns KM, Nesbitt BJ, Laffan SW, Letnic M, Crowther MS. 2020.** Geographic hot spots of dingo genetic ancestry in southeastern Australia despite hybridisation with domestic dogs. *Conservation Genetics* **21**: 77–90.
- Claridge AW, Spencer RJ, Wilton AN, Jenkins DJ, Dall D, Lapidge SJ. 2014.** When is a dingo not a dingo? Hybridisation with domestic dogs. In: Glen AS, Dickman CR, eds. *Carnivores of Australia: past, present and future.* Collingwood: CSIRO Publishing, 151–172.
- Coltman DW, O'Donoghue P, Jorgenson JT, Hogg JT, Strobeck C, Festa-Bianchet M. 2003.** Undesirable evolutionary consequences of trophy hunting. *Nature* **426**: 655–658.
- Conover DO, Munch SB. 2002.** Sustaining fisheries yields over evolutionary time scales. *Science* **297**: 94–96.
- Conover DO, Munch SB, Arnott SA. 2009.** Reversal of evolutionary downsizing caused by selective harvest of large fish. *Proceedings of the Royal Society of London B: Biological Sciences* **276**: 2015–2020.
- Cook L, Grant B, Saccheri I, Mallet J. 2012.** Selective bird predation on the peppered moth: the last experiment of Michael Majerus. *Biology Letters* **8**: 609–612.
- Corbett LK. 1995.** *The dingo in Australia and Asia.* Sydney: University of New South Wales Press.
- Darimont CT, Carlson SM, Kinnison MT, Paquet PC, Reimchen TE, Wilmers CC. 2009.** Human predators outpace other agents of trait change in the wild. *Proceedings of the National Academy of Sciences USA* **106**: 952–954.
- Fleming P, Corbett L, Harden R, Thompson P. 2001.** *Managing the impacts of dingoes and other wild dogs.* Canberra: Bureau of Resource Science.
- Greaves JH, Ayres P. 1969.** Linkages between genes for coat colour and resistance to warfarin in *Rattus norvegicus*. *Nature* **224**: 284–285.
- Hoffmann AA, Willi Y. 2008.** Detecting genetic responses to environmental change. *Nature Reviews Genetics* **9**: 421–432.
- Hood GM. 2010.** *PopTools version 3.2.5.* Available at: <http://www.poptools.org>.
- Jones E. 1990.** Physical characteristics and taxonomic status of wild canids, *Canis familiaris*, from the Eastern Highlands of Victoria. *Wildlife Research* **17**: 69–81.
- Leo V, Reading RP, Gordon C, Letnic M. 2019.** Apex predator suppression is linked to restructuring of ecosystems via multiple ecological pathways. *Oikos* **128**: 630–639.
- Letnic M, Crowther MS. 2013.** Patterns in the abundance of kangaroo populations in arid Australia are consistent with the exploitation ecosystems hypothesis. *Oikos* **122**: 761–769.
- Letnic M, Ritchie EG, Dickman CR. 2012.** Top predators as biodiversity regulators: the dingo *Canis lupus* dingo as a case study. *Biological Reviews* **87**: 390–413.
- Manly BFJ. 2006.** *Randomization, bootstrap and Monte Carlo methods in biology.* Boca Raton: CRC Press.
- McIlroy J. 1986.** The sensitivity of Australian animals to 1080 poison. 9. Comparisons between the major groups of animals, and the potential danger nontarget species face from 1080 poisoning campaigns. *Wildlife Research* **13**: 39–48.
- McIlroy J, Cooper R, Gifford E, Green B, Newgrain K. 1986.** The effect on wild dogs, *Canis f. familiaris*, of 1080-poisoning campaigns in Kosciusko National Park, NSW. *Wildlife Research* **13**: 535–544.
- McKenzie JA, Batterham P. 1994.** The genetic, molecular and phenotypic consequences of selection for insecticide resistance. *Trends in Ecology & Evolution* **9**: 166–169.
- Millien V, Kathleen Lyons S, Olson L, Smith FA, Wilson AB, Yom-Tov Y. 2006.** Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters* **9**: 853–869.
- Milner JM, Nilsen EB, Andreassen HP. 2007.** Demographic side effects of selective hunting in ungulates and carnivores. *Conservation Biology* **21**: 36–47.
- Morris T, Letnic M. 2017.** Removal of an apex predator initiates a trophic cascade that extends from herbivores to vegetation and the soil nutrient pool. *Proceedings of the Royal Society B: Biological Sciences* **284**.
- Newsome TM, Stephens D, Ballard G-A, Dickman CR, Fleming PJS. 2013.** Genetic profile of dingoes (*Canis lupus dingo*) and free-roaming domestic dogs (*C. l. familiaris*) in the Tanami Desert, Australia. *Wildlife Research* **40**: 196–206.
- Ozgul A, Childs DZ, Oli MK, Armitage KB, Blumstein DT, Olson LE, Tuljapurkar S, Coulson T. 2010.** Coupled dynamics of body mass and population growth in response to environmental change. *Nature* **466**: 482–485.

