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Evaluating the Potential of Brood Recapping to Select *Varroa destructor* (Acari: Varroidae) Resistant Honey Bees (Hymenoptera: Apidae)

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Abstract

Several resistance traits have been proposed to select honey bees (*Apis mellifera* L.) that can survive in the presence of parasitic mite *Varroa destructor* (Anderson and Trueman) and enable a more sustainable apiculture. The interest for uncapping-recapping has recently increased following its identification in several naturally surviving honey bee populations, yet the utility of this trait for human-mediated selection is poorly known. Here, we evaluated the repeatability of recapping and its correlations with mite infestation levels, and assessed the expression of the trait in the often neglected drone brood. We also calculated correlations between recapping, mite infertility, and mite fecundity, expressed either at the level of individual brood cells or of the whole colony. Recapping measured in worker brood showed moderate repeatability (ranging between 0.30 and 0.46). Depending on sample, recapping slightly correlated negatively with colony infestation values. Recapping was also measured in drone brood, with values often comparable to recapping in worker brood, but no significant correlations were obtained between castes. At cell level, recapped cells in drone brood (but not in workers) were significantly less infested than nonrecapped cells, whereas in workers (but not in drones), recapped cells hosted mites with significantly lower fecundity. At colony level, with a few exceptions, recapping did not significantly correlate with mite infertility and fecundity, caste, sample, or number of infested cells considered. These results indicate limited possibilities of impeding mite reproduction and possibly mite infestation of honey bee colonies by recapping, which would need to be confirmed on larger, different populations.

Key words: honey bee, resistance, recapping, selection, varroa mite

Honey bees (*Apis mellifera*) are threatened by the parasitic mite *Varroa destructor* in many regions worldwide. This invasive pest, originating from the Asian honey bee *Apis cerana* Fabricius (Hymenoptera:Apidae) (Koeniger et al. 1981, Rath 1999) and spread by honey bee trade (Crane 1978, de Jong et al. 1982b, Owen 2017), is a key contributor to honey bee colony losses (Guzman-Novoa et al. 2010, Le Conte et al. 2010, Neumann and Carreck 2010). Mites feed on and therefore weaken developing and adult honey bees (de Jong et al. 1982a, Amdam et al. 2004, Aronstein et al. 2012), and transmit deadly viruses (Bowen-Walker et al. 1999, Dainat et al. 2012), leading to colonies usually succumbing within 1–4 yr in absence of varroacidal treatments (Korpela et al. 1992, Büchler 1994). The latter significantly improve colony survival (Beyer et al. 2018, Haber et al. 2019, Hernandez et al. 2022) but are not a reliable long-term management strategy (Dietemann et al. 2012), as they are costly, can harm the bees (Rademacher et al. 2017, Gashout et al. 2020), can generate treatment-resistant mites (Elzen et al. 2000, Hernández-Rodríguez et al. 2021), and contaminate bee products (Bogdanov 2006, Kast et al. 2021). To improve colony health, one privileged approach is the selection of honey bees capable of coping with the parasite by means of resistance mechanisms expected to limit the mite infestation level (Dietemann et al. 2012). The objective beyond this approach is to limit or suppress the need to treat colonies, enabling a more sustainable apiculture.

Observations in several naturally surviving populations or populations selected for resistance against *V. destructor* have shown adult workers uncapping (opening) and recapping (re-sealing) brood cells containing honey bee pupae, especially when the latter were mite-infested (Villa et al. 2009; Kirrane et al. 2015; Buchegger 2018; Oddie et al. 2018a,b, , 2021; Martin et al. 2019; Mondet et al. 2020a; Grindrod and Martin 2021a; Hawkins and Martin 2021). Recapping can be easily visualized on brood cell caps manually cut out and placed upside down: some caps display a brownish, granular wax surface of variable size, as opposed to the shiny and smooth aspect of cappings from nonrecapped cells, displaying remains of the silk produced by the honey bee during pupation (Büchler et al. 2017, Oddie et al. 2018b).

Researchers have hypothesized that uncapping-recapping could have a protective effect on honey bee colonies, as cell opening could lead to founder mites escaping the cell (Boecking 1994), or disrupt the mite reproductive cycle by either leading to a diminished fecundity (Buchegger 2018, Oddie et al. 2021) or to an elevated mite offspring mortality (Harris et al. 2012). This behavior can increase with higher *V. destructor* infestation levels (Villegas and Villa 2006, Grindrod and Martin 2021b, Hawkins and Martin 2021), suggesting that it can be triggered or amplified at a specific infestation threshold. It could target cells containing reproducing mites (Harris et al. 2012). However, given the contradictory results found by others (Sprau et al. 2021), this point is still debated. Uncapping-recapping is expected to be less costly for the colony than complete brood removal, as performed within the frame of varroa sensitive hygiene (VSH), because the host pupa is not destroyed and continues its development (Oddie et al. 2018b). As recapping has been found in association with VSH on several occasions (Villa et al. 2009, Harris et al. 2012, European Commission 2022) but not systematically (Kirrane et al. 2015), it has been described as an incomplete, less efficient form of VSH or as an independent resistance trait (Arathi et al. 2006, Oddie et al. 2018b, Van Alphen and Fernhout 2020, Hawkins and Martin 2021).

The fact that recapping is also observed in varroa-susceptible populations (Kirrane et al. 2015, Grindrod and Martin 2021a, Oddie et al. 2021) makes implementation of human-mediated selection conceivable to improve this trait (European Commission 2022). In this view, the utility of recapping should be verified before its adoption in a selection program. To be deemed useful for selection, a potential trait should fulfill certain conditions (Guichard et al. 2020): its measures should be accurate and repeatable, it should be heritable to ensure rapid genetic progress, and it should be associated with colony infestation and colony survival. This association could, in theory, be either a negative correlation (colonies with more recapping should have fewer mites), a positive correlation (colonies with more mites should recap more), or a threshold response (above a certain infestation, colonies should recap more). In the field, the trait should still effectively protect colonies and be selectable broadly, at acceptable costs, in different populations.

