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METHODS & TECHNIQUES

A new, practicable and economical cage design for experimental studies on small honey bee colonies

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ABSTRACT

Bees are in decline globally as a result of multiple stressors including pests, pathogens and contaminants. The management of bees in enclosures can identify causes of decline under standardized conditions but the logistics of conducting effect studies in typical systems used across several colonies is complex and costly. This study details a practicable, new and economical cage system that effectively houses live honey bee colonies to investigate the impact of physical conditions, biological factors and environmental contaminants on honey bee health. The method has broad application for a range of effect studies concerning honey bee development, physiology, survival and population dynamics because it enables entire colonies, as opposed to individual workers, to be managed well in captivity.

KEY WORDS: *Apis mellifera*, Pollinators, Contaminants, Pesticides, Effect studies, Cage system

INTRODUCTION

Insect pollinators are ecologically and economically important for both wild and cultivated plants (Klein et al., 2007; Ollerton et al., 2011). Both managed and unmanaged pollinator taxa are currently in decline as a result of pests, pathogens, contaminants and change in land use (Ollerton et al., 2014; Potts et al., 2010; Rundlöf et al., 2015; Woodcock et al., 2016). Being central-place foragers, bees in particular are vulnerable to these environmental stressors. Central-place foraging demands advanced capacities of learning, memory and navigation on highly variable floral resources. Hence, even at low intensity levels, many stressors can disrupt key cognitive functions needed for effective foraging, with dramatic consequences for brood development and colony survival (Klein et al., 2017).

Honey bees are the most ubiquitous and widely studied central-place foragers. Given their ease of management and significance as pollinators, many studies have assessed honey bee responses to environmental stressors in field- and laboratory-based studies (Table 1). While individual worker responses to pests, diseases and contaminants are well investigated, colony-level responses to environmental stressors are less well understood (Table 1) (Bakker and Calis, 2003; Schur et al., 2003). This is because of the difficulties in managing live honey bee colonies in cages effectively for an extended duration. Studies that remove worker bees from their colonies can be influenced by stress factors which confound the response observed (Winston, 1987).

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Studies focusing on colonies provide critical predictive and multi-generational information about long-term changes to the population that cannot be gained from experiments on individuals (Khoury et al., 2011; Torres et al., 2015; Uzunov et al., 2015). The effect of stressors on honey bees is intrinsically linked to the whole-colony response (Klein et al., 2017). Colony dynamics are influenced by environmental cues, the fecundity of the queen and the introduction of contaminants, pests and disease (Khoury et al., 2011; Seeley, 1982; Torres et al., 2015; Uzunov et al., 2015). Environmental factors such as temperature and resource availability strongly influence colony behaviour and physiology, and may reduce a colony's capacity to survive exposure to contaminants, disease, altered habitat and poor nutrition (Hedtke et al., 2011; Morimoto et al., 2011; Simon-Delso et al., 2014).

Field-based testing of exposure to contaminants on bee performance, longevity and colony dynamics has provided valuable insights into the impact of neonicotinoids on honey and bumble bees (Kessler et al., 2015; Rundlöf et al., 2015; Simon-Delso et al., 2014; Tsvetkov et al., 2017; Wu et al., 2011). Chronic effects of exposure to sub-lethal contaminant concentrations that may accumulate in pollen, in nectar, on leaf surfaces and in hive components can only realistically be fully evaluated through field assessment (Ravoet et al., 2015; Sanchez-Bayo and Goka, 2014; Wu et al., 2011). Identifying all the factors that influence the observed result, however, is challenging in the highly dynamic field environment because of the range of variables that exist (Table 1) (Kessler et al., 2015; Sponsler and Johnson, 2017; Tsvetkov et al., 2017).

Different enclosure systems including outdoor tents, flight cages and indoor flight rooms have been used for colony-level assessment attempting to control field variability (Bakker and Calis, 2003; Schur et al., 2003). These can provide an environment in which all known variables are introduced, eliminated or monitored, allowing for a specific set of variables against which the result can be measured (Decourtye et al., 2005; Köhler et al., 2015; Medrzycki et al., 2015; van den Heever et al., 2015; Williams et al., 2013). While some of these enclosed systems have enabled the investigation of colony-level impacts such as the effect of contaminants upon the queen's reproductive capacity and brood development (Bakker and Calis, 2003; Schur et al., 2003), significant limitations exist. For example, the number of treatment replicates is constrained, as well as the capacity to control some environmental conditions and exposure chemicals. These systems also require complex and costly infrastructure and climate control (Table 1).

