Many social animals display collective activity cycles based on synchronous behavioural oscillations across group members. A classic example is the colony cycle of army ants, where thousands of individuals undergo stereotypical biphasic behavioural cycles of about one month. Cycle phases coincide with brood developmental stages, but the regulation of this cycle is otherwise poorly understood. Here, we probe the regulation of cycle duration through interactions between brood and workers in an experimentally amenable army ant relative, the clonal raider ant. We first establish that cycle length varies across clonal lineages using long-term monitoring data. We then investigate the putative sources and impacts of this variation in a cross-fostering experiment with four lineages combining developmental, morphological and automated behavioural tracking analyses. We show that cycle length variation stems from variation in the duration of the larval developmental stage, and that this stage can be prolonged not only by the clonal lineage of brood (direct genetic effects), but also of the workers (indirect genetic effects). We find similar indirect effects of worker line on brood adult size and, conversely (but more surprisingly), indirect genetic effects of the brood on worker behaviour (walking speed and time spent in the nest).

1. Introduction

Cycles are ubiquitous across scales of biological organization, from cells contracting to generate cardiac rhythm [1] to entire animal populations migrating seasonally [2]. Social and gregarious animals often display collective activity cycles, which vary in period from seconds (e.g. fireflies flashing in unison [3,4]) to years (e.g. cicada brood emergence [5]). Collective cycling in turn requires behaviour to be synchronized across group members [6,7]. These synchronized behavioural oscillations can come about through two basic mechanisms: a global group wide variable that influences each group member or a local interaction between group members with no global connection among them [8].

An iconic collective cycle is that of army ants, whereby colonies made up of thousands or millions of individuals undergo stereotypical biphasic reproductive and behavioural cycles of about one month. This cycle closely tracks brood development: in the nomadic phase, colonies emigrate almost daily, hunting for prey to feed growing larvae. Larval pupation coincides with the entry into the statary phase, in which the queen lays eggs and the colony stays in one location [9,10]. While these iconic cycles have long fascinated naturalists [11–14], the basis of their regulation and maintenance cannot be probed experimentally, in part because most species displaying such cycles are notoriously difficult to keep under laboratory conditions.
The clonal raider ant (Oecophylla smaragdina) is a doryline ant displaying stereotypical army ant like colony cycles. Unlike army ants, however, this species is uniquely experimentally amenable. Colonies of the clonal raider ant are queenless and consist of genetically identical female workers that all reproduce synchronously, meaning that all colony members undergo reproductive and behavioural cycles. The colony cycle consists of alternating brood care and reproductive phases [15], characterized by the presence and absence of larvae, respectively [16–18]. In the brood care phase, workers nurse the larvae in the nest but also leave the nest to forage; in the reproductive phase in contrast, all workers stay in the nest to lay eggs, and no foraging takes place [17]. Brood stage controls colony phase: in the brood care phase—corresponding to the nomadic phase of army ant cycles—the presence of larvae suppresses worker ovarian activity and induces worker foraging activity [16,17]. When larvae become prepupae (i.e. shed their meconium and stop feeding), ovarian inhibition is released and workers synchronously lay eggs, while foraging activity stops. The colony switches back to the brood care phase when those eggs hatch into new larvae.

While brood stage controls cycle phase, it is unclear what controls cycle length. Although cycle length was initially assumed to be fixed [9], recent work has shown that it is highly plastic with respect to at least one group trait, colony size [18]. It is not known whether cycle length can vary with other basic colony features and in particular, whether it varies with the genotype of the colony. If cycle length varies with genotype, it is also unclear whether the genotype of the brood, of the workers, or both, drive the effect. In principle, cycle length could be controlled by the brood alone, if e.g. brood develops at a fixed, genetically determined speed, which in turn determines cycle length. However, because brood in social insects fully depend on workers for food and care, worker behaviour (e.g. foraging efficiency) is also likely to affect brood development. If worker behaviour varies across genotypes, then worker genotype could affect cycle length via effects on brood development.

