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Author for correspondence:
Clint A. Penick
e-mail: cpenick1@kennesaw.edu

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Reversible plasticity in brain size, behaviour and physiology characterizes caste transitions in a socially flexible ant (*Harpegnathos saltator*)

Clint A. Penick[1,2], Majid Ghaninia[2], Kevin L. Haight[2], Comzit Opachaloemphan[3], Hua Yan[3,4,5,6], Danny Reinberg[3,4] and Jürgen Liebig[2]

[1]Department of Ecology, Evolution, and Organismal Biology, Kennesaw State University, Kennesaw, GA 30144, USA
[2]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA
[3]Department of Biochemistry and Molecular Pharmacology and [4]Howard Hughes Medical Institute, New York University School of Medicine, New York, NY 10016, USA
[5]Department of Biology, and [6]Center for Smell and Taste, University of Florida, Gainesville, FL 32611, USA

CAP, 0000-0002-5368-507X

Phenotypic plasticity allows organisms to respond to changing environments throughout their lifetime, but these changes are rarely reversible. Exceptions occur in relatively long-lived vertebrate species that exhibit seasonal plasticity in brain size, although similar changes have not been identified in short-lived species, such as insects. Here, we investigate brain plasticity in reproductive workers of the ant *Harpegnathos saltator*. Unlike most ant species, workers of *H. saltator* are capable of sexual reproduction, and they compete in a dominance tournament to establish a group of reproductive workers, termed 'gamergates'. We demonstrated that, compared to foragers, gamergates exhibited a 19% reduction in brain volume in addition to significant differences in behaviour, ovarian status, venom production, cuticular hydrocarbon profile, and expression profiles of related genes. In experimentally manipulated gamergates, 6–8 weeks after being reverted back to non-reproductive status their phenotypes shifted to the forager phenotype across all traits we measured, including brain volume, a trait in which changes were previously shown to be irreversible in honeybees and *Drosophila*. Brain plasticity in *H. saltator* is therefore more similar to that found in some long-lived vertebrates that display reversible changes in brain volume throughout their lifetimes.

1. Introduction

Phenotypic plasticity provides a flexible response to changing environments within an organism's lifetime [1,2]. Despite the benefits plasticity provides, phenotypic changes are rarely reversible, which implies there are limitations and costs [3–6]. For this reason, reversible plasticity is thought to occur primarily in long-lived organisms for which the benefits of maintaining plasticity outweigh the costs [7]. Notable examples include seasonal changes in brain size that occur in songbirds and other vertebrate species in advance of the breeding season or during hibernation [8–10]. Interestingly, age- and caste-dependent changes in brain size have also been reported in social insects [11–13], but the degree to which these changes are reversible is unknown. Determining whether or not changes in brain plasticity are reversible in social insects could help identify drivers and constraints of phenotypic plasticity in both short- and long-lived species.

In social insects, phenotypic plasticity underlies the development of distinct queen and worker castes [14,15]. Caste specialization in social insects has been compared to the epigenetic changes of cell differentiation and their reversibility or respective lack thereof [16,17]. In highly eusocial species, reproductive castes emerge during the larval stage through the production of morphologically distinct queens and workers. Adult workers of some ant species, however, have re-evolved the ability to mate and reproduce [18]. These reproductively totipotent workers are called 'gamergates' [19], and they undergo an internal transformation that activates their ovaries and causes a cascade of associated physiological and anatomical changes [20–24]. While morphological specialization is irreversible in ant, bee, and wasp societies, the physiological and behavioural changes associated with the gamergate transition are not [25–28]. The gamergate transition therefore provides a useful opportunity to investigate the reversibility of phenotypically plastic traits in insects.

Previous investigations of caste transitions in social insects have demonstrated a wide range of phenotypically plastic traits and some evidence of reversibility, though not changes in brain size. In honeybees, foragers can revert back to nurse workers and show subsequent changes in hormones [26], behaviour [26,29], learning [30], and immunocompetence [31], but they notably do not exhibit reversible changes in brain size [32]. Brain size increases as honeybee workers transition from nurses to foragers, but brain size is not reduced after foragers are reverted back to nurses. Similarly, research on *Drosophila* has shown that brain regions that were experimentally stunted during early adult life were unable to recover in mature adults [33]. The majority of research on brain plasticity in insects has focused on unidirectional changes rather than reversibility [34,35].

The lack of reversibility in brain plasticity in insects contrasts with that found in vertebrates, though previous work has focused on the nurse–forager transition rather than the gamergate transition. In contrast with gamergates, foragers generally live for only a matter of weeks or months, and there may be little selective pressure for them to maintain brain plasticity. In the rare event that a forager does revert to nurse status, it seems unlikely that there would be a strong need to reduce brain size to that of a typical nurse worker, especially given that the same individual will likely return to forager status eventually. In contrast with the increase in brain size associated with the nurse–forager transition, gamergates exhibit a decrease in brain size compared to non-reproductive workers [12]. A decreased brain size is thought to allow reproductives to divert additional resources to egg production, as brain tissue is metabolically expensive to maintain [36]. In the event that a gamergate reverts to non-reproductive status, re-expansion of specific brain regions, such as the optic and antennal lobes, may be required to perform foraging tasks.

