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RESEARCH ARTICLE

Arrival sequence and diet mediate interspecific competition in an ant community

R. F. Barbieri · J. Grangier · P. J. Lester

Abstract The arrival sequence of organisms in a habitat and their diet are two factors that are thought to modulate animal performance, affect the outcome of behavioural interactions, and shape communities. In New Zealand, two species that seldom co-occur in field populations are *Prolasius advenus* and *Monomorium antarcticum*. Herein we tested the hypotheses that arrival sequence and diet influence the strength of interactions between these two species. These ant species presented asymmetric responses to arrival sequence and diet variations. When arriving first *P. advenus* displayed increased aggression and *M. antarcticum* a defensive reaction. Changes in carbohydrate and protein availability modulated colony activity rates of both species. Colonies of *M. antarcticum* fed on a high carbohydrate and low protein diet displayed higher activity rates than colonies fed on a low carbohydrate and high protein diet. In contrast, control colonies of *P. advenus* fed on a high carbohydrate and low protein diet displayed lower activity rates than colonies fed on a low carbohydrate and high protein diet. These results indicate that arrival sequence can modulate the agonistic reaction displayed by interacting species in situations of conflict. This work also demonstrates that species adjust activity rates in response to diet, but different species do so differently. Therefore, arrival sequence and diet could explain species mutually exclusive distribution patterns observed in nature.

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Introduction

Species typically arrive into a community at different times. The arrival sequence of species in the colonization process often favours the species that arrives first (the "primary species") and reduces establishment success of subsequent species ("secondary species"), thus influencing the fitness of organisms and community assembly (Cole, 1983a, b; Weslien et al., 2011). When the primary species is able to negatively impact the secondary species, this process is called priority effect (Victorsson, 2012). The mechanisms conferring competitive advantage to the primary species and, consequently, determining the outcome of future interspecific interactions can include: numerical advantage (Louette and De Meester, 2007), exploitative resource competition (Miller-Pierce and Preisser, 2012; Victorsson, 2012), larger body size (Alford and Wilbur, 1985), defensive strategies (Wilson, 1974), aggressive behaviour (Cole, 1983b), or even familiarity with the local environment (Ridley et al., 2010). Additionally, nutrient availability might also affect the outcome of interspecific interactions and shape communities (Blüthgen et al., 2004).

The availability of suitable food sources is also crucial to ensure animal nutrient balance and fitness (Davidson, 1997; Jervis et al., 2008; Dussutour and Simpson, 2012). Changes in the dietary intake have been shown to elicit distinct activity rates and behavioural responses in a number of organisms (Kay et al., 2010; Kohler et al., 2012). A poor diet can influence multi-species communities throughout the

biological hierarchy, affecting individuals, populations, and finally the entire community (Wagner et al., 2013). Species feeding on higher concentrations of energetic substrates could accelerate their metabolic rates and display behaviours that further enhance their abilities to secure resources (Kay et al., 2010; Kay et al., 2012). Alternatively, the abundance or scarcity of essential food sources could modulate the tempo of species and tune their foraging activity rates (Oster and Wilson, 1978).

Here we studied the interspecific interaction between two ant species, *Monomorium antarcticum* and *Prolasius advenus*. Both are native species frequently found throughout New Zealand. They are able to tolerate a wide range of abiotic conditions (Don, 2007). Both *P. advenus* and *M. antarcticum* use venom during aggressive interactions (Don and Jones, 1993; Grangier and Lester, 2011); are generalist foragers and nest in the soil, under logs or rocks (Brown, 1958; Don, 2007). *Monomorium antarcticum* is a ubiquitous species in open habitats (Brown, 1958) and *P. advenus* is widely dispersed in beech forest (*Nothofagus* sp.) (Burne, 2012). In open habitats, colonies of *M. antarcticum* were observed to have high number of workers ($\sim$1,800 workers; median number) (Wang and Lester, 2004). In forest areas, colonies of *P. advenus* were observed to have nests containing up to 6,000 workers (Burne, 2012). However, these species seldom co-occur out of their natural range, but likely co-exist on transitional zones between forest and open habitats. When occasionally occurring out of their usual range, both species present lower colony size ($\sim$300 workers) than those observed in their usual range (Barbieri, in prep.).

In this study, we subjected *P. advenus* and *M. antarcticum* to interspecific interactions in controlled conditions to test the hypothesis that arrival sequence and diet can modulate the outcome of behavioural interactions. We investigate whether the primary species deploys strategic behaviours that enhance their ability to secure resources and persist in the presence of a competitor arriving in the territory after. Also, we assess the effects of diet variation on modulating species aggressive and foraging behaviour. Thus, arrival sequence and nutrient availability are two factors that could potentially shape New Zealand ant communities and explain the negative co-occurrence patterns between *P. advenus* and *M. antarcticum* we observe in nature.

Materials and methods

Ant colony collection and preparation

Colonies of *P. advenus* were collected in New Zealand from forest areas in Nelson Lakes National Park (41°48′S;

172°38′E) and Kaitoke Regional Park (41°4′S; 175°11′E) between 2010 and 2011. *Monomorium antarcticum* colonies were excavated from open areas in Nelson Lakes National Park during the same time period. The ant *M. antarcticum* likely represents a species complex that may be composed of 3–5 species (Brown, 1958; Don and Jones, 1993; Wang and Lester, 2004). Therefore, we collected colonies of *M. antarcticum* with similar morphologies from a single location. We prepared colonies containing 200 (small colony) or 1,000 (large colony) workers and two queens, according to the desired population size (see below). Colonies were placed in plastic containers (13 × 9 × 6 cm) with the sidewalls painted with Fluon^{TM} (polytetrafluoroethylene PTFE-30; BioQuip Products, Inc.), and a segment of plastic tubing (0.5 cm internal diameter; 10 cm length) connected as a nest exit. The tubing exit was initially plugged with aluminium foil to stop ants leaving the artificial nest while not connected with the foraging area. In each container we placed three 10-mL nesting tubes one-third filled with water, plugged with cotton wool and covered with aluminium foil. For acclimation sub-colonies were kept at 20 ± 1 °C for 1 week and fed three times (2-day interval) via soaked cotton wool with 20/80 % honey/water (volume/volume) and a mealworm (*Tenebrio molitor*) larva cut into three parts.

