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ORIGINAL PAPER

Geographic variation in caste ratio of trematode colonies with a division of labour reflect local adaptation

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Abstract Similarly to the division of labour in social insects, castes of morphologically distinct individuals exist within colonies of some species of parasitic trematodes. These colonies occur in the first intermediate host of the trematode's complex life cycle and are composed of clonal individuals. Individuals of the reproductive caste have significantly larger bodies while non-reproductive individuals are small and appear to be specialised for defence against co-infecting trematode colonies. In parallel with colony organisation of social insects, demographic traits such as the proportion of the small, non-reproducing individuals relative to the large, reproducing individuals and colony size are expected to vary and adjust to local conditions. In the case of colonies from geographically and potentially genetically distinct populations, this variation is hypothesised to become fixed by evolutionary divergence, as reported in social insect studies. In this study, the adaptive demography theory was further tested by looking at caste ratio and colony organisation of *Philophthalmus* sp. (a parasitic trematode with a recently discovered division of labour) colonies from geographically distinct populations. Results indicate that the caste ratio from geographically distinct *Philophthalmus* sp. colonies differs; the proportion of small, defensive individuals is higher in colonies from the location where the risk of competition is highest. This is suggestive of local adaptation, as caste ratios do not change over time under standardised laboratory conditions. This is the first evidence to support the adaptive demography theory in a species with a division of labour other than social insects.

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Introduction

Division of labour is a trait which has evolved in several taxa where related individuals live in groups or colonies. It is defined as the specialisation of individuals to perform some tasks and inability to perform others (Duarte et al. 2011). In cases of reproductive divisions of labour, not all individuals in the colony reproduce. Generally, there exist two or more castes or morphotypes, one caste which can reproduce and one or more which cannot and are specialised to perform other tasks necessary to colony survival and fitness (Simpson 2012). Reproductive division of labour is seen widely across animal taxa (Simpson 2012); most notably not only in social insects (Oster and Wilson 1978) but also in naked mole rats (Jarvis 1981), snapping shrimp (Duffy 1996), sea anemones (Francis 1976) and most recently in parasitic trematodes (Hechinger et al. 2011; Leung and Poulin 2011; Miura 2012).

In cases of colonial species with reproductive divisions of labour, selection acts primarily at the level of the colony, not the individual (Wilson 1985). Colonies develop, function and are subjected to evolutionary pressures as a ‘superorganism’ (Holldobler and Wilson 2009). Therefore, colony traits such as ratios between caste members, colony size and age-frequency distribution are selected for or against based on suitability to the environment, and they are adaptive only to the extent that they optimise colony performance (Wilson 1985; Hasegawa 1997). When specifically applied to caste ratios of colony members, for example, this idea is formulated as the optimal caste ratio theory, which predicts that with highly specialised castes, variation in ratios will be linked to overall colony success. Therefore, ratios should be adaptive and optimised according to various conditions (Oster and Wilson 1978). Considering that expressed phenotypes are a compromise between phenotypic plasticity and genetic determinism, this leads to two predictions: (1) the caste ratio phenotype of single colonies should be plastic (within a given range) and should respond to changing local environments, and (2) colonies from different populations or areas separated by disrupted gene flow may exhibit different caste ratio phenotypes due to evolutionary divergence. Evidence for the first pattern comes from observations of changes in caste ratios of colonies of various social insect species in response to inter or intraspecific competition (Passera et al. 1996; Harvey et al. 2000), food availability (McGlynn and Owen 2002), seasonality (Passera 1977) or risk of predation (Shingleton and Foster 2000). Evidence for the second pattern is seen in geographically isolated ant and paper wasp colonies of the same species exhibiting different caste ratios (Bershers and Traniello 1994; Yang et al. 2004; Fucini et al. 2009). In the case of the study of ants (*Pheidolemorrisi*) of Yang et al. (2004), the differences in caste ratios between geographically distinct colonies persisted after the colonies were maintained under identical laboratory conditions, suggesting that the caste ratio phenotype was determined by evolutionary diverged genotype rather than plastic phenotypic responses to environmental conditions. This finding suggests local adaptation of caste ratios, whereas if the trait was not locally adapted, the differences in caste ratio would not have persisted after the colonies were maintained under identical conditions. In contrast, caste ratio differences are not always seen when expected by the theory of optimal caste ratios (Calabi and Traniello 1989), and similar negative results may not often be published, possibly creating inflated support for the theory.

While divisions of labour have been studied for decades in social insect systems, the trait has only recently been observed in some species of parasitic trematode (Hechinger et al. 2011; Leung and Poulin 2011; Miura 2012). Typically, trematode juveniles (rediae or sporocysts, depending on the species) live within the first intermediate host (commonly a gastropod) where they asexually reproduce, forming a clonal colony (Galaktionov and Dobrovolskij 2003). Free-swimming stages (cercariae) develop within the rediae or sporocysts and leave the host to encyst in or on the second intermediate host (for example, a crustacean or mollusk) and await ingestion by the definitive vertebrate host (Galaktionov and Dobrovolskij 2003). In colonies of trematode species with a division of labour, there exist two distinct castes among the individual rediae: a large reproducing caste and a small, non-reproducing caste. Morphological and behavioural differences between the two morphs indicate that the small, non-reproducing caste members appear specialised for defence against hetero or conspecific colonies trying to establish within the same host, similar to the organisation of many social insect colonies (Hechinger et al. 2011; Miura 2012).

