reduces the already slim likelihood that the non-occurrence of therians before ~110 Myr is due to a preservational/sampling artefact.

Finally, in offering the first well-preserved holotherian dental remains known from the Middle to Late Jurassic of Gondwana, *A. mahabo* has important paleobiogeographic implications. Mesozoic mammal faunas of Laurasia and Gondwana were long considered to have remained isolated during much of the late Mesozoic; tribosphenidans (regarded as Laurasian in origin) were thought to have been excluded from the southern continents until latest Cretaceous or early Paleocene time, because Mesozoic tribosphenidans remained undiscovered in Gondwana[4]. To the contrary, recent finds in Morocco[25] and Australia[17] indicate the occurrence of tribosphenidans on the southern continents as early as the earliest Cretaceous (that is, as early in Gondwana as in Laurasia). The discovery of *A. mahabo* inverts this biogeographic scenario even further, showing tribosphenidans to have appeared in Gondwana by the Middle Jurassic. They may have remained isolated there until earliest Cretaceous time, when they appear in North Africa, Europe and Asia. ☐

Received 15 March; accepted 28 June 1999.

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Acknowledgements

This work was supported by the National Geographic Society, Field Museum of Natural History and the Unviersity of California. We thank the Ministre de L'Energie et des Mines of Madagascar for authorizing this work, S. Goodman and M. Ravokatra for help with our field efforts and G. Ravololonarivo Randria and A. Rasoamiaramanana. Field Museum volunteers D. Kinzig and R. Chisholm found this specimen during washing and microscope sorting of the matrix; K. Mazanek and M. Donnelly produced the illustrations; R. Andriantompohavana, L. Razafimanantso, A. Toto Volahy and A. Gandie aided us in the field; and W. Clemens and A. Smith provided advice.

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Transgenerational induction of defences in animals and plants

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Predators are potent agents of natural selection in biological communities. Experimental studies have shown that the introduction of predators can cause rapid evolution of defensive morphologies and behaviours in prey[1–5] and chemical defences in plants[6,7]. Such defences may be constitutively expressed (phenotypically fixed) or induced when predators initially attack[8–10]. Here we show that non-lethal exposure of an animal to carnivores, and a plant to a herbivore, not only induces a defence, but causes the attacked organisms to produce offspring that are better defended than offspring from unthreatened parents. This transgenerational effect, referred to as a maternally induced defence, is in contrast to the more common defences induced in single individuals within a generation. Transgenerational induction of defences is a new level of phenotypic plasticity across generations that may be an important component of predator–prey interactions.

Inducible defences may provide an adaptive defensive strategy in which non-lethal cues from predators, herbivores or parasites provide a reliable indicator about the future risk of attack. In both animal and plant systems, inducible defences allow organisms to reap the benefits of defence while saving potential costs associated with investment in the defensive strategy when it is not needed. Such phenotypic benefits and costs have been demonstrated for inducible biochemical and morphological defences in plants, and for inducible morphological, life-history and behaviour defences in animals[8–11]. If the parental environment predicts the quality of the progeny's environment, then parents may further enhance their net reproductive success by differentially endowing their offspring with phenotypes to cope with potential hazards such as predation. Such maternal effects have been reported in both animals and plants[12–14]. These effects have traditionally been ascribed to passive consequences of the resource environment and have been viewed as phenotypic noise associated with thwarting adaptive evolution. More recently, however, maternal effects have been seen as being potentially adaptive[12,15–18]. Adaptive maternal effects involve responding to information in the environment to improve the fitness of offspring by altering the offspring's phenotype. We have investigated the consequences of non-lethal exposure of wild radish plants (*Raphanus raphanistrum*) and waterfleas (*Daphnia cucullata*) to their predators, and have documented the effects on the defensive phenotypes of their offspring.

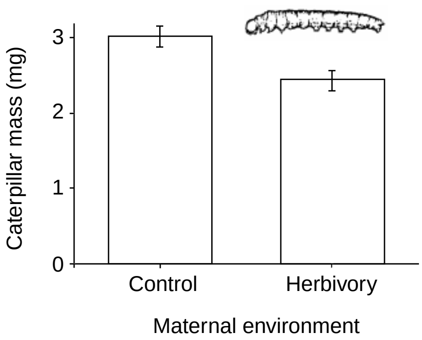

Figure 1 *Raphanus raphanistrum* resistance to herbivory as measured by growth of a specialist caterpillar, *Pieris rapae*, on the F_1 generation of seedlings from different maternal environments. Maternal plants were either subject to herbivory by *P. rapae* or left undamaged. This transgenerational induction of defences was not associated with differences in seed mass or seed concentrations of carbon or nitrogen. Error bars show s.e.