Colonies were connected by the plastic tubing to opposite sides of a foraging area, which was composed of a plastic tray (51 × 37 × 4.5 cm) with the sidewalls coated with Fluon^{TM}. The plastic tray simulated foraging conditions where ants sought food sources and where the primary species established its territory prior to the arrival of the secondary species. During the experiment different diets were offered three times a week. The energetic diet consisted of a cotton dish ($\sim$2 × 2 cm; 0.2 cm thick) soaked in 20/80 % (high carbohydrate) or 2/98 % (low carbohydrate) honey/water solution (v/v). The source of protein was a single mealworm cut in three parts ($\sim$0.09 g, high protein) or 1/3 mealworm ($\sim$0.03 g, low protein). Low and high carbohydrate concentrations were chosen based on previous observations indicating that *P. advenus*, in similar treatments, increased foraging activity in response to reduced carbohydrate resources (Duthie and Lester, 2013; Grangier, unpubl. data). Both food substrates were offered on separate pieces of aluminium foil (4 × 4 cm) randomly placed in the foraging area.

Experimental design

We used a 2 × 2 factorial design with four replicates and controls (16 experimental units) to evaluate the effects of arrival sequence and diet on the aggression rates, foraging activity and colony survival of *M. antarcticum* and *P. advenus*. The independent variables were: (1) arrival

sequence—small colonies of *M. antarcticum* used the foraging area for a week first than large colonies of *P. advenus*, and large colonies of *P. advenus* used the foraging area for a week first than small colonies of *M. antarcticum*; (2) diet— high carbohydrate-low protein (HCLP); low carbohydrate– high protein (LCHP). As a control, small colonies of *M. antarcticum* and large colonies of *P. advenus* were subjected to the same diets (HCLP and LCHP; $n = 4$), but not subjected to interspecific interactions. Therefore, we had a total of 32 experimental units.

Preliminary observations showed that small colonies of *P. advenus* subjected to interspecific interactions with large colonies of *M. antarcticum* ($n = 4$) had their nests invaded right after the confrontation initiated and were exterminated by *M. antarcticum*. When species were subjected to interspecific interactions in groups containing equal number of workers (10×10, $n = 10$; 200×200, $n = 4$), *P. advenus* was also exterminated. These preliminary results provide evidence of the superiority of *M. antarcticum* in open areas, where they are numerically dominant. The overall colony size of *P. advenus* in beech forests is higher than colony size of *M. antarcticum* found in open areas (Wang and Lester, 2004; Burne, 2012). Therefore, we chose to use colonies of *M. antarcticum* with lower size than *P. advenus* to evaluate their responses in realistic scenarios that could occur in beech forest, where nest of *M. antarcticum* are smaller than in open areas.

Interspecific aggression level

Patterns of interspecific aggression were scored using the following behavioural categories adapted from Rowles and O'Dowd (2007): *touch* (score 1) = contact followed by antennation, when one ant tapped the other ant with its antennae; *avoid* (score 2) = after contact ants retreat in opposite directions; *aggression* (score 3) = head biting, leg biting, raising up the gaster or spraying acid; and *fighting* (score 4) = prolonged aggression (>5 s) between individuals with one or both ants locking mandibles onto a body part, carrying the other with the mandibles, or gripping and flexing the gaster in an attempt to use chemical defences. Interactions between species in the foraging area were scored for 5 min every 15 min for 3 h after the first interspecific encounter. Scores were computed for the species that initiated the behavioural interaction. If at the encounter between two individuals species simultaneously reacted to each other (e.g. both species simultaneously retreat in opposite direction), both were scored. The mean aggression index was calculated based on all interactions observed for each 5-min period. Additionally, we counted the number of individuals of each species in the foraging area at the end of each 5-min period of observations, while species were being scored. Therefore, at the end of 3 h we had the level of

aggression displayed by each species and the number of individuals involved with these interactions.

Colony activity and survival

Following the observations of interspecific interactions, we recorded colony activity of both species by counting the total number of ants in the foraging area for 65 days. Counting was performed twice a day, on three occasions per week. The first reading reflects the "food-searching activity" and was performed before offering food into the foraging area. The second reading aims to evaluate the number of workers in the foraging area 2 h after the placement of the food source and is herein referred to as the "food-collecting activity". Each counting session was performed at the same time of the day for all treatments.

After 65 days of trials, all nests were opened and live workers of both *P. advenus* and *M. antarcticum* quantified. Colony survival rate was used to assess the impacts of different diets and the effects of interspecific interaction between ants in different arrival sequences on a colony level.

Statistical analyses

Interspecific aggression level was analysed using a linear mixed models (LMM) with the R package nlme (Pinheiro et al., 2013). The arrival sequence and diet were fixed effects. The number of workers from the opponent ant species present in the foraging area at each time interval of observation was accounted as a random factor, with colony included as the replicate. We used Spearman's coefficient of rank correlation to measure the linear dependence of the aggression level displayed by one species towards the opponent species and the number of workers from the opponent species.