In most social insects, brood–adult interactions are difficult to study experimentally due to the inherent complexity of colonies, which typically consist of a unique mix of one or more queens, workers of different ages and brood at different developmental stages. Consequently, the brood environment encountered by the workers, and the worker environment encountered by the brood are difficult to control and replicate. By contrast, asexual and synchronous [19] reproduction in the clonal raider ant provides precise control over the genetic background and the age of both workers and brood. Within a colony, workers belong to one of two subcastes that differ in morphology, behaviour and reproductive physiology: small, regular workers with two ovarioles and larger intercastes with four to six ovarioles, vestigial eyes and a tendency to spend more time in the nest [19–21]. Field collected clonal raider ant colonies belong to different clonal lineages (henceforth, ‘lines’) [22,23], which can readily be cross-fostered [21,24]. Differences in worker behaviour [21], anecdotal differences in brood developmental time [18], as well as differences in intercaste proportion [24] have been reported between two commonly used lines (A and B). Previous work on these two lines showed that the propensity of larvae to develop into intercastes is affected by epistatic interactions between larval and worker lines [24]. Furthermore, cross-fostering larvae of each line in colonies containing workers of both lines suggested that larvae of different lines vary in their effects on worker behaviour [21]. Collectively, this points to a role for larvae–worker genotypic interactions in driving colony cycle length.

Here, we use a cross-fostering approach to quantify the impact of genotypic interactions between larvae and workers on cycle length, worker behaviour and brood development in the clonal raider ant. We first establish that cycle length varies across lines using long-term data from laboratory-reared stock colonies. We then investigate the source of this variation by analysing the developmental and behavioural correlates of cycle length variation in a full-factorial cross-fostering experiment between four lines, combining survival, developmental and morphological analyses with automated behavioural tracking.

2. Material and methods

(a) Cycle length variation across stock colonies

To test for baseline differences in cycle length between lines, we analysed long-term data (collected from October 2017 to May 2021; electronic supplementary material, table S1) on the total cycle length and the duration of each brood developmental stage (egg, larva, prepupa, pupa) in seven laboratory-reared stock colonies belonging to four lines (A, B, D, M) [22,23]. All stock colonies were maintained at 27 ± 1 °C in airtight plastic containers with water-saturated plaster of Paris floors. During the brood care phase, stock colonies were fed with frozen ant (Messor or Camponotus) larvae and houseflies. Prior to September 2020, data were collected five times a week (from Monday to Friday) and thereafter, data were collected three times a week (on Mondays, Wednesdays and Fridays). If a change in brood developmental stage occurred on a day when data were not recorded, the initial stage was consistently assumed to last until the subsequent brood stage was observed. Only complete cycles (i.e. cycles in which the brood successfully developed from eggs to adults) were included in analyses. Cycle length was defined as the time (in days) elapsed between two successive larval hatching events.

(b) Cross-fostering experiment

To remove sources of variation other than line, we used workers and larvae of the same age across all treatments. To obtain age-matched workers across all lines, we synchronized reproductive cycles across lines by separating 1000 workers from each of four stock colonies (C16, STC1, BC9, BG14) from different lines (A, B, D, M, respectively) to create four experimental colonies. Separated workers initiated a new cycle by laying eggs within 1–3 days. New workers eclosed from these eggs 26–28 days later (i.e. within 2 days of each other across experimental colonies) and were used as focal ants in the cross-fostering experiment. All focal ants were tagged with colour marks on the thorax and abdomen using oil-paint markers, so as to be individually recognized using automated tracking (see below). To obtain age-matched larvae across all lines, workers from the experimental colonies that had reared the focal ants were transferred to new nests (without brood) to initiate a new event of synchronized egg-laying. New eggs were laid by these workers within 4–5 days, which hatched into larvae on the same day across all lines (8–9 days after egg-laying).