Wild radish plants damaged by a specialist caterpillar, *Pieris rapae*, induced tenfold higher concentrations of indole glucosinolates (mustard oil glycosides) and 30% higher densities of setose trichomes on newly formed leaves of damaged plants compared with undamaged control plants[19,20]. The induction of these putative defences protected them against subsequent herbivory in several field experiments, and induced plants had 60% higher lifetime seed production than uninduced controls[11,19].

To examine the consequences of herbivory for the next generation of plants, we grew unmanipulated control plants and plants with 50% of each leaf consumed by a caged *P. rapae* larva, a natural herbivore of *R. raphanistrum*. Defensive glucosinolate profiles shifted in seeds from damaged plants compared with undamaged controls (multivariate analysis of variance; Wilks' $\lambda = 0.699$, $F_{4,26} = 2.803$, $P = 0.046$). Hydroxylated glucosinolates increased in concentration, whereas other classes of glucosinolates (aliphatic and indole) decreased. Herbivory on the maternal plant also induced changes in the number of trichomes per leaf in seedlings (A.A.A., unpublished observations). Seedling progeny from damaged and undamaged plants were challenged with *P. rapae* larvae. Caterpillars gained 20% less weight on seedlings whose parents were damaged than on seedlings whose parents were not damaged (Fig. 1, Table 1). This transgenerational induced plant resistance was not explained by seed mass variation or investment in primary metabolites (Table 1). Wild radish plants with induced defences produced seeds that did not differ in percentage composition of nitrogen or carbon compared with seeds from undamaged plants (nitrogen: control, 4.37 ± 0.07 (mean ± s.e.); maternal herbivory, 4.46 ± 0.06; $t = 0.988$, d.f. $= 33$, $P = 0.330$; carbon: control, 56.93 ± 0.30; maternal herbivory, 56.77 ± 0.19; $t = 0.454$, d.f. $= 33$, $P = 0.653$). Thus, induction of plant defences persisted in the progeny of infested plants. This effect may be the result of both a maternally induced defence and a greater rapidity of induction of plant defences in the offspring of damaged mothers.

Induced chemical defences have been best studied in plants, but induced morphological defences have been best studied in animals. Helmet formation in *Daphnia cucullata* (Fig. 2) is a textbook example of cyclomorphosis[21] (seasonal variation in morphology), although the agents that maintain this polyphenism have not been adequately demonstrated[22,23]. Morphological defences in cladocerans are induced by chemicals known as kairomones that are released by predators[24]. In the presence of caged predaceous cladocerans, *Leptodora kindtii*, the relative helmet length of *D. cucullata* almost doubles (control, 15.53 ± 0.35; induced, 29.71 ± 0.49; $t = 23.72$, d.f. $= 303$, $P < 0.001$). Similar results were obtained for *D. cucullata* in the presence of caged aquatic larvae of the dipteran phantom midge, *Chaoborus flavicans* (control, 13.93 ± 0.15; induced, 27.88 ± 0.28; $t = 44.29$, d.f. $= 470$, $P < 0.001$). The induction of helmets acts as a defence by lowering the capture success by both predators: in controlled experiments, daphnids with induced helmets suffered lower mortality from both predators (mean (± s.e.) number of prey captured per feeding trial; *Leptodora*: control, 6.43 ± 0.48; induced, 0.71 ± 0.42; $n = 7$, $P = 0.018$; *Chaoborus*: control, 1.86 ± 0.33; induced, 0.71 ± 0.22; $n = 14$, $P < 0.001$). These results indicate that a proximate cue used is a chemical released by predators, and defence against invertebrate predators is a major selective force favouring cyclomorphosis in *D. cucullata*.

To examine transgenerational induction of defences in *D. cucullata*, we raised daphnids in either control environments or environments with the *Chaoborus* kairomone. We then imposed four treatments and compared helmet size across the treatments in the F_1 and F_2 generations: (1) control daphnids (C), which, like their mothers, had not been in contact with the predator kairomone; (2)

Table 1 Effects of maternal environment (herbivory or no herbivory) and grandmaternal and maternal family on growth of caterpillars feeding on seedling plants

Source	d.f.	MS*	*F*	*P*
Maternal environment (ME)	1	1.503	7.516	0.009
Grandmaternal family (GF)	7	0.090	0.452	0.863
Maternal family nested within ME × GF	43	0.200	1.157	0.288
Seed mass	1	0.017	0.099	0.754
Error	74	0.173		

* MS, mean square.