One species of trematode which uses a division of labour is *Philophthalmus* sp. (Leung and Poulin 2011). The first intermediate host of this species is the common New Zealand mudsnail, *Zeacumantus subcarinatus* (Martorelli et al. 2008). This snail is the first intermediate host to five other described trematode species, the most common being *Maritrema novaezealandensis* (Martorelli et al. 2004). Multiple trematode colonies (either hetero or conspecific) can infect the snail at the same time, and in such cases, competition for resources of space and food is expected (Sousa 1992; Poulin 2007). The outcome of this competition, in the case of double-species infections by *Philophthalmus* sp. and *M. novaezealandensis*, is decreased as cercarial production by colonies of both species, resulting in lower colony fitness (Lloyd and Poulin 2012; Lloyd and Poulin 2013). The small, non-reproducing rediae are hypothesised to be specialised for defence against co-infecting colonies (Hechinger et al. 2011). This, taken with the optimal caste ratio theory, leads to the prediction that the ratio of small to large rediae in *Philophthalmus* sp. colonies should be adaptive, with increasing numbers of small, non-reproducing rediae in relation to large, reproducing rediae when the host is co-infected (or at risk of being co-infected) by another trematode colony. Therefore, it is predicted that the average caste ratios of geographically distinct *Philophthalmus* sp. populations will vary in parallel with variation in the prevalence of other potentially co-infecting trematode species.

Very little is known about the evolutionary or environmental control of the caste ratio phenotype in trematode colonies with a division of labour. The small-to-large caste ratio has been studied previously in *Philophthalmus* sp. colonies from one population (Lower Portobello Bay, Otago Harbour). In that single population, *Philophthalmus* sp. can infect between 3–8 % of snails while *M. novaezealandensis* can be found in up to 80 % of snails. Here, the small-to-large caste ratio in *Philophthalmus* sp. colonies varies widely (0.24–3.27 small rediae/large rediae) (Leung and Poulin 2011; Kamiya and Poulin 2013; Lloyd and Poulin 2013) and can increase in response to interspecific competition by *M. novaezealandensis* when colonies are kept for 10 weeks in laboratory conditions (Lloyd and Poulin 2013). However, field-collected *Philophthalmus* sp. that infected snails with or without *M. novaezealandensis* does not exhibit any difference in small-to-large caste ratios as would be expected according to the theory of optimal caste ratios (Leung and Poulin 2011; Kamiya and Poulin 2013). The extent to which caste ratio responds to environmental conditions in nature is unknown, as is the potential for this trait to be subjected to different local selective pressures leading to evolutionary divergence.

The aim of this study was to examine whether colony demography of *Philophthalmus* sp. colonies differs between geographically distinct locations around the South Island of New Zealand. This was done by, first, quantifying colony demographic traits (small-to-large caste ratio and colony size) from the colonies in three locations. Locations that vary in total trematode prevalence and *Philophthalmus* sp. prevalence were ideal because the division of labour is predicted to play a role in competitive interactions (and thus a variation in caste ratio would be most likely observed from these sites). Secondly, the colonies from different locations were kept long-term under identical laboratory conditions to determine if any difference found in these demographic traits was due to evolutionary divergence (local adaptation) or phenotypic plasticity. Evidence suggestive of evolutionary divergence would be obtained if, first, a difference in demographic traits is observed among colonies from geographically distinct locations and, second, if this difference persists after colonies are kept long-term in identical conditions. In contrast, evidence suggestive of phenotypic plasticity would be obtained if, first, a difference in demographic traits is seen among colonies from geographically distinct locations and, second, this difference is plastic and does not persist long-term after colonies are kept in identical conditions.

We predicted that caste ratios would be different at different sites and that they would reflect the local risk of competition (i.e. where infection prevalence is high, the ratio of small-to-large rediae should also be high). The risk of interspecific competition was measured as the local infection prevalence of all trematode species, and the risk of intraspecific competition was measured as the prevalence of *Philophthalmus* sp. If these differences in caste ratios were results of locally adapted colonies, they would persist in colonies collected from different locations kept in identical conditions.

Methods

Field study sites and snail collection

Approximately 2,000–3000 snails were collected (between approximately 9–18 mm in shell length) haphazardly by hand from each of 5 sites in an attempt to find at least 20 snails infected with *Philophthalmus* sp. per site. Snails were collected between September and November 2012. The five sites were on the South Island of New Zealand where infected *Z. subcarinatus* have been found previously. They were the following: Otago Harbour, Lower Portobello Bay (45° 52′ S, 170° 42′ E); Bluff, Greenpoint Domain (46° 34′ S, 168° 18′ E); Christchurch, McCormacks Bay Reserve (43° 33′ S, 172° 43′ E); Marlborough Sounds, Ngakuta Bay (41° 16′ S, 173° 57′ E) and Waitati, Blueskin Bay (45° 44′ S, 170° 35′ E) (see Supplementary Material for a map of these sites). Unfortunately, none of the collected snails from the two latter sites were infected by *Philophthalmus* sp., and therefore, these sites were not included in the study. Snails were kept in plastic containers (17×17 cm) in aerated seawater and fed with sea lettuce (*Ulva lactuca*) for 2 days before screening and dissection.