Colony activity rate was analysed using generalized estimating equations (GEE) with the R package geepack (Højsgaard et al., 2006). First, the effects of diet on the food searching and collecting activity of control colonies was evaluated and then compared with different arrival sequences. For the grouped GEEs analysis, fixed factors were "group" (which include the controls and colonies in different arrival sequence—Pf; Mf) and "diet" (HCLP; LCHP). The number of workers from the opponent ant species searching or collecting activity food over 65 days of interactions was accounted as a covariate on the grouped analysis (e.g. the number of *M. antarcticum* workers seeking or collecting food in the foraging area simultaneously to *P. advenus* at the moment counting was performed). We used a Poisson response function and chose an autoregressive correlation structure, where the correlation between observations is modelled as a function of the distance (time)

between observations. Prior to analysis, foraging activity data were log transformed.

Colony survival rate was analysed using generalized linear models (GLM) with Gamma error distribution. Fixed factors were diet (HCLP; LCHP) and group—which included species in different arrival sequence (Mf; Pf) and controls. As colonies of *M. antarcticum* blocked their nest entrances, we also included nest blocking as a binary factor affecting colony survival. Data were square root transformed prior to analysis as gamma distributions need values >0. Akaike's Information Criteria (AIC) was adopted to evaluate the relative goodness of fit of our models.

All statistical analyses described were performed per species and the reference category was the experimental set in which *M. antarcticum* arrived first, fed on an HCLP diet. A full factorial model design was initially utilized for all analyses. However, after accounting for different sources of variability, we choose the most appropriate model structure. All data analyses presented were performed in R version 2.15.1 (R Development Core Team, 2012) and significance for all tests was set at $P < 0.05$.

Results

Interspecific aggression level

The effects of arrival sequence and diet on the ability of species to persist when in the presence of a competitor were tested by subjecting *P. advenus* and *M. antarcticum* to interspecific interactions. When *P. advenus* was the first to arrive in the territory, both species displayed higher levels of aggression (Fig. 1; Table 1; $P < 0.011$), indicating a significant effect of arrival sequence on the intensity of agonistic reactions between species. However, each species did not react in the same fashion to the abundance of an opponent. Greater numbers of *M. antarcticum* workers increased the aggression of *P. advenus* ($r_s^{(142)} = 0.39$, $P < 0.0001$). Conversely, *M. antarcticum* decreased their aggression level in response to the number of *P. advenus* workers ($r_s^{(142)} = -0.17$, $P = 0.047$). There was no significant effect of diet on the aggression level displayed by both *P. advenus* and *M. antarcticum* (Fig. 1; Table 1; $P > 0.269$).

Colony activity

After interspecific confrontations, workers of *P. advenus* invaded 8 out of 16 *M. antarcticum* nests on the first day of interaction. The invaded colonies were probably killed as no activity was observed after the initial invasion. Conversely, all colonies of *P. advenus* that faced interspecific interactions did not appear to be hindered in their searching or
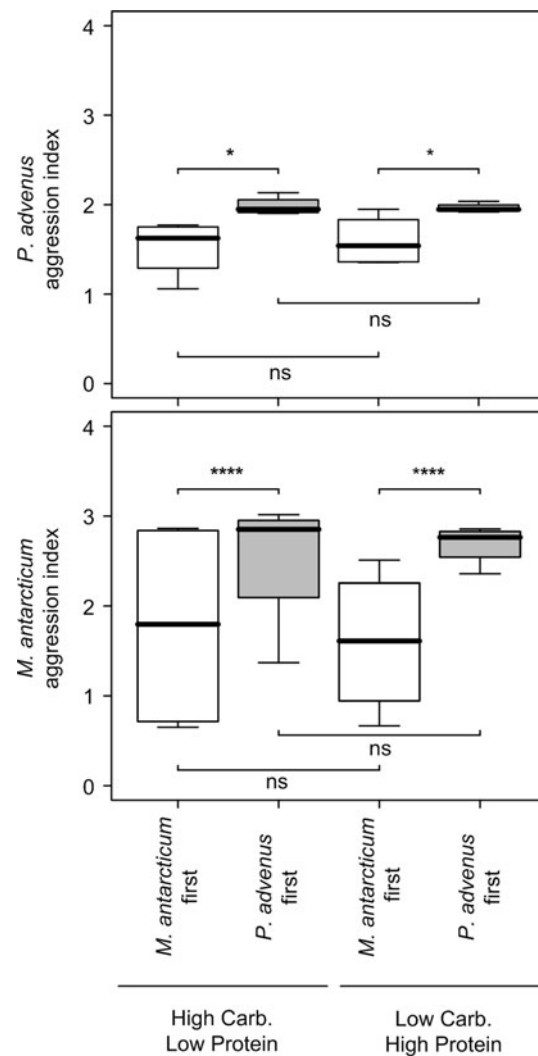

Fig. 1 Boxplots comparing the levels of aggression displayed by *P. advenus* (*top panel*) and *M. antarcticum* (*bottom panel*) in different arrival sequences and food treatments. *White boxes* are the aggression levels of workers when *M. antarcticum* was the first to arrive. *Grey boxes* are the aggression level of workers when *P. advenus* was the first to arrive. The two diets are high carbohydrate-low protein diet and low carbohydrate-high protein diet. Aggression index on *y*-axis represents the average level of aggression between species ranging from 0 (not aggressive) to 4 (highly aggressive). *Boxes* represent the lower and upper quartile, the *bold line* is the median and *whiskers* represent extreme values observed during interspecific confrontations. Aggression levels of *P. advenus* and *M. antarcticum* were significantly affected by arrival sequence, but not diet. For detailed LMM information see Table 1. *ns* $P > 0.05$; *$P < 0.05$; and ****$P < 0.0001$. In each treatment, $n = 4$ colonies

collecting of food sources (Figs. 2, 3). The eight nests of *M. antarcticum* that resisted raids of *P. advenus* did so by blocking and displaying sentinel behaviour at their nest entrance. Workers of *M. antarcticum* from colonies that blocked their nest entrance displayed low activity over the 65 days and appeared to be hindered in their use of the foraging area (Figs. 2e, f, 3e, f).