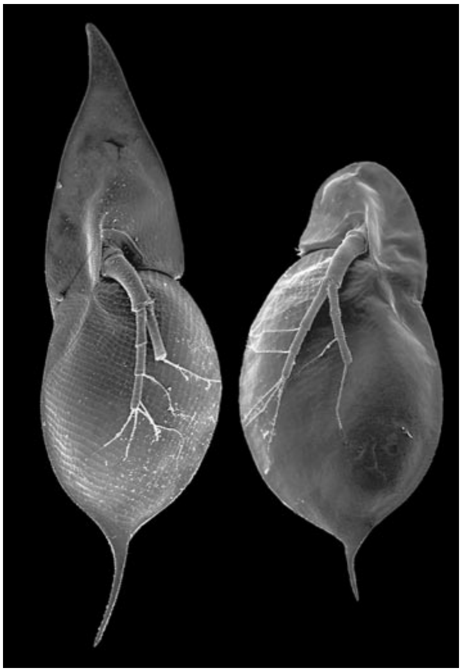

Figure 2 Scanning electron micrograph showing typical and predator-induced morphs of *Daphnia cucullata* of the same clone.

Table 2 Helmet lengths of *Daphnia cucullata* progeny compared with ANOVA within each brood and age class

Source	Age	d.f.	MS	*F*	*P*
F_1, first brood	Neonate	3,295	0.144, 0.004	40.51	<0.001
	Adult	3,239	0.603, 0.002	340.61	<0.001
F_1, second brood	Neonate	3,266	0.170, 0.004	47.57	<0.001
	Adult	3,208	0.414, 0.002	193.44	<0.001
F_1, third brood	Neonate	3,197	0.134, 0.002	71.65	<0.001
	Adult	3,176	0.297, 0.002	170.90	<0.001
F_2, generation	Neonate	3,241	0.334, 0.003	132.11	<0.001
	Adult	3,215	1.202, 0.002	486.14	<0.001

MS and d.f. are shown first between groups and second within groups (error).

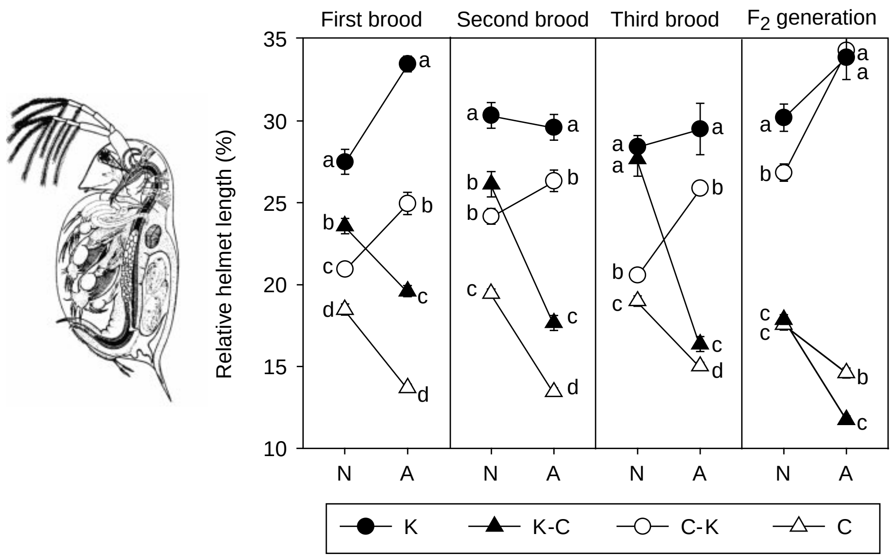

Figure 3 Relative helmet length (mean ± s.e.) of *Daphnia cucullata* (F$_1$ and F$_2$ generation) of four treatments, organized by brood number. K, kairomone treatment (*Chaoborus*); C, control; K → C, mother (F$_0$) had been transferred from kairomone to control treatment; C → K, mother (F$_0$) had been transferred from control to kairomone treatment. Mothers were transferred between environments after they had become pregnant. Daphnids are sensitive to the kairomone starting in a late embryo stage and in the juvenile stages (R.T., unpublished data). We analysed the first three broods of the F$_0$ mothers (for example, in the transfer treatment, three broods following the transfer). Thus, only the first brood shared the maternal environment for a very short time. Lines between neonates (N) and adults (A) show relative helmet growth within each treatment. Results of each overall ANOVA are shown in Table 2. Homogeneous groups within each brood and age class are indicated by vertical ordered letters (Bonferroni adjusted). The F$_2$ generation is the first brood offspring of the F$_1$ first brood. Where error bars are not shown, they were smaller than the symbol.

kairomone daphnids (K), which, like their mothers, had been in permanent contact with the kairomones; (3) daphnids which had been born from control mothers and were transferred to the kairomone environment (C → K); and (4) daphnids which had been born from kairomone mothers and were transferred to the control environment (K → C). The latter two treatments were used to distinguish between a maternally induced defence and environmental induction in the progeny. Pregnant mothers were transferred between environments shortly after they had deposited their first clutch into their brood pouch. At this stage, young daphnids are not yet sensitive to the kairomone (R.T., unpublished data).