Snail screening and caste ratio measurement

Between 345–858 snails from each site were screened to quickly identify those infected with *Philophthalmus* sp. Generally, snails infected with *Philophthalmus* sp. are slightly larger than uninfected snails (the smallest uninfected snails are <1 mm and the smallest snail infected with *Philophthalmus* sp. found was 11.9 mm). Therefore, snails selected for screening were chosen from all collected snails based on size (snails under approximately 11 mm were not screened). Infection screening was achieved by forcing cercarial release by incubating snails individually overnight at 26 °C in wells of a 12-well culture plate filled with natural seawater. From the pool of screened snails, approximately 300 snails per site were dissected to ensure that infections were identified correctly (not all infections will shed cercariae when incubated, and estimated infection prevalence based on shed cercariae alone can underestimate true infection prevalence). Trematode infection prevalence for each site was estimated based on the infections of these 300 dissected snails. Since the snails that were selected for screening and dissection were chosen from the total pool based on size, infection prevalence for each site is an estimate. However, since the prevalence was estimated in the same way for each site, it is suitable to make comparisons between sites.

The prevalence of *Philophthalmus* sp. infections in snails from Bluff and Christchurch was low: 2.71 and 2.67 %, respectively. Only 8 (out of ~300) snails infected with *Philophthalmus* sp. were found from each site. Therefore, additional snails infected with *Philophthalmus* sp. found among the remaining screened snails were used to determine caste ratio from these sites (see below). However, infection rates (of all trematode species) were estimated from the ~300 dissected snails only.

Maximum shell lengths of snails infected with *Philophthalmus* sp. were measured to the nearest 0.1 mm before each snail was dissected. The snail visceral mass was dissected out of the snail shell and teased apart to release *Philophthalmus* sp. rediae. The rediae were dyed with neutral red and pressed between two glass slides. The rediae were classified as either small or large based on the obvious size difference, the proportion of pharynx size to body size and the absence of germ balls or developing cercariae in small rediae. The pharynges of the rediae and developing cercariae stain dark red by Neutral Red dye, making the differentiation between the two castes even more clear-cut than based on size alone. *Philophthalmus* sp. small and large rediae from each infection were counted separately (38 colonies from Lower Portobello Bay, 12 from Bluff and 10 from Christchurch). The caste ratio in each infected snail was calculated as the number of small rediae divided by the number of large ones. The few snails infected with *Philophthalmus* sp. with co-infections by *M. novaezealandensis* were also included in this part of the study because previous reports indicate that co-infection by *M. novaezealandensis* have no effect on the measured colony demographic traits (Keeney et al. 2008a; Leung and Poulin 2011).

Laboratory experiment

Additional 2,000-3,000 snails were collected from Bluff and Lower Portobello Bay in January–February 2013, screened for infection by incubation and measured as above. A subset of snails infected with *Philophthalmus* sp. (nine from Lower Portobello Bay and five from Bluff) were dissected immediately, and their small-to-large caste ratio and total number of rediae were determined, to provide baseline data on these demographic traits at the onset of the experiment. The remaining infected snails (11 from Lower Portobello Bay and 9 from Bluff) were kept in laboratory conditions for 12 weeks. A previous experiment indicates that this period of time is sufficient for a turnover of rediae and alteration of caste ratio under the conditions of the present experiment (Lloyd and Poulin 2013). Snails from each locality were placed into plastic cups (one snail per cup; cups were 4.5-cm diameter × 5-cm height) filled with 65 ml of filtered seawater and fed with pieces of sea lettuce (5 cm^2) replenished every week. In the trematode *Philophthalmus* sp., cercariae produced by reproductive rediae leave the snail to quickly encyst on hard substrates after only a brief free-swimming period. Therefore, to quantify individual colony fitness, encysted cercariae (metacercariae) were quantified from each colony throughout the experiment as they are the free-swimming stage which continues the life cycle to the definitive host. To do this, a standard glass microscope slide was placed in each cup. Once weekly, encysted metacercariae on the glass slides were counted under a dissecting microscope. This method has been used in the past to measure relative, if not absolute, cercarial output of snails infected with *Philophthalmus* sp. kept in laboratory conditions (Lei and Poulin 2011). Since the plastic cups were small, encysted metacercariae on the bottom of the cups were also easily counted. The slides were replaced, water was changed and plastic cups were cleaned weekly. At the end of the 12-week experiment, snails were measured and dissected, and the small-to-large caste ratio and total number of rediae were determined.