Table 1 Results of the LMMs to evaluate the effects of the arrival sequence (Pf; Mf), diet (HCLP or LCHP) and the number of interactive opponent species workers on the level of aggression displayed by colonies of *P. advenus* and *M. antarcticum* subjected to interspecific interaction

	Predictors	df	Coefficient (SE)	t value	P
P. advenus	Intercept	1, 105	1.479 (0.071)	20.700	<0.0001
	Arrival sequence	1, 105	0.256 (0.099)	2.585	**0.011**
	Diet	1, 105	0.065 (0.078)	0.827	0.410
	M. antarcticum abundance	1, 105	0.006 (0.002)	2.529	**0.013**
M. antarcticum	Intercept	1, 105	1.806 (0.290)	6.218	<0.0001
	Arrival sequence	1, 105	0.893 (0.201)	4.416	**<0.0001**
	Diet	1, 105	0.003 (0.201)	0.013	0.989
	P. advenus abundance	1, 105	−0.001 (0.004)	−0.189	0.850

In each treatment, $n = 4$ colonies. Significant results are highlighted in bold ($P < 0.05$)

Pf P. advenus first, *Mf M. antarcticum* first, *HCLP* high carbohydrate-low protein, *LCHP* low carbohydrate-high protein
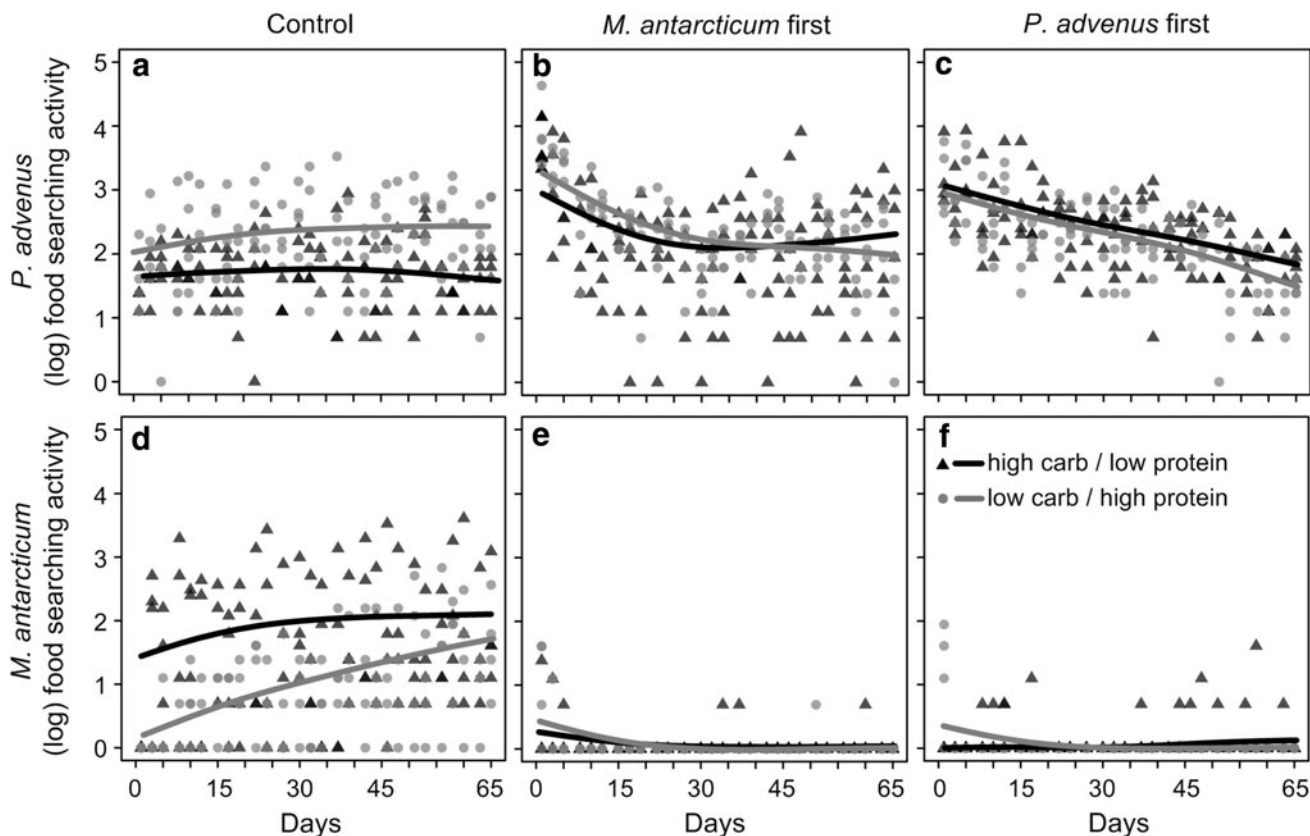

Fig. 2 Food searching activity displayed by *P. advenus* (*top panels*) and *M. antarcticum* (*bottom panels*) in different arrival sequences, food treatments and the activity of controls colonies. Food searching activities of *P. advenus* on panels are: **a** control, **b** *M. antarcticum* first, **c** *P. advenus* first. Food searching activities of *M. antarcticum* on panels are: **d** control, **e** *M. antarcticum* first, **f** *P. advenus* first. Colonies in control treatments were fed with different diet and utilized the foraging area, but did not face interspecific interactions. The *black triangles* and *grey circles* are different food treatments. The *black elements* are the activity of colonies fed on a high carbohydrate-low protein diet. The *grey elements* are the activity of colonies fed on a low carbohydrate-high protein diet. Food searching activity was log transformed. *Solid lines* indicate the smoothed spline ($df = 3$) for different food treatments. In each treatment, $n = 4$ colonies