Age-matched workers and larvae were used in a full-factorial cross-fostering experiment with 16 treatments (AA, AB, AD, AM, BA, etc. where the first letter of each treatment indicates worker line, and the second letter indicates brood line). Cross-fostering
colonies were composed of eight age-matched (26 to 28 days old) focal ants and seven age-matched (5 days old) larvae housed in airlight Petri-dishes (5 cm in diameter) with a plaster of iris floor. Colonies of this size show high fitness and normal behaviour in the clonal raider ant [18]. Each treatment was replicated six to eight times (depending on the availability of age-matched larvae and workers), resulting in 109 cross-fostering colonies in total. The colonies were placed randomly in a setup [18] equipped with cameras to record worker behaviour throughout the brood care phase (8–16 days, depending on the colony), itself housed in a climate room kept at 29 ± 1°C. Colonies were given 48 h to settle, after which any dead larvae were replaced, and the experiment started. For each colony, the experiment ended when all the original larvae had either eclosed into new adults or died. Every second day for the duration of the experiment, all colonies were cleaned (food debris and dead ants were removed), fed with frozen Messor pupae proportionally to estimated O. binoi larval biomass (one minor pupa for every third 3rd instar clonal raider ant larva and one major pupa for every third 4th instar clonal raider ant larva), and the plaster was humidified. Additionally, brood developmental stage and survival, as well as worker survival were quantified. At the end of the experiment, all newly eclosed adults (523 out of the original 763 larvae) were frozen at −80°C, and later used for dissections (to count ovariole number) and morphometric measurements (total body length, head with, gastric width and presence/absence of vestigial eyes), measured from images acquired with an Olympus Dual-Sensor Monochrome and Colour Camera (DP80) coupled to the software Olympus cell-ment, all colonies were cleaned (food debris and dead ants were removed), fed with frozen Messor pupae proportionally to estimated O. binoi larval biomass (one minor pupa for every third 3rd instar clonal raider ant larva and one major pupa for every third 4th instar clonal raider ant larva), and the plaster was humidified. Additionally, brood developmental stage and survival, as well as worker survival were quantified. At the end of the experiment, all newly eclosed adults (523 out of the original 763 larvae) were frozen at −80°C, and later used for dissections (to count ovariole number) and morphometric measurements (total body length, head with, gastric width and presence/absence of vestigial eyes), measured from images acquired with an Olympus Dual-Sensor Monochrome and Colour Camera (DP80) coupled to the software Olympus cell-Sens Standard (v.1.15; electronic supplementary material, figure S1). Individuals that had eyes and/or four or more ovarioles were categorized as intercastes.

(c) Automated behavioural analyses

Videos were recorded for 10 min every 2 h throughout the brood care phase of each cross-fostering colony, starting 24 h after the start of the experiment. The positions of individual ants were extracted from videos using the automated tracking software anTraX v.1.0.2 [25]. Tracking performance was validated manually with the built-in procedure of anTraX, based on 100 automatically made assignments per camera. In social insect colonies, tasks are spatially segregated (e.g. nursing occurs in the nest whereas foraging occurs outside the nest) [26], meaning that spatial distribution can be used as a proxy for task performance [18,27]. We, therefore, used three spatial behavioural traits to characterize the tendency of individual ants to leave the nest to forage versus stay in the nest with the brood: (i) proportion of time active (henceforth ‘activity’), defined as the proportion of frames in which an ant was moving at a speed greater than 1 mm s⁻¹; (ii) walking speed, defined as the mean walking speed (in mm s⁻¹) of an active ant, were both used as proxies for foraging activity. Activity and walking speed were averaged across videos for each ant; (iii) proportion of time in the Past, defined as the proportion of frames in which an ant was in the nest area, was used as a proxy for individual nursing behaviour. In ant colonies, workers cluster around their brood, and for each colony, the nest area (i.e. the brood pile) was thus identified as the time (in days) elapsed between the appearance of a given brood stage (e.g. larvae) until the appearance of the next brood stage (e.g. prepupae). To compare cycle length and developmental stage durations between pairs of stock colonies, we conducted Dunn’s tests (function dunns_test of package pppstats) with Benjamini–Hochberg adjustment for multiple testing.

In one cross-fostering colony (treatment AB), all workers died due to insufficient humidity and the colony was, therefore, excluded from all analyses. In one additional colony (treatment DA), all brood failed to develop into workers and this colony was thus excluded from analyses of developmental time, caste fate and body length, but retained in analyses of survival and worker behaviour. Furthermore, in one colony of each of the treatments AB, AD, BB, BD, DM and MA, the assignment rate of automated tracking was low (less than 60%) and these colonies were, therefore, excluded from behavioural analyses. After the exclusion of the seven above-mentioned colonies, an additional 29 individual ants were removed due to a low (less than 60%) assignment rate of automated tracking. After exclusion of these colonies and ants, automated tracking assigned 85.17% of the focal ant’s locations with an error rate of less than or equal to 10%.

