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Caste ratios affect the reproductive output of social trematode colonies

T. KAMIYA & R. POULIN

Department of Zoology, University of Otago, Dunedin, New Zealand

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Abstract

Intraspecific phenotypic diversification in social organisms often leads to formation of physical castes which are morphologically specialized for particular tasks within the colony. The optimal caste allocation theory argues that specialized morphological castes are efficient at specific tasks, and hence different caste ratios should affect the ergonomic efficiency, hence reproductive output of the colony. However, the reproductive output of different caste ratios has been documented in few species of insects with equivocal support for the theory. This study investigated whether the ratios of nonreproductive and reproductive morphs affect the reproductive output of a recently discovered social trematode, *Philophthalmus* sp., in which the nonreproductive members are hypothesized to be defensive specialists. A census of natural infections and a manipulative *in vitro* experiment demonstrated a positive association between the reproductive output of trematode colonies and the ratio of nonreproductive to reproductive morphs in the presence of an intra-host trematode competitor, *Maritrema novaezealandensis*. On the contrary, without the competitor, reproductive output was negatively associated with the proportion of nonreproductive castes in colonies. Our findings demonstrate for the first time a clear fitness benefit associated with the nonreproductive castes in the presence of a competitor while illustrating the cost of maintaining such morphs in noncompetitive situations. Although the proximate mechanisms controlling caste ratio remain unclear in this trematode system, this study supports the prediction that the fitness of colonies is influenced by the composition of specialized functional morphs in social organisms, suggesting a potential for adaptive shifts of caste ratios over evolutionary time.

Introduction

Division of labour is a common feature of colonial and social organisms, which is often facilitated by high genetic relatedness and resource sharing among colony members (Simpson, 2012). Phenotypic diversification of colony members often leads to formation of physical castes, which are morphologically specialized for particular tasks (Oster & Wilson, 1978). With notable ecological similarities, physical castes have evolved independently in a number of insects (Oster & Wilson, 1978), snapping shrimps (Duffy, 1996), sea anemones (Ayre & Grosberg, 1996), mole rats (Jarvis, 1981) and parasitic trematodes (Hechinger *et al.*, 2011; Leung & Poulin, 2011).

Although the physiological mechanisms of caste differentiation in social insects have received extensive scrutiny (Dewilde & Beetsma, 1982; Laine & Wright, 2003; Vargo & Husseneder, 2009), the relationship between ecological factors and the evolutionary success of colonies with different caste ratios remains understudied (Hasegawa, 1997). Research on caste ratios in social organisms was investigated by Oster & Wilson's (1978) optimal theory of caste allocation. They argued that highly specialized castes are efficient at specific

Correspondence: Tsukushi Kamiya, Department of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand.
Tel.: +64 03 479 5848; fax: +64 03 479 7584;
e-mail: philophthalmus@gmail.com

tasks, and hence different caste ratios should result in differences in colony efficiency as a whole. Therefore, under a given set of environmental conditions, there should exist an optimal ratio within a colony, according to which caste members are allocated.

Although the optimal theory (Oster & Wilson, 1978) assumes an optimal caste ratio that maximizes colony reproductive output, the fitness consequence of different caste allocation decisions has been documented in only a few species of ants with equivocal support for the theory (e.g. Porter & Tschinkel, 1985; Calabi & Traniello, 1989; Tsuji, 1994; Hasegawa, 1997). Nonetheless, empirical evidence for shifts in short-term ergonomic efficiency of colonies is essential to understand the relationship between colony composition and their responses to environmental variations. Assessing the reproductive output of different caste ratios across environmental gradients can serve to identify factors that have contributed to the origin and maintenance of physical castes. So far theoretical models and their empirical tests have been restricted to a few ant taxa (e.g. Porter & Tschinkel, 1985; Calabi & Traniello, 1989; Tsuji, 1994; Hasegawa, 1997), while simpler clonal social systems may offer a more straightforward test for the association between reproductive output and caste ratios. Caste ratio is a property whose optimum should be determined by selection acting upon the colony level (Hasegawa, 1997). Thus, clonal organisms that preclude genetic conflicts of interest at the individual level should provide better systems to study such relationships than insects with haplodiploidy.