Control colonies of *P. advenus* fed on LCHP diet displayed significantly higher activity rates than colonies fed on HCLP diet in both food searching (Fig. 2a; GEE, $b = 0.70 \pm 0.09$; $W = 64.6$; $P < 0.0001$) and collecting activity (Fig. 3a; GEE, $b = 0.42 \pm 0.07$; $W = 36.6$;

$P < 0.0001$). The food searching activity of control colonies of *M. antarcticum* was also affected by changes in diet. When alone, *M. antarcticum* displayed a significant increase in food searching activity when fed on an HCLP diet (Fig. 2d; GEE: $b = -1.07 \pm 0.17$; $W = 38.5$; $P < 0.0001$), but
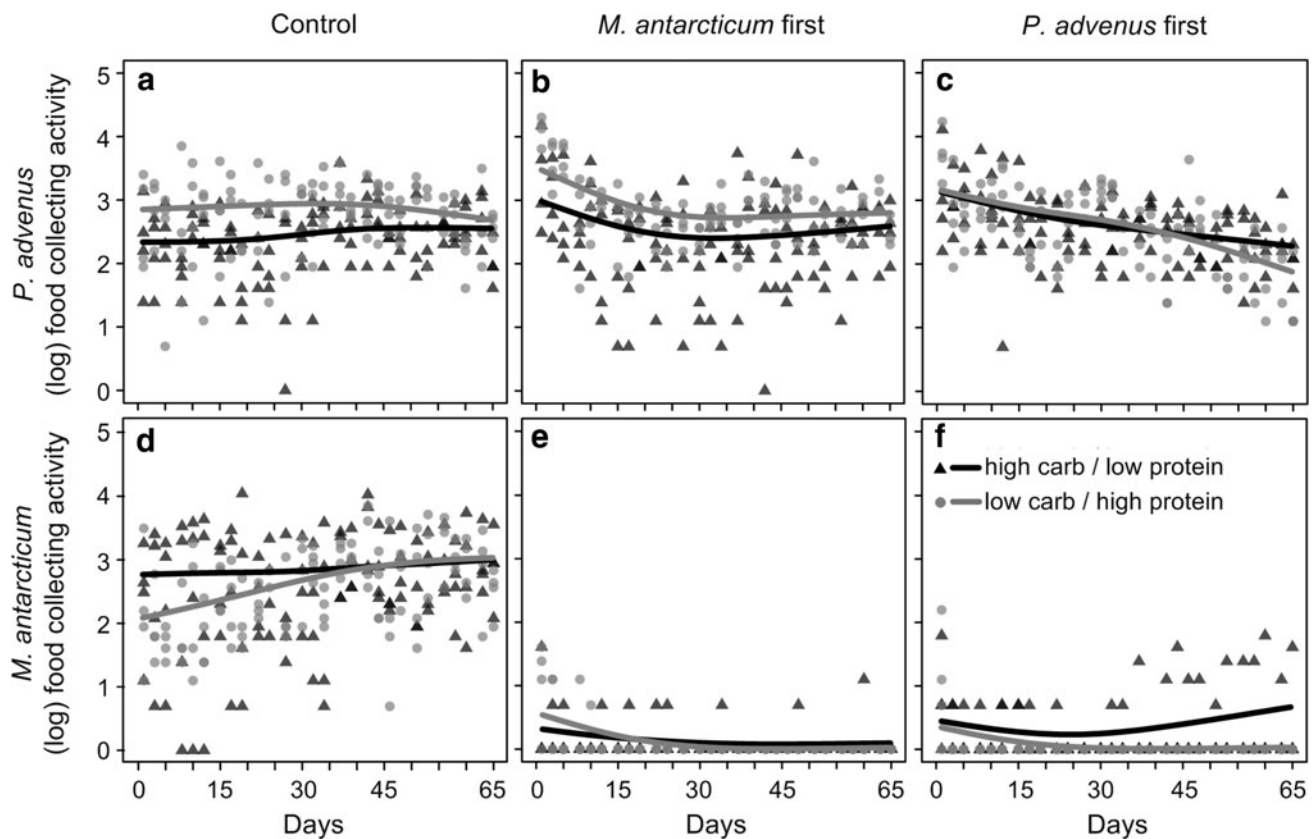
Fig. 3 Food collecting activity displayed by *P. advenus* (*top panels*) and *M. antarcticum* (*bottom panels*) in different arrival sequences, food treatments and the activity of controls. Food collecting activities of *P. advenus* on panels are: **a** control, **b** *M. antarcticum* first, **c** *P. advenus* first. Food collecting activities of *M. antarcticum* on panels are: **d** control, **e** *M. antarcticum* first, **f** *P. advenus* first. Colonies in controls treatments were fed with different diet and utilized the foraging area, but did not face interspecific interactions. The *black triangles* and *grey circles* are different food treatments. The *black elements* are the activity of colonies fed on a high carbohydrate-low protein diet. The *grey elements* are the activity of colonies fed on a low carbohydrate-high protein diet. Food collecting activity was log transformed. *Solid lines* indicate the smoothed spline (*df* = 3) for different food treatments. In each treatment, $n = 4$ colonies

there was no significant difference in their food collecting activity, regardless of diet (GEE: $b = -0.15 \pm 0.09$; $W = 2.45$; $P = 0.12$) (Fig. 3d).

The activity of control colonies of *M. antarcticum* was clearly higher than the activity of colonies subjected to interspecific interactions with *P. advenus* (Figs. 2, 3; Table 2; $P < 0.0001$). The grouped analyses, which includes different arrival sequences and controls, indicate that the food searching activity of *M. antarcticum* did not present significant changes in different arrival sequences (Table 2; $P = 0.220$). However, their food collecting activity was higher when *P. advenus* arrived first (Table 2; $P = 0.004$). Diet alone did not cause significant effects on the food searching and collecting activity of *M. antarcticum* (Table 2; $P > 0.290$). However, the number of *P. advenus* workers in the foraging area significantly affected food searching and collecting activity of *M. antarcticum* (Table 2; $P < 0.0001$).

