large pieces of prey whole[14,24]. Such observations of modern feeding behaviours have led to speculation that extinct theropods did little bone-crushing[18,25] and wasted a significant proportion of the food available from carcasses[26]. Tyrannosaur teeth appear to be stout enough to damage bone[27], however, and analyses of bite marks on *Triceratops* and *Edmontosaurus* bones indicate that *Tyrannosaurus* pulverized bones during feeding[28] and probably consumed bone fragments[29].

Although a single coprolite cannot be construed as representative of diet, this rare example of fossilized dietary residues helps to refine our understanding of theropod feeding behaviour by providing physical evidence that a tyrannosaur crushed, consumed, and incompletely digested large quantities of bone when feeding on a subadult dinosaur. It also presents a new search image for future discoveries of theropod faeces that will help us to elucidate the food habits of these giant meat-eaters. □

Methods

Bulk chemical analyses of the coprolite and host sediment (Table 1) were made on a Rigaku 3370 spectrometer by the staff of the GeoAnalytical Laboratories, Washington State University, using described procedures[30].

Mineralogical compositions of the bone and ground mass were examined with a Phillips V2.0 diffractometer at the University of California, Santa Barbara (scanning from 2–80° θ). Elemental analyses of these components (Table 2) were made on a JEOL 8900 microprobe at the US Geological Survey, Menlo Park. A 15 kV, 20 nA beam defocused to produce a spot size of 15 μm was used to analyse bone and ground mass; a 10 nA current was used for lacunae analyses. Natural minerals (Wilberforce apatite, Tiburon albite, strontianite, barite, San Carlos olivine, and sodalite) and synthetic materials (faylite, Mn_2O_3, An100, and GSC glass) were used as standards.

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Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems

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The importance of inbreeding depression in theoretical considerations of mating-system evolution[1–5] and its potential impact on the persistence of small populations[6] has renewed interest in the genetic basis of this phenomenon. Inbreeding increases homozygosity. This can produce inbreeding depression for two different reasons: first, deleterious recessive or partially recessive alleles that are masked at heterozygous loci by dominant alleles become fully expressed in homozygotes; and second, alleles may interact in an overdominant manner, such that the fitness of either type of homozygote is lower than that of heterozygotes. These two mechanisms produce different long-term effects in populations experiencing increased levels of inbreeding. Inbreeding depression resulting from deleterious alleles can be removed by selection, but inbreeding depression produced by overdominance cannot be removed without lowering the mean fitness of the population[1–5]. Using a North Carolina 3 breeding programme[7], the most powerful quantitative genetics technique available[8–10], we show here that deleterious recessive alleles are mainly responsible for inbreeding depression in two closely related annual plants, the primarily selfing *Mimulus micranthus* and the mixed-mating *M. guttatus*. Estimates indicate that deleterious alleles in *M. micranthus* are more nearly additive than they are in *M. guttatus*.

The genetic basis of inbreeding depression (or its converse, heterosis) has been examined primarily in crop plants. There is evidence for both dominance-based[11–13] and overdominance-based[13–16] inbreeding depression. However, the relative importance of dominance-based versus overdominance-based inbreeding depression in natural plant populations is largely unknown. Studies of *Eichhornia paniculata*[17] (Pontederiaceae) and two *Amsinckia* species[18] (Boraginaceae) have found indirect evidence for dominance-based inbreeding depression.

