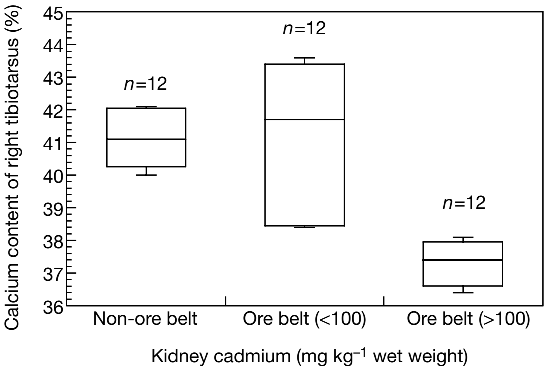

Figure 4 Bone-calcium content as a function of kidney cadmium levels in adult ptarmigan in ore-belt and non-ore-belt birds.

metals gradient. Densities were lowest in calcium-poor, acidic, metals-rich habitats and highest in calcium-poor, pH-normal, habitats with low metals levels. Our lowest-density habitat contained fewer ptarmigan than any population reported by other investigators throughout the range of the species[9].

Cadmium exists in natural ecosystems worldwide and can be mobilized by a number of activities including mining. Willow has been shown in this study to biomagnify cadmium. Many herbivores, such as wapiti (*Cervus elaphus*), moose (*Alces alces*), mule deer (*Odocoileus hemionus*), beaver (*Castor Canadensis*) and snowshoe hare (*Lepus americanus*), eat large quantities of willow[29,30]. Therefore, we postulate that chronic cadmium poisoning may be more widespread than shown in this study. Herbivores—particularly those that eat willow—should receive priority in future studies of cadmium toxicity in the Rocky Mountains, and elsewhere around the world where natural cadmium levels are high. We speculate that demographic and physiological effects of naturally occurring and/or anthropogenically mobilized cadmium might explain some distributional gaps or range boundaries in certain herbivorous species, and that cadmium—especially where anthropogenically mobilized—has a role in keeping some of these wildlife populations below numbers that otherwise could be supported. ☐

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Competition induces adaptive shifts in caste ratios of a polyembryonic wasp

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An important transition in insect life-history evolution was the shift from a solitary existence to living in groups comprising specialized castes. Caste-forming species produce some individuals that reproduce and others with worker functions that have few or no offspring[1]. Morphologically specialized castes are well known in eusocial species like ants and termites[1], but castes have also evolved in less-studied groups like thrips, aphids and polyembryonic wasps[2–5]. Because selection acts at both the individual and the colony level, ratios of investment in different castes are predicted to vary with environmental factors like competition and resources[6–8]. However, experimental evidence for adaptive shifts in caste ratios is limited[9] owing to the experimental difficulty of manipulating factors thought to influence caste ratios[10–13], and because some species produce behaviourally flexible castes that switch tasks in response to colony needs[14,15]. Unlike other caste-forming species, the broods of polyembryonic wasps develop clonally, so that increased production of one caste probably results in decreased production of the other[16]. Here we show that the polyembryonic wasp *Copidosoma floridanum* alters caste

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ratios in response to interspecific competition. Our results reveal a distinct trade-off by *C. floridanum* between reproduction and defence, and show experimentally that caste ratios shift in an adaptive manner.

C. floridanum is a small (1 mm long) parasitoid wasp that oviposits one egg (female or male) or two eggs (1 female and 1 male) into the egg stage of the moth *Pseudoplusia includens*[17]. After parasitism, the host egg hatches and the larva develops to its final (fifth) instar. During this period, the *C. floridanum* egg proliferates clonally to form approximately 1,200 embryos[16]. Some embryos develop during the host's first–fourth instar into precocious larvae (Fig. 1). One or two precocious larvae are present in first instar hosts and from 2 to 10 precocious larvae develop, respectively, during the host's second to fourth instars. As a result, the number of precocious larvae progressively increases as the host caterpillar grows, to an average maximum of 30 (ref. 18). When the host reaches the fifth instar, the remaining embryos (> 1,000) develop into reproductive larvae that have smaller mandibles and a distinctly more rounded body than precocious larvae (Fig. 1). Reproductive larvae consume the host, pupate and emerge as adult wasps that disperse to locate new hosts. Precocious larvae in contrast function as a sterile soldier caste that defends its reproductive siblings from competitors[4,5]. Precocious larvae never pupate and always die from desiccation when the host is consumed by the reproductive larvae[16]. We viewed the absence of task switching, ability to manipulate experimentally a restricted environment (the host), and clonal development as key attributes favouring polyembryonic wasps for studies on caste ratio evolution.