F$_1$ neonates varied in helmet length between the four treatments (Table 2, Fig. 3), with offspring from mothers in the kairomone environment always having larger helmets than offspring from mothers in the control environment. Significant differences between daphnids with the same F$_1$ environment but different maternal environments (compare C with K → C, and K with C → K) demonstrate the importance of the maternal environment in helmet formation. This pattern persisted in subsequent broods that were initiated after the mothers had been transferred, which demonstrates the lasting influence of the maternal environment on the transgenerational induction of helmet defences (Fig. 3). A comparison of treatments with the same maternal environment but different offspring environments (compare K with K → C, and C with C → K) indicates that the late embryonic environment also had an influence on helmet induction, albeit a smaller effect than that of maternal environment (Fig. 3).

Postnatal helmet development in *D. cucullata* is induced by the juvenile environment, as can be seen from the interactions between helmet length and age (Fig. 3). The K → C daphnids, which were born with large helmets, reached maturity with relatively small helmets. In contrast, neonates from C → K mothers were born with small helmets but reached maturity with relatively large helmets. However, the juvenile environment could not induce phenotypes that matched those of maternally induced individuals. For example, the helmet size of adult daphnids from the kairomone treatment (K) could not be reached by C → K daphnids, which also spent their whole lives in contact with the kairomone. Similarly, K → C daphnids reached maturity with larger helmets than control (C) daphnids.

The F$_2$ generation from C → K daphnids had significantly smaller helmets than did K daphnids, indicating that grandmaternal environment constrained the size of helmets in the F$_2$ neonates (Fig. 3). However, the F$_2$ generation from K → C daphnids did not have a different helmet size than C daphnids. In both cases, the maternal (F$_1$) environment influenced helmet induction in the F$_2$ generation.

The greater defences of organisms whose parents were threatened by predation provides a case of adaptive phenotypic plasticity across generations. Adaptive maternal effects are distinct from other adaptations, however, in that the genes responsible are activated in the maternal generation, whereas the phenotypes on which natural selection may act are expressed in the progeny. This lagged link between parental environment and offspring phenotype may amplify or subdue responses to natural selection[18,25].

Transgenerational effects may provide tools to overcome developmental constraints acting on individuals at critical stages in the life cycle. For example, the later development of a maximally large helmet for initially short-headed daphnids might not be possible, but transgenerational effects imposed by the maternal environment may allow for such maximal defences. Furthermore, progeny born with a maternally induced defence avoid a major disadvantage of inducible defences: that the defence is not initially present when needed (there is a lag phase[26]). The immediate development of plant resistance in wild radish seedlings is important because the establishment phase has a strong influence on plant fitness[11,27]. Maternally induced progeny will be just as well protected as permanently defended individuals, and better protected than undefended individuals or individuals that respond to threats early in life. Because predation can be such a strong selective force, we posit that transgenerational induced defences are a logical extension of fitness-enhancing strategies of organisms under threat. Several other systems are strong candidates for having maternally induced defences[28–30]. That such effects are present in both animals and plants reinforces the view that the expression of adaptive phenotypes in prey may be driven by predation from higher trophic levels. □

Methods

Wild radish experiments.

We used *R. raphanistrum* seeds from a second generation of untreated greenhouse-grown plants. We germinated about 10 seeds from each of 13 maternal families in a greenhouse. At the four-leaf stage, each plant was randomly assigned to one of the two treatments (3–5 plants per treatment per family). The caterpillar herbivory treatment was maintained throughout the growth of the plant. Effects of herbivory on seed set are reported elsewhere[19]. Seeds from 8 of the original 13 families were chosen for the transgenerational experiment because they spanned the range of tolerance to herbivory[19], and 2–4 seeds from each of 58 maternal plants within 8 grandmaternal families (a total of 126 plants) were grown in a greenhouse and inoculated with a single newly hatched *P. rapae* larva. Caterpillars were not caged. After four days, the caterpillars were weighed.