Recently, distinct castes have been discovered in the clonal intermediate stage of two trematode parasite species, in which one morph produce cercariae, the next motile larval stage that leaves the host, while the second is considerably smaller and reproductively inactive (Leung & Poulin, 2011; Hechinger *et al.*, 2011; see fig. 1 in Leung & Poulin, 2011). Several lines of empirical evidence presented by Hechinger *et al.* (2011) indicate that the nonreproductive morphs of *Himasthla* sp. are defensive specialists: they have larger mouthparts, are more active than reproductives, readily attack members of foreign colonies, and are particularly common at invasion fronts within the host's body. In addition, direct consumption of co-infecting species by the nonreproductives has been reported in *Philophthalmus* sp. (Leung & Poulin, 2011). Furthermore, 'the soldier hypothesis' is consistent with the widely held view that interspecific competition for resources and space represents a potentially strong selection pressure for trematodes infecting gastropod intermediate hosts (Kuris & Lafferty, 1994; Poulin, 2001), as recently demonstrated in our study system by Lloyd & Poulin (2012).

In *Philophthalmus* sp., Leung & Poulin (2011) have shown that caste ratios observed *in situ* did not vary according to the presence or absence of a competing trematode species in the same host individual, yet they varied widely in a single population (nonreproductive: reproductive = 0.76–3.27). Nevertheless, the optimal theory claims that the reproductive output of a colony should reflect the fitness associated with its composition in a given situation even if allocation decisions to different morphs have a strong genetic basis. Thus, the evolutionary success of a particular caste ratio should fluctuate as a function of ecological factors such as competition. Having two physical castes (reproductives and nonreproductives) with no size overlap (Hechinger *et al.*, 2011), one should also expect little overlap of tasks in social trematode colonies, which should lead to steep responses in colony reproductive output (Hasegawa, 1997).

This study investigates whether the ratios of the nonreproductive and reproductive castes affect the reproductive output of *Philophthalmus* sp. colonies. Although the defensive role of nonreproductive morphs in trematodes has been suggested recently (Hechinger *et al.*, 2011; Leung & Poulin, 2011), the fitness costs and benefits of maintaining such colony members have not been evaluated in natural populations. Assuming they play a defensive role, the ergonomic theory of caste allocation predicts higher efficiency for colonies with higher proportions of defensive morphs under imminent competition. In contrast, excess production of nonreproductives at the expense of shared resources should hinder short-term ergonomic efficiency of colonies free of competitive pressure. As colonies should achieve their highest reproductive output when they display the appropriate composition for a given set of conditions, we predicted that colonies with high proportions of 'soldier' nonreproductive morphs should achieve higher reproductive outputs in the presence of competitors, while the opposite should be true in the absence of competitors. These predictions were tested by examining reproductive outputs of parasites in live hosts, as well as in *in vitro* culture experiments where caste ratios and the presence of competitors were artificially manipulated. Testing such predictions should further enhance our understanding of the evolution of multiple morphological castes in clonal trematodes, while expanding our general knowledge of the evolutionary consequences of caste allocation decisions.

Materials and methods

Study organisms, screening and maintenance

Philophthalmus sp. is one of many trematode parasites with complex life cycles that infect the mud snail, *Zeacumantus subcarinatus*, as their first intermediate host. It is the second most common trematode parasite at our study site with up to 8% prevalence, second only to *Maritrema novaezealandensis* which accounts for up to 60% of parasite infections in the snail population (Keeney *et al.*, 2008; Martorelli *et al.*, 2008). Both trematode species multiply asexually in the gonadal tissues of the snail, forming masses of clonal stages collectively known as parthenitae that produce free-swimming dispersal stages known as cercariae, which then leave the snail. Parthenitae of *Philophthalmus* sp. are called rediae and possess mouthparts, and occur as nonreproductive and reproductive morphs; those of *M. novaezealandensis* are of a different type known as sporocysts and lack mouthparts. *Philophthalmus* sp. provides an ideal system to study sociality of trematodes because its proximate ecological properties such as life cycle history (Martorelli *et al.*, 2008), larval dispersion characteristics (Koprivnikar & Poulin, 2009), thermal tolerance (Bates *et al.*, 2011) and co-infecting species (Martorelli *et al.*, 2008) in the gastropod host have been relatively well documented. Furthermore, *in vitro* culture techniques have been optimized for this particular species (Lloyd & Poulin, 2011).

Mud snails, *Z. subcarinatus,* were collected in February and March 2012 from Lower Portobello Bay, Otago Harbor, South Island, New Zealand (45°520 S, 170°420 E). The snails were individually incubated in wells of a 12-well culture plate filled with filtered saltwater at 25 °C for 24 h to induce the trematode parasites to release cercariae, which were identified using a stereomicroscope. Incubation also served to ‘empty’ cercariae stored in the host, to standardize the cercarial content of each colony to approximately zero at the start of the experiments. Snails found shedding cercariae of *Philophthalmus* sp. or both *Philophthalmus* sp. and *M. novaezealandensis* were individually transferred to separate plastic containers filled with filtered saltwater and supplied with *Ulva lactuca*. One-off incubations of snails do not guarantee accurate assessments of infection status. Therefore, snails were re-examined following the same procedure at least 48 h after the initial incubation. Snails that showed signs of infection from other trematode species were excluded. The infection status of the hosts was re-confirmed upon dissection at the end of the *in vivo* census.