Each host attacked by *C. floridanum* represents a limited resource that can support development of only a finite number of wasps. As only reproductive larvae develop into adults, increased investment in this caste directly increases brood fitness to a maximum determined largely by the amount of host resources available for consumption[18]. Because precocious larvae have only soldier functions, they should increase in abundance only under heightened risk from competitors that threaten brood survival or usurp host resources. The main interspecific competitors to *C. floridanum* are other parasitic wasps like *Microplitis demolitor* that parasitize *P. includens* during the larval stage[19,20]. These larval parasitoids possess large fighting mandibles and readily engage in combat to eliminate competitors[21]. Moreover, only one species of parasitoid usually survives when hosts are parasitized by more than one species of wasp[21]. To determine how *C. floridanum* responds to competition, we compared hosts parasitized by both *C. floridanum* and *M. demolitor* (that is, multiparasitized hosts) to hosts parasitized by each wasp alone (control). To ensure that all *C. floridanum* offspring in each host were genetically identical, broods were established from a wasp laying a single female egg into the host egg. *M. demolitor* parasitized hosts when they were first–fourth instar larvae.

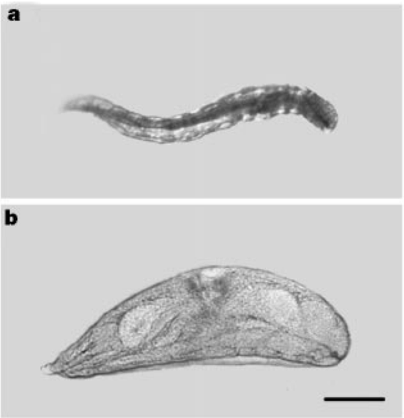

Figure 1 Light micrograph of a *C. floridanum* precocious larva (**a**) and first instar reproductive larva (**b**). Larvae were dissected from a fifth instar *P. includens* larva. The anterior (head) of each larva is oriented to the right. Scale bar, 100 μm.

Dissection of a subsample of hosts immediately after oviposition by *M. demolitor* confirmed that this competitor always laid 1–3 eggs per host regardless of whether it contained *C. floridanum* ($n = 15$) or had not been previously parasitized ($n = 15$) ($t = 0.5$, $P > 0.1$). If these species were equal competitors, we would expect a similar proportion of multiparasitized hosts to produce *C. floridanum*, *M. demolitor*, or to die without producing any parasitoid. However, we found that 63% of multiparasitized hosts produced *C. floridanum* adults, 8% produced an *M. demolitor* adult, and 29% of hosts died without producing any parasitoid ($n = 120$) ($\chi^2 = 57.1$, d.f. = 2, $P < 0.001$). In comparison, 86% ($n = 80$) and 83% ($n = 80$) of hosts parasitized by only *C. floridanum* or *M. demolitor* respectively produced adult wasps ($\chi^2 = 0.43$; d.f. = 1, $P > 0.5$). Combined, these results indicated that *C. floridanum* was an intrinsically superior competitor to *M. demolitor*. To assess how exposure to *M. demolitor* affected caste production, multiparasitized hosts and hosts parasitized by *C. floridanum* only were dissected at the end of the fifth instar. Multiparasitized hosts contained significantly more precocious larvae ($F_{4,93} = 12.7$, $P < 0.001$) and fewer reproductive larvae ($F_{4,93} = 24.5$, $P < 0.0001$) than control hosts (Fig. 2). The shift in caste ratio and average brood size was most extreme when *M. demolitor* oviposited in first instar larvae. These hosts contained an average total of 390 *C. floridanum* larvae and 24% soldiers, whereas control hosts contained 1,034 larvae and 4% soldiers.