Currently, the capacity of the recapping trait to meet these conditions is poorly known (Guichard et al. 2020). For instance, information is lacking regarding the heritability and repeatability of this trait, with only a few values published for each parameter (Buchegger 2018, Büchler et al. 2020, Eynard et al. 2020), making responses to selection and genetic progress difficult to predict. The efficacy of this trait in impeding mite development is currently intensively debated (Oddie et al. 2019, van Alphen and Fernhout 2019): the impact of this trait on mite populations varies depending on populations and by study conditions. Some publications report an association between a higher recapping and a lower infestation level of brood or adult honey bees (Villa et al. 2009, Buchegger et al. 2018, Büchler et al. 2020), whereas others did not find one (European Commission 2022).

The importance of recapping as a key mechanism to explain the survival of nontreated honey bee populations is nuanced by the fact that this trait is present in some but not in all of them (Mondet et al. 2020a, Moro et al. 2021). In addition, recapping has a high variability among colonies, even in surviving populations or populations selected for other resistance traits (Villa et al. 2009, Büchler et al. 2020, Kovačić et al. 2020, Hawkins and Martin 2021, Oddie et al. 2021, Sprau et al. 2021, European Commission 2022). Recapping could therefore be facultative for colony survival, rather than the primary mechanism responsible for colony survival (Hawkins and Martin 2021). These findings, along with the available low heritability values (Buchegger 2018), suggest that recapping is highly affected by environmental effects (European Commission 2022), questioning its utility in protecting honey bee colonies irrespective of location and season.

The limited knowledge about the utility of recapping for selection has several origins. First, at the scale of the whole colony, as for other putative resistance traits (Guichard et al. 2020), links between recapping and colony infestation are incomplete, as recapping is only marginally measured in drone brood. Drone brood is preferentially infested by *V. destructor* (Schulz 1984, Fuchs 1990) and enables production of more mite offspring per brood cycle (Martin 1994, 1995), so recapping drone brood could have a major effect on mite populations, and thus needs to be assessed. Measuring recapping in drone brood could also simplify phenotypic evaluation in the frame of selection programs, as measurements could start earlier in the spring, a period when worker brood only contains a very small number of mites. A single observation reported that drone brood recapping is rare, even in naturally surviving colonies (Martin et al. 2019), which requires broader confirmation in samples that include different honey bee populations.

Second, the unestablished utility of the trait before its implementation in a selection program can result from the variable impacts of uncapping-recapping on mites infesting honey bee brood. The divergent effects of recapping on mite populations in individual cells or in the whole colony have been reported. At the cell level, researchers have associated recapping of infested brood cells with a lower fecundity (number of viable mated offspring produced by female founders) or a higher infertility (% of female founders producing no offspring) (Buchegger et al. 2018; Oddie et al. 2018b, 2021). However, some studies reported only a reduction in the number of live offspring (Harris et al. 2012), found no impact on mite fecundity or infertility (Hawkins and Martin 2021, Moro et al. 2021, Sprau et al. 2021), or even observed more offspring in recapped versus nonrecapped cells (Kirrane et al. 2015). To gain clarity, the relationship between recapping, mite fecundity, and mite infertility should be analyzed in more detail at the cell and colony levels. This requires the retrieval of mite infertility and fecundity, usually referred to as suppressed mite reproduction (SMR) (Büchler et al. 2020), or, more recently, covered by the more inclusive term of decreased mite reproduction (DMR) (von Virag et al. 2022), from the same colonies.

To address current knowledge gaps and overcome apparent contradictions between existing publications, we first evaluated the utility of recapping measured in worker brood for selection by gathering information on repeatability and correlations between traits and colony infestation. For this, phenotypes obtained at colony level were transformed into three different, fine-tuned variables: the recapping rate of infested cells, the recapping rate of all cells, independent of their infestation status (i.e., infested, or not), and the

selectivity of recapping, defined as the ratio of the two latter rates. Second, for a finer understanding of the trait, we compared the expression of recapping in drone brood with that in worker brood. Subsequently, we inspected associations between recapping and mite reproductive outputs (infertility-based and fecundity-based DMR). The last two phases were performed at both the cell and whole colony levels. To extend the range of populations covered by the literature, this analysis was performed on a population of 100 *Apis mellifera mellifera* colonies reared in Switzerland.

Materials and Methods

Honey Bee Colonies

The experiment was performed from spring to summer 2019 and 2020 on *Apis mellifera mellifera* colonies reared at the Swiss Bee Research Centre in Switzerland. These colonies, which descended from queens obtained from the Swiss beekeepers association mellifera.ch (www.mellifera.ch), were also involved in another study centered on the utility of DMR for resistance selection, in which the experimental setup was presented in detail (von Virag et al. 2022). Briefly, the queens heading the experimental colonies belonged to four maternal lineages, displaying either high or low hygienic behavior toward pin-killed brood or mite infestation levels. The aim of this divergent selection was to favor the presence of colonies with a broad diversity of resistance phenotypes in the experimental population. In summer 2019, queens for the colonies to be tested in 2020 were reared from four (one of each lineage) colonies tested in 2019 and were mated with drones of mellifera.ch-selected *A. m. mellifera* drone-producing colonies at a mating station. The experimental colonies were kept on a single apiary in 2019 ($N = 40$), and in 2020, the colonies ($N = 60$) were separated into two groups of 30, where one group was transferred to a new apiary.

To favor sufficient drone brood production for trait recording, the experimental colonies were stimulated by repeated sugar water feeding, and the queens were caged on drone combs about three weeks before the sampling date. Worker brood as well as drone brood were collected from the experimental colonies just ahead of summer treatment to maximize mite infestation. Depending on the egg laying dynamics of the queen, up to three combs sampled per colony were collected between 0 and 7 d apart, usually within 5 d, and stored at –20°C until dissection.

Recapping Phenotyping

Recapping was evaluated twice in worker brood cells aged between 7 and 12 d postcapping, as described in Büchler et al. (2017). Brood cell caps were carefully detached one by one with forceps and flipped to enable examination of their inner side under a stereomicroscope. Caps with either no silk (complete recapping) or at least an area without silk (partial recapping) were counted as recapped. In each sample, cell caps were evaluated in both infested and noninfested cells until 35 singly infested cells, targeted to evaluate mite fecundity and infertility (Büchler et al. 2017, Mondet et al. 2020b), were found. The total number of cells inspected per colony, therefore, depended on the brood infestation rate. Repeatability of recapping was calculated from two measures obtained on two distinct worker brood areas, which constitute biological replicates, hereafter designed as Worker1 and Worker2. Depending on the number of infested samples found, each replicate could cover one or a maximum of two of the sampled combs.