In vivo trematode census

Comparisons of colonies within live snails allows for assessment of the link between colony composition and colony success in an undisturbed setting, in which each colony has naturally settled on a given ratio of reproductive to nonreproductive rediae. Following the infection checks, the snails were maintained in wells (16.8 cm^3) of 6-well culture plates filled with filtered saltwater with a piece of *U. lactuca* for 14 days at room temperature. This allowed the parasites to produce cercariae ‘from scratch’ for a set period of time and all *Philophthalmus* sp. cercariae produced over the 2 week period to be counted. Following their emergence from the snail, *Philophthalmus* sp. cercariae rapidly encyst on the inner surfaces of the wells (see (Lei & Poulin, 2011)), and are thus easily counted. Prior to dissection, the maximum shell length of each snail was measured to the nearest 0.1 mm using a set of callipers. To minimize differences between infection groups, the size of individual snails were matched as closely as possible between *Philophthalmus*-only (mean shell length = 13.20 mm) and *Philophthalmus–Maritrema* double infected snails (12.95 mm; Welch Two Sample *t*-test: *T*-value = 0.749, d.f. = 45, *P*-value = 0.458). In total, 28 *Philophthalmus*-only snails and 30 *Philophthalmus –Maritrema* double infected snails were included in this study. After cracking shells and removing the host tissue, the parasite tissues were dyed with Neutral Red, pressed between two glass plates and examined under a stereomicroscope. The total number of cercariae was assessed as the sum of cercariae produced and released over 14 days and mature cercariae found inside the host upon dissection. The number of reproductive rediae and nonreproductive rediae were also counted. We were unable to count the number of *M. novaezealandensis* sporocysts as they cluster together in tight clumps, hence they were recorded as either present or absent. In addition, the average size of a subset of reproductive ($N = 10$) and nonreproductive rediae ($N = 3–10$) in each colony was calculated as the mean flattened body areas of rediae in micrographs. The variation in the sample size of nonreproductive rediae across colonies arose due to overlaps with other structures and unreliable staining which resulted in unclear images. The images were then analysed with ImageJ 1.45s (U.S. National Institutes of Health, Bethesda, MD, USA) for area measurements.

Linear models, using data sets with and without outliers, were constructed to estimate the most representative effect sizes and provide conservative significance testing in the analysis of the relationship between cercarial production, and caste ratio and competition. Outliers were defined when the morph ratio exceeded 1.5 times the interquartile range below the first quartile or above the third quartile (i.e. below 0.13 or above 2.735). Three outliers above the third quartile were detected; two in *Philophthalmus*-only (4.19, 3.34) and one in *Philophthalmus–Maritrema* snails (4.05). The number of cercariae produced was log-transformed. Predictor variables included infection status (i.e. *Philophthalmus*-only or *Philophthalmus–Maritrema*), nonreproductive to reproductive rediae ratio, the interaction between infection status and the ratio, average sizes of reproductive and nonreproductive rediae, and maximum shell length of the host. Independent variables were centred in the case of continuous variables. Further interactions were excluded from the model because adding any one of the first-order interaction terms which included our factor of interest, infection status (i.e. infection by *Philophthalmus*-only or *Philophthalmus–Maritrema*), failed to improve model fit any further based on AICc. To control for the absolute abundance of each caste, linear models were also constructed using the absolute number of nonreproductive and reproductive rediae, instead of the ratios (see Table S1). All statistical analyses were conducted in R 2.14.1 (R Development Core Team, 2011).

In vitro experiment

The *in vitro* study allowed artificial manipulation of caste ratios in a controlled environment. A critical advantage of the *in vitro* approach was that any given colony could be split into smaller colonies that were then placed in each treatment of the experiment, so that each genotype was examined under the full range of conditions, allowing the effect of reproductive to nonreproductive caste ratio to be disentangled from that of genotype per se (ratio effects and genotype effects are inescapably confounded in live snails). In addition, the *in vitro* approach standardized a range of other confounding variables, such as nutrient availability, which vary from snail to snail.