Unless stated otherwise, we used regression models (described below) to analyse the effects of worker line (a four-factor variable), brood line (a four-factor variable) and their interaction on all response variables in the cross-fostering experiment. In all models performed at the individual ant level, cross-fostering colony was used as a random effect to account for the non-independence of ants from the same colony. Model assumptions were verified using the function simulateResiduals of package DHARMa. Significance of model terms was assessed by sequential deletion of terms and model comparison (function Anova of package car). Terms that did not significantly contribute to model fit ($p > 0.05$) were deleted and we report the results of reduced models here. If the interaction between brood and worker line was significant, pairwise comparisons of interest with user defined contrasts were conducted using the function glht of package multcomp. Comparisons of interest were those between pairs of treatments that shared either worker line (e.g. AB–AD) or brood line (BA–DA), as well as comparisons between pairs of control treatments (AA, BB, DD, MM). If the interaction was not significant, Tukey post hoc tests (function glht of package multicomp) were conducted between levels of significant main effects. In all cases, $p$-values were adjusted for multiple testing with the Benjamini–Hochberg method.

For each cross-fostering colony, larval stage duration was defined as the time (in days) elapsed between the start of the experiment (when larvae were 5- day old) and the appearance of the first prepupa. Pupal stage duration was defined as the time (in days) elapsed between the appearance of the first pupa and the eclosion of the first adult. Time to eclosion was defined as the time (in days) elapsed between the experiment start until the eclosion of the first adult. Linear models (LM, function lm from package stats) were used to analyse genotypic effects on time to eclosion and larval stage duration in the cross-fostering experiment. Time to eclosion and larval stage duration were rank transformed to satisfy model assumptions. Pupal stage duration data did not meet model assumptions every 4 h) for each colony, thereby capturing changes in the nest shape and position.

(d) Statistical analyses

Statistical analyses were performed in R v.4.0.5 [28].

Kruskal–Wallis tests were used to compare cycle length and the duration of each brood developmental stage (eggs, larvae, prepupae, pupae) across stock colonies. Developmental stage durations were defined as the time (in days) elapsed between the appearance of a given brood stage (e.g. larvae) until the appearance of the next brood stage (e.g. prepupae). To compare cycle length and developmental stage durations between pairs of stock colonies, we conducted Dunn’s tests (function dunns_test of package pppstats) with Benjamini–Hochberg adjustment for multiple testing.

In one cross-fostering colony (treatment AB), all workers died due to insufficient humidity and the colony was, therefore, excluded from all analyses. In one additional colony (treatment DA), all brood failed to develop into workers and this colony was thus excluded from analyses of developmental time, caste fate and body length, but retained in analyses of survival and worker behaviour. Furthermore, in one colony of each of the treatments AB, AD, BB, BD, DM and MA, the assignment rate of automated tracking was low (less than 60%) and these colonies were, therefore, excluded from behavioural analyses. After the exclusion of the seven above-mentioned colonies, an additional 29 individual ants were removed due to a low (less than 60%) assignment rate of automated tracking. After exclusion of these colonies and ants, automated tracking assigned 85.17% of the focal ant’s locations with an error rate of less than or equal to 10%.

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Figure 1. Colony cycles in stock colonies. Boxplots represent the median (bold horizontal line), the first and third quantiles (hinges) and the 95% confidence interval of the median (whiskers). Data points and sample sizes represent colony cycles analysed per stock colony. Colours indicate line (orange: A, pink: B, blue: D, green: S). (a) Total cycle length. (b) Duration of each brood developmental stage. (Online version in colour.)

and were therefore analysed using an aligned rank transform (ART) ANOVA (function art from package ARTool). The egg developmental time was not considered because the experiment began with larvae and the prepupal stage duration was not analysed because its mean duration (3.98 ± 0.24 days) was short compared to the data’s temporal resolution (2 days).

Binomial generalized linear mixed models with Template Model Builder (glmmTMB, function glmmTMB of package glmmTMB) were used to analyse genotypic effects on worker and brood survival. Gaussian glmmTMB were used to analyse genotypic effects on individual body length. Spearman’s rank correlation tests were used to analyse the relationship between variables of interest at the colony level (larval stage duration versus intercaste proportion, larval stage duration versus mean body length).