Statistical analysis

All statistical analyses were completed in R version 2.14.0 (R Development Core Team 2011). Infection prevalence between sites was compared using a test of equal or given proportions in the stats package (Wilson 1927; Newcombe 1998a, b). Comparisons of infection prevalence were performed on three measures from the field study: the prevalence of *Philophthalmus* sp. infections (number of snails infected with *Philophthalmus* sp. found among the ~300 dissected snails, divided by that number), proportion of snails infected with *Philophthalmus* sp. co-infected by *M. novaezelandensis* (number of double infections divided by the total number of infected snails with *Philophthalmus* sp.) and overall trematode prevalence (number of snails infected by any trematode found among the ~300 dissected snails, divided by that number).

Demographic traits of field-collected Philophthalmus sp. colonies from three sites were compared using separate linear models (LMs). Predictor variables in the model comparing small-to-large caste ratio included the site, total number of rediae per snail and infection type (single or double-species infection, the latter being snails also harbouring M. novaezealandensis). Snail length and total number of rediae are correlated (r=0.4818) and were thus not both used as predictor variables for any models. Colony size, here measured as the total number of rediae, has been reported to affect division of labour in social insects (Wilson 1983; Dornhaus et al. 2012) and was therefore included in this model. Infection type and total number of rediae were not significant factors, and removing them increased the fit of the model (according to Akaike Information Criterion, AIC, values); in this and in subsequent models, only the reduced version is presented in the 'Results' section. For these analyses, small-to-large caste ratios were log(x) transformed to fit assumptions of normality.

The predictor variables included in the LM comparing the total number of rediae included site, snail length, infection type and the small-to-large caste ratio. Here too, the infection type and caste ratio were not significant factors, and removing them increased the fit of the model (according to AIC values). For this model, the total number of rediae was log(x) transformed to fit assumptions of normality. Post-hoc tests were performed to detect differences in either caste ratio or total number of rediae between site pairs (i.e. Lower Portobello Bay vs Bluff, Lower Portobello vs Christchurch and Bluff vs Christchurch) using the multcomp package (Hothorn et al. 2008).

Similarly, the demographic traits of *Philophthalmus* sp. colonies included in the long-term laboratory experiment were compared using two LMs. The model comparing the small-to-large caste ratio included site of origin, timing (whether the demographic traits of a colony were determined immediately upon collection or after the 12-week period), the interaction between site and timing, snail length and the total number of rediae. In this data set, the snail length and the total number of rediae were not correlated (r=−0.06), and both were therefore initially included in the model. Removing non-significant variables increased the fit of the model; these included the interaction between site and timing and the total number of rediae. The model comparing the total number of rediae in these colonies included the site, the timing, the interaction between site and timing, the snail length and the small-to-large caste ratio. Again, removing non-significant factors (small-to-large caste ratio and the interaction between site and timing) increased the fit of the model. All linear models were performed with the stats package in R (Wilkinson and Rogers 1973; Chambers 1992).

Lastly, differences in numbers of *Philophthalmus* sp. encysted metacercariae produced during the experiment by individual colonies in snails from Bluff or Lower Portobello Bay were tested using a linear mixed model (LMM) performed in R version 2.14.0 (R Development Core Team 2011) using the package LME4 (Bates et al. 2011). Fixed effects included the site of origin, the small-to-large caste ratio, the snail length, the total number of rediae, and the interaction between caste ratio and site. To account for temporal pseudoreplication, snail identity was included as a random effect. Non-significant factors (the small-to-large caste ratio, total number of rediae and the interaction between caste ratio and site) were removed to improve the fit of the model. Numbers of encysted metacercariae were $\log(x+1)$ transformed to meet assumptions of normality.

Results

Trematode infection prevalence per site

Snails infected with *Philophthalmus* sp. were only found at three of the five sites visited: Lower Portobello Bay, Bluff and Christchurch. Due to low infection prevalence at Bluff and Christchurch sites, sample sizes were smaller than desired (Table 1). The prevalence of *Philophthalmus* sp. was significantly higher at Lower Portobello Bay than both Bluff and Christchurch, indicating a higher chance of conspecific double infections at this site (Table 2). The proportion of snails infected with *Philophthalmus* sp. co-infected with *M. novaezealandensis* was zero at Bluff, 15.2 % at Lower Portobello Bay and 40.0 % at Christchurch (though the difference between Lower Portobello Bay and Christchurch was statistically non-significant) (Table 2). The proportion of infected snails (infected by any trematode) was significantly higher at Lower Portobello Bay and Christchurch compared to Bluff; however, total trematode prevalence at Lower Portobello Bay and Christchurch were not significantly distinct (Table 2).