There was no significant effect of arrival sequence on the colony activity of *P. advenus* (Table 2; $P > 0.416$), but colonies in different arrival sequences differed from controls (Table 2; $P < 0.0001$). This indicates that the presence of small colonies of *M. antarcticum* affected food searching and collecting activity of *P. advenus*, regardless of arrival sequence. Furthermore, the activity of small colonies of *M. antarcticum* that persisted exerted significant influence on the activity of large nests of *P. advenus* (Table 2; $P < 0.0001$). Independent of arrival sequence and diet, *P. advenus* displayed a peak activity on the first days of interspecific interaction (Figs. 2b, c, 3b, c). However, after approximately 35 days *P. advenus* reduced their food-searching activity to similar levels presented by control colonies that were not subjected to interspecific interactions (Figs. 2a, 3a). Surprisingly, there was no significant effect of diet on the food searching activity of *P. advenus* when comparing groups in different arrival sequences and controls (Table 2; $P = 0.368$). This result suggests that colonies of *P. advenus* fed on different diets searched for food in a similar fashion. Conversely, the grouped analysis indicates significant main effects of diet (Table 2;

Table 2 Results of generalized estimating equations (GEE) evaluating the effects of arrival sequence (Pf; Mf), diet (HCLP; LCHP), and the activity of the opponent species on the food searching and collecting activity of *M. antarcticum* and *P. advenus*

Predictors		Coefficient (SE)	Wald	P
P. advenus colony activity				
Food searching activity	Intercept	2.348 (0.100)	554.330	<0.0001
	P. advenus first	0.100 (0.123)	0.660	0.416
	Control	−0.842 (0.117)	51.980	**<0.0001**
	Diet	0.125 (0.139)	0.810	0.368
	M. antarcticum activity	0.030 (0.006)	22.070	**<0.0001**
	P. advenus first × diet	−0.266 (0.172)	2.380	0.123
	Control × diet	0.458 (0.168)	7.450	**0.006**
Food collecting activity	Intercept	2.521 (0.077)	1067.240	<0.0001
	P. advenus first	0.054 (0.098)	0.300	0.582
	Control	−0.374 (0.100)	13.920	**<0.0001**
	Diet	0.384 (0.099)	15.200	**<0.0001**
	M. antarcticum activity	0.018 (0.003)	34.080	**<0.0001**
	P. advenus first × diet	−0.326 (0.134)	5.950	**0.015**
	Control × diet	−0.061 (0.121)	0.260	0.612
M. antarcticum colony activity				
Food searching activity	Intercept	−3.558 (0.653)	29.720	<0.0001
	P. advenus first	0.785 (0.646)	1.480	0.220
	Control	4.910 (0.595)	67.990	**<0.0001**
	Diet	−0.473 (1.491)	0.100	0.750
	P. advenus activity	0.061 (0.014)	20.510	**<0.0001**
	P. advenus first × diet	0.245 (1.703)	0.020	0.890
	Control × diet	−0.600 (1.512)	0.160	0.690
Food collecting activity	Intercept	−2.588 (0.411)	39.670	<0.0001
	P. advenus first	1.328 (0.456)	8.480	**0.004**
	Control	4.867 (0.411)	140.410	**<0.0001**
	Diet	−0.107 (0.574)	0.030	0.852
	P. advenus activity	0.034 (0.005)	56.470	**<0.0001**
	P. advenus first × diet	−1.509 (1.425)	1.120	0.290
	Control × diet	−0.005 (0.582)	0.000	0.993

We also compare the activity of colonies in different arrival sequences to control colonies. Control colonies were fed with different diets and utilized the foraging area, but were not subjected to interspecific interaction. The reference category for the analysis was the group in which *M. antarcticum* arrived first, fed in a HCLP diet. In each treatment, $n = 4$ colonies. Significant results are highlighted in bold ($P < 0.05$)

Pf P. advenus first, *Mf M. antarcticum* first, *HCLP* high carbohydrate-low protein, *LCHP* low carbohydrate-high protein

$P < 0.0001$) and a significant diet × arrival sequence interaction effect on the food collecting activity of *P. advenus*, indicating they displayed distinct food collecting activity rates when fed on different diets and in distinct arrival sequences (Table 2; $P = 0.015$).

Colony survival

Although numerically disadvantaged, three colonies of *M. antarcticum* fed on a HCLP diet and one colony fed on an LCHP diet persisted for the duration of the experiment. Colonies of *M. antarcticum* that persisted did so by blocking their own nest entrances and displaying constant sentinel behaviour. Workers of *M. antarcticum* appear to stand guard

and displayed offensive postures with the gaster while defending their nest entrances. The colonies of *M. antarcticum* that did not prominently protect their nest entrances were invaded, exterminated, and had their nests posteriorly occupied by *P. advenus*.

The survival probability of *M. antarcticum* was analysed with (GLM; $b = 1.384$, $P < 0.0001$, AIC $= -23.975$) and without (GLM; $b = 0.156$, $P = 0.047$, AIC $= 129.04$) nest blocking as a predictor of colony survival. Given this change in AIC values when the nest blocking ability was added to the GLMs, the improvement of the model's predictability is noticeable. However, the models evaluating the survival probability of *P. advenus* did not show any significant improvement after the insertion of *M. antarc-*

Table 3 Results of the generalized linear models (GLM) to evaluate the effects of arrival sequence (Pf; Mf[†]), diet (HCLP or LCHP*) and nest blockage behaviour of *M. antarcticum* on the colony survival of *P. advenus* on *M. antarcticum*