Snails (different individuals from those used in the *in vivo* study) were carefully cracked using a hammer and *Philophthalmus* rediae and *M. novaezealandensis* sporocysts were dissected out of separate singly infected snails. Parthenitae were cultured in a 12-well culture plate filled with 1 mL culture medium, which has been previously developed and optimized for use with both species of trematodes (see Lloyd & Poulin (2011). The culture medium consisted of 400 mg L-15 powder (Sigma L4386; St. Louis, MO, USA) dissolved in 50 mL sterile water, 1.56 g Instant Ocean powder (Aquarium Systems, Sarrebourg, France), 10 mL chicken serum (Invitrogen, Carlsbad, CA, USA) and 2 mL of the penicillin–streptomycin–neomyacin solution (Sigma P4083). The dissected rediae were allocated to six different treatments all with a fixed number of reproductives (six rediae), but differing in the ratio of nonreproductive to reproductive rediae (i.e. 2:6, 6:6, 18:6) and in the presence of *M. novaezealandensis* sporocysts (i.e. approximately 30 sporocysts, or 0 sporocysts). Rediae from a single snail were used for one replicate of each treatment and *M. novaezealandensis* sporocysts were also taken from a separate single snail for each replicate, to homogenize genotypes across treatments. Although we cannot preclude the possibility of multiple-clone infection of *Philophthalmus* sp. within a single snail, most colonies examined in preliminary assessments in our laboratory were of single clones (M. Lloyd, personal communication). Although we recognize the potential confounding effect of different densities of individuals per treatment, this effect should be minimal as the volume of an individual nonreproductive was, on average, only about 1.35×10^{-6} of the volume of the culture medium in a well. The cultures were maintained for 28 days at 18 °C in an incubator under ambient light and replenished with new media every 3–4 days. The numbers of newly produced cercariae were counted and then removed daily. The experiment was replicated 14 times using 14 independent colonies.

A generalized linear mixed model (negative binomial errors) was constructed with the number of cercariae, that is, reproductive output, as the response variable for cultures with and without the presence of *M. novaezealandensis* sporocysts using the package *glmmADMB*, to account for over-dispersion in the count data (Lawless, 1987). The number of nonreproductive rediae per culture was the main factor of interest while differences in the initial volume of reproductive rediae, calculated at the start as cylinders, were controlled by including it among fixed effects after centring. In addition, the potential influence of differences in the genotype of the rediae was taken into account by including clone identity as a random effect.

Results

In vivo trematode census

The mean caste ratios of *Philophthalmus* sp. did not differ between colonies with and without the presence of the intra-host competitor, *M. novaezealandensis,* when the host size was held constant, as previously reported for this species (*Philophthalmus*-only: 1.505, *Philophthalmus–Maritrema*: 1.491; $T_{55} = 0.124$, $P = 0.902$). In addition, the cercarial output did not vary significantly between single infection colonies and colonies that co-occurred with *M. novaezealandensis* (Table 1). However, the reproductive output of *Philophthalmus* sp. was positively related to the absolute number and the proportion of nonreproductive morphs in the presence of the competitor, while the reproductive output of colonies was negatively associated with the absolute number of nonreproductives and the ratio of nonreproductive to reproductive morphs in the absence of the intra-host competitor, *M. novaezealandensis* (Fig. 1, Table 1, Table S1). In addition, the shell length of the host was a statistically significant predictor of the reproductive output of *Philophthalmus* sp. colonies in the live host (Table 1). Although analyses were carried out with and without outliers for both the ratios and absolute numbers, the results were qualitatively identical (Table 1a,b, Table S1a,b).

In vitro experiment

The ratio of the two functional morphs did not affect the reproductive output when *M. novaezealandensis* was absent (Fig. 2; Table 2). When the competitor was present, however, the reproductive output of colonies increased with the proportion of nonreproductive members (Fig. 2; Table 2).

Table 1 Results of linear models evaluating the relationship between the reproductive success and the ratio of nonreproductive to reproductive castes in colonies of the trematode *Philophthalmus* sp., (a) with and (b) without outliers. *P*-values in bold type indicate $P < 0.05$.

	Estimate	SE	*T*-value	*P*-value
(a)				
(Intercept)	5.6525	0.1093	51.696	**<0.001**
Co-infection	−0.0364	0.1581	−0.230	0.819
Caste ratio	−0.2932	0.1031	−2.843	**0.006**
Host shell length	0.1504	0.0742	2.028	**0.048**
Size of reproductives	0.0314	0.0817	0.385	0.702
Size of nonreproductives	−0.1009	0.0800	−1.263	0.212
Co-infection × Caste ratio	0.3854	0.1649	2.337	**0.023**
(b)				
(Intercept)	5.6638	0.0930	60.883	**<0.001**
Co-infection	−0.0364	0.1330	−0.274	0.785
Caste ratio	−0.3761	0.0926	−4.061	**<0.001**
Host shell length	0.1482	0.0628	2.361	**0.022**
Size of reproductives	−0.0097	0.0674	−0.144	0.886
Size of nonreproductives	−0.1190	0.0671	−1.773	0.082
Co-infection × Caste ratio	0.6803	0.1345	5.058	**<0.001**