In each colony, for inter-caste trait comparison, recapping was also evaluated once by the same protocol in drone brood cells aged between 9 and 15 d postcapping (measurement hereafter referred to as Drone). A single trained person performed all examinations (Worker1, Worker2, and Drone) to standardize sampling conditions as much as possible.

Mite Reproduction and Infestation Phenotypes

In addition to recapping, DMR values, evaluated through mite infertility and mite fecundity, as well as mite infestation measures, were obtained on the experimental colonies. These data have already been published (von Virag et al. 2022).

Concisely, for DMR, after recapping phenotyping, the pupae were removed by means of forceps and, in case of single infestation, the number of offspring mites, the age of the offspring, and the age of the host were evaluated. For multiple infestations, only the number of founders was recorded. In singly infested cells, infertility was evaluated as the percentage of nonreproductive female founders, whereas fecundity corresponded to the predicted number of viable mated female offspring at emergence.

Mite infestation data were obtained by three complementary means: 1) the sum of all naturally fallen mites from the beginning of the beekeeping season, evaluated on the hive bottom board once or twice a week and hereafter designated as cumulated natural mite fall (unit: mites); 2) the percentage of infested workers obtained by washing a sample of approximately 300 adult workers on the day when the queen was caged on the drone comb (unit: mites/100 adult workers); and 3) the percentage of brood infestation for the three biological replicates (Worker1, Worker2, and Drone) obtained during DMR evaluation (unit: % of infested cells).

Data Analysis

To provide detailed data analyses, recapping phenotypes were transformed into three different variables. The first variable was the recapping rate of infested cells, the second was the recapping rate of all cells (infested or not), and the third was the selectivity of recapping. Selectivity of recapping was obtained as a ratio of both latter rates, and qualified the preference for infested cells versus randomly chosen cells. Selectivity values close to zero indicate that infested cells were avoided, whereas values greater than one indicate that infested cells were preferentially recapped. The two first variables are hereafter indistinctly referred to as 'recapping', except for instances of specific variable descriptions.

As the first step, the utility of recapping for colony selection was analyzed. After a rapid comparison between the three considered recapping variables, the repeatability of recapping between worker samples and correlations between recapping, mite reproduction, and mite infestation at the colony level were calculated. For this, given that not all variables were normally distributed, as confirmed by a Shapiro–Wilk's test, rank correlations between traits or samples were obtained by Kendall's tau b method after correction for fixed effects (year-apiary). For correlations between recapping and other traits, either all data or only those of colonies in which 35 singly infested cells could be found, which corresponds to a recommended minimal number of single infested cells for mite reproduction analyses (Büchler et al. 2017, Mondet et al. 2020b), were included. Later, both worker brood replicates (Worker1 and Worker2) were pooled (Worker1 + Worker2), with the aim of improving the reliability of the correlations. Given the low number of available colonies, we did not calculate heritabilities, that would not have resulted in meaningful estimates.

In the second step, to compare the impact of castes on the phenotype of the entire colony, rank correlations between recapping

measured in drone versus worker brood cells were obtained, as detailed above. To better estimate the potential effect of recapping as a resistance trait against *V. destructor*, the association between recapping and the mite content of the individual cells—that is, the infestation rate, mite infertility, and mite fecundity—was evaluated. The effect of recapping on the infestation rate and mite fecundity in individual cells (quantitative data) was verified by a Kruskal–Wallis rank sum test, and between-measurement differences were analyzed by pairwise Wilcoxon tests (Bonferroni-adjusted). The effect of recapping on mite infertility (categorical data) was analyzed using Pearson's chi-squared test. Rank correlations between DMR and recapping at the scale of the whole colony were calculated as described above. All analyses were performed with R (R-Core-Team 2018).

Results

With the exception of colonies that requeened or swarmed during the evaluation period, brood samples were taken from 83 colonies; this corresponds to 82, 79, and 80 measurements for Worker1, Worker2, and Drone, respectively. In 28, 34, and 13% of these measurements, respectively, the minimum number of 35 singly infested cells could not be reached. In total, 86,394 cappings were observed in Worker1 measurements (including 2,719 from infested cells), 56,224 for Worker2 (including 2,376 from infested cells), and 12,747 for Drone (including 3,620 from infested cells) brood.

To improve readability, only significant (p <0.05) correlation values are presented in Tables 1–3, with 'ns' (nonsignificant) displayed for the other values. Detailed statistics (value, corresponding sample size, p values) for all calculated correlations are provided as supplementary material. The density plots of the different variables (recapping of infested cells, recapping of all cells, and selectivity of recapping) for each type of sample (Worker1, Worker2, and Drone) are also provided (Supp Fig. 1 [online only]).

Comparison of Recapping Variables

Recapping of infested cells correlated relatively strongly with the recapping of all cells in all three samples (Worker1: $\tau = 0.79$, $N = 82$, $p < 2.2e^{-16}$; Worker2: $\tau = 0.89$, $N = 79$, $p < 2.2e^{-16}$; Drone: $\tau = 0.95$, $N = 80$, $p < 2.2e^{-16}$). There were markedly low correlations observed between the recapping of infested cells and the selectivity of recapping (Worker1: $\tau = 0.28$, $N = 75$, $p = 0.01$; Worker2: $\tau = 0.06$, $N = 67$, $p = 0.63$; Drone: 0.15, $N = 53$, $p = 0.28$), and between the recapping of all cells and the selectivity of recapping (Worker1: $\tau = -0.05$, $N = 75$, $p = 0.70$; Worker2: $\tau = -0.08$, $N = 67$, $p = 0.51$; Drone: $\tau = 0.01$, $N = 53$, $p = 0.96$).

Repeatability of Recapping Phenotyping

Rank correlation between Worker1 and Worker2 biological replicates revealed a medium, significant repeatability for recapping of infested cells when all data were included ($\tau = 0.33$, $N = 78$, $p = 5.1e^{-5}$), which was higher when restricting the dataset to only the colonies for which a minimum of 35 singly infested cells could be found ($\tau = 0.45$, $N = 49$, $p = 1.7e^{-5}$). Similar values were obtained for recapping of all cells ($\tau = 0.30$, $N = 79$, $p = 8.1e^{-5}$ and $\tau = 0.46$, $N = 49$, $p = 3.2e^{-6}$, respectively), whereas the selectivity of recapping had much lower repeatability values ($\tau = 0.08$, $N = 64$, $p = 0.37$ and $\tau = 0.13$, $N = 38$, $p = 0.28$, respectively).