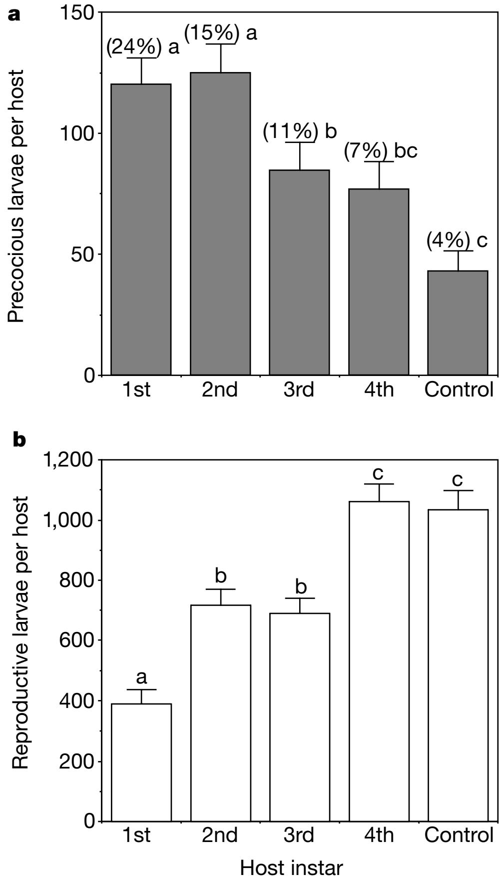

Figure 2 The effects of multiparasitism by *M. demolitor* on caste formation by *C. floridanum*. *M. demolitor* oviposited in first–fourth instar larvae, whereas control hosts contain *C. floridanum* only. All hosts were dissected in the fifth instar. **a,** The mean number of precocious larvae (± s.e.) per host (means with the same letter were not significantly different ($P > 0.05$, Tukey–Kramer method). The percentage of the total brood represented by precocious larvae is indicated in parentheses above each bar. **b,** The mean number of reproductive larvae per host (means with the same letter were not significantly different; $P > 0.05$, Tukey–Kramer method).

Comparison of soldier abundance on a daily basis further indicated that the number of precocious larvae per host increased significantly faster in multiparasitized hosts than in control hosts (Fig. 3).

To confirm that precocious larvae were responsible for elimination of *M. demolitor*, we observed *C. floridanum* and *M. demolitor* larvae *in vitro*. Sixty eight per cent of precocious larvae ($n = 30$) attacked *M. demolitor* during the assay period, whereas no reproductive larvae ($n = 30$) attacked *M. demolitor* during the same period ($P < 0.0001$; Fisher's Exact Test). We also determined that the abundance of soldiers critically influenced the outcome of competition by injecting from 1 to 30 precocious larvae into hosts parasitized 24 h earlier by *M. demolitor*. Hosts were dissected 7 days later and scored for the presence of a mature (final instar) *M. demolitor* larva. No *M. demolitor* survived when 15 or more precocious larvae were injected ($n = 31$), but 97% of *M. demolitor* survived when less than 5 precocious larvae were injected ($n = 30$) ($G^2 = 75.4$, $P < 0.001$, d.f. = 5). In contrast, 90% of *M. demolitor* survived in hosts injected with 1–30 reproductive larvae ($G^2 = 6.9$, $P = 0.23$, d.f. = 5).

Caste determination in insects is usually regulated by the interaction between environmental factors and endogenous developmental pathways[1,22]. Polyembryonic wasps differ from other caste-forming species in that both castes develop from the same egg, coexist in the same environment (the host), and develop in close proximity to one another during embryogenesis[23]. Proliferation and timing of morphogenesis of *C. floridanum* embryos is influenced by both host endocrine physiology and endogenous factors that result in two embryonic lineages fated to develop into precocious or reproductive larvae[16,24,25]. The shift in caste ratio that occurs after exposure to *M. demolitor* suggests that *C. floridanum* embryos also perceive environmental cues associated with competitors. These cues could be emitted directly from the competing parasitoid larva. Larval parasitoids like *M. demolitor*, however, also inject polyDNAviruses in calyx fluid and venom proteins at oviposition that alter host physiology[26,27]. These alterations could indirectly indicate the presence of a competitor. To examine the role of these cues in caste formation, third instar hosts containing *C. floridanum* were injected with 2–3 *M. demolitor* eggs, calyx fluid plus venom, or saline (control). Significantly more precocious larvae were produced in hosts injected with either eggs only (75.9 ± 6.1a, $n = 20$) or calyx fluid plus venom (86.2 ± 5.4a, $n = 20$) than saline (31.0 ± 4.4b, $n = 20$) ($F_{2,57} = 21.2$; $P < 0.0001$; means with the same letter were not significantly different; Tukey–Kramer method). These results reaffirmed that caste ratios change in response to competition and suggest that cues from both the competing adult parasitoid and its progeny are involved in causing this shift. Increased production of precocious larvae could be due either to enhanced proliferation of embryos already fated to develop into soldiers or to a change in the fate of totipotent embryos not yet committed to one caste or the other.