Table 1 Snails infected with *Philophthalmus* sp. found at each site during the initial field collection

Site	Snails dissected	Infected snails from dissections	Further screened snails	Infected snails from screening	Total colonies in analysis
LPB	345	46	0	NA	38
Bluff	295	8	563	4	12
ChCh	300	8	554	2	10

The number of total colonies in analysis indicates the number of *Philophthalmus* sp. colonies used to quantify demographic traits in the field study

LPB Lower Portobello Bay, *ChCh* Christchurch, NA not applicable

Table 2 Prevalence of *Philophthalmus* sp. at each site and co-occurrence with *Maritrema novaezealandensis* during the initial field collection

Site	*Philophthalmus* sp. prevalence (%)	Per cent of snails with *Maritrema* infected with *Philophthalmus* sp. (%)	Total trematode prevalence (%)
LPB	13.3 a	15.2 c	53.9 e
Bluff	2.7 b	0 d	4.8 f
ChCh	2.7 b	40.0 c	59.3 e

Letters refer to statistical differences ($P<0.05$) as determined by a test of equal proportions

Colony demographic traits

Field-collected snails

The small-to-large caste ratios in field-collected *Philophthalmus* sp. colonies ranged from 0.45 to 2.44 across all 60 snails dissected. According to the linear model, site was a significant factor explaining the difference between the caste ratio of colonies collected from three sites (Table 3). Post-hoc tests, which compared the sites in individual pairs, indicated that the small-to-large caste ratio was significantly higher in colonies from Lower Portobello Bay than colonies from Bluff, but there was no difference between colonies from Christchurch and Bluff or colonies from Christchurch and Lower Portobello Bay (Fig. 1). The total number of rediae

Table 3 Results of two separate linear models comparing the small-to-large caste ratios and the total number of rediae of *Philophthalmus* sp. colonies from three different sites

Response	Factor	Estimate	Standard error	*t* value	*P* value
Caste ratio	Intercept[a]	0.790	0.034	23.159	<0.0001
	Site Bluff	−0.215	0.070	−3.082	0.002
	Site Christchurch	−0.036	0.075	−0.485	0.747
Total number of rediae	Intercept[a]	3.336	0.609	5.482	<0.0001
	Site Bluff	−0.600	0.203	−2.950	0.005
	Site Christchurch	−0.010	0.208	−0.048	0.962
	Snail length	0.148	0.040	3.728	0.0005

[a] The effect of Lower Portobello Bay is included in the intercept

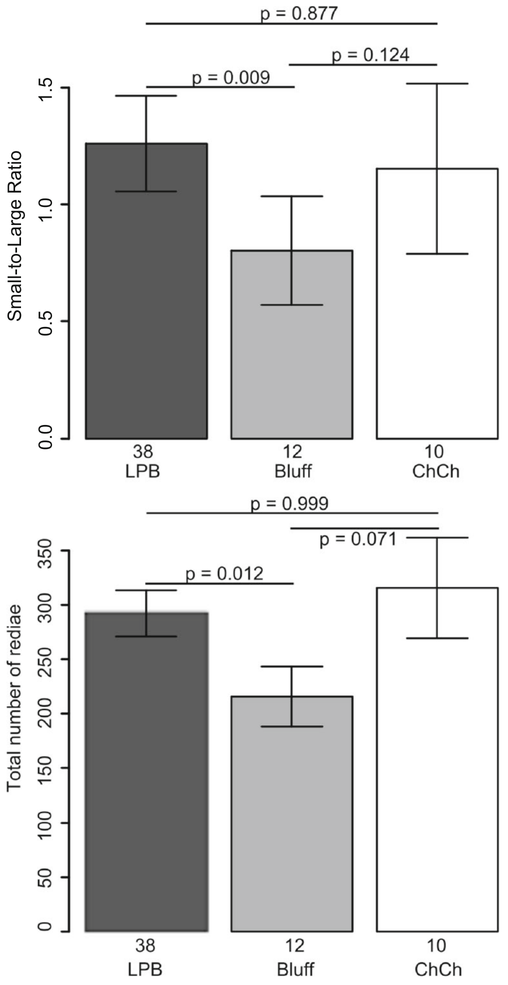

Fig. 1 Average (±S.E.) caste ratio of small to large rediae (*top*) and total number of rediae (*bottom*) in *Philophthalmus* sp. colonies from three sites. *LPB* Lower Portobello Bay, *ChCh* Christchurch. Sample size is indicated at the bottom of each *bar. P* values from post-hoc comparisons between each possible pair

ranged from 12 to 613 per infected snail, and, again, site was a significant factor in the linear model comparing the total number of rediae in colonies from three sites (Table 3). Post-hoc tests showed that the total number of rediae was significantly higher among colonies from Lower Portobello Bay than colonies from Bluff, but there was no difference between colonies from Christchurch and Bluff or colonies from Christchurch and Lower Portobello Bay (Fig. 1).

Snails in long-term laboratory experiment

Fourteen snails infected with *Philophthalmus* sp. were found when re-visiting Bluff, and 20 were found when re-visiting Lower Portobello Bay. Of the snails found from Bluff, a subset of five were dissected immediately to quantify initial colony demographic traits; of the snails found from Lower Portobello, a subset of nine were initially dissected. As seen previously, colony demographic traits were different in colonies from Bluff and Lower Portobello. Both the small-to-large caste ratio and the total number of rediae from colonies from Bluff were lower than in colonies from Lower Portobello Bay (Figs. 2 and 3, Table 4). Furthermore, these differences between colonies from different sites remained consistent with those colonies which were kept long-term under identical laboratory conditions (Figs. 2 and 3, Table 4). Timing, i.e. whether colony measurements were taken at the time of collection or at the end of the 12-week laboratory maintenance, was not a significant factor in either of the linear models comparing colony demographic traits, indicating that they did not change over time even when living conditions were standardised (Table 4). Furthermore, an interaction term between timing and site of origin was initially included in the models but later removed because it increased the fit of the models, indicating that the difference in demographic traits between Bluff and Lower Portobello remained the same at the two time points.