The genus *Mimulus* (Scrophulariaceae) has been the focus of many studies aimed at understanding the processes responsible for the evolution of plant mating systems[19–24]. *Mimulus guttatus*, the common monkey flower, has large, bee-pollinated flowers, and measured outcrossing rates for three populations, including one used in this study, ranged from 0.68–0.80 (ref. 25). *Mimulus micranthus* is a

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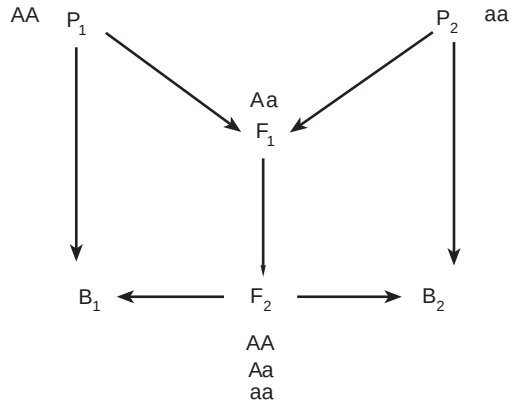

Figure 1 The North Carolina 3 breeding design. Starting with field-collected seed, inbred lines of *M. guttatus* were produced through five sequential generations of selfing in a glasshouse. Maternal lineages from selfing *M. micranthus* were allowed to self for three generations. Inbred lines (P_1 and P_2) were crossed to produce hybrid F_1 progeny. F_1 individuals were selfed to produce the F_2 generation, representing all possible assortments of linkage groups from the parental inbred lines as well as many recombinant genotypes. Backcross progeny (B_1 and B_2) were produced by crossing randomly selected F_2 pollen parents to each of the original inbred lines (P_1 and P_2).

Table 1 Representative ANOVA of backcross progeny from a North Carolina 3 breeding design

Source of variation	d.f.	MS	Exp (MS)
Set	$s-1$	–	–
Replication (set)	$s(r-1)$	–	–
Inbred lines (set)	s	–	–
F_2 parents (set)	$s(n-1)$	M_{31}	$Ve+r\alpha$
$F_2 \times$ line (set)	$s(n-1)$	M_{32}	$Ve+r\delta$
Error	$s(2n-1)(r-1)$	M_{33}	Ve

Expected mean squares (Exp (MS)) are as presented in ref. 10, where Ve includes part of the genetic variance as well as the environmental variance. α, genetic variation among F_2 (male) parents (additive component); δ, interaction between F_2 genotypes and inbred parents (non-additive or dominance component); d.f., degrees of freedom; s, set of progeny, equivalent to the number of paired inbred lines mated to form the F_1 sets; r, number of progeny measured per cross, represents level of replication of each resultant backcross progeny set.

Table 2 Estimates of average dominance ($\bar{a}$) responsible for inbreeding depression in traits from two populations (S and T) of mixed-mating *M. guttatus* and primarily selfing *M. micranthus*

	Average dominance		
	M. guttatus		*M. micranthus*
Trait	S	T	
Total flower production	0.778*	0.741*	0.436***
Above-ground biomass	0.747*	0.692**	0.577***
Ovule production	0.613***	0.601***	–
Pollen viability	0.972	0.931	–
Pollen production	0.174***	1.321*	–

Significance levels follow the convention of * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ (ref. 26). No inbreeding depression was detected for ovule and pollen traits in *M. micranthus*[24].

small-flowered, primarily selfing derivative of *M. guttatus* with an estimated outcrossing rate of 0.16 (ref. 20). *M. guttatus* is found widely throughout western North America whereas *M. micranthus* is restricted to the Coastal Ranges of northern California. The magnitude of inbreeding depression in mixed-mating *M. guttatus* is much greater than in selfing *M. micranthus*[24] with respect to above-ground biomass, pollen production, and ovule production. Here we present estimates for the average dominance levels responsible for the inbreeding depression in these same traits as well as for total flower production and pollen viability.