	Predictors	df	Coefficient (SE)	t value	P
P. advenus colony survival	Intercept	23	0.037 (0.002)	21.329	<0.0001
	P. advenus first	1, 21	0.004 (0.002)	1.953	0.068
	Control	1, 21	0.003 (0.002)	−1.190	0.250
	Diet	1, 20	−0.002 (0.002)	−0.956	0.353
	M. antarcticum block	1, 19	0.000 (0.001)	0.058	0.955
	P. advenus first × diet	1, 17	−0.001 (0.003)	−0.406	0.690
	Control × diet	1, 17	0.002 (0.003)	0.659	0.519
M. antarcticum colony survival	Intercept	23	1.384 (0.021)	64.716	<0.0001
	P. advenus first	1, 21	0.063 (0.007)	9.620	**<0.0001**
	Control	1, 21	−1.311 (0.021)	−61.147	**<0.0001**
	Diet	1, 20	0.019 (0.005)	3.774	**0.002**
	M. antarcticum block	1, 19	−1.302 (0.021)	−61.052	**<0.0001**
	P. advenus first × diet	1, 17	−0.051 (0.037)	−1.391	0.182
	Control × diet	1, 17	−0.019 (0.005)	−3.440	**0.003**

We also compare the survival rates of colonies in different arrival sequences to control colonies. Control colonies were fed with different diets and utilized the foraging area, but were not subjected to interspecific interaction. The reference category for the analysis was the group in which *M. antarcticum* arrived first, fed in a HCLP diet. In each treatment, n = 4 colonies. Significant results are highlighted in bold (P < 0.05)

Pf P. advenus first, *Mf M. antarcticum* first, *HCLP* high carbohydrate-low protein, *LCHP* low carbohydrate-high protein

ticum nest blocking ability (with blocking: GLM; $b = 0.037$; $P < 0.0001$, AIC = 111.86; without blocking: GLM; $b = 0.037$; $P < 0.0001$, AIC = 113.85). Thus, we selected the models including *M. antarcticum* nest blocking ability as a factor influencing colony survival for both *P. advenus* and *M. antarcticum*.

All nests of *P. advenus* persisted for the duration of the experiment and neither diet nor the nest blocking behaviour of *M. antarcticum* affected their survival (Table 3; $P > 0.353$). The survival rates of *P. advenus* colonies that faced interspecific interaction did not differ from the controls (Table 3; $P = 0.250$). However, there was a tendency towards a significant effect of arrival sequence and colonies of *P. advenus* utilizing the territory first experienced higher mortality rates (Fig. 4; Table 3; $P = 0.068$).

Nest blocking ability increased the survival probability of *M. antarcticum* (Table 3; $P < 0.0001$). Also, colonies of *M. antarcticum* utilizing the territory first presented higher survival probability than colonies that accessed the foraging area after *P. advenus* (Fig. 4; Table 3; $P < 0.001$). We also found significant main effects of diet on the survival rates of *M. antarcticum* ($P = 0.002$) and colonies of *M. antarcticum* that faced interspecific interactions presented higher survival rates when fed on a HCLP diet (Fig. 4).

Discussion

Previous works have indicated that multiple factors give competitive advantages to the primary species (Cole,

1983b; Louette and De Meester, 2007; Victorsson, 2012; Kardol et al., 2013). It is also known that diet can modulate interspecific interactions between competitors (Kay et al., 2010; Wagner et al., 2013). We demonstrated that the arrival sequence influences aggression level and affects colony survival of *P. advenus* and *M. antarcticum*. Diet modulated their colony activity. Interestingly, reactions displayed by both species in different arrival sequence and diets were asymmetrical in nature. Large colonies of *P. advenus* displayed increased aggression when arriving first, and small colonies of *M. antarcticum* prominently displayed a defensive reaction when arriving first. Colonies of *M. antarcticum* that were not subjected to interspecific interactions and fed on an HCLP diet displayed higher activity rates than colonies fed on an LCHP diet. On the other hand, control colonies of *P. advenus* fed on a HCLP diet displayed lower activity rates than colonies fed on an LCHP diet. Thus, our laboratory study indicates that arrival sequence and diet should have an effect on the probability of these two ant species co-occurring.

The abundance of a competitor influenced both aggression level and colony activity of both ant species, suggesting that workers are evaluating risks (Robinson et al., 2009). The sentinel behaviour displayed by colonies of *M. antarcticum* increased their survival probability. It is known that sentinel behaviour effectively prevents fitness loss of animals in the presence of risks; however, this behaviour can also restrict their access to food sources and should affect colony fitness (Wilson, 1974). The triggering of sentinel activity protected small nests of *M. antarcticum*
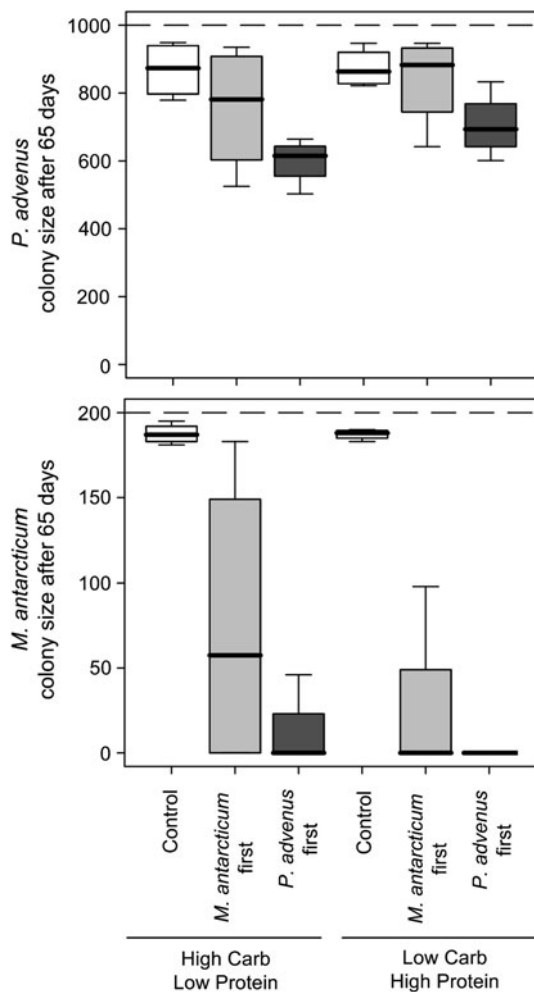
Fig. 4 Boxplots comparing the number of living workers of *P. advenus* (*top panel*) and *M. antarcticum* (*bottom panel*) at the end of 65 days in different treatments. *Dashed lines* indicate the colony size of each species at the beginning of the experiment. Boxes on different colours are different treatments: control (*white boxes*), *M. antarcticum* first (*light grey boxes*), *P. advenus* first (*dark grey boxes*). Colonies in control treatments were fed with different diets and utilized the foraging area, but did not face interspecific interactions. *Boxes* represent the lower and upper quartile, the *bold line* is the median and *whiskers* represent extreme values of colony survival rates. For significant values see Table 2. In each treatment, $n = 4$ colonies