Fitness of geographically separated colonies

In order to determine whether the differences in demographic traits between sites were adaptive or simply neutral, in regards to colony function, we quantified the cercarial output (one component of fitness) of colonies kept in laboratory conditions by counting metacercarial cysts over 12 weeks. The number of encysted metacercariae produced by individual colonies from Lower Portobello Bay exceeded those from the Bluff (Fig. 4, Table 5). Factors originally included in this model but removed to increase model fit were caste ratio, total number of rediae, and the interaction between caste ratio and site. Not surprisingly, host snail size also had a significant positive effect on cercarial production.

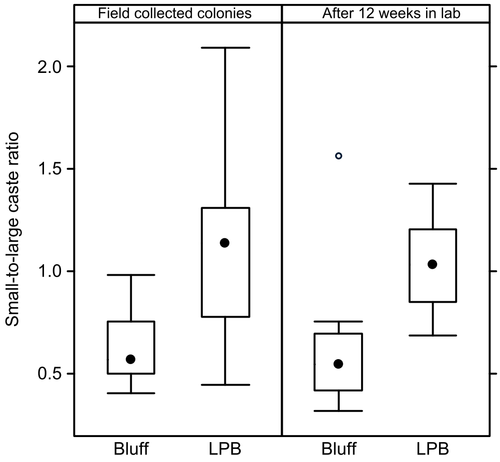

Fig. 2 Box plot showing the median small-to-large caste ratio of *Philophthalmus* sp. colonies collected from Bluff or Lower Portobello Bay before (Field-collected colonies) or after being kept 12 weeks in laboratory conditions

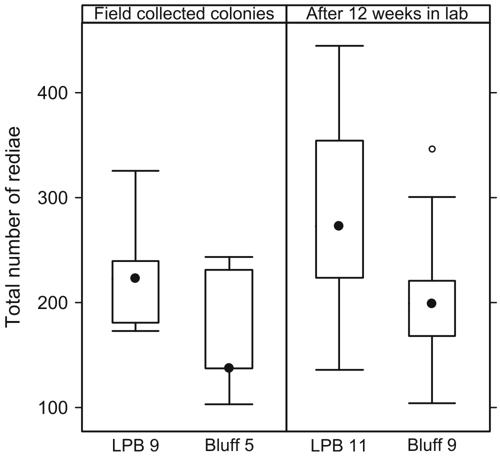

Fig. 3 Box plot showing the median total number of rediae from *Philophthalmus* sp. colonies collected from Bluff or Lower Portobello Bay before (Field-collected colonies) or after being kept 12 weeks in laboratory conditions

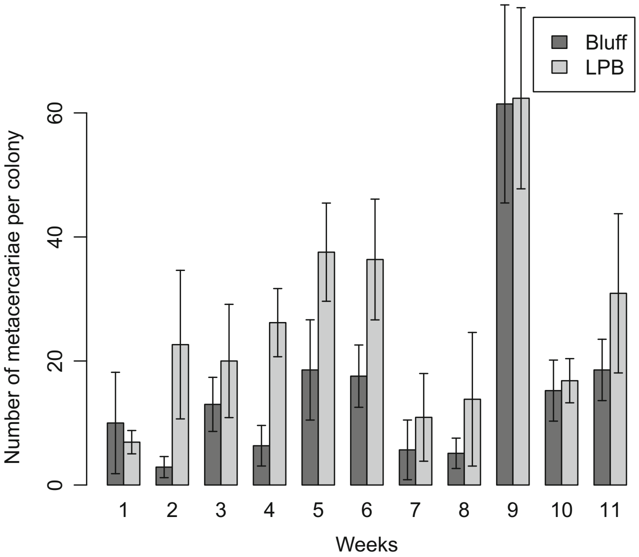

Fig. 4 Average (±S.E.) metacercariae counted from each snail infected with *Philophthalmus* sp. from either Bluff or Lower Portobello Bay over 12 weeks under laboratory conditions

Discussion

Although previously documented for social insects, this is the first study of geographical variation in caste ratios for a social trematode species with a division of labour. Our results demonstrate that there is a significant variation in caste ratios of *Philophthalmus* sp. colonies among three distant localities, and this may be the product of local adaptation through genetic divergence, as the caste ratios do not change over time under standardised laboratory conditions.