3. Results

Cycle length varied across stock colonies (Kruskal–Wallis rank sum test, $\chi^2 = 48.09$, d.f. = 6, $p = 1.13 \times 10^{-6}$; figure 1a), with almost a week difference between the longest (colony C17: mean ± s.e. 39.23 ± 0.74 days) and the shortest (colony BG9: 33 ± 1.66 days) mean cycle lengths. Mean cycle length in all three stock colonies of line A was longer than in the four stock colonies belonging to other lines (Dunn’s test, C16, C17, OIST1 versus STC6, STC1, BG9, BG14, all $p < 4.3 \times 10^{-2}$; electronic supplementary material, table S2). Breaking down the cycle in different brood developmental stages revealed that differences in cycle length stemmed from differences in larval stage duration between stock colonies ($\chi^2 = 65.24$, d.f. = 6, $p = 3.85 \times 10^{-12}$; figure 1b), and not from differences in the duration of the egg, prepupal or pupal stages (eggs: $\chi^2 = 10.69$, d.f. = 6, $p = 0.1$; prepupae: $\chi^2 = 4.96$, d.f. = 6, $p = 0.05$; pupae: $\chi^2 = 10.18$, d.f. = 6, $p = 0.001$).
Indeed, larvae of all three stock colonies of line A took longer to develop than larvae of all other stock colonies from different lines (Dunn’s test, C16, C17, OIST1 versus STC6, STC1, BG9, BG14, all $p < 1.11 \times 10^{-2}$; electronic supplementary material, table S2). Thus, variation in cycle length across stock colonies is associated with clonal lineage and arises from variation in larval stage duration.

In the cross-fostering experiment, both worker survival (mean ± s.e.: 0.92 ± 0.01) and brood survival (0.68 ± 0.02) were consistently high across treatments. Neither worker survival nor larval survival were affected by worker line (glmMQR, worker survival: $\chi^2 = 0.56$, d.f. = 5, $p = 0.91$; brood survival: $\chi^2 = 1.59$, d.f. = 3, $p = 0.66$; electronic supplementary material, figure S2), brood line (worker survival: $\chi^2 = 2.08$, d.f. = 3, $p = 0.56$; brood survival: $\chi^2 = 2.45$, d.f. = 3, $p = 0.48$) or their interaction (worker survival: $\chi^2 = 7.16$, d.f. = 9, $p = 0.62$; brood survival: $\chi^2 = 9.91$, d.f. = 9, $p = 0.35$). The lack of effect of worker line on brood survival supports the view that workers cannot differentiate or do not favour brood of their own line over brood of other lines.

Brood time to eclosion was affected by both worker line (LM, $F_{3,100} = 3.71$, $p = 1.41 \times 10^{-2}$) and brood line ($F_{3,100} = 4.69$, $p = 4.16 \times 10^{-3}$; figure 2a). Brood of line A, irrespective of the worker line rearing them, took longer to develop into adults than brood of other lines (post hoc tests: A versus B, D, M, all $p < 2.41 \times 10^{-2}$; electronic supplementary material, table S3). Additionally, brood reared by workers of line A, irrespective of their own line, took longer to develop into adults than brood reared by workers of line M (M-A: $t = -3.12$, $p = 1.44 \times 10^{-2}$; figure 2a). As in stock colonies (figure 1), variation in developmental time across cross-fostering colonies was driven by variation in larval stage duration. Larval stage duration was affected by both worker line (LM, $F_{3,100} = 4.81$, $p = 3.56 \times 10^{-3}$) and brood line ($F_{3,100} = 10.62$, $p = 4 \times 10^{-4}$; figure 2b). Larvae of line A took longer to become prepupae than larvae of all other lines (post hoc tests, A versus B, D, M, all $p < 7.7 \times 10^{-2}$; electronic supplementary material, table S3) and larvae of line B took longer to develop than larvae of line D (D-B: $t = -2.54$, $p = 1.89 \times 10^{-2}$), irrespective of the line of the workers rearing them. In addition, larvae took longer
Worker activity were associated with differences in worker behaviour. To develop, we next asked whether differences in cycle length
0.85 days) across treatments.
workers and line A larvae had the longest larval stage (12 ±
0.56; figure 2)
line or their interaction (ART, worker line:
2.88, \( p = 1.47 \times 10^{-2} \); M–A, \( t = -3.44, p = 5.06 \times 10^{-5} \)). Unlike the larval stage, the duration of the pupal stage did not vary with either brood line, worker line or their interaction (ART, worker line: \( f_{3,91} = 1.39, p = 0.25 \); brood line: \( f_{3,91} = 0.72, p = 0.54 \); interaction: \( f_{4,91} = 0.87, p = 0.56 \); figure 2c). Thus, variation in cycle length arises from variation in larval stage duration and is determined by both larval and worker lines. In line with this, colonies composed of line A workers and line A larvae had the longest larval stage (12 ± 0.85 days) across treatments.