We used the socially flexible ant, *Harpegnathos saltator*, to test whether gamergates were capable of reversible changes in brain size and correlated behavioural and physiological traits. Like other gamergate species, workers of *H. saltator* retain the ability to mate and lay fertilized eggs [37,38]. Initially, colonies are founded by a morphologically distinct queen [39,40], but after queen senescence, workers compete to establish a reproductive hierarchy [41]. Once a colony is socially stable, three main behavioural roles can be distinguished: gamergates, inside workers, and foragers. Gamergates exhibit a range of physiological changes [20,21,42], including increased lifespan [43] and reduction in brain volume [12]. Workers that do not become gamergates progress through an age-based division of labour, whereby young workers perform in-nest tasks and shift to foraging after approximately 50 days of adult life [44], which is also associated with changes in behaviour and physiology [20,21]. We experimentally reverted established gamergates to non-reproductive status to determine the extent to which changes in brain size, as well as a suite of correlated behavioural and physiological traits, remained plastic. Based on our results, we discuss brain plasticity in *H. saltator* within the context of previous work on the reversibility or irreversibility of phenotypic change in insects and vertebrates.

2. Methods

(a) Study species and rearing conditions

Colonies of the Indian jumping ant, *Harpegnathos saltator*, were originally collected from Jog Falls in Karnataka State, India, and have been reared in the laboratory for more than 20 years. Colonies were maintained at 25°C and fed live crickets (*Acheta domesticus* and *Gryllus assimilis*) twice per week. Laboratory colonies were housed in plastic boxes (27 × 19 cm) with a plaster base that featured a preformed nest chamber (12 × 15 cm) covered by a clear glass plate. All areas of the nest were exposed to similar light levels over a 12 : 12 h light/dark cycle.

(b) Gamergate reversion

We experimentally induced gamergates to revert back to non-reproductive status to quantify changes in behaviour and physiology associated with this reversal. We identified two mature gamergates (estimated to be at least 1 year old) per colony based on well-established methods [20–22], and each gamergate was marked on their thorax using an identifying code with Testors Pactra™ enamel (Rockford, Illinois, USA). For each gamergate pair, one individual was chosen at random and removed from her colony and placed in isolation for 3–4 weeks in a 10 × 10 cm plastic box with a plaster base, while the second gamergate was left in place in her original colony. All isolated gamergates were observed laying eggs within 3 days of removal, which confirmed their previous status as gamergates. Isolated gamergates were provided pre-stung crickets (paralysed by workers from a separate nest) two times per week and were otherwise maintained under standard conditions.

We expected social isolation would reduce gamergate fertility status due to a lack of social interaction and care, and that they would revert to worker status when reintroduced to their former nests. To confirm this, we observed whether or not isolated gamergates were policed by their nest-mates within 24 h after they were returned to their former nests. Policing is a behaviour in which workers physically bite and hold individuals in a colony that display a fertility signal intermediate between a non-reproductive worker and an established gamergate [45,46]. Worker policing is thought to activate stress pathways that induce a physiological cascade to revert reproductives to worker status [21]. In total, we isolated gamergates from 30 colonies, but not all measurements were performed on every colony (sample sizes and colonies used for comparison are reported in electronic supplementary material, table S1).

(c) Behavioural trait measurements

We observed the behaviour of reverted and control gamergates during the 8 weeks after isolated gamergates were returned to their nests to determine the extent to which reverted gamergates displayed non-reproductive worker-like behaviour. First, we

noted the position of reverted and control gamergates as either inside the nest or outside in the foraging chamber using scan sampling conducted every 2 days on average until the individuals were removed for sampling (ranging from 42 to 55 days). Second, we tested whether or not reverted and control gamergates would engage in hunting behaviour when provided with a live cricket 2–6 weeks after reintroduction. We placed isolated reverted and control gamergates in 10 × 10 cm boxes with a single cricket (approx. 6 mm in length) and recorded whether or not the cricket had been stung and paralysed after 1 h. Finally, we tested whether reverted and control gamergates displayed defensive behaviour when provoked. Non-reproductive workers display defensive behaviour towards nest intruders, while gamergates generally display a 'flight response' when confronted by intruders [21]. We tested the defensive behaviour of reverted and control gamergates 2–6 weeks after their reintroduction by placing them in a 19 × 13 cm test arena and clicking forceps 2–5 mm in front of their heads. Ants were allowed to acclimate to the arena for 2 min before testing, and once a trial began, we recorded whether or not individuals displayed 'mandible gaping' and/or 'biting with an attempt to sting' over 2 min. All trials were performed blind so that experimenters did not know whether the test individual was a reverted or control gamergate.

(d) Physiological trait measurements

We sacrificed reverted and control gamergates 6–8 weeks after isolated gamergates were reintroduced to their colonies, and we analysed their cuticular hydrocarbon (CHC) profiles, ovarian development, venom content, brain size, and gene expression of two candidate genes in the fat body associated with reproduction in *H. saltator* (*vitellogenin* [*Vg*] and *elongase of very long fatty acid* [*ELOV*]). We sampled inside workers and foragers at the same time for comparison. Individuals were incapacitated on ice, and their head, thorax, and gaster were dissected for subsequent analyses. Each colony in our study contributed a single ant for each role sampled.