The construction, operation and maintenance of an enclosure to manipulate and maintain colony dynamics must be practicable and economical, and enable sufficient replication. Here, we describe a new, simple, economical cage system which can be used to conduct standardized trials of honey bee colonies for short periods and for extended durations. This method enables assessment of colony responses to one or more variables of interest, resulting in a better representation of bee responses in the external environment. The

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Table 1. A comparison of the strengths and weaknesses of honey bee colony study systems used for effect studies

Study type	Description	Strengths	Weaknesses	References
Field study	Research conducted in the natural context of the subject of study	• Bees remain in the colony • Bees are studied in their natural environment • Chronic exposure can be assessed • Enables assessment of interaction between variables • Enables long-term studies • Cost effective	• Unable to control environmental conditions • Potential interactions between variables • Difficulty identifying all contributing factors to result obtained • Difficulty monitoring response to all variables • Control sites may be contaminated • Limited replication	Kessler et al., 2015; Ravoet et al., 2015; Rundlof et al., 2015; Sponsler and Johnson, 2017; Tsvetkov et al., 2017; Wu et al., 2011
Semi-field study	Research conducted in the field with the bees confined to a specific area in a tent or cage	• Bees remain in the colony • Enables control of bee foraging area • Enables assessment of a combination of realistic field conditions within a controlled area and exposure period • Medium cost	• Unable to control some aspects of environment conditions • Potential interactions between variables • Difficulty monitoring response to all variables • Control sites may be contaminated • Increased cost due to enclosures • Limited replication	Bakker and Calis, 2003; Schur et al., 2003
Flight room/ enclosure study	Research conducted in a room with artificial lighting and climate control	• Bees remain in the colony • Enables study of colony dynamics • Enables close manipulation of variables • Enables assessment of interaction between variables	• Costly • Specialised infrastructure required • Limited replication	Elzen et al., 2015; Goblirsch et al., 2013; Kefuss, 1978; Nye, 1962; Pernal and Currie, 2001; van Praagh, 1972

system permits testing of the effects of contaminants, pests, pathogens, diseases, nutritional deficiencies and supplements, as well as climate change impacts on honey bee colonies of varying size, age and composition.

MATERIALS AND METHODS

Cage design and cost

We housed entire honey bee colonies in purpose-built cage systems designed with knowledge of successful husbandry and existing flight rooms (expert advice from Doug Somerville, NSW Department of Primary Industries, personal communication; Table 1). Cages were constructed from untreated plywood, timber beams, aluminium insect screen, wood glue and zinc-plated steel screws (cage dimensions 1.2×1.2×1.7 m; Fig. 1A). Cages were installed in a fully enclosed glasshouse bay (dimensions 6×3×6 m). Insect mesh screen was used to cover all side and roof frames to allow light to enter the cages, but the floor and door were constructed entirely from plywood. The door (Fig. 1B; dimensions 1.5×8 m) allowed safe and easy access, and an insect mesh insert (dimensions 80×37 cm) allowed smoke to be directed into the cage to disperse bees from the door area when required. The door was secured externally with five timber toggles, with a timber toggle also located inside the cage to allow closure from the inside (Fig. 1B). The door was fitted into the front wall of the cage with three galvanised steel butt hinges, ensuring it was flush with the cage front frame and tightly sealed when closed (Fig. 1C). Replaceable clear plastic contact lined the floor of the cages to enable easy cleaning. The cost of materials for each cage was approximately 400 AUD.