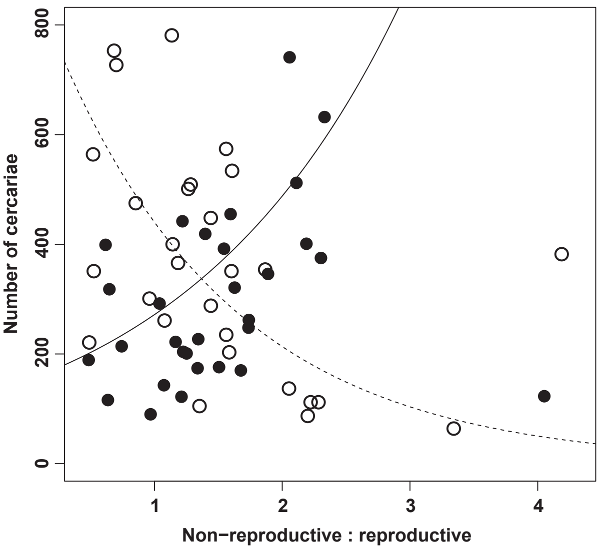

Fig. 1 The number of cercariae produced over 14 days in live snail hosts plotted against the ratio of nonreproductive to reproductive morphs. Open circles with the dashed regression line represent *Philophthalmus* sp. colonies without competitor (i.e. $N = 28$ *Philophthalmus*-only), whereas solid circles with the solid line represent *Philophthalmus* sp. colonies with the intra-host competitor *Maritrema novaezealandensis* (i.e. $N = 30$ *Philophthalmus–Maritrema*). The regression lines were exponentially converted from log-transformed data used in a linear model (refer to Table 1b for results).

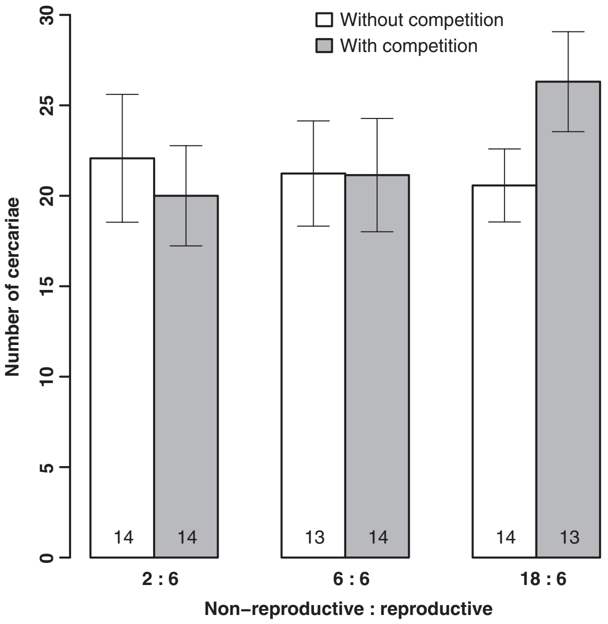

Fig. 2 The mean number of cercariae produced over 28 days by six *Philophthalmus* sp. reproductive morphs in *in vitro* culture with different ratios of nonreproductive to reproductive morphs, with and without the presence of the intra-host competitor *Maritrema novaezealandensis*. Sample sizes are shown inside each bar. Standard error bars are also shown.

Table 2 Results of a generalized linear mixed model with negative binomial errors evaluating the relationship between the reproductive output and the interaction between the presence of competitor and the number of nonreproductives in *in vitro* cultures of the trematode *Philophthalmus* sp. *P*-values in bold type indicate $P < 0.05$.

	Estimate	SE	*T*-value	*P*-value
(Intercept)	3.0599	0.1252	24.442	**<0.001**
Competitor	−0.1512	0.1146	−1.321	0.187
Nonreproductives	−0.0043	0.0074	−0.593	0.557
Rediae volume	0.1382	0.0635	2.181	**0.030**
Competitor × Nonreproductives	0.0230	0.0105	2.193	**0.029**

The genotypes of the rediae, included as a random effect, explained 12.8% of the variance unaccounted for by the main effects.

Discussion

Our findings demonstrate a clear fitness benefit of maintaining the nonreproductive castes in the presence of a competitor, while illustrating the cost of such morphs in a naturally infected population when the competitor is absent; this supports our hypothesis that reproductive output of *Philophthalmus* sp. colonies is influenced by the interaction of caste ratios and an environmental factor, namely intra-host competition.