CHCs were extracted from the thorax in 200 µl hexane (Sigma-Aldrich, St. Louis, MO) for 10 min. Extracts were dried to 3–5 µl using N_2 gas, and 1 µl of this solution was injected by autosampler into an Agilent 6890 N gas-chromatograph (GC) (Agilent, Santa Clara, CA, USA) coupled with an Agilent 5975 mass selective detector operated in the electron impact ionization mode (detailed methods in electronic supplementary material). For each individual, the relative peak area of 13,23-dimethylheptatriacontane, a fertility-related hydrocarbon, was calculated as the quotient of its peak area over the average area of the five largest other CHC peaks in the chromatogram.

Individual gasters were dissected in 95% ethanol under a Leica MZ125 microscope to determine ovarian activity and venom sac volume. The number of yolky oocytes in the ovaries was counted as a measure of ovarian activity. The venom sac was removed and digitally photographed against a 1 mm grid for later volume measurements using ImageJ software (v. 1.44, 2010). The long and short axes of venom sacs were measured and their volumes calculated as per Haight [44].

(e) Brain imaging and volume calculations

Brains were imaged using confocal microscopy, and volume measurements were based on digital reconstructions. Heads were imaged under a microscope in dorsal view as per Haight [44] using ImageJ software. For visualization using confocal microscopy, we followed established protocol for staining insect brains [47]. Briefly, freshly decapitated heads were soaked in a fixative at 4°C overnight. The brains were then dissected and cleared by washing three times in phosphate-buffered saline solution with Tween™ (PBS-T). Brains were stained by incubating overnight at 4°C in a diluted (1 : 10 from an original concentration of 5 µg ml^{-1}) monoclonal antibody nc82 (DSHB, Iowa City, IA). Brain dissections and preparation were randomized so that workers of different roles were processed in a random order to remove any potential bias (detailed methods in electronic supplementary material).

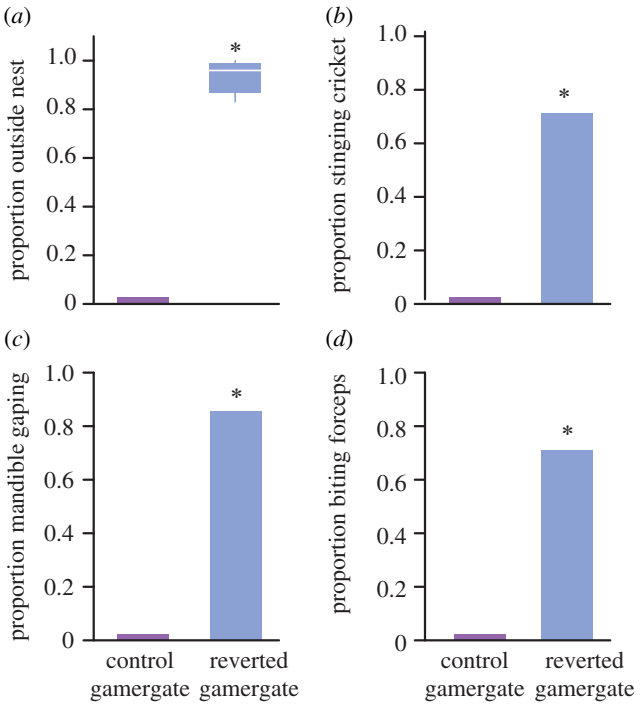

Figure 1. Changes in behaviour. (*a*) Proportion of observations (median, 25–75%, and non-outlier range) when control and reverted gamergates were in the foraging arena outside the nest. (*b*) Proportion of control and reverted gamergates that stung and subdued a cricket, (*c*) opened their mandibles when provoked with forceps, and (*d*) bit and stung forceps when provoked. Asterisks indicate significant differences ($p < 0.001$). (Online version in colour.)

Confocal images were obtained using a Leica TCS SP5 confocal laser scanning microscope (Leica, Bensheim, Germany) with a Leica HC PLAPO 10×/0.40 CS objective. Confocal image stacks of 14 ants from each role (control gamergate, reverted gamergate, inside worker, and forager worker) were analysed and compared. To overcome the limited field of view and to reduce inaccuracy due to dissection damage, we scanned only one hemisphere of each specimen, choosing the one with minimal or no damage. For volumetry, we measured one optic lobe, one antennal lobe, and the remaining portion of the brain hemisphere (henceforth 'central brain' [48]), which included the mushroom bodies, lateral horns, and half of the central complex. We used AMIRA (v. 4.1.1, TGS, San Diego, CA, USA) software for three-dimensional reconstruction. The image files for each ant were given a file code so the measurer would be blind to the role of the individual brains during reconstruction and measurement. We doubled all brain volume measurements in the analyses and figures to represent the total brain volume rather than the volume of a single-brain hemisphere.