Influence of Recapping on *V. destructor* Infestation at the Colony Level

At the colony level, significant, low correlations were obtained between recapping evaluated in Worker1 and Drone and estimated colony infestations, while none was obtained for recapping measured in Worker2 (Table 1, Supp Table 1 [online only]). The sample pool Worker1 + Worker2 occasionally correlated significantly with colony infestation (Table 1, Supp Table 2 [online only]). The absolute values of the significant correlations ranged between 0.16 and 0.25. Recapping of infested cells, recapping of all cells, and selectivity of recapping exhibited eight, six, and five significant correlation values, respectively, when compared with infestation, but these values did not necessarily correspond to the same couples of variables. Recapping never significantly correlated with adult infestation, although it significantly correlated with the brood infestation rate of Worker 1 on eight occasions, with the cumulative natural mite fall on five occasions, and with the brood infestation rate of Drone on three occasions. Selectivity of recapping in Worker1 + Worker2 positively correlated with infestation variables on two occasions, whereas all other correlations were negative (Table 1, Supp Table 2 [online only]).

Recapping Values Acquired From Drone Brood Compared to Worker Brood

On average, at the colony level, recapping rates of infested cells were lower in Drone (5.4%) than in Worker1 and Worker2 (9.4 and 11.2%, respectively) (Fig. 1). A Kruskal–Wallis rank sum test indicated that the recapping rate of infested brood significantly differed between measurements ($p = 0.049$). However, following the pairwise Wilcoxon rank sum tests, no p-value was below the significance threshold of 0.05, so the three samples did not provide significantly distinct values (pairwise Wilcoxon rank sum tests: Drone versus Worker1: $p = 0.056$, Drone versus Worker2: $p = 0.186$, Worker1 versus Worker2: $p = 1.00$, p_{adjust}: Bonferroni). Recapping rate of all cells obtained at colony level was lower (means = 3.2% for Worker1, 5.2% for Worker2, and 4.7% for Drone) and did not significantly differ according to sample following the Kruskal–Wallis rank sum test ($p = 0.83$) (Fig. 1). Selectivity of recapping was lowest in Drone (mean = 1.17) compared to Worker1 (mean = 3.43) and Worker2 (mean = 3.40), and significantly differed according to sample (Kruskal rank sum test, $p = 0.03$) (Fig. 1). Significant correlations, ranging between 0.23 and 0.37, were obtained for recapping of all cells and selectivity of recapping when Worker1 and Drone were compared. However, the recapping of infested cells obtained either from Worker1 or Worker2 replicates did not significantly correlate with the Drone measurement (Table 2).

Influence of Recapping on DMR and *V. destructor* Infestation at the Cell Level

The influence of recapping on brood infestation, as well as on mite infertility and mite fecundity measured at the cell level, is presented in Table 4. In Drone, the average number of founders per infested brood cell was significantly ($p = 0.02$) lower in recapped cells (mean = 1.23, $N = 194$) as opposed to nonrecapped cells (mean = 1.38, $N = 3426$), whereas it was nearly equal irrespective of cap status for Worker1 and Worker2 (means = 1.05 and 1.06, respectively).

Recapped cells showed a higher infertility rate of mites than nonrecapped cells in Worker1, Worker2, and Drone (26 vs. 19% in Worker1, 28 vs. 22% in Worker2, and 38 vs. 34% in Drone). However, these differences in infertility between recapped and nonrecapped cells were only significant (p <0.05) following Pearson's chi-squared test for Worker1. The number of viable mated female offspring per founder (fecundity) in singly infested brood cells was significantly ($p < 10^{-3}$) lower in recapped worker cells of Worker1 (mean = 1.01, $N = 229$) and Worker2 (mean = 1.01, $N =$

Table 1. Correlations of recapping of infested brood cells with *V. destructor* infestation rates, with either all data included or only samples with 35 singly infested cells. Infestations rate estimates of adult workers, brood, and colony (as cumulative natural mite fall) were used. Kendall's tau b coefficients (τ) are given, as well as *p*-values (*p*) and sample size (*N*) associated with each correlation. Correlations which significantly ($p < 0.05$) differed from zero are indicated in bold, and p values are given as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$. All correlations involving adult infestation were nonsignificant and are to be found in supplementary material (SuppTables 1 and 2 [online only])