In our field study, colony demographic traits (both small-to-large caste ratio and colony size) differed among *Philophthalmus* sp. populations from three geographically separated sites. As predicted, colonies with higher small-to-large caste ratios originated from the site with the highest infection prevalence: the average small-to-large caste ratios in colonies collected from Lower Portobello Bay were significantly higher than the caste ratios in colonies collected from Bluff. Both the total prevalence of all trematode species and the prevalence of *Philophthalmus* sp. were significantly higher at Lower Portobello Bay than Bluff (Table 2). Of the three sites, Lower Portobello Bay was the only site with high total prevalence paired with high *Philophthalmus* sp. prevalence. The non-significant difference between the caste ratio in colonies collected from Bluff (where trematode prevalence was low, see Table 2) and Christchurch (where total trematode prevalence was high but *Philophthalmus* sp. prevalence was low, see Table 2) might provide more information about the factors contributing to the control of caste ratio. Perhaps, the combined risk of high inter and intraspecific competitions, which was only present at Lower Portobello Bay, is a factor in the high caste ratio in colonies collected from that site. This would not be surprising because multi-clone *Philophthalmus* sp. colonies have been identified in *Z. subcarinatus* collected from Lower Portobello Bay, and intraspecific competition appears to be strong in these hosts (Lloyd and Poulin 2014). However, the non-significant difference in caste ratios between colonies from Bluff and Christchurch may be a result

Table 4 Results of two separate linear models comparing the small-to-large caste ratio and the total number of rediae in *Philophthalmus* sp. colonies from Bluff or Lower Portobello Bay (LPB), before and after being kept 12 weeks under laboratory conditions

Response	Factor	Estimate	Standard error	*t* value	*P* value
Caste ratio	Intercept[a]	1.133	0.371	3.055	0.005
	Site LPB	0.190	0.065	2.922	0.007
	Timing	−0.048	0.062	−0.784	0.439
	Snail length	−0.041	0.023	−1.746	0.091
Total number of rediae	Intercept[a]	4.215	0.722	5.840	<0.0001
	Site LPB	0.402	0.127	3.172	0.003
	Timing	0.232	0.120	1.934	0.063
	Snail length	0.054	0.045	1.205	0.238

[a] The effect of Bluff is included in the intercept

Table 5 Results of a linear mixed model comparing metacercarial output of *Philophthalmus* sp. colonies from either Bluff or Lower Portobello (LPB) over 12 weeks in laboratory conditions

Factor	Estimate	Standard error	*t* value	*P* value
Intercept[a]	−2.645	1.409	−1.877	0.062
Site LPB	1.317	0.303	4.354	<0.0001
Snail length	0.269	0.084	3.211	0.002

[a] The effect of Bluff is included in the intercept

of low sample size. Further work could be done to elucidate the control of caste ratio by (a) collecting more colonies from each site and (b) using previously published microsatellite markers for *Philophthalmus* sp. to identify the prevalence of multiple intraspecific infections at each site.

The total number of rediae was also different among colonies collected at these sites. The total number of rediae in colonies from Bluff was lower than in those from Lower Portobello Bay, despite snails from Bluff being larger than snails from Lower Portobello Bay. As expected, there was a positive relationship between snail maximum length and total number of rediae and no effect of co-infection by *M. novaezealandensis*, a pattern which has been seen in studies done on this system in the past (Keeney et al. 2008a; Lloyd and Poulin 2013) and in other systems (Hendrickson and Curtis 2002).

These differences in colony demographic traits mirrored trematode prevalence as expected: A higher caste ratio and larger colony size were seen at the site with the highest chance of both inter and intraspecific competition (measured by total trematode prevalence and *Philophthalmus* sp. prevalence). This result provides evidence that colony demographic traits in *Philophthalmus* sp. populations are adaptive according to the theory of optimal caste ratio of Oster and Wilson (1978). The small-to-large caste ratio is higher in colonies of populations where more small, soldier-like rediae would be advantageous due to a high likelihood of potential co-infecting trematodes. These results are very similar to those of the observational study completed by Yang et al. (2004) and perhaps, reflect parallels between divisions of labour in social insect colonies and trematode colonies.

In order to determine if the phenotypic differences observed between colonies collected from Bluff and Lower Portobello Bay are due to genetic divergence (local adaptation) or phenotypic plasticity in response to variation in environment, a subset of colonies collected from either Lower Portobello Bay or Bluff were kept long-term in identical laboratory conditions. Experimental infections, while ideal for this experiment, cannot be achieved in this system because *Philophthalmus* sp. adults live in the eyes of definitive shorebird hosts, and eggs are passed in bird tears; the birds are protected species, and the parasite's eggs cannot be found in the field. Therefore, a common garden experimental design was used instead, where colonies were transplanted from their natural environments to a common laboratory setting. After the 12-week experiment, i.e. a period sufficient for turnover of rediae, colony demographic traits (small-to-large caste ratio and total number of rediae) were the same as observed in colonies freshly collected from the field. The small-to-large caste ratio and total number of rediae were lower in colonies originating from Bluff than in those from Lower Portobello Bay in both subsets of snails: those dissected immediately after collection and those kept in the laboratory for 12 weeks. In the linear model comparing the small-to-large caste ratio in these colonies, site of origin was the only significant factor. This indicates that there was no difference in caste ratio between colonies dissected immediately or after 12 weeks. Furthermore, the lack of interaction between site and time of dissection confirms that the difference in caste ratio observed between colonies from these sites remains the same over time. This is a strong indication of local adaptation through genetic divergence. However, it is possible that the 12-week period of this experiment was not sufficient for the complete turnover of rediae in these colonies.