(f) RNA extraction and quantitative reverse transcription PCR

Fat body tissue was dissected from the gasters of 10 individuals from each role under ice-cold 1 × PBS. Total RNA was purified, reverse transcribed to complementary DNA (cDNA), and mRNA expression levels were quantified using the Startagene M x3000P qPCR system. Specific quantitative primer pairs for vitellogenin

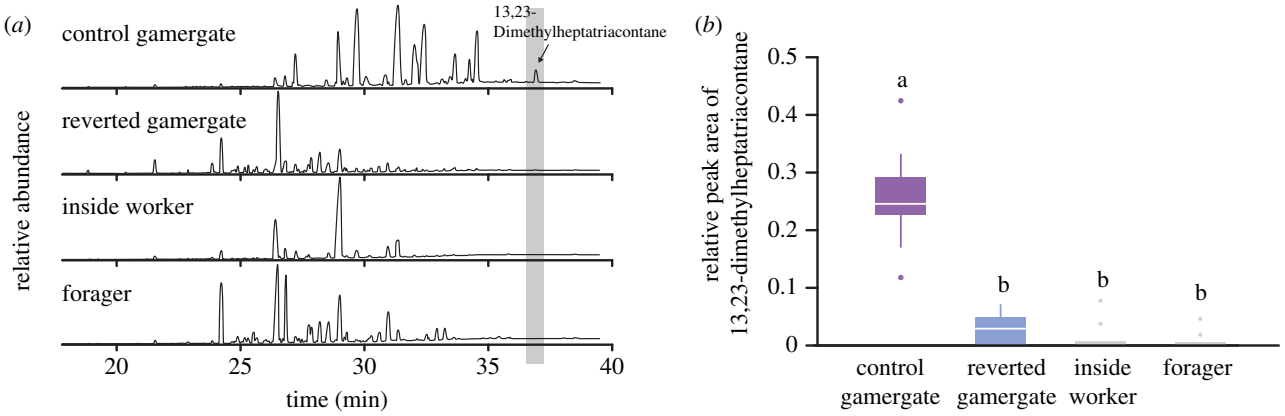

Figure 2. Cuticular hydrocarbon profiles. (*a*) Example cuticular hydrocarbon profiles of a control gamergate, reverted gamergate, inside worker, and forager (shaded area indicates the reproductive-associated compound 13,23-dimethylheptatriacontane). Non-reproductive workers and reverted gamergates exhibit intracolonial profile variation typical in this species [22]. (*b*) Relative proportion of 13,23-dimethylheptatriacontane present in the cuticular profiles of all roles (median, 25–75%, and non-outlier range). Letters indicate significant differences between roles ($p < 0.0001$; electronic supplementary material, table S3). (Online version in colour.)

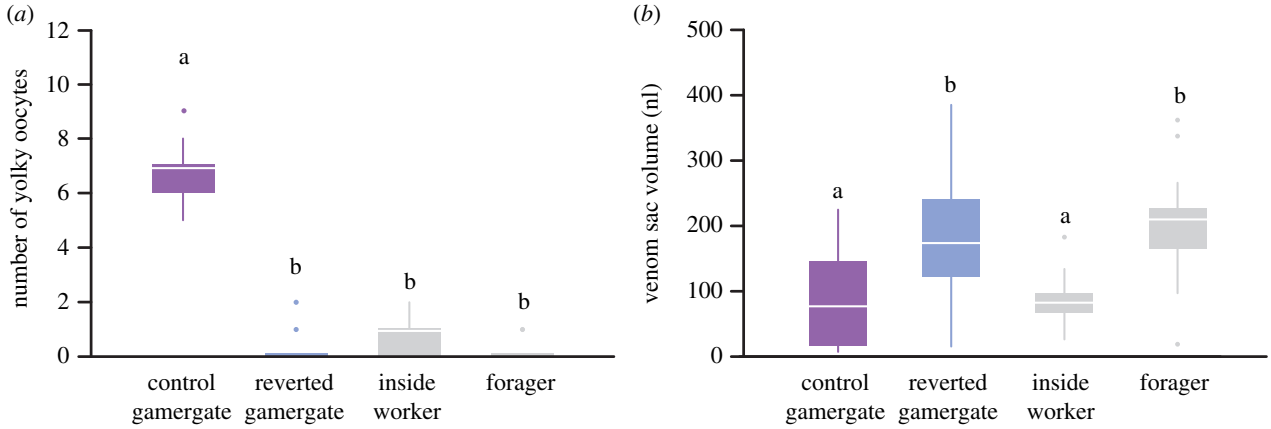

Figure 3. Changes in internal anatomy. (*a*) Number of yolky oocytes present in the ovaries and (*b*) venom sac volume of control gamergates, reverted gamergates, inside workers, and foragers (median, 25–75%, and non-outlier range). Letters indicate significant differences between roles ($p < 0.05$; electronic supplementary material, table S3). (Online version in colour.)

(*Vg*), elongation of very long-chain fatty acids protein AAEL008004 isoform X2 (*ELOV*), and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) genes were used (electronic supplementary material, table S2). RNA levels of *GAPDH*, a housekeeping gene that is often stably and constitutively expressed at high levels in most tissues and cells, were used as a normalization control (detailed methods in electronic supplementary material).