			Cumulative natural mite fall	Worker1 brood infestation	Worker2 brood infestation	Drone brood infestation	Worker1 + Worker2 brood infestation
Recapping of infested brood cells	All data included	Worker1	**−0.17** *	**−0.21** **	−0.14 (ns)	−0.13 (ns)	
		Worker2	−0.09 (ns)	−0.15 (ns)	0 (ns)	−0.04 (ns)	
		Worker1 + Worker2	**−0.16** *	**−0.21** **	−0.11 (ns)	−0.08 (ns)	**−0.20** **
		Drone	−0.14 (ns)	−0.13 (ns)	0.02 (ns)	−0.15 (ns)	
	35 singly infested cells/sample	Worker1	**−0.22** *	**−0.24** *	**−0.23** *	−0.17 (ns)	
		Worker2	−0.12 (ns)	−0.19 (ns)	−0.12 (ns)	−0.08 (ns)	
		Worker1 + Worker2	−0.18 (ns)	−0.18 (ns)	−0.15 (ns)	−0.05 (ns)	−0.16 (ns)
		Drone	−0.17 (ns)	−0.09 (ns)	0.12 (ns)	−0.16 (ns)	
Recapping of all brood cells	All data included	Worker1	−0.07 (ns)	**−0.16** *	−0.04 (ns)	−0.06 (ns)	
		Worker2	0.01 (ns)	−0.12 (ns)	0.10 (ns)	0.08 (ns)	
		Worker1 + Worker2	−0.06 (ns)	**−0.16** *	−0.01 (ns)	0.00 (ns)	−0.14 (ns)
		Drone	−0.04 (ns)	**−0.21** **	−0.06 (ns)	**−0.23** **	
	35 singly infested cells/sample	Worker1	−0.06 (ns)	−0.16 (ns)	−0.08 (ns)	−0.02 (ns)	
		Worker2	−0.06 (ns)	−0.14 (ns)	−0.05 (ns)	0.04 (ns)	
		Worker1 + Worker2	−0.07 (ns)	−0.13 (ns)	−0.05 (ns)	0.04 (ns)	−0.10 (ns)
		Drone	−0.06 (ns)	**−0.25** *	−0.05 (ns)	**−0.21** *	
Selectivity of recapping (% rec infested cells/%rec all cells)	All data included	Worker1	**−0.18** *	−0.13 (ns)	−0.13 (ns)	**−0.19** *	
		Worker2	−0.13 (ns)	−0.12 (ns)	−0.10 (ns)	−0.12 (ns)	
		Worker1 + Worker2	0.02 (ns)	**0.23** **	0.13 (ns)	0.03 (ns)	**0.20** *
		Drone	−0.18 (ns)	−0.02 (ns)	0.02 (ns)	−0.11 (ns)	
	35 singly infested cells/sample	Worker1	**−0.22** *	−0.11 (ns)	−0.07 (ns)	−0.19 (ns)	
		Worker2	−0.11 (ns)	−0.13 (ns)	−0.18 (ns)	−0.14 (ns)	
		Worker1 + Worker2	−0.02 (ns)	0.17 (ns)	0.14 (ns)	0.02 (ns)	0.16 (ns)
		Drone	−0.22 (ns)	−0.01 (ns)	0.02 (ns)	−0.14 (ns)	

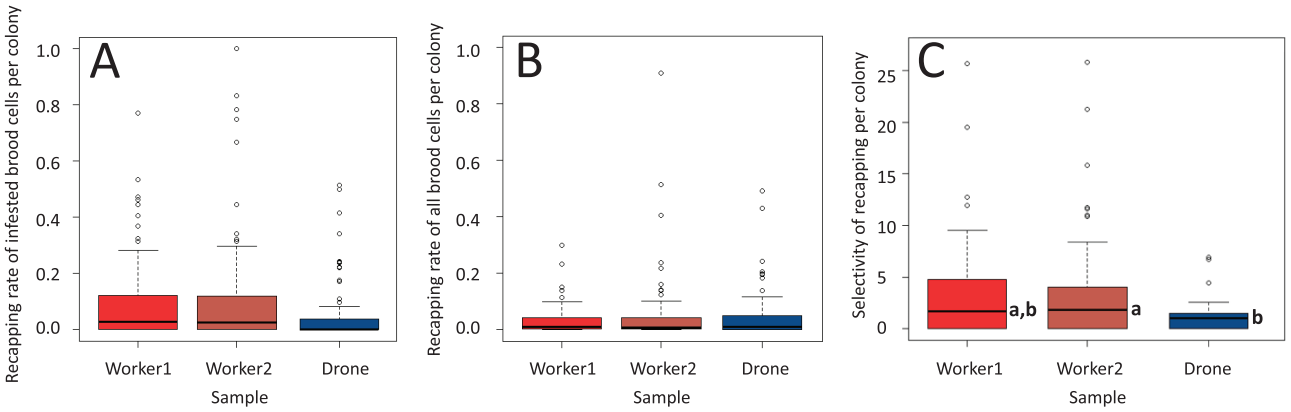

Fig. 1. Recapping rate of infested brood cells per colony (A), defined as the number of recapped infested cells divided by the total number of infested cells investigated; recapping rate of all brood cells per colony (B), defined as the number of recapped cells (infested or not infested) divided by the total number of cells investigated (infested or not infested) and selectivity of recapping per colony (C), defined as a ratio where the recapping rate of infested brood cells per colony is divided by the recapping rate of all brood cells. Selectivity of recapping indicates to what extent colonies tend to preferentially recap cells which are infested. For each trait, the data is presented for the three brood samples (Worker1, Worker2, and Drone). Box plots represent minimum value, first quartile, median, third quartile, and maximum values. Dots indicate points located more than 1.5 times above or below the interquartile range. Different letters indicate significant ($p < 0.001$) differences between groups following a pairwise Wilcoxon test.

Table 2. Correlations for recapping of infested brood cells compared between worker samples (Worker1 and Worker2) and Drone sample, with either all data included or only samples with 35 singly infested cells. Kendall's tau b coefficients (τ) are given. Correlations which significantly ($p < 0.05$) differed from zero are indicated in bold, and p values are given as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, nonsignificant (ns) $p > 0.05$

			Drone
Recapping of infested brood cells	All data included	Worker1	0.10 (ns)
		Worker2	0.11 (ns)
	35 singly infested cells/sample	Worker1	0.19 (ns)
		Worker2	0.16 (ns)
Recapping of all brood cells	All data included	Worker1	**0.23** **
		Worker2	0.12 (ns)
	35 singly infested cells/sample	Worker1	**0.38** ***
		Worker2	0.14 (ns)
Selectivity of recapping (% rec infested cells/%rec all cells)	All data included	Worker1	**0.29** **
		Worker2	0.09 (ns)
	35 singly infested cells/sample	Worker1	**0.37** **
		Worker2	0.19 (ns)

242) compared to nonrecapped cells (means = 1.12 (N = 2,359) and 1.08 (N = 1,997), respectively). Capping status had no significant (p = 0.13) impact on fecundity in Drone (mean = 2.14 (recapped, N = 153) to 2.31 (nonrecapped, N = 2,484)).

Influence of Recapping on DMR at the Colony Level

At the colony level, only a rare significant rank correlation was obtained between recapping and DMR, expressed either as infertility or fecundity (Table 3, detailed in Supp Tables 4 and 5 [online only]). This was the case on two occasions with the recapping rate of all cells (−0.18 to −0.16) and four times with the selectivity of recapping (|τ| comprised between 0.22 and 0.24), but never with the recapping rate of infested cells (Table 3).

Discussion

The aim of our study, whose subject was a Swiss population of 100 *A. m. mellifera* colonies, was first to evaluate the utility for the selection of recapping, evaluated through three different variables: recapping of infested cells, recapping of all cells, and selectivity of recapping of mite-infested cells. Second, we compared recapping in worker and drone brood to gain a more precise understanding of the trait and define the best caste for trait recording. We further investigated the impact of recapping on mite infestation and reproduction (DMR). The results were obtained both at the level of individual cells and at the colony level.