Because larvae rely on food and care provided by workers to develop, we next asked whether differences in cycle length were associated with differences in worker behaviour. Worker activity—a proxy for foraging behaviour—was affected by the workers’ own line (glmmTMB, \( \chi^2 = 149.25, \) d.f. = 3, \( p < 2.2 \times 10^{-16} \); figure 3a). Apart from one comparison (post hoc tests, M–B, \( z = 2.29, p = 0.01 \)) the activity of all worker lines differed from each other (D > M, B > A, all \( p < 1.27 \times 10^{-2} \); electronic supplementary material, table S4).

Another proxy for foraging, worker walking speed was influenced by an interaction between worker line and brood line (glmmTMB, \( \chi^2 = 226.83, \) d.f. = 3, \( p < 2.2 \times 10^{-16} \); figure 3b) and by the line of the larvae they were rearing (\( \chi^2 = 8.47, \) d.f. = 3, \( p = 3.72 \times 10^{-3} \)). All worker lines differed in the proportion of time they spent in the nest (post hoc tests, A > B > M > D, all \( p < 9.12 \times 10^{-3} \); electronic supplementary material, table S6). Furthermore, workers that reared brood of line M spent more time in the nest than workers that reared brood of line B (M–B: \( z = 2.74, p = 3 \times 14^{-2} \)).

Figure 3. Worker behaviour in cross-fostered colonies. Boxplots show the median (bold horizontal line), the first and third quantiles (hinges), and the 95% confidence interval of the median (whiskers). Points and sample sizes represent individual workers. Colours indicate lines (orange: A, pink: B, blue: D, green: M). (a) Activity as a function of brood and worker lines. (b) Walking speed as a function of brood and worker lines. (c) Proportion of time in the nest as a function of brood and worker lines. (Online version in colour.)
Thus, worker behaviour was not only affected by their own genotype, but also by the genotype of the larvae they reared.

Because developmental plasticity is often linked to caste in social insects, we then asked whether differences in brood developmental time affected the morphology (caste, body size) of the resulting adults (figure 4a).

We found substantial variation in caste fate across treatments, both within and between lines, showing extensive developmental plasticity in this system. The proportion of intercastes ranged from 2.38 ± 2.38% in treatment BA to 56.47 ± 13.91% in treatment MB (figure 4b). Intercaste proportion was influenced by an interaction between worker line and brood line (glmmTMB: χ^2 = 17.51, d.f. = 9, p = 4.14 × 10^{-2}). Differences in intercaste production stemmed from the propensity for brood of line B to grow into smaller adults than brood of other lines, even when reared by the same workers (AM–AA, AM–AB, AM–AD, BM–BA, BM–BB, BM–BD, DM–DA, DM–DB, DM–DD, MM–MA, MM–MB and MM–MD; p < 4.81 × 10^{-2}; electronic supplementary material, table S8 and figure S3b). Brood line influenced adult body length in only one other case (MD–MB; z = −2.35, p = 4.81 × 10^{-2}). Interestingly, worker line also affected the adult size of the brood they reared. In two cases, A workers produced larger adults than other workers from the same larvae (post hoc tests, BA–AA, z = −3, p = 8.55 × 10^{-3}; MD–AD, z = −2.51, p = 3.26 × 10^{-2}), while in two other cases, B workers produced smaller adults than other workers from otherwise identical larvae (DA–BA, z = 2.81, p = 1.41 × 10^{-2}; MB–BB, z = 3.09, p = 6.84 × 10^{-4}). Thus, while adult morphology was influenced primarily by the brood’s own line, we also detected indirect genetic effects arising from the rearing environment (worker line). Despite variation in brood developmental time and adult morphology, we find no clear relationship between these two traits across colonies (Spearman’s rank correlation tests: larval stage duration – intercaste proportion: S = 188 903, p = 0.19; larval stage duration – body length: S = 139 478, p = 0.175).
4. Discussion