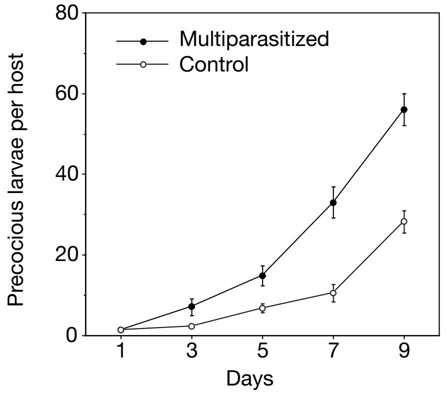

Figure 3 Mean number (± s.e.) of precocious larvae in hosts multiparasitized by *M. demolitor* and control hosts parasitized by *C. floridanum* only. Following oviposition by *M. demolitor*, hosts were dissected every other day ($n = 8$) and the number of precocious larvae per host was counted. Overall, significantly more precocious larvae were produced in multiparasitized hosts than in control hosts ($F_{9,70} = 43.1$, $P < 0.0001$). The significant interaction between treatment and time ($F_{4,70} = 12.3$, $P < 0.0001$) indicates that the difference in soldier production between multiparasitized and control hosts increases with time.

The response of *C. floridanum* to interspecific competition is a dramatic example of adaptive phenotypic plasticity in a caste-forming species. As precocious larvae are incapable of reproducing, soldier fitness is determined solely by the success of their genetically identical reproductive siblings. In the absence of any competitor, caste ratios are strongly biased toward reproductive larvae, but when challenged by a competitor, caste ratios rapidly shift towards increased production of precocious larvae. This response decreases the average number of reproductive larvae produced, but enhances the probability of brood survival. Contact with one competitor may also indicate to *C. floridanum* that the abundance of interspecific competitors in the field is high, and that increased investment in defence is warranted. Similarly, colonies of the ant *Pheidole pallidula* facultatively increased soldier production when foragers regularly encountered conspecifics from neighbouring colonies[9].

Shifts in the ratios of morphologically specialized castes are most likely to evolve when changes in an environmental factor, like competition, occur on a timescale longer than is required to produce more of a given caste (such as soldiers) to counter the threat[6–8,22]. This situation clearly exists in *C. floridanum*, where development of precocious larvae is rapid (~24 h) compared to the amount of time that hosts are exposed to competitors before being consumed by reproductive larvae (15–17 days). ☐

Methods

P. includens and parasitoids were reared at 27 °C and a 16 h light: 8 h dark photoperiod as previously described[18,26]. Hosts were parasitized by *C. floridanum* as 12-h-old eggs. Like most Hymenoptera, *C. floridanum* is haplodiploid with unfertilized eggs producing males and fertilized eggs producing females. All ovipositions were observed and only hosts containing a single female wasp egg were used in experiments. Identifying hosts containing a female egg was easily accomplished, because adult females exhibit different behaviours when ovipositing a fertilized and unfertilized egg[17]. *M. demolitor* females were allowed to oviposit once in hosts after they had become first–fourth instar larvae. Hosts were then reared until adult parasitoids emerged or were dissected to count the number and type of larvae in the haemocoel. Cohorts of hosts multiparasitized in the second instar were also dissected daily, and the number of precocious larvae present was compared to control hosts parasitized only by *C. floridanum*.