In order to quantify how the difference in demographic colony traits between sites was related to colony fitness, we quantified the cercarial output of colonies kept in laboratory conditions by counting metacercarial cysts over the 12-week period. The numbers of metacercarial cysts per colony from Lower Portobello Bay greatly exceeded those shed from colonies collected at Bluff (Table 5, Fig. 4). This provides evidence that colonies from Lower Portobello Bay have a higher fitness, despite having a higher small-to-large caste ratio, i.e. a relatively greater proportion of non-reproductive individuals per colony. This is a pattern that has been seen before in another species: *M. novaezealandensis* colonies within *Z. subcarinatus* collected from different sites also shed different quantities of cercariae (Koprivnikar and Poulin 2009). The disproportionate investment in the production of small non-reproductive rediae in the Lower Portobello Bay population does not come at a fitness cost relative to colonies in the Bluff population, possibly because Lower Portobello Bay colonies consist of more total rediae. However, in the model comparing the metacercarial output from these colonies, total number of rediae was a non-significant factor (and removed to increase model fit); therefore, colonies from Lower Portobello Bay do not merely produce more metacercariae because they have more rediae. Other non-significant and removed factors included the caste ratio and the interaction between ratio and site. This was somewhat surprising because in previous similar experiments, caste ratio has been a significant factor indicating that a higher small-to-large caste ratio increases colony fitness (Lloyd 2013).

Results from this study provide evidence that colony demographic traits such as the small-to-large caste ratio and colony size may be under geographically different selective pressures in *Philophthalmus* sp. colonies, and perhaps, adaptive divergence has occurred between populations at Bluff and Lower Portobello Bay. Further genetic evidence supports this indication of local adaptation. Gene flow between *Philophthalmus* sp. populations is not disrupted between Lower Portobello Bay and Christchurch but is low between Lower Portobello Bay and Bluff (and also low between Christchurch and Bluff, though not statistically significant) (Keeney et al. 2009), providing evidence that the difference in caste ratio between Bluff and the other two populations may be due to evolutionary divergence. However, based on sequence analysis of the cytochrome oxidase 1(CO1) mitochondrial gene, four prominent haplotypes exist within the *Philophthalmus* sp. populations, and all four are found at each of the three sites used in this study (Keeney et al. 2009). Therefore, populations from these sites are not truly genetically distinct. Genetic dispersal of *Philophthalmus* sp. is enabled by movement of the definitive host which is most likely a gull or other shorebird (Howell 1965; Neal and Poulin 2012). In the definitive host, adult worms develop, sexual reproduction occurs and eggs are released into the environment. The life cycle continues as the eggs hatch, releasing small miracidia which infect snails and develop into rediae (West 1961; Nollen 1995). Even though the snails and the parasites are not capable of movement across hundreds of kilometres, adult worms are transported by the definitive bird host, potentially allowing for gene flow (Dybdahl and Lively 1996; Miura et al. 2006; Keeney et al. 2008b). Despite CO1 sequence analysis indicating some gene flow among the sites studied here, selection could be acting specifically on genes that control caste ratio phenotype and not the CO1 gene. Indeed, it is possible for local populations to adapt to their particular environment even with significant gene flow if selection pressure is strong (Dybdahl and Lively 1996).

Further evidence that the caste ratio is genetically determined in these colonies comes from studies comparing the caste ratio of *Philophthalmus* sp. colonies co-infected with *M. novaezealandensis* colonies (Keeney et al. 2008a, b) or conspecific colonies (Lloyd and Poulin 2014). In both studies, there is no difference between the caste ratio of single *Philophthalmus* sp. infections and double infections (either hetero or conspecific). It is possible that this trait is genetically determined and there is not a lot of room for variation later in the colony's life.

To determine if colony demographic traits are in fact under selective pressure, further work is needed. It is possible that the colony demographic traits are determined early in the life of the infection and were not plastic at the time the colonies were collected. A common garden experiment which included infections from the start of the life cycle through maturity would clarify this further but requires challenging experimental infections. Another alternative hypothesis is that the differences in colony demographic traits observed here are responding to other differences than trematode infection prevalence. Many other ecological factors may vary between the two populations including density and quality of first intermediate hosts, density of definitive hosts, climatic factors etc., and perhaps the difference in caste ratio is due to a response to one of these factors.

In summary, results of this study provide evidence that the optimal caste ratio theory of Oster and Wilson (1978), which explains caste structure of social insects, may also explain caste structure of trematode colonies with a division of labour (Oster and Wilson 1978). The small-to-large caste ratio in *Philophthalmus* sp. colonies differs significantly among geographically distinct colonies in a way that matches exactly the expected selective pressures from the local risk of competition.

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