Effects of grandmaternal family (fixed), maternal environment (control or herbivory; fixed), maternal family (nested within grandmaternal family by treatment interaction; random) and seed mass (covariate) on caterpillar growth were analysed using a mixed-model analysis of variance (ANOVA). *F*-ratios for grandmaternal family and treatment effects were calculated with maternal family nested in grandmaternal family by treatment interaction mean-square and degrees of freedom in the denominator.

For phytochemical analysis, glucosinolates were analysed using modified procedures for determination of trimethylsilyl glucosinolate derivatives with capillary gas chromatography and flame ionization detection[20]. Seed nitrogen and carbon were determined from a separate set of seeds using dynamic flash-combustion and gas-chromatographic separation and a thermal-conductivity detection system (Division of Agriculture and Nature Resources, University of California, Davis).

Daphnia experiments.

We used a *Daphnia cucullata* clone isolated from Thaler lake, Germany. The experiments were conducted in the laboratory under constant conditions at 20 °C and fluorescent light in a synthetic medium. The F_0 generation had been synchronized by always raising the third brood offspring born within 12 h, starting from a single *Daphnia*. *Daphnia* were fed *ad libitum* daily with *Scenedesumus acutus* ($1.5\,\mathrm{mg\,C\,l^{-1}}$).

Animals with freshly deposited eggs were placed into 0.75 l medium containing a 125-μm net cage, enclosing either 10 fourth-instar larvae of *Chaoborus flavicans* or 4 *Leptodora kindtii*, or no predators for controls. The net cages prevented direct contact between predators and prey.

Predation experiments with *Chaoborus* were conducted for 0.5 h with each prey morph separately in 100 ml medium with 10 prey organisms and a single predator. Predation trials with *Leptodora* were conducted with 10 prey organisms of each morph together in 0.5 l in 24-h experiments. In each experiment, animals of the same age and body size class (0.6–0.8 mm, from the eye to the base of the tail spine) but of different morphology were compared.

We induced helmet formation in *Daphnia* by using *Chaoborus* kairomones. We placed four *Chaoborus* larvae in net cages in 1.5-l beakers and changed the water ever day. Chaoborids were fed daily with 10–15 prey (*D. cucullata* and *Ceriodaphnia* sp.). The F_0 generation had been born and raised in these beakers in either the control or kairomone treatment. We used three beakers per treatment as replicates, which did not differ and were pooled for analysis.

Daphnids were measured with a digital image-analysis system (SIS, Münster, Germany). To compensate for small changes in body length within an age class, we calculated the relative helmet length (helmet length/body length $\times$ 100). Relative helmet length is a good predictor of the defensive effect within an age class. The relative values were arcsin-transformed for analysis. In the induction treatment, differences between control and kairomone treatment were compared using *t*-tests. Effects of induced *Daphnia* phenotypes on predation were analysed by using a Mann–Whitney-*U*-test for *Chaoborus* and with a paired Wilcoxon test for related samples for *Leptodora*. To test for transgenerational effects, we compared the treatments within each brood and age class (Table 2, Fig. 3) with ANOVA and Bonferroni adjustments.

Received 29 March; accepted 28 June 1999.

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Acknowledgements

We thank R. Karban, the plant-herbivore group at Davis and W. Gabriel for advice and encouragement; M. Morra and V. Borek for help with phytochemical analyses; R. J. Mercader, M. Kredler, S. Y. Strauss, J. Kniskern and E. Hochmuth for help with experiments; and L. S. Adler, S. Diehl, H. Dingle, R. Karban, R. E. Lenski, T. W. Schoener, S. Y. Strauss, J. S. Thaler, D. A. Thiede, and T. G. Whitham for comments on the manuscript. This work was supported by the US NSF.

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Genetic enhancement of learning and memory in mice

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Hebb's rule (1949) states that learning and memory are based on modifications of synaptic strength among neurons that are simultaneously active. This implies that enhanced synaptic coincidence detection would lead to better learning and memory. If the NMDA (*N*-methyl-D-aspartate) receptor, a synaptic coincidence detector[1–4], acts as a graded switch for memory formation, enhanced signal detection by NMDA receptors should enhance learning and memory. Here we show that overexpression of NMDA receptor 2B (NR2B) in the forebrains of transgenic mice leads to enhanced activation of NMDA receptors, facilitating synaptic potentiation in response to stimulation at 10–100 Hz. These mice exhibit superior ability in learning and memory in various behavioural tasks, showing that NR2B is critical in gating the age-dependent threshold for plasticity and memory formation. NMDA-receptor-dependent modifications of synaptic