High correlations, between 0.7 and 0.95, were found between the recapping of infested cells and the recapping of all cells, irrespective of their mite content, for all three samples (Worker1, Worker2, Drone), indicating that a detailed brood investigation limited to an observation of the cell cappings could be sufficient to identify the colonies with a higher probability of recapping infested cells. This could limit the cost involved in the analysis of recapping. By contrast, the selectivity of recapping showed only low to medium correlations with the two other variables, indicating that colonies that recap brood, to a greater extent, do not necessarily target infested cells more efficiently, and vice versa.

In this study, we observed relatively low levels of recapping of infested cells in worker brood in the considered colonies, which, on average, ranged between 8.8% (Worker1) and 10.9% (Worker2) (Fig. 1, Supp Fig. 1 [online only]). However, the variation between colonies was relatively large, with values per colony ranging

Table 3. Correlations of recapping of infested brood cells with infertility- or fecundity-based DMR, with either all data included or only samples with 35 singly infested cells. Worker1 + Worker2 corresponds to the sample pool of Worker1 and Worker2. Infestations rate estimates of adult workers, brood and colony (as natural mite fall) were used. Kendall's tau b coefficients (τ) are given. Correlations which significantly ($p < 0.05$) differed from zero are indicated in bold, and p values are given as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$. All correlations involving Worker1 infertility, Worker2 infertility, and Worker2 fecundity were nonsignificant and are to be found in supplementary material (Supp Tables 4 and 5 [online only])

			Worker1 fecundity	Drone infertility	Drone fecundity	Worker1 + Worker2 infertility	Worker1 + Worker2 fecundity
Recapping of infested brood cells	All data included	Worker1	−0.09 (ns)	0.07 (ns)	−0.06 (ns)		
		Worker2	−0.06 (ns)	−0.01 (ns)	−0.06 (ns)		
		Worker1 + Worker2	−0.06 (ns)	0.05 (ns)	−0.09 (ns)	0.08 (ns)	−0.09 (ns)
		Drone	−0.01 (ns)	0.03 (ns)	−0.05 (ns)		
	35 singly infested cells/sample	Worker1	−0.11 (ns)	0.02 (ns)	−0.05 (ns)		
		Worker2	−0.15 (ns)	−0.12 (ns)	0.06 (ns)		
		Worker1 + Worker2	−0.09 (ns)	−0.02 (ns)	−0.06 (ns)	0 (ns)	0 (ns)
		Drone	−0.12 (ns)	0.06 (ns)	−0.10 (ns)		
Recapping of all brood cells	All data included	Worker1	−0.12 (ns)	0.12 (ns)	−0.12 (ns)		
		Worker2	−0.07 (ns)	−0.02 (ns)	−0.06 (ns)		
		Worker1 + Worker2	−0.10 (ns)	0.08 (ns)	**−0.16** *	0.08 (ns)	−0.08 (ns)
		Drone	−0.10 (ns)	0.12 (ns)	−0.12 (ns)		
	35 singly infested cells/sample	Worker1	**−0.18** *	0.02 (ns)	−0.10 (ns)		
		Worker2	−0.16 (ns)	−0.05 (ns)	−0.01 (ns)		
		Worker1 + Worker2	−0.17 (ns)	0.05 (ns)	−0.14 (ns)	0.09 (ns)	−0.10 (ns)
		Drone	−0.13 (ns)	0.13 (ns)	−0.15 (ns)		
Selectivity of recapping (% rec infested cells/%rec all cells)	All data included	Worker1	−0.04 (ns)	0.05 (ns)	−0.05 (ns)		
		Worker2	−0.01 (ns)	0.06 (ns)	−0.04 (ns)		
		Worker1 + Worker2	0.04 (ns)	−0.14 (ns)	0.13 (ns)	−0.09 (ns)	0.08 (ns)
		Drone	0.17 (ns)	−0.08 (ns)	0.11 (ns)		
	35 singly infested cells/sample	Worker1	−0.02 (ns)	0.02 (ns)	−0.02 (ns)		
		Worker2	−0.06 (ns)	−0.06 (ns)	0.05 (ns)		
		Worker1 + Worker2	0.12 (ns)	**−0.24** *	**0.24** *	**−0.23** *	**0.22** *
		Drone	0.10 (ns)	−0.04 (ns)	0.08 (ns)		

Table 4. relation between the capping status of brood cells (recapped or nonrecapped), the number of infesting founders per cell and the fecundity of singly infesting founders for the three sample (Worker1, Worker2, and Drone). Significant ($p < 0.05$) effects are indicated in bold

	Worker1	Worker2	Drone
N total inspected cells, across all colonies	86,394	56,224	12,747
N total cells recapped	3,077	2,494	650
% total cells recapped	3.6%	4.4%	5.1%
N total infested cells, across all colonies	2,719	2,376	3,620
N infested cells recapped	240	259	194
% infested cells recapped	8.8%	10.9%	5.4%
N total noninfested cells, across all colonies	83,675	53,848	9,127
N noninfested cells recapped	2,837	2,235	456
% noninfested cells recapped	3.4%	4.2%	5.0%
Infestation rate, across all colonies	3.1%	4.2%	28.4%
Recapping selectivity: Recapping rate infested cells/Recapping rate all cells	2.5	2.5	1.1
Sig. effect of recapping on infestation rate?	No,	No,	**Yes,**
	Kruskal test	Kruskal test	**Kruskal test**
	p -value: 0.89	*p*-value: 0.85	***p*-value: 0.02**
nb founders/cell *recapped cells*	μ = 1.05	μ = 1.06	**μ = 1.23**
	median = 1.00	median = 1.00	**median = 1.00**
	(*N* = 240)	(*N* = 259)	**(*N* = 194)**
nb founders/cell *nonrecapped cells*	μ = 1.05	μ = 1.06	**μ = 1.38**
	median = 1.00	median = 1.00	**median = 1.00**
	(*N* = 2,479)	(*N* = 2,117)	**(*N* = 3,426)**
Sig. effect of recapping on mite infertility?	**Yes,**	No,	No,
Only cells with 1 founder included	**Pearson's Chi-squared test**	Pearson's Chi-squared test	Pearson's Chi-squared
	***p*-value: 0.02**	*p*-value: 0.38	test
			p-value: 0.41
% of infertile mites *recapped cells*	**26% (60/229)**	28% (60/242)	38% (58/153)
% of infertile mites *nonrecapped cells*	**19% (455/2,359)**	22% (441/1,997)	34% (852/2,484)
Sig. effect of recapping on mite fecundity?	**Yes,**	**Yes,**	No,
Only cells with 1 founder included	**Kruskal test**	**Kruskal test**	Kruskal test
	***p*-value: 8.8×10^{-4}**	***p*-value: 1.3×10^{-3}**	*p*-value: 0.13
nb of viable mated offspring/founder *recapped cells*	**μ = 1.01**	**μ = 1.01**	μ = 2.14
	median = 1.32	**median = 1.32**	median = 2.76
	(*N* = 229)	**(*N* = 242)**	(*N* = 153)
nb of viable mated offspring/ founder *nonrecapped cells*	**μ = 1.12**	**μ = 1.08**	μ = 2.31
	median = 1.45	**median = 1.45**	median = 3.52
	(*N* = 2,359)	**(*N* = 1,997)**	(*N* = 2,484)