In vitro assays were conducted in 3-μl culture wells containing TC-100 medium (JRH). *C. floridanum* and *M. demolitor* larvae were collected from hosts immediately before the experiment. A precocious or reproductive larva was placed in the culture well with a first instar *M. demolitor* larva and observed for 2 h. An attack was recorded if the *C. floridanum* larva gripped the *M. demolitor* larva with its mouthparts for more than 30 s. When this occurred, consumption of tissue was often visible. Precocious and reproductive larvae were dissected from fifth instar hosts in physiological saline and injected into third instar hosts containing *M. demolitor* using a glass needle mounted on a micromanipulator. *M. demolitor* eggs and ovariole factors (calyx fluid plus venom) were collected by dissecting female wasps in physiological saline, and removing the ovaries and venom glands[27]. *M. demolitor* and many other larval parasitoids in the families Braconidae and Ichneumonidae carry polyDNAviruses which replicate in female wasps in a region of the ovary called the calyx. Virions are stored in the lumen of the oviduct, with the resulting suspension of virus and protein being called calyx fluid. In combination with venom proteins produced by the venom gland, polyDNAviruses are known to cause several alterations in parasitized hosts including suppression of the immune system and regulation of moulting[26,28]. Third instar hosts containing *C. floridanum* were injected with 2–3 *M. demolitor* eggs, 0.05 wasp equivalents of calyx fluid plus venom, or saline. These dosages reflect the amount of each factor normally injected into the host by *M. demolitor* during oviposition[27]. The number of precocious larvae was then determined by dissecting hosts in the fifth instar.

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Condition-dependent signalling of genetic variation in stalk-eyed flies

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Handicap models of sexual selection predict that male sexual ornaments have strong condition-dependent expression and this allows females to evaluate male genetic quality[1–5]. A number of previous experiments have demonstrated heightened condition-dependence of sexual ornaments in response to environmental stress[6–9]. Here we show that genetic variation underlies the response to environmental stress (variable food quality) of a sexual ornament (male eye span) in the stalk-eyed fly *Cyrtodiopsis dalmanni*. Some male genotypes develop large eye span under all conditions, whereas other genotypes progressively reduce eye span as conditions deteriorate. Several non-sexual traits (female eye span, male and female wing length) also show genetic variation in condition-dependent expression, but their genetic response is entirely explained by scaling with body size. In contrast, the male sexual ornament still reveals genetic variation in the response to environmental stress after accounting for differences in body size. These results strongly support the hypothesis that female mate choice yields genetic benefits for offspring.

In the Malaysian stalk-eyed fly *C. dalmanni*, males have exaggerated eye span and females show strong mating preferences for males with greater eye span[10,11]. Here we investigated how genotype and environment (and their interaction) affected the development of sexual and non-sexual traits. Experimental males were mated to virgin females to generate full and half-sibling families (see Methods). Eggs and larvae from each family were cultured on corn, spinach or damp cotton wool, which represent food environments of decreasing nutritional value. Emerging flies were sexed, and the male sexual trait (eye span), several comparable non-sexual traits (female eye span, male and female wing length), and body size (thorax length) were measured.

Food quality, as expected, had a large effect on absolute trait sizes (Table 1). For all traits and both sexes, spinach-fed flies were smaller than corn-fed flies ($P < 0.001$). However, the main change occurred with the shift to the poorest environment, flies that had fed on cotton wool being dramatically smaller than flies that had fed on spinach ($P < 0.001$, Fig. 1). Despite this strong condition-dependent environmental effect, there was clear evidence for genetic variation in all traits, in both sexes (Table 1). This was still the case when each environment was analysed separately (all $P < 0.05$).

Of greater interest were the interactions between effects due to food quality and effects due to genotype—that is, food quality by genotype interactions as these test whether there is a genetic basis for environmental condition dependence. For absolute male eye span, the interaction was significant (Table 1). Comparisons of the genetic variance in each environment showed that differences between genotypes increased under food stress, especially in the cotton-wool environment (Fig. 1; corn–wool $F_{29,125} = 1.937$, $P = 0.007$; spinach–wool $F_{31,113} = 1.664$, $P = 0.028$; corn–spinach $F_{31,67} = 1.462$, $P = 0.099$; tested using the Satterthwaite method, see Methods). Nonetheless genotype ranks were maintained across

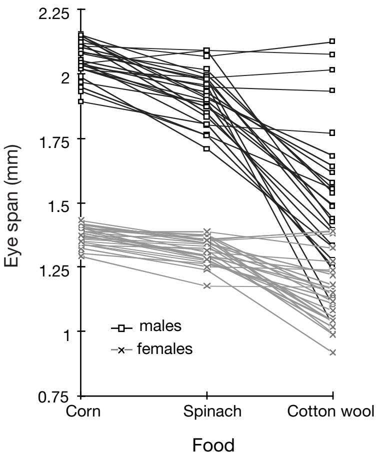

Figure 1 Genotypic responses for absolute male and female eye span in corn, spinach and cotton-wool environments. Each line represents the mean eye span of a group of full-siblings.