Colony housing and maintenance

A ventilated timber hive box with dimensions 58×29×13 cm was placed on the floor of each cage to house each honey bee colony (Fig. 1A). Each hive box had the capacity to hold three wooden full-depth beehive frames (frame area 46×24 cm) (Fig. S1). Hive boxes and frames used were as recommended in NSW state guidelines (NSW DPI, 2016). Water was provided in a chicken feeder with the water trough filled with pebbles to provide easy access and prevent bees from drowning (Fig. 1A) (Root, 1978). Sugar syrup was prepared using white sugar and boiled water and replaced weekly. We used a concentration of 55% sugar syrup because a concentration lower than 50% will ferment within a few days, causing gastrointestinal problems in the bees (Sammataro and Weiss, 2012; Somerville, 2005). An upturned 700 ml plastic bucket with the lid secured was used to supply the syrup. Syrup was allowed to seep into the lid rim through 0.8 mm holes drilled around the base (Fig. 1A). The water and syrup feeders were placed at the back of the hive boxes in the cage to prevent faecal contamination of the water and syrup during flight, with water and sugar syrup levels were monitored three times per week and replenished if required. Jarrah pollen (5 g) and Bee Build Protein Sausage™ (10 g) (Dewar Corporation Pty Ltd) were provided weekly and placed on top of the frames in the hive boxes to allow nurse bees easy access (Root, 1978; Somerville, 2005).

This experiment took place in the Australian summer months with natural light entering the cages for approximately 8 h per day, hence artificial lighting was not used for the experiment (Fluri and Bogdanov, 1987; Kefuss, 1978). The temperature in the glasshouse

Journal of Experimental Biology (2019) 222, jeb200998. doi:10.1242/jeb.200998

Fig. 1. The cage system. (A) The system includes a hive box and feeders (door not visible but located on the left side of the cage). (B) External view of the secure timber door with insect screen panel and closure toggles. (C) View of the overlapping cage door panels for secure fit with the cage frame. The cage system was developed and used to successfully house honey bee colonies for effect studies. See Materials and Methods, 'Cage design and cost' for specifications of system components.

bay was maintained between 26 and 28°C with 40–60% humidity. Hive box and ambient temperature was monitored and recorded three times per week (Jaycar Electronics Pty Ltd, Jumbo Display In/Out Thermometer).

Colony preparation, instalment and monitoring

The cage system was tested in three replicates. Three bee colonies of similar size, age structure and climatic origin including young mated queens approximately 3 months old were sourced from large healthy colonies in Newcastle, NSW, Australia. With no previous published reports available on small-scale enclosures suitable for rapid assessment and multiple-replication colony-effect studies, initial colony size for the cage design was based on expert advice from NSW Department of Primary Industries (Doug Somerville, personal communication). The colony comprised a queen bee, approximately 500 young worker bees and late-stage capped brood occupying 50% of the frame area. For each replicate cage, one colony on its brood frame was placed within the hive box, the entrance opened and positioned facing north to ensure maximum sunlight exposure (Root, 1978; Winston, 1987). Each hive box also housed two additional empty frames to allow for colony growth as required (Fig. S1) (Eckert et al., 1994; NSW DPI, 2016). Health and development were assessed as indicators of adaptation and cage efficacy to house bee colonies successfully. After 1 week, the brood frames were removed and replaced with wax comb frames so new eggs could be identified in each colony (Fig. S1). Monitoring continued over a further 4 weeks also including growth and development from week 2.

Observation of faecal deposits during week 1 showed that colony 2 displayed evidence of diarrhoea (black to dark brown compared with round, yellow and orange in the other colonies). Subsequently, all the colonies were treated with the digestive supplement HiveAlive™ (2.5 ml supplied in 1 litre of 55% sugar syrup) for 1 day (Charistos et al., 2016) and for the remainder of the trial there was no evidence of diarrhoea in colony 2 or in the other colonies.

Metrics assessed

Each of the three colonies was treated as a separate unit for data assessment. Bee adaption was assessed three times over a period of 1 h each week and included activity outside the hive box, collecting syrup and water and flying in the cage. Colony health metrics were also determined each week and included hive box temperature, evidence of disease, dead bees, pests inside the hive box and on the frames, and also characteristics of faecal deposits. From week 2, after introduction of the wax comb frame, colony development, percentage growth and percentage mortality were assessed using the number of eggs, larvae, pupae and juvenile adults (including empty cells previously containing pupae) counted each week in photographed frames, the counts of dead bees on the cage floor each week, and the initial number of bees at week 2 as:

$$g = \frac{j - d}{i} \times 100, \quad (1)$$

where g is colony growth (%), j is the number of emerged juvenile adults, d is the number of dead bees and i is the initial number of bees; and:

$$r = \frac{d}{i} \times 100, \quad (2)$$

where r is the weekly mortality (%), d is the number of dead bees and i is the initial number of bees. Colony weekly percentage mortality was used to determine the cumulative mean percentage mortality.