Using long-term data and a cross-fostering experiment, we show that the duration of a collective cycle varies across clonal lineages in a social insect. We find that differences in cycle length stem from variation in the duration of a specific brood developmental stage—the larval stage—and that the duration of that stage is influenced not only by the genotype of the brood itself, but also by the genotype of the workers rearing them. Larvae are the only feeding brood developmental stage, and *O. biroi* workers only forage in the presence of larvae [16], indicating a role for nutrient acquisition and processing in driving genotypic effects on cycle length. In line with this, the worker line that consistently prolonged larval development (A) had the lowest activity and highest time spent in the nest, both of which point to reduced foraging activity. This is supported by Piekarski et al. [29], who report reduced foraging activity in workers of line A. The finding that both larvae and workers of the same line (A) were sufficient to prolong cycle length suggests a genetic effect acting on both larval growth and worker foraging activity. Such an effect could stem, for example, from a lower metabolic rate in line A.

We find pervasive direct genetic effects on brood development (the line of the brood affected its developmental time, caste and adult body size) and on worker behaviour (the line of workers affected their activity and time spent in the nest). Additionally, we detect indirect genetic effects of workers on brood development (worker line affected brood developmental time and final size) likely reflecting the dependence of larvae on workers for food and care. Some of these findings are corroborated by Piekarski et al. [29] who report higher motility in line D workers and larger body size in brood reared by workers of line A using different metrics. More surprisingly, we detect several instances of indirect genetic effects of the brood on worker behaviour, with larval genotype affecting the amount of time workers spend in the nest as well as their walking speed. While effects of the rearing environment on brood development, and particularly on sex and caste allocation, have been extensively studied in social insects [29–33], whether and how the brood can affect worker behaviour has received comparatively little attention. Previous work in other systems has shown that the brood of social insects can produce chemical [34], behavioural [35] and acoustic [36] cues, some of which influence worker behaviour [37,38]. For example, honeybee larvae secrete a pheromone that affects worker physiology and behaviour [37,39,40]. Similarly, some ant larvae can increase their own food provisioning through body movements [35,41], akin to begging behaviour in other systems [42]. Our findings indicate that clonal raider ant larvae produce a global cue affecting worker behaviour, and that the intensity (or nature) of this cue varies across clonal lineages, as has recently been theoretically predicted [21]. They also illustrate how the brood—often overlooked colony members in social insects—can play an active role in the regulation of colony behaviour, suggesting that larvae may be able to affect their own developmental trajectory by influencing worker foraging activity.

Genotypic interactions between brood ‘begging’ intensity and worker foraging activity would in turn be expected to have far-reaching consequences on colony growth, composition and behaviour over time. We find substantial plasticity in the production of intercastes stemming from genotypic interactions between the brood and workers. Previous work with two commonly used lines [24] proposed that such plasticity reflects different reproductive strategies across lines. In particular, a line with high intercaste production was proposed to be favoured in environments where line-mixing occurs frequently. This is because by producing offspring that reproduce more and forage less, that line could outcompete the other in mixed colonies, akin to socially parasitic lineages of parthenogenetic ants [43] and bees [44]. Here, brood of line B developed into intercastes at a higher frequency than all other lines and would, therefore, be expected to outcompete these other lines in mixed colonies over time, supporting the view that it may act as a general facultative social parasite in the presence of other lines.

Our finding of extant direct genetic effects and bi-directional indirect genetic effects between brood and adults is reminiscent of classic findings in other systems [45–47], where genetically based variation in brood solicitation and adult provisioning are thought to drive complex coevolutionary dynamics [48,49]. While brood care is central to the biology and the evolution of social insects, these questions have received comparatively little attention in social insects, in part due to practical limitations linked to the complexity of colonies. The clonal raider ant, with its comparatively simple social organization (queenlessness, asexual reproduction), relatively short generation time and unique experimental amenability is a promising system to explore both the ultimate and proximate bases of brood–adult interactions in social insects.

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.n02v6ws0v [50].

The data are provided in electronic supplementary material [51].

Authors’ contributions. S.L.J.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; D.K.: data curation, methodology, writing—review and editing; Y.U.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing—original draft, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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