The benefit of maintaining a high proportion of nonreproductive morphs was evident as the reproductive output increased with the ratio of nonreproductives to reproductives when the intra-host competitor was present, in both live hosts and in the *in vitro* experiment. Our results corroborated earlier experimental studies of social insects which demonstrated changes in reproductive output with regard to caste ratios (e.g. Tsuji, 1994; Hasegawa, 1997). According to the optimal theory (Oster & Wilson, 1978), such increases in reproductive output should be due to the specialized role that the nonreproductives play in the presence of the intra-host competitor. Empirical evidence presented by Hechinger *et al.* (2011) points towards the hypothesis that the nonreproductive individuals are eusocial 'soldiers', which readily attack members of foreign colonies. The soldier hypothesis assumes that there exists intra-host competition, hence the need for evolving 'soldiers'. Such competition may take the form of exploitation competition where parties compete for limited food and space in the host, or interference competition where co-infecting species interfere with the growth and reproduction of the focal species. Consequently, the soldier hypothesis predicts that the nonreproductives are capable of damaging and decreasing the mass of the competing species sufficiently to lead eventually to competitive exclusion. Yet, despite the observation that the nonreproductives consume competing species as can be seen in the micrographs reported by Hechinger *et al.* (2011) and Leung & Poulin (2011), reduction in the mass of the competitor remains to be quantified. Furthermore, empirical evidence regarding the potential for competitive exclusion of *M. novaezealandensis* appears puzzling. Keeney *et al.* (2008) examined the extent of intra-host competition between the two trematode species. They found that snails infected with *M. novaezealandensis* only, or co-infected with both *Philophthalmus* sp. and *M. novaezealandensis,* were significantly smaller than ones infected only with *Philophthalmus* sp., indicating that *M. novaezealandensis* may be excluded from older snails. In contrast, the frequency of snails harbouring both species was roughly identical to what would be expected by chance alone from the prevalence of each species, suggesting no measureable competitive exclusion at the population level (Keeney *et al.*, 2008). In our *in vitro* experiment, *M. novaezealandensis* sporocysts were present at the end of the 28 day experiment, still producing motile cercariae. In addition, the clonal stage of the competing species, *M. novaezealandensis*, lacks mouthparts and organs; hence, interference competition is unlikely to be imposed upon *Philophthalmus* sp. by its competitor. Therefore, the increase in reproductive output over 28 days as a response to high proportions of nonreproductives in the presence of *M. novaezealandensis* cannot be explained solely by the soldier hypothesis. Instead, nonreproductives seem capable of increasing resource acquisition of reproductives when the co-infecting parasite is present. Thus, there may be an additional benefit of the nonreproductive morphs that acts beyond exclusion of competing species. Nevertheless, given our evidence that the reproductive output of a colony with a particular caste ratio was influenced by the presence of the competitor both in the natural population and *in vitro*, and in light of two recent studies (Hechinger *et al.*, 2011; Leung & Poulin, 2011), it can be conservatively deduced that the evolution of the nonreproductive morph in *Philophthalmus* sp. has been tightly associated with intra-host competition.

A limitation associated with the *in vitro* approach was evident in this study as ecologically important factors that would naturally be imposed on live hosts could not be replicated *in vitro*. For example, limited food availability and space are essential parameters for demonstrating the cost of nonreproductive individuals to the colony. However, we were logistically constrained from imposing resource limitations as low nutrient environments significantly reduce the survival of redial cultures (Lloyd & Poulin, 2011). In addition, the space provided by wells on a culture plate is enormous relative to the space available inside the gastropod hosts. Such technical difficulties of *in vitro* culture are likely to account for the discrepancy between the results from the *in vivo* study and *in vitro* culture. Nevertheless, the reduced reproductive output demonstrated in the naturally infected population indicates a fitness cost associated with high proportions of the nonreproductives in the absence of competition, probably due to necessary sharing of limited space and/or resources with the reproductives. Although Lloyd & Poulin (2012) have recently suggested some benefit of the nonreproductives without competition in *in vitro* conditions, this study did not corroborate the pattern in the natural parasite population. In addition, our findings suggest that the extent of the benefit of nonreproductives clearly depends on the ratio of the physical castes both with and without the presence of the competitor.