RESULTS AND DISCUSSION

Keeping honey bees in cages for an extended period presents multiple challenges. The physical environment must provide suitable temperature and humidity (Southwick and Heldmaier, 1987; Wang et al., 2016), adequate white and ultraviolet (UV) lighting (Nye, 1962; van Praagh, 1972), and adequate space for colony exercise and cleansing flights away from feeding stations (Winston, 1987). Previous studies indicate that the size of the cage and hive boxes will influence colony growth and bee activity (Moore et al., 2015). In this study, the small colonies responded well to the cage environment for most metrics measured. The bee colonies in each cage accepted the hive box as the new colony location, demonstrated in particular by observed egg laying. Nevertheless, colony size should be adjusted to suit the scale of the study. Adaptation of this system is possible where accommodation of larger colonies is required, which is advantageous in that larger colonies typically show greater viability as a result of the increased ability to maintain colony temperature, hygiene and longevity (Eckert et al., 1994).

The general pattern of bee behaviour was described as inactive until approximately 10:00 h, when sunlight began to fully enter the cage. From 10:00 h to 12:00 h, activity was dominated by feeding, removal of dead bodies and defecation, and from 13:00 h, with full

Journal of Experimental Biology (2019) 222, jeb200998. doi:10.1242/jeb.200998

sunlight, a greater number of bees left the hive box, with flight activity at a maximum at approximately 15:00 h, when an estimated 400 bees on average in each colony were observed in the cage, flying, walking on walls and gathering in groups of up to 25 on the wall of the cage with the most direct sunlight. This behaviour was suggestive of good adaptation being of wider scope (e.g. for exercise, social) than waste removal, collection of sugar syrup or water, or hovering above the hive entrance for orientation (Capaldi and Dyer, 1999). Colony hygiene was similar to the hygiene of a healthy colony in a non-cage environment with the regular removal of dead bees and waste material from the hive box and defecation outside the hive box (Bigio et al., 2014; Uzunov et al., 2015). There was no evidence of pests on brood frames through the trial, nor disease after the first week.

Any successful cage system must enable adequate nutrition to sustain colony health and development (van Praagh, 1972; Williams et al., 2013). Honey bee nutritional requirements include carbohydrates and a wide range of vitamins, minerals, fatty acids and amino acids (Di Pasquale et al., 2013; Somerville, 2005). The cage system allowed the provision and replacement of sugar syrup and a commercial complete bee food (Somerville, 2005) and could thus be used to study bee responses to a specific type of diet. In this study, bees located the sugar syrup within 2 h of being placed in the cage environment, with pollen and protein supplement utilized almost immediately. Sugar syrup and pollen storage were observed in all of the colonies during week 1 and this continued to the end of monitoring. Sugar syrup was not processed to capped honey, which is not unexpected within the period of the trial. Bees were observed using the water feeders in each cage on approximately two of the three observational occasions each week, except during the first week. Reduced water use in this week may have been due to favourable conditions in the glasshouse reducing the need for water, rather than an inability to source the feeders (Ohguchi and Aoki, 1983).

Honey bee colonies must be able to maintain a cluster temperature between 32 and 36°C for successful brood development (Root, 1978; Winston, 1987). The ambient glasshouse temperature was maintained at 22–28°C during the trial. Hive box temperatures in this trial were in the range 28–35°C, 24–37°C and 23–33°C for colonies 1, 2 and 3, respectively. Despite these lower than optimal temperatures recorded, the colonies developed brood demonstrating good adaptation and health (Table 2). The lower colony temperatures recorded may be explained because the colonies were small and clustered away from the temperature probes and also were unexpectedly able to move the gently secured probes (Wang et al., 2016). Temperature probe installation therefore needs consideration in temperature effect studies. In any case, we demonstrate that this system can be easily established in a glasshouse for management of bees under different temperature and humidity regimes.