- Paradis E, Claude J, Strimmer KJB. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.
- Phillips BL, Shine R. 2004.** Adapting to an invasive species: Toxic cane toads induce morphological change in Australian snakes. *Proceedings of the National Academy of Sciences, USA* **101**: 17150–17155.
- Radford CG, Letnic M, Fillios M, Crowther MS. 2012.** An assessment of the taxonomic status of wild canids in south-eastern New South Wales: phenotypic variation in dingoes. *Australian Journal of Zoology* **60**: 73–80.
- Ralls K, Harvey PH. 1985.** Geographic variation in size and sexual dimorphism of North American weasels. *Biological Journal of the Linnean Society* **25**: 119–167.
- Segers FH, Taborsky B. 2012.** Competition level determines compensatory growth abilities. *Behavioral Ecology* **23**: 665–671.
- Sheridan JA, Bickford D. 2011.** Shrinking body size as an ecological response to climate change. *Nature Climate Change* **1**: 401–406.
- Stephens D, Wilton AN, Fleming PJS, Berry O. 2015.** Death by sex in an Australian icon: a continent-wide survey reveals extensive hybridization between dingoes and domestic dogs. *Molecular Ecology* **24**: 5643–5656.
- Thomson P. 1986.** The effectiveness of aerial baiting for the control of dingoes in North-Western Australia. *Wildlife Research* **13**: 165–176.
- Thomson PC. 1992.** The behavioural ecology of dingoes in North-Western Australia: IV. Social and spatial organisation, and movements. *Wildlife Research* **19**: 543–563.
- Twigg LE, Martin GR, Lowe TJ. 2002.** Evidence of pesticide resistance in medium-sized mammalian pests: a case study with 1080 poison and Australian rabbits. *Journal of Applied Ecology* **39**: 549–560.
- Yom-Tov Y. 2003.** Body sizes of carnivores commensal with humans have increased over the past 50 years. *Functional Ecology* **17**: 323–327.
- Yom-Tov Y, Geffen E. 2011.** Recent spatial and temporal changes in body size of terrestrial vertebrates: probable causes and pitfalls. *Biological Reviews* **86**: 531–541.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Estimated number of 1080 baits and volume of 1080 solution distributed in the Kalgoorlie, Pilbara and South Australian pastoral regions per decade between commencement of baiting and 2009. Baiting commenced in 1964 in the Kalgoorlie and Pilbara pastoral regions and in 1972 in the South Australian pastoral regions.

**Figure S1.** Condylbasal length and year of collection for 554 dingo skulls held in museums from four regions of arid Australia for which data on the date of collection and provenance were available.

**Figure S2.** Predicted values of condylbasal length of dingo skulls of each sex from each administrative region ( $\pm 95\%$  confidence interval) generated from the parsimonious linear mixed effects model (sex  $\times$  baiting onset) examining factors influencing the condylbasal length of dingo skulls before and after the commencement of 1080 in three regions where baiting was conducted.