between 0 and more than 50%. For Worker2, some colonies had close to 100% recapping. Our average values for the recapping rates of infested worker brood cells were below the mean value (33%) reported in *V. destructor*-susceptible colonies from other populations, and far below the mean value obtained for resistant colonies (55%) (Grindrod and Martin 2021a). The susceptible status of our population was confirmed by the fact that the colonies had to be treated at the end of each evaluation season to enable colony survival. Therefore, our results should be interpreted knowing that our colonies do not cover the full range of theoretically observable recapping, that is, including Varroa-resistant colonies (only a few colonies had samples with recapping of more than 50% of the infested cells). Given that recapping can also vary according to the infestation levels of colonies (Villegas and Villa 2006, Grindrod and Martin 2021b, Hawkins and Martin 2021), different results could probably have been obtained on the same colonies if exposed to higher mite infestations, for example, when climatic conditions favor the build-up of high mite populations. Recapping of all brood cells was, on average, lower than recapping of infested cells, and the selectivity of recapping was, on average, higher than one for all samples (Table 4). This suggests that workers are selective in their targeting of brood cells, preferring to uncap and recap cells containing at least one mite, as already mentioned in the literature for other susceptible populations (Oddie et al. 2018b).

A main difficulty with the recapping assessment method employed here (Büchler et al. 2020), is that all cells without mite were considered as noninfested, even if they could have been initially infested and the mite could have escaped between uncapping and recapping. Therefore, we cannot exclude the possibility that we underestimate the recapping rate of infested cells (in the sense of cells that have contained a mite at least within the first hours-days after initial cell capping) and the selectivity of recapping. As we did not find any studies that accounted for cases of escaped mites, we suggest that future studies should consider them, for instance, by documenting the presence or absence of characteristic mite fecal deposits in inspected brood cells (Dietemann et al. 2013).

Recapping in worker brood showed a moderate, yet significant ($p < 10^{-4}$) repeatability, varying between 0.30 and 0.46, depending on the variable (recapping of infested cells or recapping of all cells) and on whether all data were included (i.e., including data from colonies where the recommended threshold of 35 singly infested cells was not reached) or restricted to those corresponding to colonies in which 35 singly infested cells were found. Although calculation methods vary between studies, our values are in line with a previously published value from another population (0.35 in Büchler et al. 2020) and in the same order of magnitude as that of hygienic behavior toward dead brood (0.21 in [Eynard et al. 2020], 0.33 in [Büchler et al. 2020]), another trait theoretically implementable in

honey bee selection. Based on our data, there is a relatively high probability that the colonies that recap the most can be detected by worker brood sampling, irrespective of the sampling region in the brood, and even when less than 35 singly infested cells are found per sample. This, together with the results above that recapping of infested cells is highly correlated with the recapping of all cells, suggests that a relatively easy identification of the best colonies could be done in a selection program.

To predict the effect of selecting for recapping on colony infestation, correlations between recapping and infestation were calculated (Table 1, detailed in Supp Tables 1 and 2 [online only]). Recapping of mite-infested cells as well as recapping of all cells retrieved from Worker1 brood samples showed significant correlations mainly with cumulative natural mite fall and brood infestation. Recapping of Worker2 did not significantly correlate with these infestation estimation methods, but signs of corresponding correlations (i.e., negative) were the same, and values could potentially be improved in a larger dataset. This possibility was confirmed when Worker1 and Worker2 were summed to a single observation Worker1 + Worker2 (Table 1, Supp Table 2 [online only]); recapping of the latter significantly correlated occasionally with cumulative natural mite fall of the colonies as well as with brood infestation of Worker1 + Worker2. In agreement with existing studies (Buchegger et al. 2018, Büchler et al. 2020), this supports the notion that a higher recapping level in colonies could be associated with a limited development of mite infestations. Given that many correlations were not significant and that the experimental colonies, on average, exhibited only low recapping levels, this relationship should be verified more precisely by adding more colonies with high recapping rates to the experimental population.

We also investigated differences in recapping between castes. The level of recapping in the infested drone brood was a little lower than in the worker brood (on average, 5.4% in Drone, compared to 8.8% in Worker1 and 10.9% in Worker2; Fig. 1), but the difference was not significant, whereas no significant difference was found regarding the recapping rate of all cells. This indicates that even at low occurrence, recapping of drone brood can be comparable to that of worker brood, advocating for the inclusion of the male brood in trait utility analyses. Significant, low correlation between worker and drone brood samples was obtained only for recapping of all cells and selectivity of recapping, indicating that brood type may influence the way recapping is performed (Table 2, Supp Table 3 [online only]). For example, different mechanisms or different sensitivity thresholds triggering recapping could potentially be linked to brood caste and infestation levels. This became visible in the investigation of the selectivity of recapping in worker brood, which was about three times higher than in drone brood (Table 4).