Most fitness traits are probably affected by many loci. Some of these loci could be segregating alleles that act in an overdominant manner, whereas others could be segregating alleles that act in a dominant–recessive manner. We used the North Carolina 3 breeding design (Fig. 1) to estimate average dominance, a mean of the various allelic interactions across all loci affecting particular fitness traits[7]. Average dominance ($\bar{a}$) represents the ratio of non-additive genetic variance to additive genetic variance (Table 1). A ratio of more than 1 indicates inbreeding depression due to overdominance, whereas a ratio of less than 1 indicates inbreeding depression due to partially recessive deleterious alleles. In both *Mimulus* species, inbreeding depression in most life-history traits results from partially recessive deleterious alleles (Table 2). All estimates of $\bar{a}$ for total flower production, above-ground biomass, and ovule production were significantly less than 1.0 (ref. 26). For pollen viability in both populations of *M. guttatus*, $\bar{a}$ values did not differ significantly from 1.0 and are therefore indistinguishable from complete dominance of wild-type alleles. Despite the similarity in average dominance values for most traits between populations of *M. guttatus*, estimates of average dominance for pollen production differed in each population (Table 2). Population S showed partial dominance (approaching additivity; $\bar{a}$ significantly <1.0), but population T exhibited evidence of overdominance ($\bar{a}$ significantly >1.0). If deleterious alleles are linked in repulsion, estimates from the NC3 design are biased towards overdominance[7]. Our data therefore indicate that allelic interactions are not consistent and/or that the degree of linkage disequilibrium may vary among populations. Direct estimates of the genetics underlying inbreeding depression in other natural populations are needed.

Estimates of average dominance, $\bar{a}$, for total flower production and above-ground biomass in the inbred derivative, *M. micranthus*, are consistently lower (more nearly additive) than in its outbred ancestor, *M. guttatus*. Although the 95% confidence intervals do not allow us to distinguish statistically between the *M. micranthus* and the *M. guttatus* estimates[10], models for purging genetic load[1–3] predict such differences. Selection against highly recessive alleles becomes much more efficient as selfing rates increase. Increased selfing rates will have little effect, however, on those alleles that are more nearly additive (dominance coefficient $h=0.5$; $\bar{a}=0$)[2].

Comparisons of species or populations that differ in their mating system indicate that inbreeding depression may be due to deleterious recessive alleles (presumably introduced by mutation)[22,27]. This trend is also observed for *M. guttatus* and *M. micranthus*[24]. We have now provided direct support for this long-standing inference with data from natural populations. As $\bar{a}$ is an estimate of the average dominance effects across all loci affecting a trait, we cannot preclude the action of overdominance at any particular locus. However, even after the inferred removal of deleterious alleles in highly selfing *M. micranthus*[24], we see no evidence for overdominance. Additional support for dominance-based inbreeding depression comes from a five-generation serial inbreeding and outcrossing program in *M. guttatus* that generated some maternal family lines in which the genetic load was successfully purged[28] for the same traits that we studied. A study of allozyme segregation patterns also showed little evidence for overdominance-based inbreeding depression[21]. Our intermediate levels of dominance for most life-history traits in *Mimulus* also concur with studies of two primarily selfing *Amsinckia* species[18]. One primary concern of conservation genetics is the loss of population fitness through inbreeding that is associated with reduced population size and fragmentation. Breeding programs aimed at rapidly purging a population of its inbreeding depression through inbreeding and selection will be successful only if deleterious recessive alleles are responsible[6]. Our results from a natural system indicate that such breeding programs may be worthwhile for some wild populations. □

Methods

We followed the outline of the North Carolina 3 breeding design described in ref. 7. To maximize the power of the design, we mated ten pairs of *M. guttatus* inbred lines in a negative assortative fashion with regard to overall vigour in

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each of two populations (named S and T). We restricted the mating of ten pairs of *M. micranthus* inbred lines to those with fixed alternative isozyme markers, to allow confirmation of successful crosspollination in this autogamous selfer. The degree of dominance was quantified by partitioning variance measured in the backcross (B) generation. The B_1 and B_2 families (constructed crosses between $n = 20$ F_2 sires mated to each original inbred line, P_1 and P_2) make up one set of progenies (s), and we replicated each set $r = 5$ times. For each population of *M. guttatus* and for the single population of *M. micranthus*, ~2,000 backcross progeny grew in a randomized block design with B_1 and B_2 progeny randomized within each set and sets randomized within blocks.