(g) Statistical analyses

All analyses were performed using R v. 3.6.0 [49]. We used an exact binomial test to test whether reverted gamergates were observed outside the nest more frequently than control gamergates, and we used Fisher's exact tests to test whether reverted and control gamergates differed with respect to hunting and defence behaviour. To test for differences among roles with respect to physiological traits and gene expression, we used generalized linear mixed models (GLMMs) using the packages 'lme4' [50], 'lmerTest' [51], and 'car' [52]. We specified worker role as the fixed effect and source colony as a random effect in all models (the full GLMM for yolky oocyte comparisons did not converge, so we reverted to a simpler generalized linear model without colony included as a random effect). We used a Gaussian distribution with an identity link function to compare the relative peak area of 13,23-dimethylheptatriacontane among roles, a Poisson distribution with a log link function to compare the number of yolk oocytes among roles and a Gamma distribution with a log link function for comparing head width cubed, venom sac volume, brain volumes, and *Vg* and *ELOV* expression among roles. To check whether our GLMMs met model assumptions, we created Q-Q plots of residuals to check for linearity. Significant tests were followed with Tukey tests for multiple comparisons using the package 'multcomp' [53].

Head width cubed, a measure that scales directly with body size as measured by mg dry weight in *H. saltator* [44], significantly differed among roles (electronic supplementary material, figure S1). As a result, we size standardized volumetric data by multiplication with a standardization factor calculated as the average ant size (mg dry weight approximated by head width cubed) divided by the size of each ant in question. Results did not qualitatively differ when using size standardized or non-standardized values.

3. Results

(a) Behavioural changes

All gamergates that were returned to their colonies after 3–4 weeks of isolation were observed being policed by their nest-mates within 24 h, an indication they had lost their gamergate status. 1–8 weeks after reintroduction, reverted

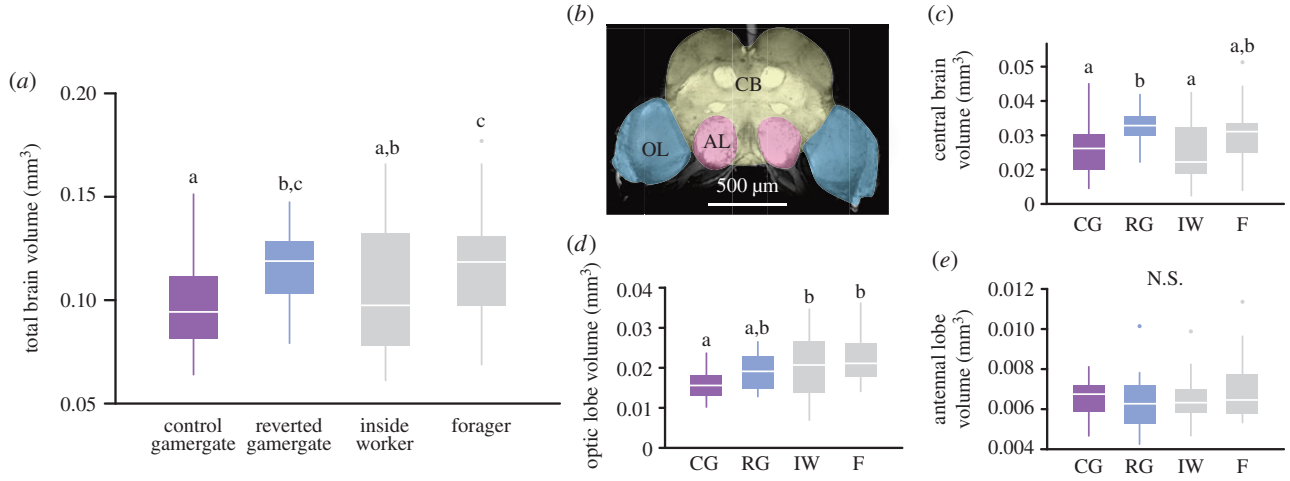

Figure 4. Changes in brain volume. (*a*) Total brain volume of control gamergates, reverted gamergates, inside workers, and foragers 6–8 weeks after reintroduction. (*b*) Brain regions (OL, optic lobes; AL, antennal lobes; and CB, central brain) shown on two-dimensional section of scanned *H. saltator* brain; (*c*) central brain, (*d*) optic lobe, and (*e*) antennal lobe volume of control gamergates, reverted gamergates, inside workers, and foragers (median, 25–75%, and non-outlier range). Letters indicate significant differences between roles ($p < 0.05$; electronic supplementary material, table S3). (Online version in colour.)