However, we cannot exclude the possibility that the lower selectivity in drone brood was due to a higher infestation level in this brood type, and to a potentially linked response threshold of the colony (workers could inspect more cells in a highly infested area). Given the lack of data on the recapping of drone brood in the literature, more information is needed to conclude on this point. The fact that recapping in drone brood and recapping in worker brood did not correlate the same way as the infestation parameters (the corresponding couples of variables differed) suggests that acquiring recapping values from drone brood may provide results different from those of worker brood. Further, as all correlations had the same magnitude, using drone brood may not be more accurate to identify less infested honey bee colonies. This result should be confirmed at different time points, for instance, in the spring instead of summer, as done here, when sampling in drone brood could be more convenient than sampling in worker brood to find a sufficient number of mites. Meanwhile, it would seem more judicious to use the worker brood to measure recapping rates.

At the level of individual cells, clear differences between castes appeared in terms of the infestation level of recapped cells as well as mite fecundity (Table 4). In the Drone samples, the infestation level of brood cells, estimated by the number of founders per cell, was significantly lower in recapped cells than in nonrecapped cells. In the two worker brood samples (Worker1 and Worker2), the mean number of founder mites per cell did not vary according to the status of the cell cap. This latter result is distinct from one reported in the literature, in which a higher recapping frequency has been observed in cases of multiple infestations (Oddie et al. 2021), possibly due to differences between populations. In our dataset, multiple infestations were, for instance, infrequent in worker brood. The lower infestation in recapped drone brood could mean that some founders escaped from the cell between the uncapping and the recapping (Boecking 1994). Differences compared to the worker brood could originate from the fact that in the case of multiple infestations, which are more frequent in drones, the probability that at least one mite leaves the cell after uncapping (Boecking 1994) is likely higher than in singly infested cells.

The mite infertility rate in recapped cells of all samples (Worker1, Worker2, and Drone) was higher than in nonrecapped cells, suggesting that the opening and re-sealing of brood cells could interfere with mite reproduction (Buchegger et al. 2018; Oddie et al. 2018b, 2021). However, for unidentified reasons, the differences were only significant for Worker1, pointing out that more data could be required to conclude on this point. Significant differences in terms of fecundity were found between nonrecapped and recapped worker cells, where it was significantly lower, but not between nonrecapped and recapped drone cells (Table 4). This result suggests that uncapping and recapping can affect mite fecundity in worker brood, which is in agreement with some previous findings (Oddie et al. 2018b, 2021) but contradicts others (Kirrane et al. 2015, Hawkins and Martin 2021, Moro et al. 2021, Sprau et al. 2021). However, the differences in fecundity between the nonrecapped and recapped worker cells were relatively small (in general, around 0.10 mite offspring less when the cell was recapped).

Differences in terms of fecundity were also found in drone brood, but the absence of significance could be explained by the proportionally lower number of recapped cells in this caste. In our analysis, we worked with frozen frames, so the impact of recapping in terms of offspring mortality, which has been analyzed in at least another study (Harris et al. 2012), could not be investigated. At the colony level, correlations between recapping, infertility-based, and fecundity-based DMR were calculated. Almost no significant correlations were found between recapping and infertility-based and fecundity-based DMR, irrespective of the dataset considered (all data versus only data from samples with 35 singly infested found) (Table 3, Supp Tables 4 and 5 [online only]). When Worker1 and Worker2 were summed to a single observation Worker1 + Worker2, some significant correlations were obtained between recapping selectivity, mite infertility, and fecundity in both drone brood and summed worker brood, but it should be noted that the corresponding data size was low (Table 3, Supp Table 5 [online only]).

In a previous analysis of the same dataset (von Virag et al. 2022), and in another study (Büchler et al. 2020), DMR had been found to be a trait with a low repeatability, so the absence of correlation with other resistance traits at colony level is not surprising. As previously mentioned by Oddie et al. (2018b), absence of clear correspondence between recapping and DMR at colony level could be due

to a bias of bees, which may more selectively target infested cells or cells containing reproducing mites, causing a reduction of fecundity in recapped cells but not in nonrecapped cells, as observed in Table 3. We created a recapping selectivity ratio to verify whether colonies recapping more selectively would have a better correlation between recapping and mite infertility or fecundity. However, this was not the case, perhaps because certain mite infertility or fecundity thresholds must be reached before any significant outcome can be observed. Therefore, colonies with an overall high DMR can display low mite fecundity, explained either by recapping (active disturbance of reproduction by workers uncapping and recapping the cells) or by other yet unidentified reasons.

Compared to DMR, recapping evaluated in worker brood appeared to be a more suitable trait for mite resistance selection; it showed higher repeatability and correlated better with infestation measurements (von Virag et al. 2022). Drone brood, which could enable an early trait evaluation due to the preference of mites for this brood type, exhibited results that differed from those obtained in worker brood, and appeared less suitable for recapping evaluations due to a lower specificity of recapping toward mite-infested cells in this brood type. However, even in worker brood, both the repeatability of recapping and its correlation with colony infestation showed moderate values. It is uncertain whether this trait can guarantee an operational protective effect for colonies, enabling at least a reduction or even a complete cessation of miticide treatments, or how rapidly such protection could be achieved. The same type of study should be repeated on a larger number of colonies, also including some with higher recapping rates. Estimating heritabilities, which was not feasible here, given the limited number of colonies, could then become possible.

Given the fraction of observed variation of additive genetic origin, heritability values are crucial to predict the success and speed of genetic progress that can be expected in a selection program (Büchler et al. 2020, Guichard et al. 2020, Uzunov et al. 2022). Even if it is less tedious than DMR or VSH, evaluating recapping remains relatively time-consuming in the field and is currently rarely implemented (Buchegger 2018, Uzunov et al. 2022). Designing simplified, field-realistic protocols is necessary. A previous attempt to propose an easier method to evaluate recapping ended with unconvincing results, such as low heritabilities and low correlations with mite infestation (Guichard et al. 2021). The development of genome-based methods, however, appears promising to gain knowledge on the genetic background of recapping and to enable the emergence of genomic marker-assisted selection (Brascamp et al. 2018, Bernstein et al. 2021, Guichard et al. 2022), which might help increase the speed of genetic progress. Our results confirm the difficulty of identifying suitable traits for successful resistance selection programs but suggest that recapping is one of the promising candidate traits that may lead to colonies with an elevated resistance against *Varroa destructor*, or at least a significant reduction in the need for miticide treatments.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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