Although Oster & Wilson (1978) have considered the potential importance of ecological factors in determining caste ratios, they assumed that proximate alteration of caste ratios should be rare in their focal taxa, the ants, due to a lack of reliable signals for environmental fluctuations and the cost of additional production required for a flexible defensive strategy (Gordon, 1996). The production of new colony members is accompanied by a time-lag as well as considerable energetic costs. Thus, plasticity of caste ratios should only be possible when the benefits, which depend on the degree of specialization of each caste and reliability of signals, outweigh the energetic costs of new production. In contrast, the short-term ergonomic efficiency of a colony may be enhanced by plasticity of its composition in response to environmental fluctuations (Herbers, 1980). For example, increased investment in defensive morphs will come at the expense of resources which could potentially be devoted to reproduction and hence colony expansion (Tyerman & Roitberg, 2004). In the presence of competitors, however, the overall efficiency of a colony should be enhanced by increased production of defensive castes. Overall, adaptive alterations of physical caste ratios are empirically supported when periods of environmental fluctuation last longer than the time it takes to implement changes and provided that the benefits of alterations outweigh their costs. (Passera *et al.*, 1996; Harvey *et al.*, 2000; Yang *et al.*, 2004). Although the competitive pressure exerted by *M. novaezealandensis* should persist for a relatively long period of time until its competitive exclusion (if that eventuates), little is known about the proximate mechanisms of caste differentiation in social trematodes. Therefore, the cost and benefit associated with flexible caste allocation cannot be quantified based on current knowledge. Nevertheless, our results corroborate an earlier suggestion by Leung & Poulin (2011) that *Philophthalmus* sp. lacks proximate mechanisms to adjust caste ratios according to intra-host competition. Perhaps, the lack of adaptive caste allocation is not surprising given that a number of recent studies have reported a strong genetic basis for caste determination in social insect species (Jaffe *et al.*, 2007; Evison & Hughes, 2011; Kraus *et al.*, 2011).

On a much longer time scale, however, the higher reproductive output of colonies with a high proportion of nonreproductive morphs in competitive situations means that adjusting caste ratios according to competitive pressure over evolutionary time should be possible given that such pressure stays relatively constant and populations are sufficiently genetically isolated. If the among-population variation exceeds the temporal fluctuations of competitive pressure, selection should favour adaptive shifts of caste ratios on an evolutionary time scale. A classic study by Davidson (1978) documented consistent shifts in caste ratios with the magnitude of competitive pressure in allopatric populations of ants. Similarly, we predict that when populations with different competitive pressures exist in relative isolation on a large geographical scale, there should be differences in colony compositions between those populations of social trematodes correlating with the extent of local competition (i.e. prevalence of the competitor). However, given the mode of parasite dispersal by the definitive host (birds in the case of *Philophthalmus* sp.), true allopatric populations with sufficient genetic isolation may only exist on a much larger geographical scale than terrestrial insects.

The strong association between the nonreproductive morph and intra-host competition in the clonal intermediate stage of *Philophthalmus* sp. presents an ideal model system to investigate the relationship between colony composition and competition, and its evolutionary consequence. This study presents the first empirical evidence for the role of caste ratios in affecting reproductive output outside of social insects.

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References

Ayre, D.J. & Grosberg, R.K. 1996. Effects of social organization on inter-clonal dominance relationships in the sea anemone *Anthopleura elegantissima*. *Anim. Behav.* **51**: 1233–1245.

Bates, A.E., Leiterer, F., Wiedeback, M.L. & Poulin, R. 2011. Parasitized snails take the heat: a case of host manipulation? *Oecologia* **167**: 613–621.

Calabi, P. & Traniello, J.F.A. 1989. Social-organization in the ant *Pheidole dentata* – physical and temporal caste ratios lack ecological correlates. *Behav. Ecol. Sociobiol.* **24**: 69–78.

Davidson, D.W. 1978. Size variability in worker caste of a social insect (*Veromessor pergandei mayr*) as a function of competitive environment. *Am. Nat.* **112**: 523–532.

Dewilde, J. & Beetsma, J. 1982. The physiology of caste development in social insects. *Adv. Insect Physiol.* **16**: 167–246.

Duffy, J.E. 1996. Eusociality in a coral-reef shrimp. *Nature* **381**: 512–514.

Evison, S.E.F. & Hughes, W.O.H. 2011. Genetic caste polymorphism and the evolution of polyandry in Atta leaf-cutting ants. *Naturwissenschaften* **98**: 643–649.

Gordon, D.M. 1996. Behavioural ecology – soldier production under threat. *Nature* **379**: 583–584.

Harvey, J.A., Corley, L.S. & Strand, M.R. 2000. Competition induces adaptive shifts in caste ratios of a polyembryonic wasp. *Nature* **406**: 183–186.

Hasegawa, E. 1997. The optimal caste ratio in polymorphic ants: estimation and empirical evidence. *Am. Nat.* **149**: 706–722.