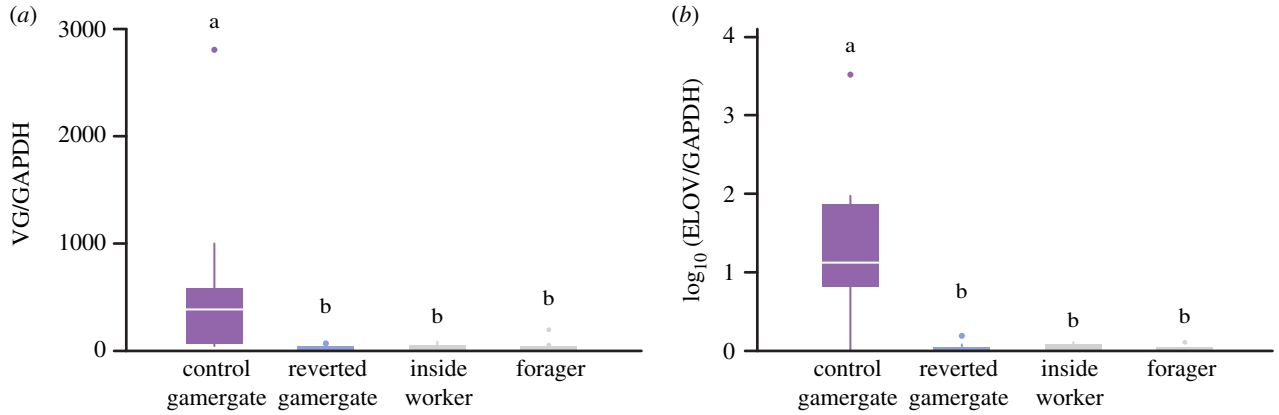

Figure 5. Gene expression changes. (*a*) Vitellogenin (*Vg*) and (*b*) Elongase (*ELOV*) expression relative to GAPDH of control gamergates, reverted gamergates, inside workers, and foragers (median, 25–75%, and non-outlier range). Letters indicate significant differences between roles ($p < 0.0001$; electronic supplementary material, table S3). (Online version in colour.)

gamergates were observed in the foraging arena during 93% of observations, while control gamergates were always observed inside the nest chamber (Exact binomial test, $N = 14$, $p = 0.0001$; figure 1*a*). With respect to hunting behaviour, reverted gamergates subdued crickets in 71% of trials, while control gamergates subdued no crickets (Fisher's exact test, $N = 14$, $p = 0.0002$; figure 1*b*). Likewise, 86% of reverted gamergates displayed mandible gaping, and 71% bit and stung forceps when provoked, while control gamergates displayed no defensive behaviours (Fisher's exact test; mandible gaping, $N = 14$, $p < 0.0001$; biting, $N = 14$, $p = 0.0002$; figure 1*c*,*d*).

(b) Cuticular hydrocarbon profiles

Gamergates display a unique CHC signature in *H. saltator* [22], but this signature changed in reverted gamergates to a more non-reproductive worker-like profile (figure 2*a*). The relative peak area of 13,23-dimethylheptatriacontane, a compound associated with reproductive status in *H. saltator* [22], was more than eightfold higher in control gamergates than in reverted gamergates, inside workers, and foragers, which did not differ from one another (GLMM, $N = 52$, d.f. = 3, $\chi^2 = 320.36$, $p < 0.0001$; electronic supplementary material, table S3 and figure 2*b*).

(c) Ovarian development and venom production

In line with predictions, control gamergates had the highest number of yolky oocytes per individual (median: 6.8 oocytes), while the majority of individuals from all other roles had fewer than one oocyte per individual (GLMM, $N = 74$, d.f. = 3, $\chi^2 = 262$, $p < 0.0001$; figure 3*a*, electronic supplementary material, table S3 and figure S2). Venom sac volume was 2–3 times higher in foragers compared with control gamergates and inside workers, and venom sac volume did not significantly differ between foragers and reverted gamergates (GLMM, $N = 74$, d.f. = 3, $\chi^2 = 31.09$, $p < 0.0001$; electronic supplementary material, table S3 and figure 3*b*).

(d) Changes in brain size

Previous work in *H. saltator* found that gamergate brains were 26% smaller than the brains of non-reproductive workers [12]. Likewise, we found that total brain volume of control gamergates was 19% smaller than forager brains, while reverted gamergate brains were larger and roughly equal in size to forager brains (GLMM, $N = 56$, d.f. = 3, $\chi^2 = 18.29$, $p = 0.0004$; electronic supplementary material, table S3 and

figure 4*a*). Central brain volume (including mushroom bodies) of control gamergates was also 15% smaller than forager brains and 20% smaller than the central brain volume of reverted gamergates (GLMM, $N = 56$, d.f. = 3, $\chi^2 = 18.28$, $p = 0.0002$; electronic supplementary material, table S3 and figure 4*c*). Optic lobe volume was largest in foragers and inside workers, lowest in control gamergates, and intermediate in reverted gamergates (GLMM, $N = 56$, d.f. = 3, $\chi^2 = 18.73$, $p = 0.0003$; electronic supplementary material, table S3 and figure 4*d*). Antennal lobe volume did not differ among roles (GLMM, $N = 56$, d.f. = 3, $\chi^2 = 3.87$, $p = 0.28$; figure 4*e*).

(e) Gene expression changes

Expression levels of two candidate genes in the abdominal fat body varied among roles and matched related physiological changes (figure 5). Expression levels of *vitellogenin* (*Vg*) were significantly elevated in control gamergates compared with all non-reproductive roles (GLMM, $N = 40$, d.f. = 3, $\chi^2 = 87.22$, $p < 0.0001$; electronic supplementary material, table S3 and figure 5*a*). Expression of *ELOV* was also significantly higher in control gamergates compared to all non-reproductive workers (GLMM, $N = 40$, d.f. = 3, $\chi^2 = 131.15$, $p < 0.0001$; electronic supplementary material, table S3 and figure 5*b*).