from raids of *P. advenus* and prevented *M. antarcticum* from losing workers. As soon as the nest entrance was blocked, few *M. antarcticum* workers left the nest and fought against *P. advenus*. A similar reaction was observed when small groups of *M. antarcticum* fought against large colonies of *Linepithema humile* (Sagata and Lester, 2009). Workers of *M. antarcticum* tried, with no success, to protect the colony against recurrent attacks of *L. humile* by standing guard at the nest entrance. In our previous observations, colonies of *M. antarcticum* that had a numerical advantage did not display sentinel activity when fighting against *P. advenus*. Neither was such behaviour observed by Sagata

and Lester (2009). These results suggest that the reaction displayed by *M. antarcticum* is colony-size dependent and triggered in situations of risk to the colony.

The triggering of aggressive behaviour as a response to quantitative or qualitative variations in diet seems to be evident in ant communities (Hölldobler, 1979; Davidson, 1997). Although other ant species can modulate their levels of aggression towards opponents when fed with distinct diets (Grover et al., 2007; Kay et al., 2010), our results do not support the hypothesis that interspecific aggression between *P. advenus* and *M. antarcticum* is a response to or is modulated by nutrient availability. The constant level of aggression displayed by both species, regardless of diet, could be a result of a not physiologically significant reduction of carbohydrate/protein dosages offered to the colonies. However, this seems to be improbable as control colonies of both species displayed distinct activity levels according to diet. A possible explanation for the increased aggression displayed by *P. advenus* when they were the first to arrive could be related to their level of territoriality. *Prolasius advenus* are abundantly found in the honeydew-rich beech forest and may exert a "level III" territoriality (Vepsalainen and Pisarski, 1982) by defending not only nest sites (level I) and resources areas (level II), but full territories constantly patrolled by workers (level III). *Monomorium antarcticum*, on the other hand, may exert an intermediate level of territoriality (level II). According to Vepsalainen and Pisarski (1982), "level II" species can be displaced by "level III" species through numerical advantage and organized recruitment of nest mates.

In the present study, the lack of carbohydrates did increase colony activity of *P. advenus*. A similar response was predicted by the "tempo hypothesis" (Oster and Wilson, 1978). The concept of "tempo" suggests ants should be classified as either low- or high-tempo species. High-tempo species are prone to a high-level performance while low-tempo species act carefully. The concept of tempo is not only related to how fast an individual moves, but to the probability of and how fast individuals find and exploit suitable food sources (Leonard and Herbers, 1986). In the beech forests of New Zealand, *P. advenus* seems to be adapted to a predictable honeydew-rich environment and may reduce tempo in response to the facility for utilizing an energy-rich food source. When deprived of an energetic food source, the foraging behaviour or tempo increases and *P. advenus* simultaneously increases the probability of finding resources and displacing competitors. The tempo hypothesis may also explain the low numbers of *P. advenus* observed in areas where the invasive wasp *Vespula vulgaris* are actively controlled by toxic baiting (Duthie and Lester, 2013). This invasive wasp is a well-established competitor of *P. advenus* in beech forests (Grangier and Lester, 2011, 2012) and also consumes high quantities of honeydew

(Moller and Tilley, 1989). A reduction in the wasp population should increase the availability of honeydew in beech forest and, as a consequence, decrease food searching and collecting activity rate of *P. advenus*.

Multiple stochastic and deterministic processes are claimed to be drivers of community assembly (Abrams, 1996; Chase and Myers, 2011; Cerdá et al., 2013). Behavioural responses likely contribute to shaping the whole community structure by creating behavioural type-dependent patterns of occurrence and regulating population dynamics (Cole, 1983a, b; Sanders and Gordon, 2000; Gravel et al., 2011; Sih et al., 2012). We demonstrated that agonistic responses, either defensive or aggressive in nature, and diet are two mechanisms that could determine competitive ability and potentially drive community structure. Clearly, our results emphasize the role of priority effects modulating agonistic behaviour, and diet as a regulatory factor modulating the probability of species to interact with competitors and their environment. Priority effects and diet are two factors that could explain the negative patterns of co-occurrence presented by *P. advenus* and *M. antarcticum* (Barbieri, unpubl. data). However, other factors such as thermal tolerance (Cerdá et al., 1997), habitat preference (Czechowski and Markó, 2005) or even parasitism (Donald and Fenner, 2000; Zhao et al., 2013) may also contribute to the patterns we observe in nature. Furthermore, the monopolization of rich food sources by territorial species may also be related to the evolution of defence mechanisms (Davidson, 1997). Future work integrating a wide range of behavioural strategies and multiple factors modulating their expression are necessary to understand how competitive interactions, at the individual and colony levels, can scale to higher levels of organization.

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