The ratio in equation (1) estimates the square of the average dominance of wild-type alleles:

$$\frac{M_{32} - M_{33}}{M_{31} - M_{33}} = \bar{a}^2 \quad (1)$$

where M31, M32, and M33 refer to the expected mean squares from the analysis of variance (ANOVA) in Table 1. This is the ratio of the interaction effect between F_2 pollen parents and the parental inbred lines to the additive genetic effects among F_2 pollen parents. If there is little or no dominance, the mean performance of progeny sired by a given F_2 pollen donor should be similar in both the B_1 and B_2 families. The pollen donor × inbred line interaction effect (the numerator in equation (1)) will be very small in this case, and the ratio will approach 0. With complete or partial dominance of wild-type alleles (that is, complete or partial recessitivity of deleterious alleles), the mean performance of progeny sired by a given F_2 pollen parent will differ between the B_1 and B_2 families because of differences in heterozygosity between progeny of B_1 and B_2 families (Fig. 1). In this case, the magnitude of the interaction effect approaches the additive effect (as dominance becomes more complete), and the ratio approaches 1. With overdominance, the performance of the backcross progeny sired by a given F_2 pollen donor will be determined almost entirely by the interaction between the pollen donor and the inbred line to which it is crossed. The cross resulting in the most heterozygous progeny will outperform the other. In this case, almost all variation among backcross families is nonadditive, and the ratio is greater than 1.

The average dominance estimate, $\bar{a}$, is related to h, the dominance coefficient used in many population genetic models[1,2], in the following way: $h = 0.5 - \bar{a}/2$, with estimates in the range $0 > \bar{a} > 1.0$. When complete directional dominance is responsible for the genetic load, $h = 0$ and equivalently $\bar{a} = 1$. In the case of pure additivity, $h = 0.5$ and $\bar{a} = 0$. In the case of overdominance, $\bar{a} > 1$ and there is no equivalent value of h.

There are several assumptions[7] for the genetic interpretation of the variance components listed in Table 1. Here the assumptions regarding linkage and epistasis are the most critical. Genetic interpretation of the ANOVA assumes that backcross progeny are in linkage equilibrium for all loci affecting the traits and that no nonallelic interactions occur. If deleterious recessive alleles are linked in repulsion, estimates of $\bar{a}$ are inflated because of pseudo-overdominance. Epistatic effects also upwardly bias estimates of $\bar{a}$, because the numerator in equation (1) will include nonallelic as well as allelic interactions. The bias caused by ignoring epistasis is minor compared with the potential bias caused by ignoring linkage disequilibrium. The few data available on the contribution of epistatic interactions to heterosis indicate that epistatic effects are minor compared with dominance effects[19,28–30].

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A nutrient-sensing pathway regulates leptin gene expression in muscle and fat

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Leptin, the protein encoded by the *obese* (*ob*) gene, is synthesized and released in response to increased energy storage in adipose tissue[1–4]. However, it is still not known how incoming energy is sensed and transduced into increased expression of the *ob* gene. The hexosamine biosynthetic pathway is a cellular 'sensor' of energy availability[5–8] and mediates the effects of glucose on the expression of several gene products[9–12]. Here we provide evidence for rapid activation of *ob* gene expression in skeletal muscle by glucosamine. Increased tissue concentrations of the end product of the hexosamine biosynthetic pathway, UDP-*N*-acetylglucosamine (UDP-GlcNAc), result in rapid and marked increases in leptin messenger RNA and protein levels (although these levels were much lower than those in fat). Plasma leptin levels and leptin mRNA and protein levels in adipose tissue also increase. Most important, stimulation of leptin synthesis is reproduced by either hyperglycaemia or hyperlipidaemia, which also increase tissue levels of UDP-*N*-acetylglucosamine in conscious rodents[7]. Finally, incubation of 3T3-L1 pre-adipocytes and L6 myocytes with glucosamine rapidly induces *ob* gene expression. Our findings

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