4. Discussion

Workers of *Harpegnathos saltator* exhibited reversible changes in brain size similar to that found in relatively long-lived vertebrate species. Changes in brain volume observed in vertebrates generally track seasonal reproductive cycles and are triggered by reproductive hormone cascades [9]. Likewise, brain changes in *H. saltator* also track the reproductive status and are associated with changes in reproductive hormone levels [20,21] and the expression of key regulatory genes [42]. Changes in the vertebrate brain include the seasonal addition of new neurons [54], which we did not specifically measure here, but changes in total and region-specific brain volumes are comparable.

Task or experience-dependent plasticity of brain compartments has been demonstrated in various insects, including honeybees, ants, paper wasps, and moths (e.g. [11,34,55–57]). In *H. saltator*, gamergate brains were 19% smaller than the brains of foragers on average, which is in line with predictions that brain size should be reduced to divert metabolic resources to reproduction [12,13]. Even compared to comparatively younger inside workers, gamergate optic lobes were 24% smaller, suggesting they may not simply retain the brain size of young nurse workers, but most likely experience region-specific brain volume reduction. When gamergates of *H. saltator* were reverted back to non-reproductive status, their brains re-expanded and matched that of forager brains. Foraging requires the ability to orient towards the nest and to attack and retrieve live prey items, all of which requires higher cognitive processing. The observed reduction in central brain volume of gamergates and the subsequent expansion in reverted gamergates suggest it is used for the more demanding cognitive abilities of foraging [58]. Changes in the central brain of *H. saltator*, which includes the mushroom body, are consistent with results from other social insects [59]. In carpenter ants, foragers that perform cognitively demanding tasks exhibit an increase of more than 50% of mushroom body neuropile volume [11] and a similar pattern is found in the mushroom body of honeybees [60].

The pattern of size differences in the optic lobe of *H. saltator* suggests a programmed rather than experience-dependent change in brain volume. Gamergates displayed significantly smaller optic lobes than inside workers and foragers, both of which had equally large optic lobes. Gamergates were still exposed to light and thereby received visual stimulation from their nest-mates in our laboratory settings, so sensory deprivation is an unlikely cause for the size differences we observed. Given that gamergates do not rely on optic information under natural conditions, a reproduction-dependent size reduction seems most likely. The intermediate optic lobe size of reverted gamergates relative to gamergates and non-reproductive workers suggests a presumably slower reversion speed of the optic lobe compared to the central brain. However, the size reductions of the optic lobes and of the central brain compared to reverted gamergates both suggest this brain size reduction is an energy-saving mechanism as proposed previously [12,13].

The reversibility of changes in brain size in *H. saltator* contrasts with results in the honeybee and in *Drosophila*. Brain size in honeybees increases as nurse workers transition to foragers, but when foragers are reverted to nurse status, they do not show a decrease in brain volume [32]. Honeybee foragers in the study by Fahrbach *et al*. [32] were only reverted back to nurses for 5 days, while gamergates in the present study were reverted for 6–8 weeks, which may explain why brain changes were observed in our study but not in previous studies on honeybees. In addition, honeybee foragers typically only live for a matter of weeks, and there is no biological 'reason' for why they should fully revert to nurse status—in a colony of 50 000 bees, foragers can easily be replaced by new workers. By contrast, *H. saltator* colonies are small (usually less than 100 individuals), and each worker represents a more valuable resource in terms of their relative contribution to colony productivity. Studies in *Drosophila* have looked at region-specific changes in brain size associated with adult age, and while the medulla of the optic lobe in *D. melanogaster* increases in size with age, sensory deprived medullae do not increase in size and this lack of growth seems to be irreversible later in life [33]. This difference in brain plasticity corroborates differences in the plasticity of the antennal lobe between ants and *Drosophila*. When the *odorant receptor co-receptor* (*orco*) was knocked out in ants, the antennal lobes showed a significant reduction in two ant species [61,62]. A similar morphological change was not present in *Drosophila* when *orco* was knocked out, which suggests a hardwired mode of olfactory glomeruli formation in the *Drosophila* antennal lobe [63] and potentially major differences in brain development between *Drosophila* and ants.

Along with reversion in brain size, we found behavioural and physiological reversions that include the ovarian activity, venom production, CHC profile, and expression of associated genes (figure 6). Combined changes in physiological traits and underlying gene expression levels demonstrate that the changes we observed in reverted gamergates were not random, but instead matched a clearly defined worker phenotype. If we had observed a mix of different physiological changes that were inconsistent with the worker phenotype, then we might have expected these changes to be driven by isolation stress alone. The effects of chronic stress