Table 2. Colony development observed over weeks 2–4

	Week	Eggs	Larvae	Pupae	Juvenile adults
Colony 1	2	162	47	0	0
	3	195	62	111	0
	4	235	43	184	43
	5	212	54	135	134
Total		804	206	430	177
Colony 2	2	144	0	0	0
	3	208	74	167	0
	4	263	38	135	62
	5	244	56	123	87
Total		859	168	425	150
Colony 3	2	103	0	0	0
	3	61	15	9	0
	4	74	8	18	7
	5	0	1	17	8
Total		238	24	44	15

Data are number of individuals.

The colour, intensity and duration of light will also influence the colony response to environmental and biological factors (Carrington et al., 2007). The natural light in this study proved sufficient for colony growth and brood development (Table 2; Fig. S2). However, this cage system also allowed provision of artificial UV and white light if required (Nye, 1962; Poppy and Williams, 1999; van Praagh, 1972).

For a successful colony, the cage conditions must be optimized so that the queen lays eggs. The queens from colonies 1 and 2 were active and survived for the entire duration of the trial. The queen from colony 3 was active during week 1 but in week 2 was observed outside the cage. We presume this happened when the cage door was opened to document colony metrics (Cobey, 2005; Tew, 2011). No other bees were outside the cage, and when the queen was returned to the hive box, she survived through the remaining trial period and continued egg laying. This suggests disturbance was the cause of her exiting the cage rather than non-acceptance. Eggs, larvae, pupae and juvenile adults were observed in all colonies (Table 2; Fig. S2). The reduced development observed in colony 3 probably reflects the time (no more than 5 days) that the queen was outside the hive.

The number of eggs laid by a queen bee is largely dependent on resource availability, the number of adult workers in the colony, the availability of open cells, her own fertility and the presence or absence of disease (Eckert et al., 1994; Moore et al., 2015; Root, 1978). During the cage trial, a total of 804, 859 and 238 eggs were recorded over the 4 weeks of development monitoring for colonies 1, 2 and 3, respectively (Table 2). Colony growth was 64%, 57% and −33% for colony 1, 2 and 3, respectively. Growth was significantly less than that expected under typical outdoor bee hive conditions, which was probably a response to the reduced cage and hive box size (Moore et al., 1998). Under optimal conditions, a queen honey bee can lay up to 1500 eggs per day (Winston, 1987). Nevertheless, colonies 1 and 2 displayed a typical growth pattern expected of a small healthy colony of bees in a restricted environment (Moore et al., 1998). Colony 3 observations suggested lower queen productivity, possibly as a consequence of the brief period in week 2 in which the queen was detected outside the cage without food or water.

Cumulative mortality for colonies 1, 2 and 3 was 51.6%, 57.8% and 39.6%, with the mean cumulative colony percentage mortality at 49.7±5.3% (mean±s.e.m.) by week 5 (Fig. S3). These data represent the first quantification of bee colony mortality in a cage system. With approximately 50% mortality, colonies (at least colonies 1 and 2) sustained healthy growth. The lower mortality in colony 3 is possibly due to the observed lower productivity of the queen in colony 3, resulting in reduced brood rearing and therefore less life-taxing work for colony 3 worker bees (Behrends and Scheiner, 2010; Johnson, 2003).

In conclusion, this study demonstrates that it is possible to keep whole bee colonies, that also show development, successfully in small cages for short-term, and probably longer term, experimental studies that require estimates of colony health, growth and intergenerational effects. The cage system is practicable and cost effective, and provides several advantages over other contemporary systems including good control of environmental conditions with ease of replication. While the specifications reported here will suit many experimental effect studies, the cage environment will need to

be adapted to match the experimental questions and scale of the study. This means that cage size, materials and human access will ultimately depend on the variables under investigation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.A.S., R.R., S.C.W.; Methodology: C.A.S., R.R., S.C.W.; Formal analysis: C.A.S., R.R.; Investigation: C.A.S., S.C.W.; Data curation: C.A.S.; Writing - original draft: C.A.S.; Writing - review & editing: R.R., S.C.W.; Supervision: R.R., S.C.W.; Project administration: S.C.W.

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