Hechinger, R.F., Wood, A.C. & Kuris, A.M. 2011. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. *Proc. R. Soc. Lond. B Biol. Sci.* **278**: 656–665.

Herbers, J.M. 1980. On caste ratios in ant colonies – population responses to changing environments. *Evolution* **34**: 575–585.

Jaffe, R., Kronauer, D.J.C., Kraus, F.B., Boomsma, J.J. & Moritz, R.F.A. 2007. Worker caste determination in the army ant *Eciton burchellii*. *Biol. Lett.* **3**: 513–516.

Jarvis, J.U.M. 1981. Eusociality in a mammal – cooperative breeding in naked mole-rat colonies. *Science* **212**: 571–573.

Keeney, D.B., Boessenkool, S., King, T.M., Leung, T.L.F. & Poulin, R. 2008. Effects of interspecific competition on asexual proliferation and clonal genetic diversity in larval trematode infections of snails. *Parasitology* **135**: 741–747.

Koprivnikar, J. & Poulin, R. 2009. Effects of temperature, salinity, and water level on the emergence of marine cercariae. *Parasitol. Res.* **105**: 957–965.

Kraus, F.B., Gerecke, E. & Moritz, R.F.A. 2011. Shift work has a genetic basis in honeybee pollen foragers (*Apis mellifera l.*). *Behav. Genet.* **41**: 323–328.

Kuris, A.M. & Lafferty, K.D. 1994. Community structure – larval trematodes in snail hosts. *Annu. Rev. Ecol. Syst.* **25**: 189–217.

Laine, L.V. & Wright, D.J. 2003. The life cycle of Reticulitermes spp. (Isoptera: Rhinotermitidae): what do we know? *Bull. Entomol. Res.* **93**: 267–278.

Lawless, J.F. 1987. Negative binomial and mixed Poisson regression. *Can. J. Stat.* **15**: 209–225.

Lei, F. & Poulin, R. 2011. Effects of salinity on multiplication and transmission of an intertidal trematode parasite. *Mar. Biol.* **158**: 995–1003.

Leung, T.L.F. & Poulin, R. 2011. Small worms, big appetites: ratios of different functional morphs in relation to interspecific competition in trematode parasites. *Int. J. Parasitol.* **41**: 1063–1068.

Lloyd, M.M. & Poulin, R. 2011. *In vitro* culture of marine trematodes from their snail first intermediate host. *Exp. Parasitol.* **129**: 101–106.

Lloyd, M.M. & Poulin, R. 2012. Fitness benefits of a division of labour in parasitic trematode colonies with and without competition. *Int. J. Parasitol.* **42**: 939–46.

Martorelli, S.R., Fredensborg, B.L., Leung, T.L.F. & Poulin, R. 2008. Four trematode cercariae from the New Zealand intertidal snail *Zeacumantus subcarinatus* (Batillariidae). *N. Zeal. J. Zool.* **35**: 73–84.

Oster, G.F. & Wilson, E.O. 1978 *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, NJ.

Passera, L., Roncin, E., Kaufmann, B. & Keller, L. 1996. Increased soldier production in ant colonies exposed to intraspecific competition. *Nature* **379**: 630–631.

Porter, S.D. & Tschinkel, W.R. 1985. Fire ant polymorphism (Hymenoptera, Formicidae) – factors affecting worker size. *Ann. Entomol. Soc. Am.* **78**: 381–386.

Poulin, R. 2001. Interactions between species and the structure of helminth communities. *Parasitology* **122**: S3–S11.

R Development Core Team. 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Simpson, C. 2012. The evolutionary history of division of labour. *Proc. R. Soc. Lond. B Biol. Sci.* **279**: 116–121.

Tsuji, K. 1994. Inter-colonial selection for the maintenance of cooperative breeding in the ant *Pristomyrmex pungens* – a laboratory experiment. *Behav. Ecol. Sociobiol.* **35**: 109–113.

Tyerman, J.G. & Roitberg, B.D. 2004. Factors affecting soldier allocation in clonal aphids: a life-history model and test. *Behav. Ecol.* **15**: 94–101.

Vargo, E.L. & Husseneder, C. 2009. Biology of subterranean termites: insights from molecular studies of Reticulitermes and Coptotermes. *Annu. Rev. Entomol.* **54**: 379–403. Annual Reviews, Palo Alto.

Yang, A.S., Martin, C.H. & Nijhout, H.F. 2004. Geographic variation of caste structure among ant populations. *Curr. Biol.* **14**: 514–519.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results of linear models evaluating the relationship between the reproductive success and the number of nonreproductive and reproductive castes in colonies of the trematode *Philophthalmus* sp.

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