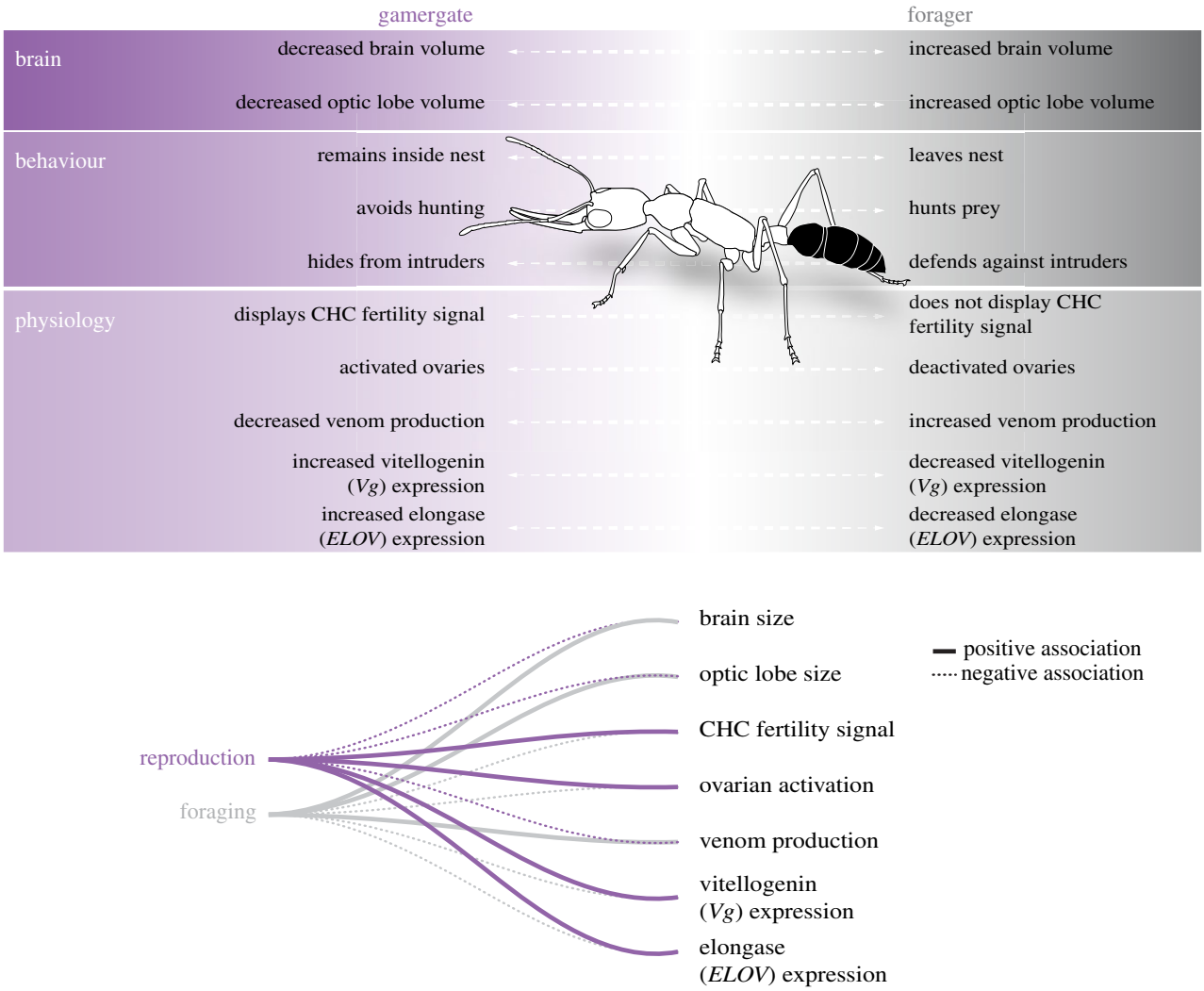

Figure 6. Correlated plasticity in brain, behaviour, and physiology between reproductive and non-reproductive workers. (Online version in colour.)

are generally expected to increase the allostatic load and result in decreases in body mass and brain function [64], yet contrary to this prediction, we observed increases in brain size and venom production in reverted gamergates. Likewise, changes in gene expression of *ELOV*, which is involved in fatty acid elongation, and *Vg*, the vitellogenin egg yolk precursor, were consistent with downstream physiological responses of CHC profiles and ovarian activity.

The observed reversibility in phenotypic plasticity in *H. saltator* gamergates that transition back to non-reproductive workers is present despite the rarity of such events. Naturally, queens and gamergates reproduce until senescence and do not substantially contribute to foraging after the loss of status. Thus, there would appear to be little selective pressure to keep reproductive specialization reversible. However, reversibility of phenotypic plasticity could be maintained to allow workers the return to forager status after they have lost a reproductive tournament. Dominance tournaments last up to 40 days in *H. saltator*, and initially up to half the workforce of a colony may compete [41]. Physiological changes begin shortly after tournaments are initiated [21,42], and reversibility may allow workers to return to forager status without suffering long-term effects associated with the early transition period to reproductive status. Among social insects, lower termites offer another rare example of reversible plasticity, in which individuals develop regressively from nymphal instars to 'worker' instars that lack wing buds [65]. The precise reason why regressive moults occur in lower termites is not understood, but it does have parallels to reversible plasticity in *H. saltator*. In both *H. saltator* and lower termites, reversible plasticity allows individuals to retain flexibility in shifting between non-reproductive and reproductive pathways.

Data accessibility. Data and R analysis are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tx95x69x0 [66].

Authors' contributions. C.A.P., M.G., K.L.H., H.R., D.R., and J.L. conceived the project. C.A.P., M.G., K.L.H., C.O., and H.Y. carried out behavioural experiments and physiological measurements. C.A.P., K.L.H., and J.L. analysed the data. All authors contributed substantially to manuscript drafts.

Competing interests. We declare we have no competing interests.

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